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Acute lung injury associated with thoracic surgery

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**Submitted in fulfilment of the requirements for the
Degree of Doctor of Medicine**

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Dedication

To Jenny, Kate, Jamie and Maisie,
with thanks for the sacrifices, apologies for the misery and
excitement about the future.

Abstract

Lung cancer is the most common cause of cancer death in the UK. In suitable cases, the best chance of cure is surgical resection. Due to high levels of co-morbidity seen in this population, lung resection is associated with high cardio-respiratory complication rates. One such complication is the development of Acute Lung Injury / Acute Respiratory Distress Syndrome (ALI/ARDS). ALI/ARDS is reported to occur in four to 11% of patients undergoing lung resection and is the major cause of hospital mortality following lung resection.

ALI/ARDS occurring following lung resection is widely interpreted to be a variant of ALI/ARDS and follows an identical clinical and pathophysiological course to that seen in the wider critical care environment. The pathophysiology of lung injury following lung resection is complex and can be broadly conceptualised as occurring secondary to insults specific to both the ipsilateral (surgical) lung, the contralateral (anaesthetic) lung in addition to those insults common to both lungs. Increased recognition of the role of ventilator induced lung injury, and peri-operative fluid prescribing in the pathogenesis of lung injury in this population has brought the prevention of lung injury to the attention of the thoracic anaesthetist. Though high quality evidence is lacking, expert opinion widely favours the adoption of lung protective ventilatory strategies and restriction of peri-operative fluids in patients undergoing lung resection.

This thesis presents the rationale, methodology and results of four discrete studies concerning the development of lung injury in the thoracic surgical population undergoing resection of primary lung cancer.

Investigation 1 is a survey of contemporary UK thoracic anaesthetic practice when anaesthetising for thoracic surgery and lung resection, with specific reference to strategies designed to prevent lung injury. Though implementation of the techniques described is far from universal, the survey results suggest that aspects of lung protective ventilation are widespread within UK thoracic anaesthetic practice.

Investigation II seeks to examine the impact of increased adoption of such strategies over time. A random effects meta-analysis and meta-regression analysis was performed to examine the trends in the incidence of and mortality from ALI and/or ARDS over time. The main findings of this study are that whilst there is no evidence to suggest the incidence of ALI and/or ARDS post-lung resection is falling, mortality due to ARDS (but not ALI) does appear to be falling over time.

Investigations III and IV examine the utility of two clinical monitoring methodologies which have potential to provide bedside clinical monitoring of lung injury development in the thoracic surgical population in order to guide clinical decision making, monitor patient progress and serve as a surrogate end point in future clinical studies.

Investigation III examines the utility of a single lung injury biomarker (long chain Pentraxin 3 - PTX3) and a panel of multiple lung injury biomarkers in the early post-operative period following lung resection. The properties of the 'ideal' lung injury biomarker are defined, against which PTX3 and the multiple biomarker panel are compared. PTX3 compared favourably to properties of the 'ideal' lung injury biomarker and appeared to identify a population of patients with elevated post-operative Lung Injury Score with high sensitivity. Conversely there is no evidence from the results presented that a 'risk of lung injury score' derived from a panel of 7 candidate lung injury biomarkers (as previously defined in a cohort of critically ill patients with ALI/ARDS) has any utility in the lung resection population.

Investigation IV tests the reproducibility and construct validity of transpulmonary thermodilution derived measurements of extravascular lung water and pulmonary vascular permeability index in patients undergoing lung resection. The study's findings are largely supportive of the reproducibility and construct validity of extravascular lung water measurement and pulmonary vascular permeability measurements after lung resection.

In combination, it is hoped that the studies presented provide greater insight into the syndrome of post lung resection lung injury. More accurate definition of standard anaesthetic practice and the incidence of and mortality from ALI/ARDS

following lung resection should serve to inform future clinical studies seeking to prevent, treat, or better understand this important clinical syndrome. The biomarker PTX3 and transpulmonary thermodilution derived measurement of extravascular lung water and pulmonary vascular permeability index are presented as surrogate endpoints suitable for use in such studies.

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Author's Declaration

All of the studies reported in this thesis were conceived and designed by the author. Funding application, ethics procedures, patient recruitment and follow-up, blood sampling, transpulmonary thermodilution measurement, data collection, analysis of data and preparation of the manuscript was performed by the author. Biomarker sample analysis was performed by Dr Lisa Jolly and Dr Damon Lowes. All of the statistical analysis was performed by the author, though I gratefully acknowledge the advice received from a variety of sources.

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No work referred to in this thesis has been submitted in support of an application for another degree or qualification in this or any other university.

A handwritten signature in purple ink, appearing to read 'Ben Shelley'.

Dr Ben Shelley
MB ChB, FRCA, DipPaed.

Abbreviations

AECC	American European Consensus Conference
ALI	Acute Lung Injury
Ang-2	Angiopoietin 2
ARDS	Acute Respiratory Distress Syndrome
AUC	Area under the curve
AUROC	Area under the receiver operating characteristic curve
BNP	B-type Natriuretic Peptide
BAL	Broncho-alveolar lavage
CI	Cardiac index
CO	Cardiac output
CO ₂	Carbon dioxide
CPAP	Continuous Positive airway Pressure
CXR	Chest X-ray
DLCO	Diffusing capacity for carbon monoxide
DSt	Down-slope time
EAA	Extravascular albumin accumulation
EGL	Endothelial glycocalyx (layer)
ELWI	Extravascular lung water index
EVLW	Extravascular lung water
FEV ₁	Forced expiratory volume in one second
FiO ₂	Fraction of inspired oxygen
GEDI	Global end-diastolic volume index
GEDV	Global end-diastolic volume
HDU	High dependency unit
HPV	Hypoxic pulmonary vasoconstriction
ICAM-1	Intracellular Adhesion Molecule 1
IL	Interleukin
ITBV(I)	Intra-thoracic blood volume (index)
ITTV	Intra-thoracic thermal volume
MDA	Malondialdehyde
(m)LIS	(Modified) Lung Injury Score
MTt	Mean transit time
NO	Nitric Oxide
NSCLC	Non-small cell lung cancer
OLV	One-lung ventilation
sICAM	Soluble intracellular adhesion molecule
STD	Single thermodilution

PAP	Pulmonary artery pressure
PAWP	Pulmonary artery wedge pressure
PBV	Pulmonary blood volume
PBW	Predicted body weight
P_c	Pulmonary capillary pressure
PCP-III	Pro-collagen peptide III
PCWP	Pulmonary capillary wedge pressure
PEEP	Positive End-Expiratory Pressure
PLR-ALI	Post-lung resection ALI
P_{peak}	Peak airway pressure
$P_{plateau}$	Plateau airway pressure
ppoFEV ₁ / DLCO	Predicted post-operative FEV ₁ / DLCO
PTV	Pulmonary thermal volume
PTX3	Pentraxin 3
PVPI	Pulmonary vascular permeability index
RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
RV	Right ventricle
RVEF	Right ventricular ejection fraction
SAPS-II	Simplified acute physiology II (score)
sICAM	Soluble intracellular adhesion molecule
SOFA	Sequential organ failure (score)
STD	Single thermodilution
TDD	Thermo-dye dilution
TNF- α	Tumour necrosis factor alpha
TLV	Two-lung ventilation
TCTD	Trans-cardiac thermodilution
TPTD	Trans-pulmonary thermodilution
VATS	Video assisted thoracoscopic surgery
VCAM-1	Vascular Cell Adhesion Protein 1
VILI	Ventilator induced lung injury
V_d	Volume of distribution
V_T	Tidal volume
ZEEP	Zero positive end-expiratory pressure

1 Introduction

The work presented in this thesis concerns the occurrence of Acute Lung Injury / Acute Respiratory Distress Syndrome (ALI/ARDS) post-operatively in patients undergoing surgical resection of lung cancer. By way of introduction, the first section of this opening chapter describes the incidence and mortality of lung cancer, the expanding role of surgical resection in its treatment and goes on briefly to describe mortality and morbidity after lung resection. The remainder of this chapter reports the findings of a detailed literature review examining the pathophysiology of pulmonary oedema, the definition and pathophysiology of ALI/ARDS and the role of ventilator induced lung injury in ALI/ARDS. Finally the syndrome of post-lung resection acute lung injury (PLR-ALI) is comprehensively discussed in terms of its definition, pathophysiology, risk factors, management and potential preventative strategies.

In the subsequent chapters, the methodology and results of four discrete ‘investigations’ are presented and discussed:

Investigation I is a survey of contemporary UK thoracic anaesthetic practice when anaesthetising for thoracic surgery and lung resection, with specific reference to the adoption of strategies to prevent PLR-ALI.

Investigation II seeks to examine the impact of increased adoption of such strategies over time. A random effects meta-analysis and meta-regression analysis was performed to examine the trends in the incidence of and mortality from PLR-ALI over time.

Investigations III and IV examine the utility of two clinical monitoring methodologies which have potential to provide bedside clinical monitoring of lung injury development in the thoracic surgical population in order to guide clinical decision making, monitor patient progress and serve as a surrogate end point in future clinical studies seeking to prevent, treat, or better understand this important clinical syndrome.

Investigation III examines the utility of a single lung injury biomarker (long chain Pentraxin 3 - PTX3) and a panel of multiple lung injury biomarkers in

the early post-operative period following lung resection. In order to provide background, a targeted literature review is provided concerning the use of lung injury biomarkers. The properties of the 'ideal' lung injury biomarker are defined, against which Pentraxin 3 and the multiple biomarker panel are compared.

Investigation IV tests the reproducibility and construct validity of transpulmonary thermodilution derived measurements of extravascular lung water and pulmonary vascular permeability index in patients undergoing lung resection. Preceding this, a further targeted literature review details the concepts involved in establishing reproducibility and validity of a clinical monitor and provides an in depth exploration of the principles of transpulmonary thermodilution, and the potential impact of both lung resection and lung injury on the results obtained.

1.1 Lung cancer

1.1.1 Incidence of and mortality from lung cancer

In the words of W. Michael Alberts (then) immediate past president of the American College of Chest Physicians (ACCP) in his introduction to the *ACCP Evidence-Based Clinical Practice Guidelines on the Diagnosis and Management of Lung Cancer*:

“The numbers are still staggering...”

W. Michael Alberts (2007)¹.

This statement is nowhere more true than in Glasgow. In 2011, there were some 43,463 new cases of lung cancer in the UK, making lung cancer the second most commonly diagnosed cancer after breast cancer². Within the UK, lung cancer is especially common in Scotland, with a European age-standardised incidence rate of 66 per 100,000 population compared to 44 in England and 50 in Wales². In Greater Glasgow, lung cancer incidence is almost a third higher than the Scotland wide average. As an area traditionally associated with heavy industry and shipbuilding, and with particularly high levels of socio-economic deprivation, lung cancer rates in Greater Glasgow are amongst the highest worldwide².

Reflecting trends in cigarette smoking prevalence, male lung cancer incidence rates have decreased overall in the UK since the mid 1970s, but continue to rise in females (where the peak in smoking prevalence came later). As such, overall incidence rates are essentially static².

“Lung cancer has an enormous impact on national mortality”

Cancer Research UK (2014)²

Lung cancer is the most common cause of cancer death in the UK, accounting for 6% of all deaths (including non-cancer deaths). One year survival following lung cancer diagnosis is approximately 30%, falling to less than 10% by five years².

1.1.2 Surgical resection of lung cancer

It is well established for early stage non-small cell cancer (NSCLC) that the best chance of cure is surgical resection^{3, 4}. The National Institute for Health and Care Excellence (NICE) describe surgical resection by lobectomy as the “*treatment of first choice*” for patients with NSCLC “*who are medically fit and suitable for treatment with curative intent*”.

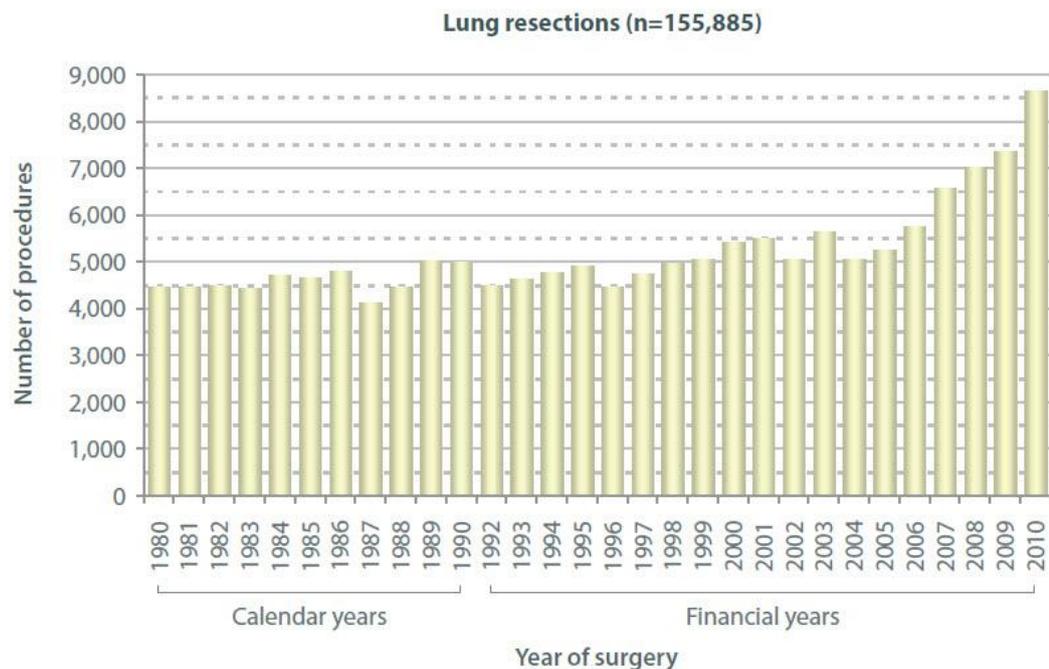


Figure 1.1. Lung resections by year in the UK.

From the Society for Cardiothoracic Surgery 2nd National Thoracic Surgery Activity and Outcomes Report, 2011⁵.

In 2010 (the most recent year for which data are available), over 8500 people underwent lung resection surgery in the UK, more than 5000 of which were for resection of primary lung cancer⁵. Since 2005 there has been a dramatic increase in surgery for lung cancer in the UK⁵ (Figure 1.1). Such an expansion probably reflects the effects of three major drivers.

Firstly, it had been recognised for some time that lung cancer outcomes in the United Kingdom lagged behind those observed in other parts of Western Europe⁶. Whilst the explanation for this was likely to be multi-factorial (with influences from socio-economic to political), lung resection rates in the UK were half those seen elsewhere in Europe^{7, 8}. Thoracic surgery in the UK was “*in crisis*”⁸; a joint working party of the Society of Cardiothoracic Surgeons and the British Thoracic

Society described the “*critical under-provision of thoracic surgery in the UK*” reporting that “*fifty extra surgeons were required to come up to European average standards*”⁷. In parallel, data was accumulating which demonstrated wide geographical variation in resection rates throughout the UK, mirrored by a corresponding variation in survival^{9, 10}. It was becoming clear that “[*there was*] a large number of lung cancer deaths that could be postponed if more patients were resected”⁹. These observations triggered a response from central government, beginning in 1998 with the Department of Health publishing guidance on commissioning services for lung cancer under the title ‘*Improving Outcomes in Lung Cancer*’, followed in 2000 by the ‘*NHS Cancer Plan*’ aiming to tackle inequalities in quality of care and treatment¹⁰. As a result thoracic surgery has seen increased investment, radical restructuring in the delivery of services and “*a welcome expansion of the thoracic surgical workforce*”⁵.

Secondly, surgeons and anaesthetists are increasingly likely to offer surgery to patients previously considered unsuitable for resection due to co-morbidity. There is increased recognition that acceptable levels of peri-operative morbidity and mortality are achievable even in patients previously considered to be ‘very-high risk’¹¹. Reflecting this, recent clinical guidelines have undergone a shift of emphasis, moving the basis of assessment of suitability for resection from rigid criteria based on the results of physiological testing, to a more global assessment of functional ability^{11, 12}. Clinicians are encouraged to “*offer patients with [baseline pulmonary function] below the recommended limit[s]... the option of undergoing surgery if they accept the risks of dyspnoea and associated complications*”¹³.

Thirdly, current evidence supports an expansion in lung cancer surgery in patients with more advanced disease and a greater uptake in patients who are willing to accept higher risks¹⁴. Though survival rates in patients with more advanced disease are lower, in patients with (regional lymph node) N1-N3 disease (stage II & III - previously considered not to be suitable for resection), surgery confers an absolute 5-year survival benefit of 11% (8% no surgery vs 17% with surgery)¹⁵.

1.1.2.1 Morbidity and mortality following lung resection

Since 1980, the Society for Cardiothoracic Surgery in Great Britain & Ireland has been auditing thoracic surgical activity in the UK. The most recent report, the Second National Thoracic Surgery Activity & Outcomes Report (2011), reports in-hospital mortality from a cumulative series of over 100,000 lung resections and reveals that “*combined operative mortality for all patients having lung cancer surgery has almost halved from 3.8% in 2001-2002 to 2.1% in 2009-2010*”⁵.

Whilst in-hospital mortality of 2% might represent ‘acceptable’ risk for what is a destructive, major operation, this is set against considerable morbidity. Almost all patients undergoing lung resection for lung cancer have been long-term smokers, many from low socio-economic backgrounds. As such, thoracic surgical patients exhibit high levels of pre-existing cardio-respiratory disease^{5, 16}. Perhaps unsurprisingly therefore, cardio-respiratory complication rates following lung resection are high. Combined cardio-respiratory complication rates (including complications ranging from supra-ventricular tachy-arrhythmias, myocardial ischaemia / infarction, right ventricular dysfunction, cardiogenic pulmonary oedema and pulmonary thromboembolism to atelectasis, sputum retention, pneumonia, bronchospasm, respiratory failure and acute lung injury / acute respiratory distress syndrome), are reported to occur in 20-65% of cases¹⁷⁻²¹. Such complications are associated with increased mortality, costs and prolonged duration of both critical care unit and hospital stay^{17, 19}.

In conclusion, lung cancer is a devastating disease, which carries high mortality. Surgical resection offers the greatest potential for cure in the approximately 15% of patients suitable for treatment with curative intent. As advances in surgical techniques and adjuvant therapies confer survival benefits; more, older and sicker patients with more advanced disease are going to present for lung resection. It is incumbent therefore on all involved in the care of such patients, to embrace this increasing demand, and strive to better understand and combat the causes of mortality and morbidity in this patient group. The occurrence of acute lung injury / acute respiratory distress syndrome following lung resection (the subject of this thesis), though not the most commonly occurring complication, represents the major cause of early, non cancer related mortality in this patient group.

1.2 Pulmonary oedema

Before exploring the pathophysiology of lung injury, I will first discuss the structure and physiology of the alveolar-capillary membrane, the mechanisms which serve to regulate microvascular fluid exchange and prevent pulmonary oedema formation, and the influence of pathology upon these mechanisms. In recent years, the increased recognition of the role played by the endothelial glycocalyx has challenged many long held beliefs.

1.2.1 Structure and function of the lung

The lung has evolved as a tremendously efficient unit to facilitate its primary function of gas exchange; uptake of oxygen and elimination of carbon dioxide. A copious network of capillaries are wrapped around approximately 300 million alveoli providing an effective surface area for gas exchange of 130 square meters, whilst occupying a volume of only approximately four litres^{22, 23}. Much of this efficiency of gas exchange may be attributed to the extremely thin nature of the air-blood barrier; as little as 0.3 micrometres in some places. Pulmonary oedema occurs when the delicate physiological balance which maintains fluid within the capillaries and keeps the alveolar air spaces free of fluid accumulation becomes disrupted.

1.2.1.1 Anatomy of the alveolar-capillary barrier

The alveolar-capillary (or air-blood) barrier is described as having 'thin' and thick portions^{22, 24} (Figure 1.2). The alveolar side of the barrier is lined predominantly with type I epithelial cells, whilst on the capillary side, the capillary wall comprises a single layer of endothelial cells.

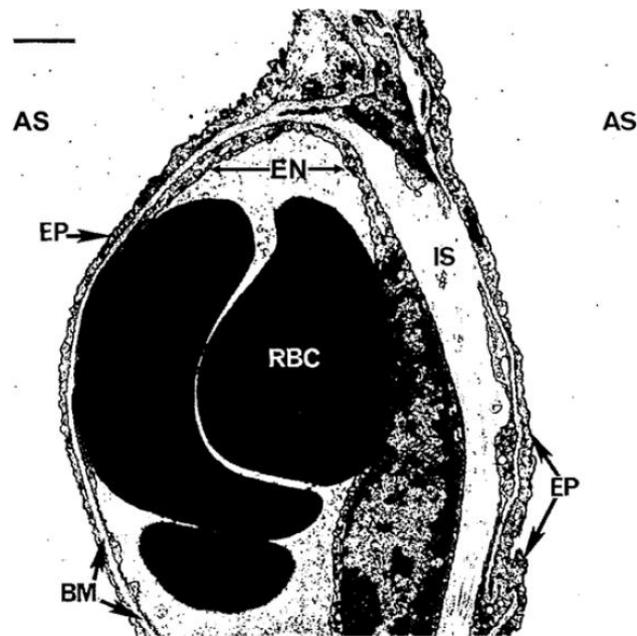


Figure 1.2. Electron micrograph of a single pulmonary capillary.

The thick portion of the alveolar-capillary barrier lies on the right where the epithelial cell (EP) and endothelial cell (EC) are separated by an interstitial space (IS). In the thin portion (lying on the left), epithelial and endothelial cells lie on a fused basement membrane (BM). RBC, red blood cell; AS, alveolar space. Horizontal bar = 1 μ m. From Murray (2010)²².

Gas exchange takes place predominantly across ‘thin’ portions of the alveolar-capillary barrier where capillary blood and air space are separated by thin cytoplasmic extensions of both alveolar and endothelial cells with their basement membranes fused into a single layer. At the other side of the capillary (the right hand side in Figure 1.2), the alveolar space and capillary lumen are further apart, separated by the bulk of the endothelial cell nucleus, and an interstitial space containing connective tissue fibrils. This is the ‘thick’ portion of the alveolar-capillary barrier across which liquid and solute exchange occurs²²⁻²⁴. Though the majority of the alveolar surface is lined by type I epithelial cells, the dominant cell type in the alveolar epithelium is in fact the smaller type II cell. Type II epithelial cells produce surfactant, a phospholipid molecule responsible for reducing surface tension and maintaining alveolar stability^{22, 23}.

1.2.2 Pathophysiology of pulmonary oedema

1.2.2.1 Starling forces

The regulation of fluid exchange across the alveolar-capillary barrier is classically described by the ‘Starling principle’ of microvascular fluid exchange. Following experiments in dogs, Starling proposed that the walls of the capillaries

are semi-permeable membranes²⁵. From these observations, hydrostatic and oncotic pressure were identified as the primary determinants of microvascular fluid exchange²⁶. The relationship between these forces is represented by the widely adopted ‘Starling’ equation:

$$\frac{J_v}{A} = K[(P_c - P_I) - \theta(\Pi_P - \Pi_I)]$$

Equation 1.1

Where, J_v is net filtration rate of fluid per unit area (A) of capillary wall,
 K is the membrane permeability,
 P_c is the hydrostatic pressure in the capillary,
 P_I is the hydrostatic pressure in the interstitium,
 θ is the reflection coefficient to plasma protein,
 Π_P is the plasma protein oncotic pressure,
 and Π_I is the protein oncotic pressure in the interstitium.

From Equation 1.1, it can be appreciated that the overall trans-vascular flow across a pulmonary capillary is determined by the balance between forces favouring outward flow (extravasation of fluid) - P_c and Π_I , and forces favouring inward flow (reabsorption of fluid) - P_I , and Π_P , and by the permeability of the endothelium to water (K) and protein (θ). Classical descriptions of the ‘Starling forces’ describe changes in the direction of microvascular fluid exchange at different sites in the capillary. At the arterial end P_c is high and so there is a net filtration of fluid, whilst at the venous end of the capillary P_c has fallen such that there is net absorption of fluid²⁷.

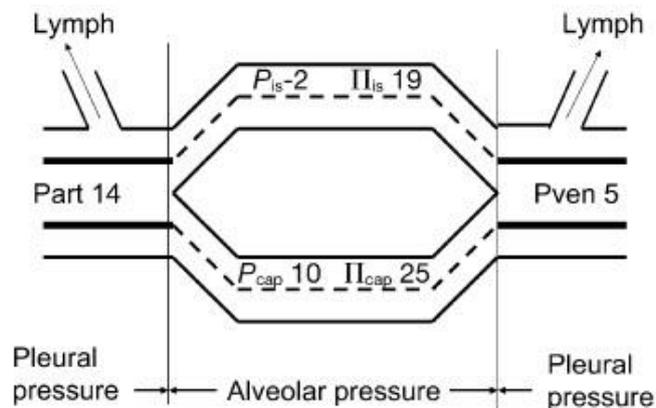


Figure 1.3. Schematic diagram showing the location and magnitude of the Starling forces in healthy human lung.

P_{art} , mean pulmonary artery pressure; P_{ven} , mean pulmonary venous pressure; P_{cap} , hydrostatic pressure in the capillary; P_{is} , hydrostatic pressure in the interstitium, Π_{cap} is the plasma protein oncotic pressure (referred to as Π_P above); Π_{is} , protein oncotic pressure in the interstitium. From Murray (2011)²⁸.

Figure 1.3 provides estimated values for P and Π in health, where it can be seen that balance of the outward hydrostatic force ($10 - -2 = 12\text{mmHg}$) exceeds the net inward oncotic force ($25 - 19 = 6\text{mmHg}$) such that a net filtration pressure of 6mmHg favours extravasation of fluid²⁹. This pressure gradient results in a continuous transvascular flow of fluid from pulmonary capillary to interstitium.

1.2.2.2 Pulmonary lymphatics

Following formation by filtration across the pulmonary capillary endothelium into the interalveolar septum, filtrate is able to flow directly through the loose interstitial tissue surrounding arterioles, venules and bronchioles from where it may pass into the terminal branches of the pulmonary lymphatics. Such flow is maintained by a pressure gradient between the interalveolar interstitium (where pressure is approximately equivalent to alveolar pressure) and the peribronchovascular interstitium (where pressure is approximately equivalent to pleural pressure)²⁸. Though technically challenging to measure, extrapolation from animal data suggests that in health lung lymph flow is $8\text{-}9\text{ml/h}$, though in pathological states lymph flow can increase 10-fold or more³⁰.

1.2.2.3 Evolution of pulmonary oedema formation

From Equation 1.1 it can easily be appreciated that increased fluid extravasation may occur in any situation where either the net filtration pressure increases, or where membrane permeability increases. In clinical practice two discrete clinical syndromes are recognised; ‘hydrostatic’ pulmonary oedema where increased filtration pressure leads to increased fluid extravasation across an essentially normal capillary endothelium, and ‘permeability’ pulmonary oedema where increased filtration occurs as a result of pathological increases in capillary permeability. Classical examples are those of ‘hydrostatic’ pulmonary oedema occurring in left ventricular failure, and ‘permeability’ pulmonary oedema occurring in acute lung injury / acute respiratory distress syndrome. In hydrostatic oedema the filtrate is watery, where in ‘permeability’ oedema, increased capillary permeability permits extravasation of protein resulting in an oedema fluid rich in protein.

The histological appearances of pulmonary oedema formation have been well described^A. As net filtration across the endothelial barrier increases, lymph flow increases in parallel such that initially interalveolar interstitial volume is not increased, but peribronchovascular lymphatics become engorged; observable as fluid 'cuffs' around small vessels and bronchioles. Fluid filtration in excess of the capacity of the lymphatics leads to interstitial water accumulation; evident as increased width of alveolar septae. Further accumulation of interstitial oedema leads to increase interstitial pressure which (especially in the presence of endothelial injury) results in alveolar flooding^{24, 29}. The degree of alveolar flooding depends on the extent of interstitial oedema, the integrity of the alveolar epithelium and the ability of the epithelium to actively remove alveolar oedema³¹.

1.2.2.4 Alveolar fluid clearance

Once oedema has progressed to the point of alveolar flooding, clearance of oedema from the alveolar spaces relies upon active transport of sodium across the alveolar epithelial barrier. Sodium crosses the apical membrane of alveolar type II pneumocytes via amiloride-sensitive sodium channels, and is then actively transported across the basolateral membrane into the interstitium by the Na⁺-K⁺-ATPase ion transporter. Water is then able to follow sodium passively; in the lung osmotic permeability to water is high^{32, 33}. Clinical studies have revealed that patients with increased permeability pulmonary oedema have impaired alveolar epithelial fluid transport³².

1.2.3 The endothelial glycocalyx and the revised Starling equation

In recent years, it has become evident that 'conventional' principles of microvascular fluid exchange (as represented by the Starling equation, Equation 1.1) are inadequate to describe microvascular fluid exchange, with several observations combining to undermine the validity of the 'Starling' equation.

Firstly, several researchers have failed to demonstrate reabsorption of interstitial fluid (as hypothesised to occur at the venous end of the capillary and

^AThis paragraph refers to the histological appearances of pulmonary oedema per se, rather than the appearances of diffuse alveolar damage, a pathognomic finding in ALI/ARDS.

in the venule by the Starling principle) as a consistent component of microvascular fluid homeostasis. In experimental models manipulating capillary pressure (P_c), when P_c falls transiently below oncotic pressure (Π_p) net absorption of fluid is observed, but at steady state (with $P_c > \Pi_p$), no absorption occurs^{27, 34, 35}. Similarly, it has been observed that net absorption of fluid cannot play any significant role in microvascular fluid homeostasis in tissues greater than ~10cm below the heart as venous P_c exceeds Π_p in this situation^{27, 34, 35}.

Secondly, if in the circumstances just described, reabsorption of interstitial fluid is unlikely to play any significant role in maintaining tissue fluid balance, then the lymphatic system becomes the principle method for returning filtrate to the circulation. Observed values of overall lymph flow are however an order of magnitude less than would be needed to account for levels of filtration suggested by the ‘Starling principle’. This observation has been described as the ‘low filtration force paradox’; to account for observed levels of lymph flow, globally averaged net filtration force should be in the region of 1mmHg, rather than the 5-10mmHg predicted by the ‘Starling equation’^{34, 35}.

Thirdly, in a series of experiments involving direct manipulation of interstitial oncotic pressure (Π_i), several researchers have demonstrated that manipulations in Π_i , even to the extent that $\Pi_i = \Pi_p$, have minimal effect on filtration rate; a finding incompatible with the ‘Starling principle’^{34, 35}. The latter observation, that filtration rate is independent of interstitial oncotic pressure intimates that microvascular fluid exchange is governed by principles other than the simple balance of capillary and interstitial hydrostatic and oncotic pressures.

1.2.3.1 Structure and function of the endothelial glycocalyx

In 1966, using electron microscopy with a ruthenium red stain, Luft identified the presence of a three dimensional network of fibrous chains adherent to the luminal surface of the capillary³⁶. As early as 1979, Michel suggested that the “molecular sieving properties of the capillary wall” (the semi-permeable membrane across which filtration takes place and is regulated), may lie within this endocapillary layer rather than within or between endothelial cells forming the capillary wall³⁷. It has not been until recently however, that the structure and function of endocapillary layer has been more fully appreciated.

The endothelial glycocalyx (as this layer has become known), is a meshwork membrane bound glycoproteins, proteoglycans and glycosaminoglycans found on the luminal side of endothelial cells (Figure 1.4)^{38, 39}.

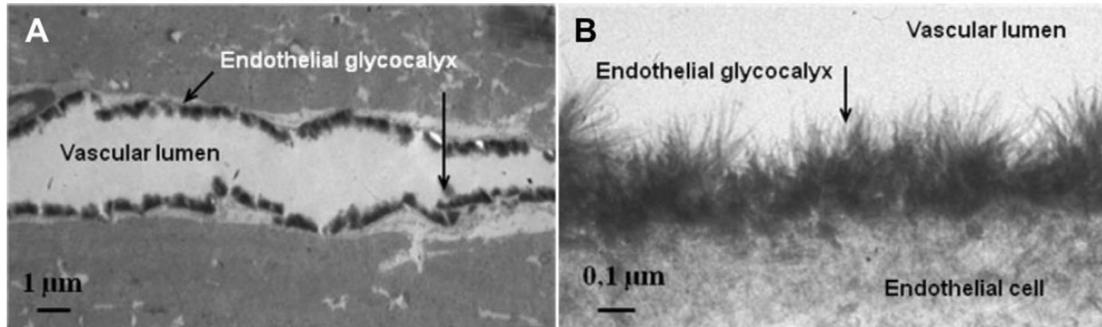


Figure 1.4. Electron microscopic pictures showing an intact endothelial glycocalyx in coronary vessels of a guinea pig heart.

From Brettner et al (2012)⁴⁰.

In non-fenestrated capillaries such as those found within the lung, the endothelial glycocalyx (EGL) forms a continuous layer lining the endothelial cell walls and filling the inter-endothelial cell clefts³⁸. The EGL is freely permeable to water, and behaves as a semipermeable membrane with respect to plasma protein molecules such as albumin. As a consequence, within the EGL lies a volume of fluid; intravascular, yet excluded from the circulating volume, devoid of red blood cells and low in protein concentration³⁵.

At the site of the endothelial inter-cellular junction (the primary fluid conducting pathway across the capillary endothelium), the sub-glycalyceal space is in direct communication with the interstitial space via the intercellular cleft. Filtration of fluid at this site therefore is not driven by the interaction between hydrostatic pressure and interstitial colloid oncotic pressure, but by the sub-glycalyceal colloid oncotic pressure (Π_G)^{34, 35, 38} (Figure 1.5).

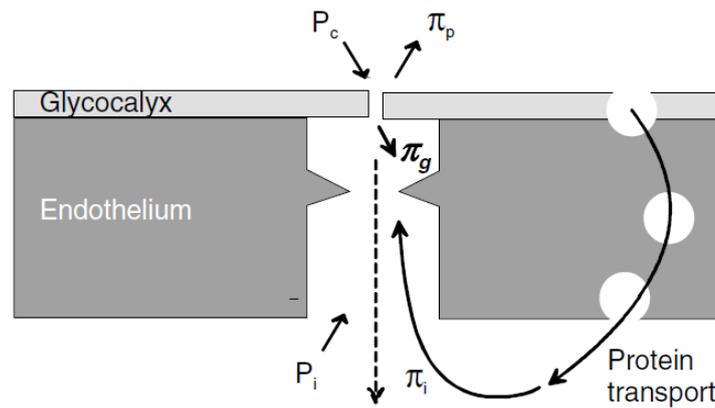


Figure 1.5. The revised Starling principle: forces acting to govern microvascular fluid transport across the endothelial semipermeable membrane.

P_c , hydrostatic pressure in the capillary; P_i , hydrostatic pressure in the interstitium; Π_p , plasma protein oncotic pressure; Π_g , protein oncotic pressure in the sub-glycocalyceal space. Modified from Levick and Michel (2010)³⁵.

Whilst protein molecules are able to reach the interstitial space by direct transport through endothelial cells, (maintaining interstitial colloid oncotic pressure) the EGL is protected from the accumulation of protein by two mechanisms³⁵. Firstly, the reflection coefficient to plasma protein (θ) of the EGL is high (in part due to the negatively charged glycosaminoglycans), preventing direct extravasation of plasma protein. Secondly, the retrograde passage of protein from the interstitium into the subglycocalyceal space is prevented by the long and tortuous path of the intercellular cleft, through which continuous and relatively high velocity flow of ultrafiltrate prevents upstream flow of protein. As such, Π_G is maintained at a considerably lower level than Π_{IS} , so low in fact, that the colloid oncotic pressure opposing fluid filtration is essentially Π_p , rather than $\Pi_p - \Pi_{IS}$ as predicted by the Starling equation. Incorporating these principals into Equation 1.1 leads to the formation of a ‘revised Starling principle’^{34, 35}:

$$\frac{J_v}{A} = K[(P_c - P_i) - \theta(\Pi_p - \Pi_G)]$$

Equation 1.2

Where, Π_G is the sub-glycocalyceal oncotic pressure.

Mathematical modelling based on this ‘revised Starling principle’ predicts reduced filtration rates to values in keeping with observed lymph flow, providing a solution to the ‘low filtration force paradox’³⁵.

In addition to the regulation of microvascular flow as discussed, the EGL has several other important functions, notably the attenuation of vascular shear stress and regulation of leucocyte and platelet adhesion^{39, 41}. Nitric oxide (NO) mediated vasodilation in response to increased endothelial shear stress is an essential regulatory mechanism within the capillary serving to couple vessel diameter to flow. Whilst the relevant mechanisms are not completely understood, it is now believed that interactions between plasma constituents and the EGL play an essential role in mechano-transduction^{39, 41}. Endothelial cell adhesion molecules such as P-selectin, Intracellular Adhesion Molecule 1 (ICAM-1) and Vascular Cell Adhesion Protein 1 (VCAM-1) are found on the endothelial cell wall, buried deep within the EGL. The presence of an intact EGL prevents interaction between leucocytes and platelets and these molecules, preventing adhesion of these cells to the endothelial wall^{39, 41}.

1.2.3.2 Pathophysiology of the endothelial glycocalyx

The EGL plays a fundamental role in determining capillary permeability, regulating blood cell - endothelial cell interaction and mediating the sensing of shear stress. In a variety of pathological situations however, the EGL can become damaged (characteristically described as 'shedding'), leading to loss of EGL constituents which can subsequently be found in plasma. Such glycocalyx degradation, inhibits these key homeostatic roles of the glycocalyx and results in the development of capillary leak, oedema formation, accelerated inflammation, platelet hyper-aggregation and loss of vascular responsiveness⁴¹. Systemic inflammatory states such as ischaemia-reperfusion injury, sepsis, trauma, atherosclerosis and diabetes are all well recognised pathophysiological syndromes in which EGL damage has been documented^{38, 41}.

The endothelial glycocalyx and pulmonary oedema formation

Greater understanding of the determinants of microvascular filtration and endothelial cell function suggest that the theoretical division of the mechanisms of pulmonary oedema formation into 'hydrostatic' (where capillary permeability is perceived to be normal) and 'permeability' (where hydrostatic pressure is normal and permeability is increased), is likely to be an over simplification. The classic 'Starling principle' suggests that increases in capillary hydrostatic pressure (as might be expected in true 'hydrostatic' pulmonary oedema), would

result in a linear increase in net filtration (Figure 1.6). Research conducted in isolated animal lung models however, reveals a non-linear relationship between capillary hydrostatic pressure and net filtration, such that as capillary hydrostatic pressure increases, capillary permeability is also increased (Figure 1.6)⁴².

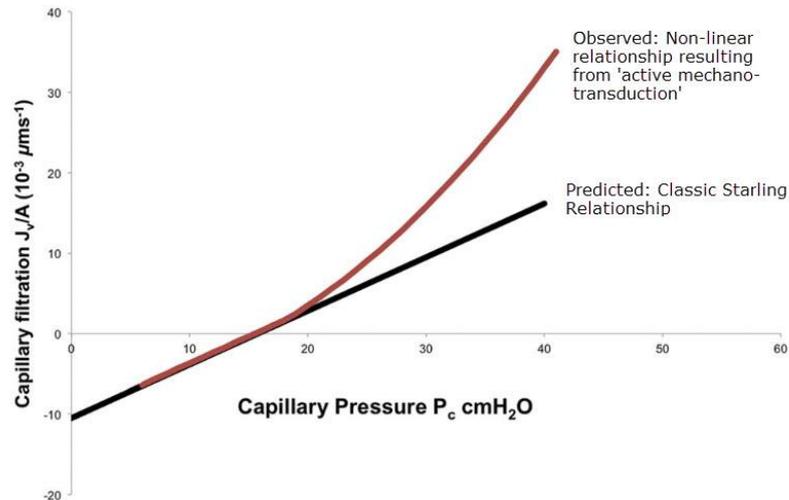


Figure 1.6. Schematic representation of the relationship between capillary pressure and capillary filtration.

Modified from Collins et al (2013)⁴².

Located at the interface of the endothelium and vascular shear stresses, there is evidence to suggest heparan sulphate chains (the predominant glycoaminoglycan chain of the EGL) act as a mechano-transducer in this process; shear stresses applied to the capillary endothelium are ‘sensed’ by heparan resulting in increased capillary permeability via intracellular changes mediated through increased nitric oxide production^{42, 43}.

The endothelial glycocalyx and lung injury

In laboratory and animal studies (of capillary endothelial cells in a variety of anatomic locations), damage to the glycocalyx has been linked to a myriad of pathogenic processes pertinent to the development of lung injury; adhesion of platelets and leucocytes to the capillary endothelial surface, activation of coagulation pathways, leakage of fluid and protein into the interstitial space and the development of tissue oedema^{42, 44}. It appears in fact that the theoretical evidence for a role for EGL injury in ALI/ARDS overwhelming yet to date there is a paucity of clinical data⁴².

In 2012, Schmidt et al published a seminal paper cataloguing how in a series of animal (mice) and human studies “*the pulmonary endothelial glycocalyx*

regulates neutrophil adhesion and lung injury during experimental sepsis"⁴⁵. The authors demonstrated that systemic sepsis led to glycocalyx degradation via TNF- α mediated activation of endothelial heparanase leading to loss of heparan sulphate. The resulting rapid thinning of the EGL facilitated neutrophil adhesion to endothelial adhesion molecules. Excitingly, inhibition of endothelial heparanase in animal models abolished degradation of the EGL, prevented neutrophil adhesion and attenuated sepsis induced ALI, highlighting the potential of the EGL as a target for therapeutic intervention^{41, 45}. In humans, increased heparan sulphate degradation activity was observed in patients with respiratory failure secondary to non-pulmonary sepsis. In a separate cohort of lung biopsy specimens with diffuse alveolar damage, heparanase immunofluorescence was eight-fold higher than in normal controls. This the authors conclude "*suggest[s] that heparanase is active in human sepsis and contributes to inflammatory lung injury*"⁴⁵. Though inconclusive, these observations provide the strongest evidence to date for a role of EGL injury in ALI/ARDS.

Endothelial glycocalyx injury after major surgery

In 2007, Rehm et al measured plasma markers of EGL degradation (syndecan-1 and heparan sulphate) in 14 patients undergoing infrarenal aortic aneurysm repair⁴⁶. Infrarenal ischemia-reperfusion was followed by 15- and 3-fold increases, in syndecan-1 and heparan sulphate respectively ($p < 0.001$ for both). In 2011, Steppan et al measured the same two markers of EGL degradation in a cohort of patients undergoing major abdominal surgery, in patients with severe sepsis and in healthy controls⁴⁷. Though patients undergoing major abdominal surgery (primary site: pancreas 46%, colon 18%, liver 7%, genitourinary 11% and other 18%) exhibited less evidence of inflammation (lower interleukin-6 levels) than patients with severe sepsis, plasma levels of syndecan-1 and heparan sulphate were markedly elevated post-operatively when compared to controls. Whilst (unfortunately) neither of these studies provides any link between EGL degradation and clinical outcomes, it suggests a role of EGL degradation in the pathogenesis of pulmonary oedema formation in the peri-operative period. To date, there have been no studies examining EGL function in patients undergoing lung resection⁴², though a potential role of EGL damage in the pathogenesis of ALI/ARDS after lung resection has been postulated^{42, 48, 49}.

1.3 Acute Lung Injury / Acute Respiratory Distress Syndrome

Before discussing the specifics of Acute Lung Injury / Acute Respiratory Distress Syndrome (ALI/ARDS) occurring in the immediate post-operative period in patients undergoing lung resection, I shall first explore the derivation and definitions of the terms ALI/ARDS, before briefly reviewing their pathophysiology and specifically, the role of ventilator induced lung injury in their pathogenesis.

1.3.1 What is ALI/ARDS?

In 2011, the European Society of Intensive Care Medicine, endorsed by the American Thoracic Society and the (US) Society of Critical Care Medicine convened a consensus panel, “The ARDS Definition Taskforce” to revise the definition of Adult Respiratory Distress Syndrome. In the process of deriving an updated definition (which will be discussed below), the ‘ARDS Definition Task Force’ (an international expert panel of clinicians and researchers active in the field of ALI/ARDS) defined a ‘conceptual model’ of ARDS⁵⁰:

“The panel agreed that ARDS is a type of acute diffuse, inflammatory lung injury, leading to increased pulmonary vascular permeability, increased lung weight, and loss of aerated lung tissue. The clinical hallmarks are hypoxemia and bilateral radiographic opacities, associated with increased venous admixture, increased physiological dead space, and decreased lung compliance. The morphological hallmark of the acute phase is diffuse alveolar damage (i.e., oedema, inflammation, hyaline membrane, or haemorrhage)”.

The ARDS Definition Task Force (2012)⁵⁰

This statement represents the most contemporary understanding of the syndrome of ALI/ARDS.

1.3.1.1 Development of a definition of ALI/ARDS

On Saturday 12th August 1967, David G Ashbaugh and colleagues from the University of Colorado Medical Center, published the first reported description of what is now known as ALI/ARDS⁵¹. In a case series of 12 patients, with

pathologies as diverse as trauma, pancreatitis and viral pneumonia, Ashbaugh et al observed that *“despite a variety of physical and possibly biochemical insults the response of the lung was similar in all 12 patients”*⁵¹. Patients exhibited a syndrome of acute respiratory distress characterised by severe dyspnoea, tachypnoea, cyanosis refractory to oxygen therapy, loss of lung compliance and diffuse alveolar infiltrates on chest radiography⁵¹.

Subsequently, in 1971, Petty and Ashbaugh went on to refine the description of a condition whose pathophysiology (they describe) *“is basically a nonspecific response to a variety of pulmonary injuries”*⁵². Petty and Ashbaugh describe the cardinal features of the syndrome as may be recognised today⁵²:

1. *Direct or indirect mechanism of injury*
2. *Diffuse alveolar infiltration on chest radiography*
3. *Hypoxaemia secondary to a large right to left shunt*
4. *Potential for resolution and recovery, or progressive pulmonary insufficiency leading to interstitial fibrosis and death*

For several decades, this description of ARDS stood (though at this time the ‘A’ stood for *‘adult’* rather than *‘acute’*), but increasingly drew criticism as being open to subjective interpretation and not being sufficiently specific⁵³.

In 1988 Murray et al published *“an expanded definition of the Adult Respiratory Distress Syndrome”*⁵⁴. This three part definition sought to differentiate the course of the syndrome (acute or chronic), characterise the severity of the pulmonary injury and identify the cause or risk factors associated with the injury^{53, 54}. Calculation of the ‘lung injury score’ (LIS - Table 1.1) allowed the severity of lung injury to be defined where a score of 0 points defined ‘no lung injury’, 0.1-2.5 points signified mild to moderate lung injury and greater than 2.5 points signified ‘severe lung injury’ or ‘ARDS’^{53, 54}.

Murray et al’s LIS has been widely used in clinical studies of patients with ALI/ARDS, providing a method of characterising the severity of ALI and ARDS on a numerical scale. As a definition of ARDS however, the three part definition advocated by Murray et al had one important shortfall; no formal criteria exist within the definition to distinguish cardiogenic from non-cardiogenic pulmonary oedema⁵³.

Table 1.1. Calculation of the lung injury score.

Component	Score
Chest radiograph	
- No alveolar consolidation	0
- Alveolar consolidation confined to 1 quadrant	1
- Alveolar consolidation confined to 2 quadrants	2
- Alveolar consolidation confined to 3 quadrants	3
- Alveolar consolidation confined to 4 quadrants	4
Hypoxaemia score	
- PaO ₂ /FiO ₂ ≥300 mmHg	0
- PaO ₂ /FiO ₂ 225-299 mmHg	1
- PaO ₂ /FiO ₂ 175-224 mmHg	2
- PaO ₂ /FiO ₂ 100-174 mmHg	3
- PaO ₂ /FiO ₂ <100 mmHg	4
PEEP score (when mechanically ventilated)	
- ≤5 cmH ₂ O	0
- 6-8 cmH ₂ O	1
- 9-11 cmH ₂ O	2
- 12-14 cmH ₂ O	3
- ≥ 15 cmH ₂ O	4
Respiratory system compliance score (when available)	
- ≥80 ml/cmH ₂ O	0
- 60-79 ml/cmH ₂ O	1
- 40-59 ml/cmH ₂ O	2
- 20-39 ml/cmH ₂ O	3
- ≤19 ml/cmH ₂ O	4

The lung injury score is calculated by dividing the cumulative scores for each component by the number of components scored in the derivation. PEEP, positive end-expiratory pressure. From Murray et al (1988)⁵⁴.

The American-European Consensus Conference definition of ALI/ARDS

In 1992, the American Thoracic Society and the European Society of Intensive Care Medicine convened a series of meetings of the “*American-European Consensus Committee on ARDS...in attempt to bring clarity and uniformity to the definition of ALI and ARDS*”, largely motivated by a desire to facilitate increased trans-Atlantic co-operation in the conduct of clinical studies⁵⁵.

At this meeting it was recognised that ARDS represented a spectrum of disease severity characterised by a continuum of arterial blood gas and chest X-ray abnormalities; the term ‘Acute Lung Injury’ (ALI) was defined as representing the entirety of this spectrum, whilst the term ‘acute respiratory distress syndrome’ (ARDS) was to be reserved for the most severe cases. The American-European Consensus Conference (AECC) criteria for ALI and ARDS were thus defined (Table 1.2)⁵¹. This definition of ALI/ARDS was subsequently widely adopted and has been utilised as recruitment criteria in a large number of international multicentre randomised controlled trials. It was not however without criticism:

Table 1.2. The ‘American-European Consensus Conference’ and ‘Berlin’ definitions of ALI and ARDS.

	<i>AECC definition</i>	<i>Berlin definition</i>
Timing	Acute onset	Within one week of a known clinical insult or new/worsening respiratory symptoms
Oxygenation impairment	ALI: $\text{PaO}_2/\text{FiO}_2 < 300$ ARDS: $\text{PaO}_2/\text{FiO}_2 < 200$	Mild: $\text{PaO}_2/\text{FiO}_2$ 200-300 with PEEP or CPAP $> 5\text{cmH}_2\text{O}$ Moderate: $\text{PaO}_2/\text{FiO}_2$ 100-200 with PEEP $\geq 5\text{cmH}_2\text{O}$ Severe: $\text{PaO}_2/\text{FiO}_2 \leq 100$ with PEEP $\geq 5\text{cmH}_2\text{O}$ ALI abolished
Chest imaging	Bilateral infiltrates consistent with pulmonary oedema (on CXR)	Bilateral opacities – not fully explained by effusions, lobar/lung collapse or nodules (on CXR or CT scan)
Origin of oedema	Pulmonary artery occlusion pressure $\leq 18\text{mmHg}$ or no clinical suspicion of left atrial hypertension	Respiratory failure not fully explained by cardiac failure or fluid overload; Need objective assessment (e.g., echocardiography) to exclude hydrostatic oedema if no risk factor present

PEEP, positive end-expiratory pressure; CPAP, continuous positive airway pressure. $\text{PaO}_2/\text{FiO}_2$ in mmHg. ased on The ARDS Definition Task Force, (2012)⁵⁰.

Time frame

The AECC definition requires that the respiratory failure be of ‘acute onset’, but provides no guidance as to what timeframe this represents (e.g. hours, days, weeks?).

Hypoxaemia criterion

The AECC classifies hypoxaemia by the ratio of partial pressure of oxygen in arterial blood (PaO_2) to the fraction of inspired oxygen (FiO_2) - the ‘ $\text{PaO}_2/\text{FiO}_2$ ratio’. Whilst $\text{PaO}_2/\text{FiO}_2$ performs the essential function of normalising arterial oxygenation for the level of inspired oxygen administered, its performance in such a function has limitations. Firstly, the relationship between PaO_2 and FiO_2 is non-linear such that $\text{PaO}_2/\text{FiO}_2$ varies substantially as FiO_2 is altered⁵⁶. Secondly, the relationship between PaO_2 and FiO_2 is dependent on the degree of pulmonary shunt⁵⁶ and it stands to reason therefore, that manipulation of positive end-expiratory pressure may alter $\text{PaO}_2/\text{FiO}_2$ without any immediate effect on the severity of lung injury. Ferguson at al described the effect of standardising ventilator settings on the eligibility of patients for recruitment to a

clinical trial⁵⁷. Forty two patients with PaO₂/FiO₂ less than 200 (assessed prior to intervention), were subjected to a standardised ventilatory protocol (tidal volume (V_T) 6-8ml/Kg, positive end-expiratory pressure (PEEP) 10cmH₂O and FiO₂ 1.0) and PaO₂/FiO₂ was reassessed 30 minutes later. In 58.5% of patients, on re-assessment PaO₂/FiO₂ was greater than 200⁵⁷.

Chest X-ray scoring

Rubinfeld et al took the opportunity to study the interobserver variability of the chest X-ray (CXR) criteria of the AECC definition in 21 ‘experts’ recruited from those attending a mechanical ventilation workshop (in Toronto, Canada in 1997) and from members of the National Institutes of Health ARDS Network⁵⁸. Participants were shown CXRs from hypoxaemic critically ill patients and asked to decide whether they “*fulfill the AECC definition for ALI and ALI-ARDS, ‘bilateral infiltrates consistent with pulmonary edema’? Note that the American-European Consensus Conference definition specifically included mild and patchy infiltrates*”. The authors report only ‘moderate agreement’ between observers with a K-statistic value of 0.55. Infiltrates limited to lower lung zones, atelectasis, small lung volumes, mild involvement, pleural effusions, and overlying monitoring devices were identified as contributing factors for high variability of radiograph interpretations.

Meade et al, performed a large study comparing the interobserver agreement between clinicians assessing CXRs at the time they were performed, and a pair of study investigators (one a radiologist, one a critical care physician) who assessed the CXRs independently, but had previously taken part in a ‘consensus process’. The ‘consensus process’ involved the investigators independently then simultaneously reviewing a training set of 63 films, allowing discussion of the reason for disagreement and development of standards and rules that would be applied when CXR interpretation was difficult. One of eight different clinicians and both study investigators reviewed 778 CXRs from 99 critically ill patients, asking “*Is this chest radiograph consistent with ARDS?*” In keeping with the findings of Rubinfeld et al, for rater pairings who had not jointly participated in the consensus process (i.e. any of the clinicians and either of the study investigators) interobserver variability was only moderate (K-statistic of 0.38-0.55). Inter-observer variability between the two study investigators (who had participated in the consensus process) was better with K=0.72-0.88.

Pulmonary artery catheterisation

As described in the ARDS conceptual model⁵⁰, ALI/ARDS concerns the presence of non-cardiogenic pulmonary oedema; ‘permeability’ pulmonary oedema not caused by heart failure. Establishing the ‘absence’ of cardiogenic pulmonary oedema can however be challenging. Provision for such a distinction is made in the AECC definition as a pulmonary artery wedge pressure (PAWP) ≤ 18 mmHg (when measured), or “*no clinical evidence of left atrial hypertension*”⁵⁵. Though the committee concluded that “*pulmonary artery wedge [pressure] measurement was not considered essential for diagnosis in all cases*”, its use “*was recognized as clearly useful in some cases, especially in which cardiac pulmonary edema is a distinct clinical possibility*”⁵⁵.

The practice of intensive care medicine has changed significantly since the 1994 AECC was convened. Whilst pulmonary artery catheterisation was a common technique in the early 1990s, in the intervening years as a result of reports suggesting at best no difference⁵⁹ and at worst increased harm⁶⁰ resulting from pulmonary artery catheterisation, the technique is currently in decline⁶¹. Furthermore increased understanding of the complex pathophysiology of the critically ill patient has led to the understanding that ARDS and cardiac failure can co-exist, or that patients with ARDS often have elevated PAWP due to elevated pleural pressures or vigorous fluid resuscitation^{60, 62}.

Lack of validity verses histological findings of diffuse alveolar damage

Unlike many disease processes, (and reflecting the nature of ALI/ARDS as a syndrome rather than specific disease), there is no reference standard for the diagnosis of ALI/ARDS. Arguably, the closest available criterion to such a standard is the morphological finding of diffuse alveolar damage (DAD). Though obtaining specimens for morphological analysis is invasive (and so rarely appropriate), there have been several studies comparing patients identified as having ALI/ARDS by clinical criteria to the pathological findings of diffuse alveolar damage, either in lung biopsy specimens or at autopsy^{63, 64}. The findings from such studies have been consistently poor; as Frohlich et al describe, “*up to half of the patients captured by the definitions do not have the disease*”⁶⁵.

For many of these reasons, “*and because all definitions should be reviewed and adjusted periodically to reflect new information and experience*”⁵⁰, the ‘ARDS

Definition Task Force' was convened to "*address the limitations of the previous AECC definition and propose revisions*"⁶⁶.

The Berlin definition of ARDS

The 'Berlin definition' of ARDS as proposed by the 'ARDS Definition Task Force' is summarised alongside the AECC definition in Table 1.2. At first glance there seems to be little difference between the two definitions, with both fundamentally being based on the co-existence of hypoxaemia and pulmonary infiltrates on chest radiography in patients not perceived to be suffering cardiogenic pulmonary oedema. The Task Force have however made significant attempts to address many of the criticisms of the AECC definition. These can be summarised as follows:

1. A time frame of one week from a known insult or clinical deterioration has been provided as a definition of 'acute onset'.
2. The term ALI has been abolished and oxygenation criteria defined as 'mild', 'moderate' or 'severe' on the basis of $\text{PaO}_2/\text{FiO}_2$ estimates made using a standardised level of PEEP of $\geq 5\text{cmH}_2\text{O}$.
3. The chest radiography criteria have been subtly re-defined to exclude the existence of bilateral infiltrates in situations where an alternative explanation exists. In addition, with the aim of improving inter-observer variability, the panel provided a training set of CXRs "*judged by the panel to be illustrative of the spectrum of images that are consistent, inconsistent, or equivocal for the diagnosis of ARDS*"⁶⁶.
4. The need for pulmonary artery wedge pressure measurement has been removed. The potential co-existence of hydrostatic and permeability oedema has been recognised; ARDS is diagnosed when respiratory failure cannot "*be fully explained by cardiac failure or fluid overload*"⁵⁰. In cases where no risk factor for the development of ARDS can be identified, hydrostatic oedema must be objectively ruled out.

The underlying question of whether the revised 'Berlin definition' is superior to the 'AECC definition' is yet to be resolved. The initial description of the Berlin definition included a retrospective 'empirical evaluation' against data from nearly 5000 patients included in multi- and single-center clinical data sets. Whilst the definition appeared to behave appropriately in so much as mortality, ventilator free days and duration of mechanical ventilation differed as

anticipated between patients with mild, moderate and severe ARDS, the predictive values for mortality of both the AECC definition and Berlin definition were relatively poor (AUROC of 0.577 vs 0.536 respectively). It is hard to conceive that the “*better predictive validity for mortality*” observed of the Berlin definition is of any clinical significance (AUROC of 0.041, 95% CI 0.030-0.050)⁵⁰. Hernu et al attempted to perform a prospective validation of the Berlin definition and could find no relationship between severity of ARDS as defined by the Berlin definition and mortality, concluding that their study “*did not validate the Berlin definition of ARDS*”⁶⁷.

A recent study by Thille et al compared the Berlin definition of ARDS with autopsy findings of diffuse alveolar damage⁶⁸. Whilst no direct comparison was made with the AECC definition, the findings were similar to previous studies; diffuse alveolar damage was found in just 45% of patients with clinical criteria for ARDS as defined by the Berlin definition⁶⁸. Frohlich et al have been damning in their interpretation, suggesting the Berlin definition “*to be no superior to its predecessor*”⁶⁵. Without a “*biological definition*” capable of distinguishing “*hypoxemic respiratory failure as a result of alveolar inflammation (ARDS) from other pulmonary conditions*”, they argue, significant progress in the treatment of ARDS is unlikely⁶⁵.

1.3.1.2 Alternative diagnostic techniques

Frohlich et al have not been alone in their criticism of the Berlin definition of ARDS, with many authors concluding both that reform was needed, and that the Berlin definition ‘does not go far enough’^{65, 69-72}.

For many years the late Daniel Schuster (formerly Chair in Respiratory Intensive Care Medicine and a professor of medicine, University of Washington)^B made a consistent and vocal argument for the inclusion of some objective measurement of pulmonary oedema and increased vascular permeability in the diagnostic criteria for ALI/ARDS⁷³⁻⁷⁶. ARDS he describes “*should not be a diagnosis of exclusion but should instead depend on some direct measure of lung injury*”⁷³. In

^B Much is made in this section of the opinions of the late Daniel Schuster; it must be emphasised, that his work was widely cited, and his opinions shared by a significant number of the wider critical care community.

a patient with acute bilateral radiographic infiltrates, the diagnosis of ARDS could then be made based on the (objective) presence of both pulmonary oedema and increased pulmonary vascular permeability.

It is inherent, that both at the clinical bedside and in the conduct of clinical research, in addition to the ability to diagnose ALI/ARDS, the ability to characterise the severity would be advantageous. The AECC definition categorises patients into ALI or ARDS, whilst the new Berlin definition makes provision for mild, moderate and severe categories of ARDS. Clinicians / researchers wishing to quantify severity beyond these broad categories, or indeed to monitor change over time, will often do so on the basis of oxygenation ($\text{PaO}_2/\text{FiO}_2$) or Lung Injury Score (LIS). As Schuster describes:

“It seems reasonable to assume that as an injury becomes more severe, recovery becomes less likely. Thus, implied in any attempt to quantify injury is really an attempt to determine prognosis.”

Schuster D.P. (1998)⁷⁵

Yet neither the $\text{PaO}_2/\text{FiO}_2$ nor the LIS are good predictors of mortality^{77, 78}. It is arguable that in addition to the need for a ‘biological definition’ and appropriate and objective ‘diagnostic criteria’, what is also required is a novel ‘measurement of severity of lung injury’. Though emphasising the important distinctions between ‘definition’, ‘diagnostic criteria’ and ‘severity of injury’, in the case of ALI/ARDS Schuster further argues;

“It seems natural, in the case of lung injury, to assume that measures of pulmonary edema and increased vascular permeability could be used for this purpose as well as for diagnosis”.

Schuster From, Schuster D.P., (1998)⁷⁵

To date, two broad streams of research have come closest to offering a quantifiable, ‘biological’ measure of lung injury. Though both were considered by the ARDS Definition Task Force but discounted “because of current feasibility and lack of data on operational characteristics”^{50, 66}, the measurement of extravascular lung water and pulmonary vascular permeability index by transpulmonary thermodilution^{69, 70, 73, 74, 79}, and the measurement of plasma biomarkers of lung injury⁸⁰⁻⁸³ have been advocated by many commentators as

potential candidates for addition to the diagnostic criteria of ALI/ARDS. Investigations III and IV concern the application of these technologies in the post-operative period following lung resection. A detailed literature review concerning each topic is provided alongside.

1.3.2 Pathophysiology of ALI/ARDS

It is outwith the scope of this work to provide a detailed examination of the pathogenesis of ALI/ARDS. In this section, I shall discuss the patho-physiology of ALI/ARDS with specific reference to the contribution of ventilator induced lung injury (VILI), and the development of peri-operative lung injury.

The pathophysiological hallmark of ALI/ARDS is increased permeability at the alveolar-capillary interface, which is manifest morphologically as diffuse alveolar damage. Histological examination in established ARDS reveals neutrophil, macrophage and erythrocyte infiltration of the alveolar space, hyaline membrane formation, the presence of protein rich oedema fluid and disruption of the alveolar epithelium⁸⁴. Many years of experimental studies have defined the contribution of neutrophils, cytokines, reactive oxygen and nitrogen species and dysregulation of the coagulation cascade to the pulmonary inflammatory injury sustained in ALI/ARDS. As can be observed in the now iconic figure from Ware and Matthay, lung injury results from a complex cascade of simultaneously occurring pathological processes (Figure 1.7) occurring both at the endothelial and epithelial sides of the alveolar-capillary barrier.

Pulmonary endothelial injury leads to formation of intracellular gaps between endothelial cells and necrosis, fragmentation and sloughing of the pulmonary endothelium. It is (as Ware describes) *“this focal and reversible gap formation that is accepted as the ultra-structural basis for increased microvascular permeability”*⁸⁵. Whilst endothelial injury has long been recognised as a mechanism of increased permeability and consequent pulmonary oedema formation, in ALI/ARDS, the role of epithelial injury has more recently been described. In addition to contributing to the development of increased permeability of the alveolar-capillary barrier, epithelial injury further compromises alveolar epithelial fluid transport, preventing reabsorption alveolar fluid and increasing the severity and duration of the oedema^{32, 84, 85}. In

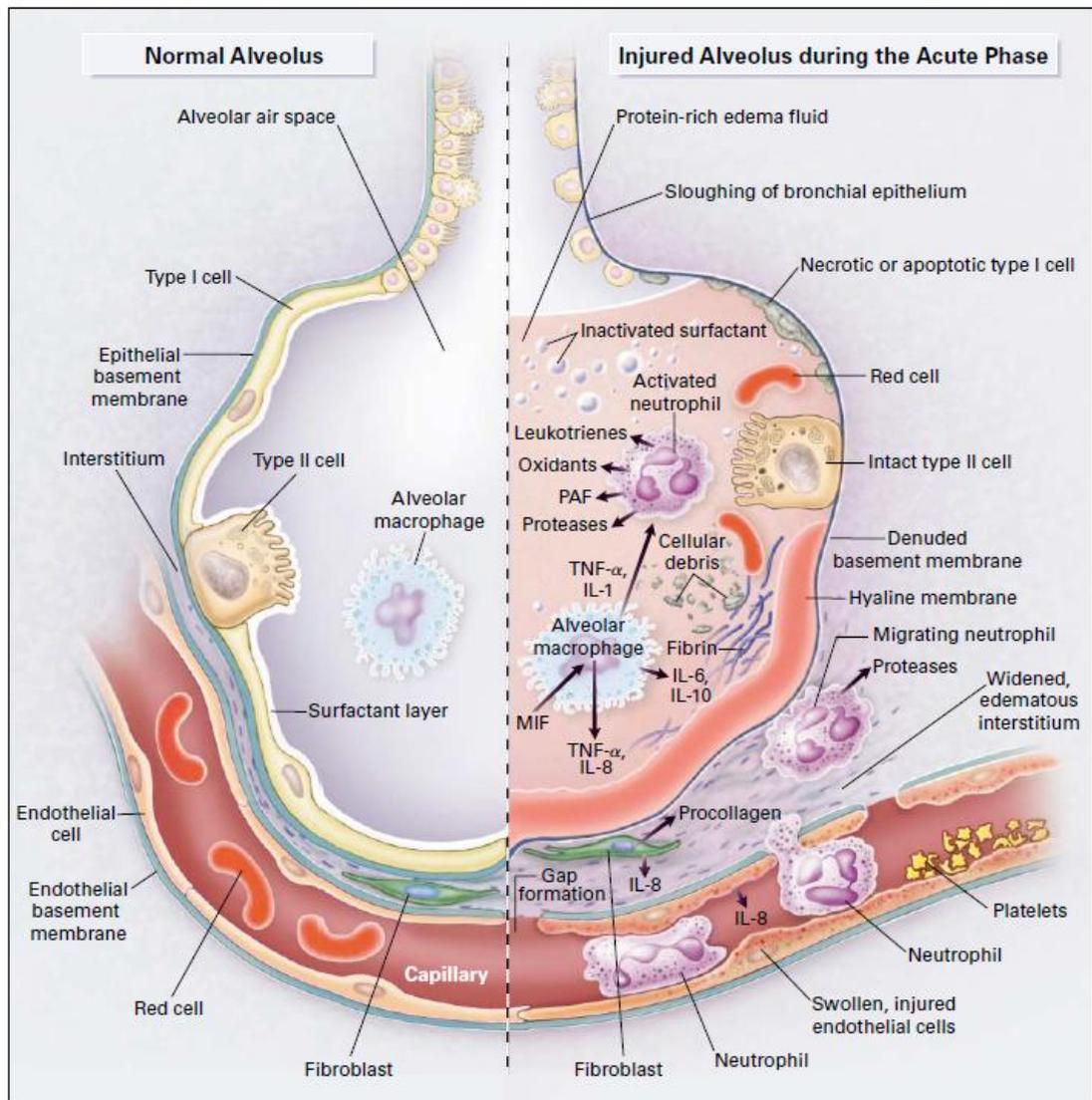


Figure 1.7. The normal and injured alveolus in the acute phase of acute lung injury / acute respiratory distress syndrome.

Simultaneous neutrophil, cytokine and oxidative mediated injuries to both the pulmonary endothelial and epithelial cells leads to formation of intracellular spaces between pulmonary endothelial cells, denudement of the basement membrane, sloughing of bronchial epithelium and necrosis and apoptosis of epithelial cells resulting in accumulation of protein rich oedema fluid and development of hyaline membranes. From Ware and Matthay (2000)⁶⁴.

in addition, reduced surfactant production by type II pneumocytes contributes to the altered lung mechanics and gas exchange abnormalities observed.^{20, 21}

1.3.3 Ventilator induced lung injury in the pathogenesis of ALI/ARDS

Since its introduction to critical care medicine, born of necessity during the 1952 polio epidemic, mechanical ventilation has long since been recognised as the main stay of supportive therapy in patients with ALI/ARDS; 7 of 12 patients in Ashbaugh's initial description of the syndrome received 'respirator' support⁵¹.

Whilst undoubtedly a life saving supportive therapy, in the years since its introduction the potentially harmful effects of mechanical ventilation have increasingly been recognised.

1.3.3.1 Barotrauma in the pathogenesis of ventilator induced lung injury

As early as the 1950's, clinicians were recognising the potential for mechanical ventilation to cause injury to the lung⁸⁵, and by the 1970's, the term 'barotrauma' was in recognised use⁸⁶. Gross barotrauma refers to the appearance of air leaks manifest as pneumothoraces, pneumomediastinum, surgical emphysema and gas embolism. Whilst such macroscopic trauma is easily recognisable, it became clear to researchers that a more subtle physiological and morphological syndrome can result from mechanical ventilation with *"alterations of lung fluid balance, increases in endothelial and epithelial permeability and severe ultrastructural damage"*⁸⁷.

Webb and Tierney were in 1974 the first to demonstrate that mechanical ventilation could induce lung injury in otherwise normal lungs⁸⁸. By ventilating rats for one hour with increasing levels of peak airway pressure (P_{peak}) the authors observed that at a P_{peak} of 14 cmH₂O there was no histological evidence of lung injury, at P_{peak} of 30cmH₂O there was evidence of perivascular oedema whilst at 45cmH₂O all the rats ventilated developed severe pulmonary oedema, with histological evidence of marked perivascular and alveolar oedema; all of the animals died before the end of the hour. Similar findings were demonstrated by others both in rats and a variety of other animal species⁸⁸.

1.3.3.2 Volutrauma in the pathogenesis of ventilator induced lung injury

In the late 1980's, Dreyfuss et al suggested that it was the effects of high tidal volumes, rather than pressures that was responsible for causing lung injury and coined the term 'volutrauma'⁸⁹. To discriminate between the effects of pressure and volume, Dreyfuss et al ventilated rats at high inflation pressures and varied the resulting tidal volume by limiting chest expansion by application of thoracoabdominal strapping. Rats exposed to high-pressure, high-volume ventilation rapidly developed pulmonary oedema whilst those in which tidal volume was limited did not (Figure 1.8). Adding further weight to this

hypothesis, Dreyfuss et al included another group of animals in which high tidal volumes were achieved, but following application of negative pressures in an iron lung. Similar levels of oedema were observed in both high-pressure, high-volume and low-pressure, high-volume groups (Figure 1.8)⁸⁹.

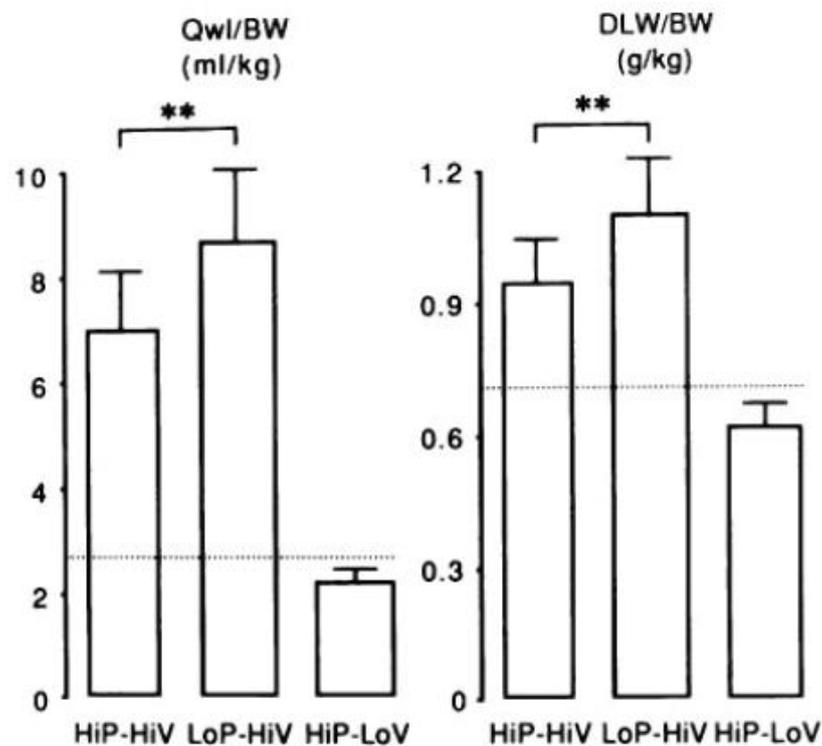


Figure 1.8. Comparison of the effects of high-pressure, and high volume ventilation on the development of lung injury.

Lung injury is assessed by determination of extravascular water content (Qwl/BW) and permeability alterations by bloodless dry-lung weight (DLW/BW). HiP, high-pressure; HiV, high volume; LoP, low-pressure (iron lung ventilation); LoV, low volume (thoracoabdominal strapping). Dotted lines represent the upper 95% confidence limit for control values. From Dreyfuss et al (1988)⁸⁹, as reproduced by de Prost et al (2011)⁹⁰.

1.3.3.1 Biotrauma in the pathogenesis of ventilator induced lung injury

To this point, the mechanism of barotrauma and volutrauma in the causation of lung injury has been assumed to be purely mechanical. Where the mechanical effects of high pressure and overdistention at high volumes are extreme enough to ‘break’ the lung structure, there is little doubt that lung injury by such a mechanism can occur. Since the early 1990s however, the potential for the mechanical forces described to lead to release of inflammatory mediators has been understood. Such an effect may occur ‘directly’ by injury to pulmonary epithelial and endothelial cells or ‘indirectly’ by transduction of these forces leading to activation of cell-signalling pathways in epithelial, endothelial and

inflammatory cells⁹¹. Evidence for such 'biotrauma' has been provided by the observation of neutrophil infiltration in animals ventilated with high peak pressures, and the recognition of a systemically deleterious effect of mechanical ventilation⁹⁰. In addition there have been countless animal and human studies reporting increased levels of both pulmonary and systemic cytokine levels in proportion to the intensity of the mechanical ventilation. To cite one such example, Tremblay et al demonstrated increased levels of tumour necrosis factor- α , interleukin-1 β (IL-1 β), IL-6 and macrophage inflammatory protein-2 in unperfused rat lungs subjected to high tidal volume ventilation when compared to controls⁹².

1.3.3.2 Atelectotrauma in the pathogenesis of ventilator induced lung injury

In addition to the injurious effects of high lung volumes, it is also evident that lung injury occurs at low lung volumes^{90, 91}. Application of positive end-expiratory pressure (PEEP) has been demonstrated by many to protect against lung injury. In Webb and Tierney's seminal paper discussed above, application of 10cmH₂O PEEP resulted in a less severe lung injury and indeed survival of all of the animals subjected to ventilation at P_{peak} of 45 cmH₂O⁸⁸. Prevention of cyclical recruitment / de-recruitment of distal lung units has become accepted as a mechanism by which PEEP prevents lung injury^{90, 93}. Rather than absolute lung volume or pressure, it appears that large cyclical changes in lung volume / pressure may lead to the development of lung injury⁹⁰. This is supported by the findings of Cobridge et al, who demonstrated in hydrochloric acid injured dog lungs that at equivalent peak inspiratory pressure, low tidal volume and high PEEP resulted in reduced lung injury in comparison to high tidal volume and low PEEP⁹⁴. Similar effects have been demonstrated by others in a variety of models.

In addition to the effects of cyclical recruitment / de-recruitment the presence of atelectatic lung regions has been demonstrated to contribute to the pathogenesis of lung injury by a number of other mechanisms⁹⁵: Localised alveolar hypoxia in atelectatic regions has been shown to induce lung inflammation through macrophage recruitment⁹⁶, whilst the presence of atelectatic lung regions which do not undergo tidal recruitment leads to increased mechanical strain on adjacent ventilated lung units. Mead et al

calculated that at an airway pressure of 30 cmH₂O, a pressure as high as 140 cmH₂O may be exerted locally at the interface between closed and open lung units⁹⁷.

1.3.3.3 Lung protective mechanical ventilation in the prevention of ventilator induced lung injury

Gattinoni has described the lung in established ARDS as resembling that of a baby⁹⁸. In studies using computed tomography in patients with lung injury, the volume of normally aerated lung tissue he reports, has the dimensions of that of a 5-6 year-old child (300-500g aerated lung tissue). Furthermore, Gattinoni demonstrated that the intrinsic elasticity of the aerated lung units was normal, suggesting that in ARDS the lung should be thought of as ‘small’ rather than ‘stiff’⁹⁸. From these observations and those discussed above under the headings baro-, volu-, bio- and atelecto- trauma it becomes easy to understand what needs to be done in order to provide safe, ‘lung protective’ mechanical ventilation. Reducing tidal volume to the dimensions of the hypothesised baby lung and limiting peak inspiratory pressures should prevent baro- and volu-trauma, whilst institution of appropriate levels of PEEP should serve to maximise the functional volume of the ‘baby lung’ whilst preventing harmful recruitment / de-recruitment. Clearly avoidance of baro-, volu- and atelecto- trauma should in turn limit bio-trauma.

In 2000, a large body of pre-clinical and early phase clinical study data culminated in the publication of the US ARDS Network’s multicentre randomised clinical trial on “*Ventilation with Lower Tidal Volumes as Compared with Traditional Tidal Volumes for Acute Lung Injury and the Acute Respiratory Distress Syndrome*”⁹⁹. In this study of 861 patients with ARDS (defined according to the AECC definition), the authors compared the effects of ‘traditional’ tidal volumes ($V_T=12\text{ml/kg}$) versus ‘lower’ tidal volumes ($V_T=6\text{ml/kg}$) alongside a standardised approach to setting PEEP. The number of ventilator free days and mortality was lower in the group treated with lower tidal volumes than in the group treated with traditional tidal volumes (31.0 vs. 39.8% mortality respectively, $P=0.007$, Figure 1.9)⁹⁹.

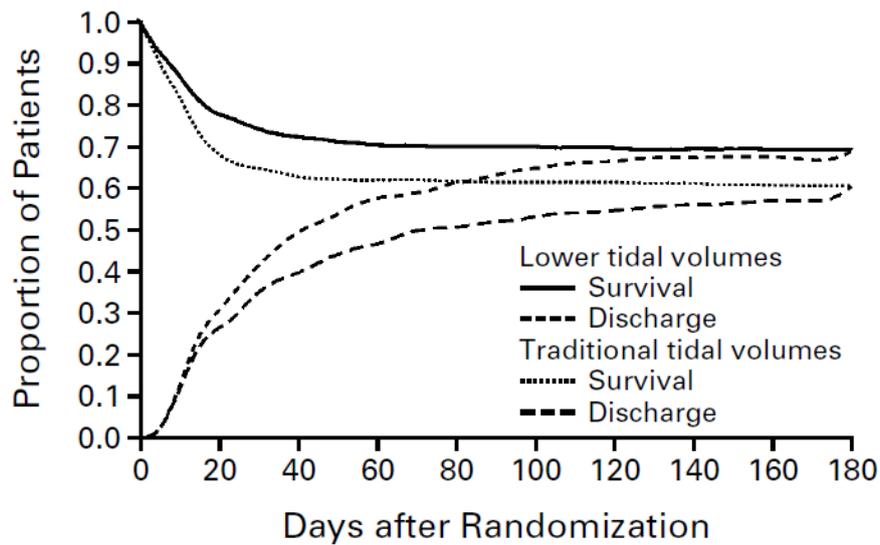


Figure 1.9. Probability of survival and hospital discharge in the US ARDS Network's 'lower tidal volume ventilation' trial.

'Lower' tidal volume = 6ml/kg, 'traditional' tidal volume = 12ml/kg. 'Discharge' reflects being discharged home and breathing without assistance, during the first 180 days after randomization. From the US ARDS Network, (2000)⁹⁹.

At the time of writing (13th October 2014), this study has been cited 3128 times (source: <http://www.nejm.org/doi/full/10.1056/NEJM200005043421801>), and it is no exaggeration to suggest that no other study has had greater influence on the practice of critical care medicine. Indeed the study's effects have been wider reaching, triggering alterations in the practice of mechanical ventilation in the operating theatre environment.

1.3.3.4 Ventilator induced lung injury and the 'multiple-hit' hypothesis of ALI/ARDS pathogenesis

Many of the animal studies discussed in this section have so far, have been concerned with the deleterious effects of mechanical ventilation at extremes of pressure or volume on the development of lung injury in normal lungs. It is important to discuss however a parallel body of work suggesting that pre-existing injury may sensitise the lungs to the deleterious effects of mechanical ventilation⁹⁰. For example, Hernandez et al studied an ex-vivo rabbit model of oleic acid induced injury¹⁰⁰. Whilst neither low doses of inhaled oleic acid nor mechanical ventilation (P_{peak} 25cmH₂O) alone were sufficient to cause lung injury, the combination of oleic acid inhalation *and* mechanical ventilation led to increased pulmonary capillary permeability¹⁰⁰.

Such a concept has become embodied in the ‘multiple-hit’ hypothesis of ALI/ARDS. It has been hypothesised that mechanical ventilation may serve as a ‘second-hit’, increasing the risk of lung injury development in mechanically ventilated patients who at the time of ventilation, do not have ALI/ARDS, but whose lungs have been primed for injury by a physiologic insult such as pneumonia, aspiration or sepsis¹⁰¹⁻¹⁰³. Given the great success of instituting lung protective ventilation in patients with established ALI/ARDS⁹⁹, it seems a logical step therefore to apply the principles of lung protective ventilation to patients ‘at risk’ of ALI¹⁰⁴.

1.3.3.5 Peri-operative lung injury

Two large retrospective cohort studies have reported a 2.6-7% incidence of ALI/ARDS in the post-operative period^{105, 106}. Patients with pre-existing sepsis, undergoing emergency procedures or those involving aortic-cross clamping, cardiopulmonary bypass and one-lung ventilation have been identified as being at particularly increased risk^{105, 106}. It is intuitive therefore to suggest that peri-operative lung injury may also be the consequence of a similar ‘multiple-hit’ model where the deleterious effects of mechanical ventilation may be sufficient to induce lung injury in patients already at increased risk due to the specifics of the type of surgery, or their pre-existing condition. From here, it appears a further logical step, to apply the principles of lung protective ventilation to patients at increased risk of ALI/ARDS in the peri-operative period. Indeed, data from a meta-analysis (incorporating many patients undergoing lung resection surgery) has demonstrated a reduction in the incidence of post-operative ALI/ARDS in patients ventilated to a lower tidal volume / higher PEEP protocol (risk ratio 0.40 (95% CI 0.22-0.70) for lower VT, 0.29 (0.14-0.60) for PEEP)¹⁰⁷.

1.4 Post-lung resection acute lung injury

The occurrence of pulmonary oedema in the early post-operative period following lung resection was first reported by Gibbon and Gibbon in 1942¹⁰⁸. In this publication, the authors report the case histories of two young patients (aged 19 and 24) undergoing pulmonary lobectomy for bronchiectasis. The patients died in the early post-operative period and in both cases the only significant finding on autopsy was of oedema of the remaining lobes. The most frequently cited case series of patients with oedema following lung resection was published in 1984 by Zeldin et al¹⁰⁹. The authors reported a case series of 10 patients who developed oedema following “*otherwise uncomplicated pneumonectomies*”; “*Post-pneumonectomy pulmonary oedema* [also the title of the paper] *has become a worldwide problem*” they report. Though the term ‘post-pneumonectomy pulmonary oedema’ was in widespread use in 1984, it is evident from others (including Gibbon et al who first reported the syndrome¹⁰⁸), that the syndrome of pulmonary oedema after lung resection is not restricted to pneumonectomy, but also occurs after lesser resections^{110, 111}.

1.4.1 Nomenclature and definitions

Variably known as ‘post-pneumonectomy pulmonary oedema’^{109, 112-114}, ‘post-thoracotomy acute lung injury’¹¹⁵, ‘permeability pulmonary oedema’¹¹⁶, ‘acute lung injury after thoracic surgery’¹¹⁷ and ‘low pressure oedema’¹¹⁸, the clinical syndrome of pulmonary oedema formation following thoracic surgery for lung resection has been well documented. To date, however, there is no established definition nor nomenclature for what will be discussed from here on in this thesis as ‘post-lung resection acute lung injury’ (PLR-ALI). A number of definitions have been reported:

“Oedema formation following pneumonectomy characterized by normal cardiac filling pressures, high pulmonary artery pressures and high cardiac output”.

Peters et al (1989)¹¹²

“Respiratory distress in a patient with pulmonary infiltrates on chest radiography but in the absence of evidence of cardiac failure, pneumonia, sepsis or aspiration”.

Turnage et al (1993)¹¹⁹

“Severe and often lethal respiratory failure secondary to non cardiac pulmonary edema shortly after resection of the lung”.

Shapira et al (1993)¹²⁰

“Pulmonary oedema and hypoxaemia developing after lung resection”.

Jordan et al (2000)¹²¹

Following the adoption of the 1994 American-European consensus conference (AECC) definition for ALI/ARDS, and the recognition that the pulmonary oedema occurring following lung resection *“follows a clinical and histopathological course indistinguishable from ARDS”* (discussed below)¹²¹, in recent years many authors have used the AECC definition to define PLR-ALI^{115, 122}.

1.4.2 What is post-lung resection ALI?

A large number of authors have used the AECC definition of ARDS⁵⁵ to identify a population of patients with ALI/ARDS post-operatively following lung resection. By definition therefore, evidence of hypoxaemia with radiological evidence of bilateral^c radiographic opacities is evident in a cohort of patients following lung resection. The question which remains however is whether these clinical appearances reflect the same underlying pathophysiological processes which are seen in ALI/ARDS?

Jordan et al suggest that *“In its extreme form, [post-pneumonectomy pulmonary oedema] follows a clinical and histopathological course indistinguishable from acute respiratory distress syndrome”*¹²¹. In agreement with these authors, post-lung resection ALI (PLR-ALI) is widely interpreted to be a variant of ALI/ARDS following an identical clinical and pathophysiological course^{115, 117, 118, 122-124}. As discussed previously, the ARDS definition task force describe ARDS as a syndrome of:

^c Though strictly the AECC definition describes *bilateral* opacities, this definition is also widely applied to patients who have undergone pneumonectomy.

*“acute diffuse inflammatory lung injury... [characterised by] increased pulmonary vascular permeability [and the] morphological hallmark... diffuse alveolar damage. [Furthermore] patients may qualify as having ARDS as long as they have respiratory failure not fully explained by cardiac failure or fluid overload”*⁵⁰.

‘ARDS Definition Task Force’ (2012)⁵⁰

To support Jordan et al’s hypothesis therefore, evidence is required of pulmonary oedema formation following lung resection, in the absence of cardiac failure, with increased pulmonary vascular permeability and diffuse alveolar damage. The evidence for each is presented in turn.

1.4.2.1 Evidence of non-cardiogenic pulmonary oedema following lung resection

In addition to the presence of hypoxia and pulmonary infiltrates on chest radiography, diagnosis of acute lung injury according to the American-European consensus conference (AECC) definition of ARDS⁵⁵ requires the absence of cardiac failure. Whilst by strict (AECC) definition this requires documentation of pulmonary artery wedge pressure of less than ≤ 18 mmHg, this is generally interpreted to reflect the absence of clinical evidence of left atrial hypertension⁵⁰. By definition therefore, all of the reports of PLR-ALI made using the AECC definition as diagnostic criteria are reporting the presence of oedema without evidence of cardiac failure.

Several authors have gone further to confirm the non-cardiogenic nature of the oedema. In Zeldin et al’s, cohort of 10 patients with post-pneumonectomy pulmonary oedema discussed above, the authors report that central pressure measurements (pulmonary artery wedge pressure) revealed no evidence of left ventricular failure or cardiogenic pulmonary oedema¹⁰⁹. Mathru et al, reported five cases of non-cardiogenic pulmonary oedema occurring after lung resection. They reported that the *“non-cardiac origin of the oedema is suggested by the presence of normal filling pressure, [and] normal or high cardiac output”*¹¹⁶. Turnage et al, report pulmonary artery catheter data from 21 patients with PLR-ALI; mean pulmonary artery occlusion pressure was in the region of 10-14mmHg for the duration of the monitored period¹¹⁹.

1.4.2.2 Evidence of increased pulmonary vascular permeability following lung resection

Waller et al, performed lung scintigraphy in 21 men following lung resection¹²⁵. By use of a radio-labelled albumin technique to observe a statistically significant accumulation of pulmonary albumin in the first six hours post-operatively, Waller et al demonstrated evidence of increased pulmonary vascular permeability post-operatively in 9 out of 10 patients who had undergone pneumonectomy. In a further 11 patients who had undergone lobectomy, 6 patients demonstrated increased permeability but this was not statistically significant. No changes in either pulmonary artery wedge pressure nor pulmonary capillary pressure were observed peri-operatively, mitigating against a hydrostatic component to the protein extravasation. Only one patient displayed clinical evidence of PLR-ALI in this case series suggesting that subclinical changes in pulmonary vascular permeability are occurring in the majority of patients.

Mathru et al investigated the aetiology of pulmonary oedema in five patients suffering “*severe respiratory distress and.. [demonstrating] x-ray evidence of diffuse interstitial pulmonary oedema within 12 hours*” of lung resection (4 following pneumonectomy, 1 following lobectomy). They report that the mean ratio of oedema fluid protein to serum protein was 0.6 or greater suggesting an oedema caused by increased permeability rather than transudation¹¹⁶.

1.4.2.3 Morphological evidence of diffuse alveolar damage following lung resection

Kozian et al studied a porcine model of one lung ventilation and thoracic surgery. Animals were subject to one-lung ventilation, left lateral thoracotomy and repeated handling of lung tissue. Following euthanasia and tissue harvesting, the authors observed histologic evidence of alveolar oedema, interstitial oedema, microatelectasis, microhaemorrhage, neutrophil infiltration and alveolar overdistention; the “*pathomorphologic*” features of diffuse alveolar damage (DAD) in both lungs¹²⁶.

Turnage et al reported autopsy findings from 17 patients who died from 'post-pneumonectomy pulmonary oedema'.¹¹⁹ Post-pneumonectomy pulmonary oedema was defined in this study (which precedes the AECC) as respiratory distress in a patient with pulmonary infiltrates on chest radiography but in the absence of evidence of cardiac failure, pneumonia, sepsis or aspiration. Of the 21 patients with PPE by these criteria, Turnage et al observed characteristic histological findings of ARDS (no definition provided) in 15 of 17 cases.

In summary, there appears to be good evidence to suggest that a syndrome exists following lung resection of hypoxia, radiological findings of pulmonary oedema, where the oedema appears to be one of non-cardiogenic origin with evidence of increased pulmonary permeability and with histological findings in both animal models and autopsy specimens of DAD. It seems reasonable therefore to conclude that PLR-ALI is a variant of ALI/ARDS and that lung resection simply serves to trigger the same pathophysiological and clinical syndrome in the susceptible individual.

1.4.3 Incidence and mortality of post-lung resection ALI

Investigation II of this thesis is a meta-regression analysis of the incidence and mortality of PLR-ALI. More extensive discussion of the incidence and mortality is therefore provided in that section (chapter 3).

The incidence of PLR-ALI is variously quoted as being between 2 and 11%¹²⁷⁻¹³⁰.

The overall (all cause) mortality following lung resection in the UK has been falling over time. Mortality following lobectomy for primary lung cancer has fallen from in excess of 4% in the 1980s to current levels of just under 2%⁵. It is interesting however to note a changing trend in the causes of mortality following lung resection. Historically, surgical complications (for example broncho-pleural fistula and empyema) were the leading cause of post-operative death in patients undergoing lung resection. As improvements in surgical technique have led to a reduction in surgical complications, in recent years respiratory complications have been reported to be the major cause of mortality¹³¹.

Though the incidence of PLR-ALI may be low, the mortality of PLR-ALI is high. The mortality from PLR-ALI is generally quoted as being in the region of 40 to 60%^{110, 111, 129, 130, 132}. In some series mortality in excess of 80% has been reported¹³³⁻¹³⁵. As with the incidence of PLR-ALI, mortality appears to be related to size of resection; in a study of 50 patients developing PLR-ALI and requiring post-operative mechanical ventilation, Dulu et al reported a 50% mortality following pneumonectomy, 42% following lobectomy and 22% following sub-lobar resections¹¹¹. Though no direct comparisons have been made, the mortality from PLR-ALI appears to be higher than the 44% mortality reported for ALI/ARDS in the general intensive care population (outwith clinical trials)¹³⁶.

Ruffini et al report the incidence and mortality of PLR-ALI in a case series of 1221 patients undergoing lung resection in the 1990s. PLR-ALI developed in 2.2% of cases and carried a 52% mortality. This meant that PLR-ALI accounted for 41% of the overall hospital mortality of 1.2% and that PLR-ALI was the major cause of mortality following lung resection¹²⁹. Kutlu et al¹³⁰ reached the same conclusion:

“ALI and ARDS are the major causes of mortality after lung resection”.

1.4.4 Pathophysiology of post-lung resection lung injury

1.4.4.1 PLR-ALI as a single disease process?

In 2003 Licker et al published one of the seminal papers exploring the risk factors for PLR-ALI¹²⁸. In a cohort of 879 consecutive patients from a single institution undergoing thoracic surgery for lung cancer, Licker et al prospectively collected clinical, anaesthetic, surgical, radiological, biochemical and histopathologic data. The overall incidence of PLR-ALI was 4.2% (n=37). The authors reported a bimodal distribution of PLR-ALI (Figure 1.10).

In the majority of cases (n=27), PLR-ALI developed within the first three days post-operatively, whilst a lesser proportion (n=10) developed ALI after the third post operative day. Licker et al defined the early cases of PLR-ALI as ‘primary ALI’, attributing them to the pathophysiological processes discussed below, whilst the latter cases, defined as ‘secondary ALI’ appeared to occur following

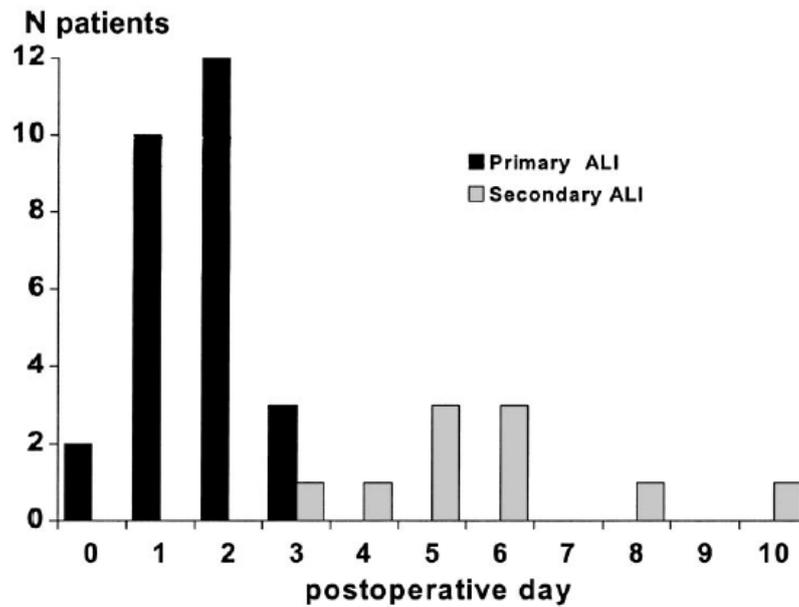


Figure 1.10. Time-related distribution of ALI after lung resection.
The bimodal distribution is clearly evident. From Licker et al, 2003¹²⁸.

the development of other complications (e.g. bronchopneumonia, gastric aspiration or broncho-pleural fistula)¹²⁸.

The remainder of this discussion concerning the pathophysiology of PLR-ALI (and to a great extent the rest of thesis), concerns the development of primary PLR-ALI.

1.4.4.2 Pathophysiology of primary PLR-ALI

Given the discussions above, it is inherent that the cellular pathology resulting in pulmonary oedema formation in PLR-ALI is the same as in any other form of ALI; endothelial barrier function is disrupted and there is extravasation of plasma. Gothard succinctly describes that PLR-ALI “*probably represents the pulmonary manifestations of a panendothelial inflammatory vascular injury*”^{121, 122}. Copious evidence has been presented documenting increases in both pro- and anti-inflammatory cytokine levels and the generation of reactive oxygen and nitrogen species (ROS & RNS) both systemically and in the lung in patients undergoing lung resection. As with ALI/ARDS in the non-lung resection population, it is unlikely that PLR-ALI occurs as a result of any single aetiological factor, but rather as the result of a ‘multiple-hit’ sequence of deleterious events^{115, 117, 118, 123, 126, 137}. In the following discussion a number of potential triggers are examined.

As the thoracic anaesthetist is forced to separate the lungs and ventilate one independently of the other, when considering the pathophysiology of PLR-ALI the lungs must similarly be thought of separately. The dependent, ventilated, 'anaesthetic' lung and the non-dependent, non-ventilated, 'surgical' lung are subject to parallel insults, each potentially resulting in, or contributing to the development of lung injury.

Pathophysiology of injury to the dependant, ventilated lung

In 2002, Padley et al reported the results of a retrospective review of intensive care admissions of patients with ALI after lung surgery¹³⁸. Nine of the 17 patients subsequently identified as having sustained post-operative ARDS following lobectomy or sub-lobar resection had both pre-operative and post-operative CT scans available for analysis. In 8 of these 9 patients the authors observed lung density to increase more in the non-operated lung than in the operated lung, causing the authors to conclude that "*following lobectomy, there appears to be a truly asymmetric form of ARDS*", with "*relative sparing of the lung that underwent lobectomy*" (Figure 1.11)¹³⁸.

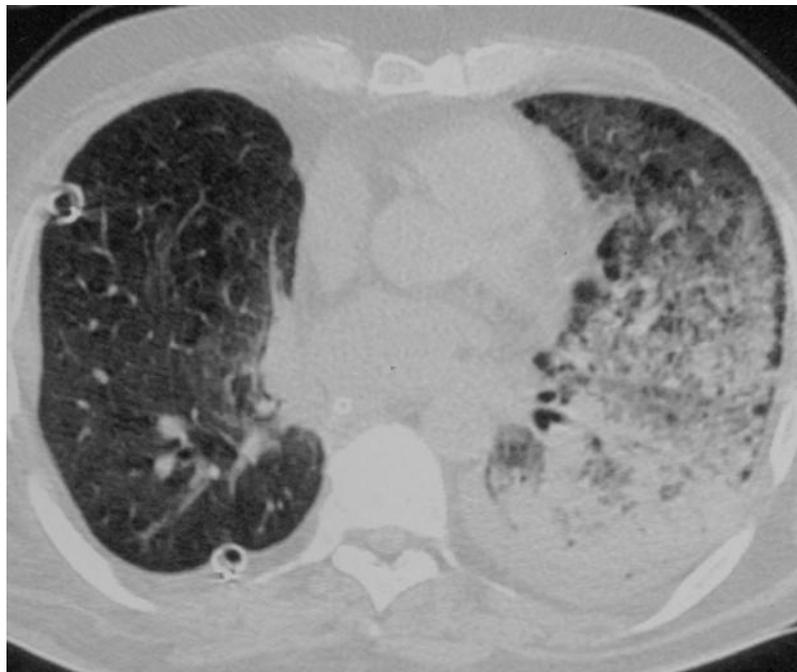


Figure 1.11. Transverse computed tomography image obtained post-operatively following lung resection.

Following right sided resection (left side of the image, evidenced by presence of chest drains), there is marked asymmetry of parenchymal opacity, with relative sparing of the operative lung. Ground glass parenchymal opacification, increased prominence of interlobular septa and an anteriorposterior opacity gradient are evident in the non-operative lung consistent with development of lung injury. From Padley et al (2002)¹³⁸.

Factors implicated in the development of lung injury in the ventilated, dependant lung are ventilator induced lung injury, oxygen toxicity and hyperperfusion. Each will be considered in turn.

Ventilator Induced Lung Injury

The concept and pathophysiology of ventilator induced lung injury (VILI) has been discussed in detail (section 1.3.3). It is reasonable to expect that VILI may equally be a problem during one lung ventilation as during two; indeed if during OLV the ventilated lung is exposed to increased pressures, tidal volumes or fraction of inspired oxygen then the potential for VILI during OLV may be increased.

Traditional teaching of thoracic anaesthesia has described OLV with a target tidal volume of 10 mL/kg, an FiO₂ of 1.0, zero positive end expiratory pressure (ZEEP) and an intention to maintain normocapnia^{124, 139-141}. Such advice was provided primarily to guard against hypoxaemia, with the belief that “*relatively large tidal volumes are needed to recruit alveoli in the dependent ventilated lung*”¹⁴⁰. Katz et al had previously demonstrated that higher tidal volumes and ZEEP were associated with improved oxygenation during OLV¹⁴².

In recent years however, in parallel with the greater understanding of the role of ventilator associated lung injury in the general intensive care population, and the recognition of the striking similarities between the single ventilated lung and the baby lung concept described by Gattioni in ARDS⁹⁸ (a smaller total lung volume, with decreased oxygenation and V/Q mismatch from increased shunt and atelectasis^{124, 143}), it became apparent use of high tidal volumes during OLV may be harmful^{122, 124, 141}.

Whilst ventilating one lung with the same tidal volume as two intuitively provides the potential for volutrauma, applying such a large tidal volume to a single lung also holds the potential for development of barotrauma. Szegedi et al reported that with a fixed tidal volume of 10ml/kg, peak airway pressure increased (P_{peak}) by 55% and plateau pressure by 42% (P_{plateau}) when switching from two- to one-lung ventilation¹⁴⁴. Whilst Szegedi et al's observations were made in the supine position¹⁴⁴, the potential for barotrauma is increased further as a result of reduced compliance of the dependant ventilated lung intra-

operatively. Larsson et al report a fall in compliance from 29 to 23 ml/cmH₂O ($p < 0.05$; $n = 8$) on turning from supine to the lateral decubitus position¹⁴⁵.

Evidence from animal models of one-lung ventilation

Gama de Abreu et al used an isolated, perfused rabbit lung model to examine the effects of tidal volume (V_T) on the development of lung injury during OLV¹⁴⁶. Animals were randomised to non-protective OLV (no V_T reduction on institution of OLV (~8ml/kg), and zero positive end-expiratory pressure (ZEEP)), or to protective OLV (V_T 50% of pre-OLV values (~4ml/kg), with PEEP) and controls (two-lung ventilation). Non-protective OLV resulted in significantly increased P_{peak} (13.5 vs 5.1 cmH₂O; $p < 0.001$) compared to protective OLV. After ninety minutes of OLV, preparations exposed to non-protective OLV demonstrated increased lung injury as evidenced by significantly greater lung weight gain and thromboxane B₂ concentration in perfusate compared to protective OLV and controls¹⁴⁶.

Kuzkov et al reported significantly reduced extravascular lung water post-operatively following pneumonectomy in sheep that were ventilated with protective ventilation (V_T 6ml/kg, PEEP 2cmH₂O) compared with controls (V_T 12ml/kg, ZEEP¹⁴⁷)^D.

Evidence from human studies of one-lung ventilation

Due to the relative infrequency of ALI development, several human studies have examined pulmonary and systemic cytokine levels as a surrogate marker for lung injury. Schilling et al reported increased numbers of intra-alveolar cells, and protein, albumin, interleukin-8 (IL-8), elastase, IL-10, tumour necrosis factor alpha (TNF- α), soluble intracellular adhesion molecule (sICAM) concentrations in (dependant lung) broncho-alveolar lavage (BAL) specimens after one-lung ventilation with V_T of 10ml/kg in humans¹⁴⁸. BAL concentrations of TNF- α and sICAM were significantly lower in a second group randomised to receive OLV with a V_T of 5ml/kg¹⁴⁸. In a similar study, Michelet et al randomised patients undergoing oesophagectomy with OLV to protective ventilation (V_T 5ml/kg, PEEP 5cmH₂O) during OLV or 'conventional ventilation' (V_T 9ml/kg, ZEEP)¹⁴⁹. Plasma levels of IL-1 β , IL-6, and IL-8 were lower at the end of the period of OLV and 18

^D The role of extravascular lung water measurement in the diagnosis of ALI/ARDS is discussed in detail in chapter 5.

hours later in the lung protective ventilation group (mean duration 85 and 89 minutes in the protective and conventional ventilation group respectively)¹⁴⁹.

Together the studies of Gama de Abreu¹⁴⁶, Kuzkov¹⁴⁷, Schilling¹⁵⁰ and Michelet¹⁴⁹ and colleagues provide evidence of pulmonary and systemic inflammation (presumed surrogate markers of lung injury) secondary to OLV which can to an extent be ameliorated by reduced tidal volumes. In order to establish a link between ventilation and the development of ALI in human patient populations however, evidence from much larger cohorts is required. Such evidence has been provided in a number of observational studies (Table 1.3), which have reported association between ventilatory variables and the development of PLR-ALI. Both increased peak airway pressure (P_{peak}) intra-operatively or during the period of OLV^{151, 152}, and tidal volume during the period of OLV¹⁵² have been shown to be independent risk factors for the development of PLR-ALI (Table 1.3).

Duration of one-lung ventilation

Intuitively, the duration of OLV is also likely to be important in determining the degree of VILI to which the lung is exposed. In Licker et al's 2003 case series, in order to represent the cumulative effect of baro-trauma throughout the duration of the period of OLV, the authors constructed a 'ventilatory hyperpressure index', defined as the "product of inspiratory pressure $>10\text{cmH}_2\text{O}$ and the duration of OLV". In multivariate logistic regression analysis, ventilatory hyperpressure index represented the strongest risk factor for the development of PLR-ALI observed in this cohort (851 patients with a complete data set), with an odds ratio of 3.53 (95% CI 1.71-8.45; $p<0.001$); representing an approximately three-fold increased risk of PLR-ALI if peak inspiratory pressure is $\geq 25\text{cmH}_2\text{O}$ versus $15\text{cmH}_2\text{O}$ ¹²⁸. The importance of OLV duration on the development of PLR-ALI is supported by an animal study in which rats were exposed to OLV for between one and three hours; biochemical and histological evidence of pulmonary tissue damage increased with the duration of OLV¹⁵⁶. In humans, Misthos et al have demonstrated higher plasma malondialdehyde level (a marker of oxidative stress) intra- and post-operatively as the duration of OLV increases¹⁵⁷.

Table 1.3. Risk factors for development of post-lung resection acute lung injury identified following multivariate analysis.

Study	N	Popn.	Risk factor	Magnitude
Patient factors				
Parquin et al. 1996 ¹⁵³	146	P	Perfusion fraction of remaining lung (<55%)	Not given
Licker et al. 2003 ^{128,C}	851	P, L, SL	Chronic alcohol consumption (≥60g ethanol per day)	OR = 1.87 (CI 1.09-4.56)
Alam et al. 2007 ¹⁵⁴	1428	P, L, SL	ppo-FEV1	OR = 1.10 (CI 1.01-1.2)
Sen et al. 2010 ¹²⁷	143	P, L, SL	ASA score	OR = 1257 (CI 17.8-88604)
Kim et al. 2010 ¹³⁴	425	P	Alcohol abuse Perfusion fraction of resected lung ppo-FEV1	OR 39.6 (CI 6.4-645.2) RR = 1.1 (CI 1.03-1.17) RR = 0.93 (CI 0.90-0.99)
Ventilatory parameters				
van der Werrf et al. 1997 ¹⁵¹	197	P	Highest ventilation pressure (≤40 vs ≥ 40cmH ₂ O)	OR = 3.0 (CI 1.2-7.4)
Licker et al. 2003 ^{128,C}	851	P, L, SL	Ventilator hyperpressure index (PIP>10cmH ₂ O x duration of OLV)	OR = 3.53 (CI 1.71-8.45); p<0.001
Jeon et al. 2009 ¹⁵²	146	P	V _T – OLV Ppeak - OLV	OR 3.37 per ml/kg CI (1.65-6.86) OR 2.32 (CI 1.46-3.67) per 1cmH ₂ O increase
Peri-operative fluid administration				
Parquin et al. 1996 ^{153,C}	146	P	Total fluid load > 2L	Not provided
Licker et al. 2003 ¹²⁸	851	P, L, SL	Fluid infused intra-op and in first 24h (<4L vs >4L)	OR = 2.91 (CI 1.87-7.38)
Alam et al. 2007 ¹⁵⁴	1428	P, L, SL	Peri-operative fluid administration	OR = 1.2 per 500ml increase
Licker et al. 2009 ^{155,C}	1091	P,L,S L	Cumulative peri-op. fluid infused	OR = 1.42 (CI 1.09-4.32) per ml/kg/hr
Blood product administration				
van der Werff et al. 1997 ¹⁵¹	197	P	Receipt of FFP;(yes / no)	OR 4.7 (CI 1.4-16.3)
Sen et al. 2010 ¹²⁷	143	P, L, SL	Receipt of FFP;	OR 28.6 (CI 1.2-1.9)
Side of resection				
Kim et al. 2010 ¹³⁴	425	P	Right sided resection; yes /no	RR 4.8 (CI 1.6-14.4)
Size of resection				
Licker et al. 2003 ^{128,E}	851	P, L, SL	Pneumonectomy; yes/no	OR 2.78 (CI 1.5-6.3)
Sen et al. 2010 ¹²⁷	143	P, L, SL	'Pulmonary resection type'	OR 1.4 (CI 1.2-1.9)

N, number of patients; ppo-FEV₁ – predicted post-operative FEV₁; ASA – American Society of Anaesthetists; FFP, fresh frozen plasma; P, pneumonectomy; L, lobectomy; SL, sub-lobar resection; VT, tidal volume; OLV, one lung ventilation; PIP, peak inspiratory pressure.

^E It is likely that some patients in the 'historical group' (n=533) in Licker et al's 2009 publication are common to their 2003 publication.

Post-operative hyperinflation

The injurious effects of hyperinflation may not be restricted to the period of one-lung ventilation^{114, 122, 147, 158, 159}. Following lung resection, residual lung tissue inflates and there is mediastinal shift towards the resected side. Larsson et al documented functional residual capacity (FRC) to be 10% greater in the non-operative lung following (lobar / sub-lobar) lung resection, (though this finding was not statistically significant in this small sample; n=8) and 35% greater following pneumonectomy (no statistical comparison; n=2)¹⁴⁵. Slinger suggests that hyperinflation leads to widening of intracellular junctions; a pathophysiological process similar to the pulmonary capillary stress failure seen with high intravascular pressures¹¹⁴. Such hyperinflation could therefore contribute to the pathogenesis of PLR-ALI, a hypothesis which is supported by the findings of Alvarez et al¹⁵⁸. These authors performed a retrospective review, examining two cohorts of patients undergoing pneumonectomy before and after the introduction of a novel 'balanced' chest drainage system. In comparison to a conventional under-water seal, the balanced systems seek to limit the extremes of positive and negative pressure that can be generated within the operative hemithorax, so preventing post-operative mediastinal shift and hyperinflation. With close to 30 patients in each group, the authors observed that the rate of PLR-ALI fell from 4 cases (14.3% incidence) in the conventional under-water seal group to zero in the balanced drainage group ($p < 0.001$)¹⁵⁸.

Oxygen toxicity

In response to inflammatory stimuli, lung endothelial cells, alveolar cells, and airway epithelial cells, as well as activated alveolar macrophages produce reactive oxygen and reactive nitrogen species (ROS & RNS)¹⁶⁰. In toxic levels these reactive species can cause molecular and cellular damage by oxidation and nitration of proteins, lipids and DNA¹⁶¹. The generation of ROS following lung resection has been demonstrated by a number of authors; oxidative injury has been demonstrated in humans undergoing lung resection and OLV as reduced plasma thiol levels / increased plasma carbonyl levels¹⁶² and increased levels of exhaled hydrogen peroxide^{163, 164} and malondialdehyde (MDA) in both plasma^{157, 165, 166} and urine¹⁶³.

The toxic effects of high fractions of inspired oxygen (FiO_2) resulting in the development of ROS and subsequent lung injury have been well described, both

in animal models and in humans. Exposure to high FiO_2 causes histopathological changes similar to those seen in ARDS^{167, 168}; in addition, evidence from animal models suggests hyperoxia can exacerbate, or predispose to lung injury from another aetiology^{167, 168}.

In light of increased understanding of the potentially harmful effects of high FiO_2 and the demonstration of oxidative stress following lung resection, it has been widely suggested that exposure of the lung to high FiO_2 during OLV (as per 'conventional practice' described above), may contribute to the development of PLR-ALI¹⁶⁹⁻¹⁷². The evidence for such an assertion is limited however; the majority of studies reporting generation of ROS after lung resection are small and there is little if any hard evidence to link ROS generation with development of PLR-ALI. In the study of Lases et al, one of 28 patients undergoing lung resection developed PLR-ALI; in this patient exhaled hydrogen peroxide and urinary MDA were 'significantly elevated' compared to others¹⁶³. Misthos et al studied a cohort of 132 patients undergoing lung resection for non-small cell lung cancer¹⁶⁵. The authors reported highest plasma MDA levels in the subgroup of patients exposed to OLV for the longest duration (120 minutes); in this group the incidence of respiratory failure, cardiac arrhythmias and pulmonary hypertension was significantly greater than in patients subjected to shorter durations of OLV. The authors go on to perform a multivariate analysis seeking risk factors for post-operative complications, but unfortunately make no attempt to assess the role of oxidative stress in the causation of post-operative complications independently of OLV duration¹⁶⁵.

Though hard evidence against the detrimental effects of high FiO_2 is lacking, several commentators make the point that in the absence of good evidence for the need to ventilate with high FiO_2 during OLV, the practice of ventilation with $\text{FiO}_2=1.0$ in a population undoubtedly at significant risk of lung injury should be questioned^{169, 170}.

Hyperperfusion

During OLV, the combined effects of gravity, collapse of the non-dependent lung, and hypoxic pulmonary vasoconstriction (HPV) within the non-dependent lung vascular bed serve to divert blood flow towards the dependent lung, minimising shunt and maintaining oxygenation. In a porcine model of OLV / ALI,

Kozian et al determined the distribution of pulmonary perfusion during OLV by performing single-photon emission computed tomography (SPECT) following the administration of ^{99m}Tc labelled macro-aggregated albumin¹⁷³. During two lung ventilation (TLV) perfusion was split 48% to the non-dependent lung and 52% to the dependent lung. During OLV, perfusion of the non-dependent lung was described as falling to “*only a minimal percentage of whole perfusion*”. Histopathological examination after 90 minutes of OLV and simulated surgical manipulation, followed by a further 90 minutes of TLV revealed diffuse alveolar damage bilaterally, but more pronounced in the dependent ventilated lung; reflecting (according to the authors) the combined insults of hyperperfusion and hyperinflation¹⁷³.

Though non-dependent lung collapse and HPV reduce shunt and improve oxygenation, the preferential perfusion of the single dependent lung appears not to be without cost. Pulmonary artery pressure and pulmonary vascular resistance are increased during the period of OLV and in the immediate post-operative period (observed in Kozian et al’s porcine model¹⁷³ but also reported in humans^{125, 174}). Several authors have suggested that increased blood flow and increased pulmonary artery pressure in the dependent lung during the period of OLV (and indeed the remaining lung immediately post-operatively) may promote disruption of the capillary endothelial cell barrier^{114, 116, 119, 169}. Such alveolar-capillary barrier disruption may occur due to a number of mechanisms.

Firstly, as originally described by West et al in a series of studies examining the pathophysiology of high altitude pulmonary oedema¹⁷⁵, pulmonary capillary stress failure refers to mechanical failure of the pulmonary alveolar-capillary barrier in response to increased transmural capillary pressure. West reports disruption of the alveolar-capillary barrier occurring at transmural pressures as low as 24mmHg¹⁷⁵. It is not known whether capillary transmural pressure increases to beyond 24mmHg during OLV; several authors have reported unchanged pulmonary artery wedge pressures (PAWP - of the order of 10-12mmHg) during OLV^{174, 176}, yet rises in pulmonary artery pressure (PAP) have been well documented^{125, 174}. Waller et al determined pulmonary capillary pressure (P_c - NB. representing hydrostatic pressure within the capillary, not transmural pressure) from the equation $P_c = \text{PAOP} + 0.4(\text{meanPAP} - \text{PCWP})$, reporting no increase in P_c during OLV nor the immediate post-operative

period¹²⁵. It might be hypothesised however that local capillary transmural pressures in dependent areas, and areas of alveolar hypoxia may will increase to injurious levels. Interestingly, and of undoubted relevance to one-lung ventilation, West et al describe the combined effects of increased capillary transmural pressure, lung hyperinflation and alveolar hypoxia as being circumstances particularly conducive to stress failure¹⁷⁵.

Secondly, injury to endothelial cells may not occur secondarily to increased pressures, but as a result of increased linear velocity of blood flow^{114, 123} (hypothesised, but not proven to occur as the same or greater cardiac output is required to pass through a lower volume vascular bed). Staub describes the potential for inertial injury (direct impact of blood against endothelium), and frictional injury (increased wall shear stress) occurring at vulnerable places within the pulmonary microcirculation (such as capillary junctions)¹⁷⁷. Staub's group subsequently demonstrated increased pulmonary lymph flow in an ovine model of hyperperfusion, where conditions of hyperperfusion of the left lower lobe were created by resection of right lung and left upper lobe whilst maintaining cardiac output. In this experiment however, increased oedema was hydrostatic in origin, as evidenced by reduced lymph:plasma protein concentration. Whilst increased linear velocity of blood flow in the residual vascular bed could potentially be a contributory factor, it seems that PLR-ALI may not purely be a syndrome of increased capillary permeability as conventionally described.

Finally, in laboratory experiments in a perfused rat lung model, pressure elevation in the lung venular capillary has been shown to have a pro-inflammatory effect. Kuebler et al demonstrated pressure induced increases in endothelial cell intracellular calcium concentration associated with enhanced luminal expression of P-selectin (a key mediator of neutrophil adhesion in inflammatory lung injury)¹⁷⁸. It seems plausible that in addition to the direct pro-inflammatory effect of mechanical ventilation during OLV, increased capillary pressures may constitute a further pathway by which endothelial inflammation might be triggered.

Pathophysiology of injury to the non-dependent, non-ventilated lung

Yin et al performed lung biopsies of the non-dependent, non-ventilated lung in a series of pigs undergoing 60 minutes of OLV, before resumption of TLV¹⁷⁹. Histological analysis of biopsy specimens revealed the presence of ‘vascular congestion’, with ‘cuffing of the blood vessels’ and ‘alveolar wall thickening’ in specimens obtained 30- and 60-minutes after return to TLV, changes the authors interpret as providing evidence of injury to the non-dependant, non-ventilated lung¹⁷⁹. In a porcine model of OLV and ALI, Kozian et al also reported evidence of lung injury in the non-dependent lung, but to a lesser extent than in the dependent lung¹⁷³.

Direct injury due to surgical manipulation and ischaemia-reperfusion injury due to collapse and re-inflation of the operative lung are proposed mechanisms by which the non-dependent, non-ventilated lung may become injured during lung resection.

Surgical manipulation

It is widely suggested that intra-operative surgical manipulation of lung tissue leads to a degree of localised parenchymal injury, potentially triggering an inflammatory reaction^{117, 121, 143}. Such is the perceived importance of the surgery in the pathogenesis of PLR-ALI, researchers constructing animal models of OLV have simulated surgical manipulation of the lung in order to adequately mimic the clinical situation¹⁷³. There have however, been no studies examining the influence of surgical technique on PLR-ALI, with the possible exception of video assisted thoracoscopic surgery (VATS).

VATS surgery has been demonstrated to lessen the systemic inflammatory response following lung resection, as evidenced by reduced C-reactive protein and IL-6 levels in plasma when compared to open resection¹⁸⁰. In addition, neutrophil and monocyte reactive oxygen species generation was reduced following VATS surgery¹⁸⁰. It is uncertain however to what degree the observed changes in systemic inflammation and oxidative stress influence the pulmonary inflammatory response. Studies directly comparing patient outcome after lung resection via VATS versus open thoracotomy, have not shown any reduction in the incidence of PLR-ALI, though overall complication rate appears to be reduced and these studies have not been powered to study the incidence of PLR-

ALI¹⁸¹⁻¹⁸³. In one case series of 1100 VATS lobectomies, the post-operative incidence of 'ARDS' was less than one percent which compares favourably with reported rates of PLR-ALI after open resection¹⁸⁴.

Ischaemia-reperfusion injury

Ischaemia-reperfusion injury of the non-dependent surgical lung constitutes a further mechanism by which the lung may become susceptible to oxidative injury, and is widely described as a cause of PLR-ALI^{121, 143, 147, 169}. Ischaemia occurs once oxygen delivery to a tissue falls below a threshold concentration. The lung (uniquely) has three potential sources of oxygenation; pulmonary arteries, bronchial arteries and alveolar ventilation. Experimental models suggest that interruption of any one of these supplies is sufficient to cause ischaemia¹⁸⁵. Whilst ischaemia in itself leads to inflammatory cell activation and subsequent lung injury, there is evidence to suggest that reperfusion plays a significantly more important role in the causation of lung injury. Reperfusion leads to further inflammatory cell activation and neutrophil infiltration. In addition, it appears that ischaemia 'primes' lung tissue (possibly by activation of xanthine oxidase) such that re-oxygenation leads to generation of further ROS¹⁸⁵.

Williams et al examined the role of ROS in the pathogenesis of lung injury in an isolated, blood perfused rodent lung model of pulmonary resection¹⁸⁶. Animals were divided into three groups; control, OLV followed by pneumonectomy and OLV followed by reinflation of the collapsed lung. Lung injury was quantified by estimation of extravascular albumin accumulation (EAA) and ROS production was quantified by measurement of hydroxylation of phenylalanine by hydroxyl radical in plasma. Increased EAA was observed bilaterally both following collapse and resection and after collapse and reinflation, though EAA was greater in the collapse-reinflation group. In addition, EAA was greater in the right (collapsed) lung in the collapse and reinflation group than in either the ventilated lung or the resected lung in the pneumonectomy group. These findings suggest that not only is ischaemia / hypoperfusion detrimental to pulmonary vasculature, but that reperfusion appears to provide an additional insult. This is supported by the finding of increased ROS production in the collapse-reinflation group and the observation that ROS production could be attenuated (and lung injury ameliorated) by co-administration of ROS scavenger (superoxide dismutase or nitric oxide synthase inhibition)¹⁸⁶.

Misthos et al determined ROS activity during and after lung resection by measurement of plasma malondialdehyde (MDA) in 212 patients undergoing lung resection for non small cell lung cancer. MDA levels were significantly elevated in all patients subjected to one lung ventilation, with peak MDA levels evident at the time of reventilation of the operative lung. Patients undergoing pneumonectomy by comparison exhibited no such evidence of ROS activity. Ahn et al observed a similar peak in plasma MDA on resumption of TLV¹⁶⁶.

The effect of FiO₂ during reperfusion has not been examined in the context of OLV. Hypoxaemic reperfusion has been shown to attenuate the histopathological and inflammatory consequences of intestinal injury¹⁸⁷; perhaps providing further incentive to use lower FiO₂ during thoracic surgery¹⁴³.

Risk factors for lung injury common to both lungs

Patient factors, peri-operative fluid administration, impaired lymphatic drainage and blood product transfusion are all risk factors common to both lungs which may influence the development of PLR-ALI.

Patient factors

A number of patient factors have been identified as independent risk factors for the development of PLR-ALI. These include alcohol consumption, American Society of Anaesthetists (ASA) physical status classification and pre-operative lung function (Table 1.3).

Alcohol consumption

Both Licker et al¹²⁸ and Sen et al¹²⁷ identified alcohol consumption to be a risk factor for the development of PLR-ALI. Whilst alcohol is well understood to be associated with a two to five fold increase in post-operative complications (including increased need for high dependency / intensive care unit admission and prolonged hospital stay) following major surgery¹⁸⁸, there are further reasons why chronic alcohol abuse may be a risk factor for PLR-ALI.

In the general intensive care environment, chronic alcohol abuse is an independent risk factor for development of ARDS in at risk patients (relative risk 1.96; 95% CI 1.32-2.85). In addition in patients that develop ARDS, chronic alcoholics are more likely to die than non alcoholics (p=0.003)¹⁸⁹. In order to

study this increased susceptibility to ALI/ARDS in chronic alcohol abusers, Guidot and Roman developed a rat model of ethanol mediated susceptibility to ALI¹⁹⁰. As in humans, Guidot and Roman observed that chronic ethanol ingestion increased the susceptibility to endotoxin mediated lung injury in isolated perfused rat lungs *ex-vivo*. Furthermore, these authors were able to demonstrate that deficiencies in glutathione (a key antioxidant molecule) in alveolar lining fluid and type-II pulmonary epithelial cells, occurring secondary to chronic ethanol ingestion led to altered surfactant synthesis and secretion, altered epithelial cell permeability and reduced cell viability. This evidence implicating oxidative stress in the increased susceptibility of alcoholics to ALI was further strengthened by the finding that glutathione supplementation could reduce lung injury¹⁹⁰. It is plausible that the increased susceptibility of alcohol abusers to PLR-ALI is mediated in exactly the same way, with glutathione deficiency rendering patients increasingly susceptible to oxidative stress during the period of OLV.

Pre-operative lung function

Assessment of predicted post-operative pulmonary function (derived from pre-operative function and adjusted based on the size of the proposed lung resection) forms the basis of risk assessment for patients undergoing lung resection in all major clinical guidelines currently available^{12, 191, 192}. Low pre-operative or predicted post-operative forced expiratory volume in one second ((ppo)FEV₁) and diffusing capacity for carbon monoxide ((ppo)DLCO) have been associated with development of cardio-respiratory complications and mortality following lung resection in a large number of studies^{16, 132, 193-197}. It is perhaps unsurprising therefore that several studies have found ppo-FEV₁ to be an independent risk factor for ALI development (Table 1.3). What is surprising perhaps is the relatively modest association observed (with odds ratios / relative risk confidence intervals falling only just on the side of significance (Table 1.3)^{134, 154}, and the finding that several studies (some of them large) found no evidence of respiratory function being a risk factor for PLR-ALI^{115, 129, 198}. Though pre-operative respiratory function is a well accepted predictor of cardio-pulmonary complications following lung resection, it appears to be only weakly predictive of PLR-ALI.

The pre-operative distribution of pulmonary perfusion was identified to be a risk factor for PLR-ALI after pneumonectomy by two studies (Table 1.3). Where the resected lung was in receipt of a high proportion of total pulmonary perfusion this increased the risk of lung injury^{134, 153}. It is likely that injury secondary to hyperperfusion would be magnified in such circumstances where the residual lung tissue is less accustomed to high perfusion.

Peri-operative fluid administration

In their case series of 10 patients with PLR-ALI, Zeldin et al reported that large peri-operative fluid load, and high intra-operative and post-operative urine outputs were risk factors for the condition¹⁰⁹. By comparing the fluid intake and output data for just four patients who developed pulmonary oedema following right pneumonectomy with that from six patients who underwent uncomplicated right pneumonectomy, Zeldin et al reported that the four patients with pulmonary oedema had significantly higher mean absolute fluid inputs and urine outputs and a higher ratio of input and urine output to body weight than the six uncomplicated cases. Interestingly, the net 24 hour fluid balances were not significantly different.

Zeldin et al went on to construct an animal model of PPPE. Right pneumonectomy was performed on a population of 13 dogs, randomised to receive pre-operative infusion of Ringer's lactate solution at either 50 or 100ml/kg. Five of eight dogs receiving the high volume fluid regimen suffered post-operative pulmonary oedema compared to one of five dogs in the low volume group ($p=0.08$ (Fishers exact test performed by the author (B Shelley), no statistical comparison offered by the authors)). Contrary to the observations seen in humans, it appeared that the ability of dogs to clear the high fluid load was important to the development of oedema; dogs able to maintain a net fluid load of less than 100ml/kg appeared to be less likely to develop oedema. Based on this laboratory data from 13 dogs, and the results of fluid balance data from just 4 patients, Zeldin et al boldly conclude that "*post-pneumonectomy pulmonary oedema appears to result from infusion of excessive volumes of fluid*"¹⁰⁹.

Whilst it is questionable that Zeldin et al had the evidence to make such a conclusion, subsequent reports have supported their assertions; the volume of

intravenous fluid administration has been implicated in the development of PLR-ALI in both univariate and multivariate analyses (Tables 1.4 and 1.3 respectively). The incidence of PLI-ALI has been linked to both the total volume of fluid infused intra- or peri-operatively and to 24 hour fluid balance in the peri-operative period.

As Slinger writes in his 1995 review article entitled “*the puzzle of post-pneumonectomy pulmonary oedema*”, whilst there appears to be a wealth of (predominantly) retrospective and anecdotal evidence suggesting some association between PLR-ALI and fluid overload, a clear cause-effect relationship has not been demonstrated¹¹⁴. Indeed, there appear to several factors which question the nature of this relationship:

Firstly, the finding of such association between fluid administration and the development of PLR-ALI is not universal. Both Turnage and Lunn (24 cases of PLR-ALI in 806 patients undergoing pneumonectomy¹¹⁹), and Waller et al (11 cases in 402 resections²⁰¹) were unable to show any such association. It would appear that the influence of fluid balance on the development of PLR-ALI is lost when fluid input is limited; in the series reported by Turnage and Lunn, mean 24 hour fluid balance was restricted to approximately one litre (yet PLR-ALI still occurred with an incidence of 2.6%)¹¹⁹.

This is the second factor arguing against the cause-effect relationship between fluid administration and PLR-ALI; the observation that PLR-ALI still occurs even in profoundly fluid restricted patients. In keeping with the findings of Turnage and Lunn¹¹⁹, in 1991 Mathisen and Grillo reported that they “*scrupulously restrict intra-operative and post-operative fluids but still see the problem*” [of PLR-ALI]²⁰².

On this subject, Margolis et al succinctly conclude that:

“Perhaps individual variations in pre-operative hydration, cardiac reserve, residual pulmonary lymphatic capacity, and pulmonary endothelial permeability affect the fluid volume that may be safely given”.

Margolis et al (1990)²⁰⁰.

Table 1.4. Univariate analyses reporting association between intra- and peri-operative fluid balance and the incidence of PLR-ALI.

<i>Study, year</i>	<i>Pop.</i>	<i>N</i>	<i>Incidence ALI</i>	<i>Comparator</i>	<i>ALI group</i>	<i>No ALI group</i>	<i>p-value</i>	<i>Findings robust to multivariate analysis?</i>
Zeldin et al, 1984 ¹⁰⁹ .	P	10	N/A	24h input 24h balance	67ml/kg 37ml/kg	46ml/kg 27ml/kg	0.10 NS	Not performed
Verheijen-Breemhaar et al, 1988 ¹⁹⁹ .	P	243	4.5%	24h balance	R: 1800ml ^F L: 2600ml ^F	R: 1050ml ^F L:1100ml ^F	0.01 0.001	Not performed
Margolis et al, 1990 ²⁰⁰ .	P	13	23%	24h balance	86.4ml/kg	47ml/kg	0.008	Not performed
Parquin et al, 1996 ¹⁵³ .	P	146	15%	Total fluid load>2L	45%	20%	<0.01	Yes
Licker et al, 2003 ¹²⁸ .	P,L,SL	879	4.2%	Input intra-op 24h input 24h balance Cumulative input intra & post-op	9.1ml/kg/h 2.1L 2.0L 2.6L	7.2ml/kg/h 1.85L 1.52L 2.0L	0.023 0.075 0.026 0.003	Yes
Alam et al, 2007 ¹⁵⁴ .	P,L,SL	152	N/A	Peri-operative fluids	2775ml	2500ml	0.05	Yes

Pop., study population; P, pneumonectomy; L, lobectomy; SL, sub-lobar resection. In Verheijen-Breemhaar et al, 1988¹⁹⁹, figures are provided for right (R) and left (L) sided resections separately.

^F Approximate values, extrapolated from a figure in the text.

Impaired lymphatic drainage

The essential role of the pulmonary lymphatic system in preventing the accumulation of pulmonary oedema has been discussed in Section 1.2.2. Impaired lymphatic drainage has been reported as a contributory factor to the development of PLR-ALI by a number of authors¹²¹. Ipsilateral lymph drainage is inevitably compromised during lobectomy or pneumonectomy¹²¹, especially when (as current guidelines dictate) surgical resection for malignancy routinely includes systematic lymph node sampling in order to provide accurate pathological staging¹³.

Several authors have reported that PLR-ALI occurs more commonly following right sided lung resection than left^{109, 119, 134, 199}, an observation which remained significant following multivariate analyses in one large series of patients undergoing pneumonectomy¹³⁴ (Table 1.3). In the opinion of many commentators, this can be explained anatomically by the observation that a significant proportion of the lymphatic drainage of the left lung is via the right, whilst the right lung relies very little on left sided lymphatics^{114, 121}. Nohl-Oser examined mediastinoscopy and scalene node biopsy specimens in a series of 749 patients with bronchogenic carcinoma²⁰³. By charting the pattern of mediastinal spread of carcinoma, Nohl-Oser concluded that whilst the lymphatic drainage of the right lung to the superior mediastinum is ipsilateral, that of the left lung “*is as frequently contralateral as ipsilateral*”²⁰³. Contralateral spread predominantly originated from the left lower lobe.

Allen et al reported the results of a large multicentre study examining the effects of two different lymph node sampling strategies in patients undergoing lung resection²⁰⁴. There was no difference in the incidence of PLR-ALI between patients randomised to complete mediastinal lymph node dissection versus a more conservative lymph node sampling, (n=1023; p=0.28; Fishers exact test performed by the author (B Shelley), no statistical comparison offered by the study authors)²⁰⁴.

Blood product transfusion

Blood product transfusion has long been understood to be a risk factor for ALI/ARDS^{205, 207}; it is plausible therefore that peri-operative transfusion in patients undergoing lung resection could contribute to the development of PLR-

ALI^{117, 118, 121}. Gibbon and Gibbon's first descriptions of PLR-ALI were in two patients who had undergone blood transfusion¹⁰⁸. These authors went on to demonstrate in cats that whilst residual lung became congested (increased lung weight compared to controls) following (extensive) lung resection, following lobectomy and blood or plasma transfusion, frank pulmonary oedema was almost universally observed^{108, 208}. Both van der Werff et al¹⁵¹ and Sen et al¹²⁷ have more recently demonstrated fresh frozen plasma transfusion to be an independent risk factor for the development of PLR-ALI (Table 1.3).

Size of resection

The observation of increased incidence of PLR-ALI in patients undergoing pneumonectomy in comparison to lobectomy and in turn sub-lobar resection is a near universal finding^{110, 111, 127, 129, 130, 198}. Such an observation potentially provides some further insight into what are the most important causative mechanisms for PLR-ALI. Ischaemia-reperfusion injury is not a feature of pneumonectomy, and though it may play a role in the development of PLR-ALI in lesser resection, it cannot in pneumonectomy. The duration of positive pressure OLV, will be marginally longer for pneumonectomy than in lesser resections, but the tidal volumes and airway pressures involved should be no greater. Patients undergoing pneumonectomy however are potentially subject to greater post-operative hyperinflation, whilst the residual lung is subsequently hyperperfused to a greater extent. It is perhaps reasonable to hypothesise therefore that these factors may be some of the more important in the aetiology of PLR-ALI.

1.4.5 Management of post-lung resection lung injury

As with ALI/ARDS in the general critical care environment, there is no specific therapy for PLR-ALI and management is largely supportive. Oxygen, chest physiotherapy, inhaled bronchodilators and close attention to fluid balance with consideration of induced diuresis are the mainstay²⁰⁹. Positive pressure ventilation in the early post-operative period following lung resection carries increased risk of pneumothorax, bronchial stump disruption and bronchopleural fistula formation and as such, avoidance of mechanical ventilation is an early goal. To this end there is some evidence to suggest that early institution of non-invasive ventilation may prevent progression to invasive ventilation and reduce

mortality²¹⁰. A study examining the utility of high-flow nasal oxygen in patients undergoing lung resection is in progress²¹¹.

The roles of corticosteroid therapy²¹² and nitric oxide administration²¹³ in the treatment of ALI/ARDS are controversial; use of both has been reported in PLR-ALI. Mathisen et al report the use of nitric oxide in ten patients receiving mechanical ventilation for PLR-ALI¹³⁵. Nitric oxide administration led to an immediate improvement in oxygenation, a reduction in peak inspiratory pressure and an improvement in chest radiography appearances within 24 hours. Though this study lacked a contemporaneous control group, the authors report a 70% survival which was favourable to a historical control group (n=7) in which survival was only 14%¹³⁵. Lee et al similarly investigated the efficacy of low-dose steroid therapy in 12 patients with PLR-ALI making comparison to a historical control group²¹⁴. The authors concluded that early low-dose steroid therapy significantly reduced in-hospital post-operative mortality (88% vs 8%; p<0.001).

The use of extracorporeal carbon dioxide removal has been described in patients who have undergone broncho-pleural fistula repair following lung resection. Extracorporeal lung assist allowed airway pressures to be reduced, potentially contributing to the healing of the bronchial repair²¹⁵. Use of extracorporeal membrane oxygenation has also been described in ARDS after post-traumatic pneumonectomy²¹⁶.

1.4.6 Prevention of post-lung resection lung injury

With recognition of the poor prognosis of patients suffering PLR-ALI and the limited treatment options available, alongside increased understanding of the pathogenesis of the condition, in recent years there has been an increasing interest in methods of preventing PLR-ALI. Peri-operative fluid management, the conduct of OLV, and mode of anaesthesia are arguably the most easily modifiable risk factors that can be targeted in the prevention of PLR-ALI. Investigation I of this thesis explores the extent to which these preventative strategies have been adopted into contemporary thoracic anaesthetic practice (Chapter 2).

1.4.6.1 Conduct of one-lung ventilation

Lung protective (one-lung) ventilation

Lung protective ventilation with low tidal volume, limitation of peak airway pressures and increased levels of positive end-expiratory pressure has been the single most effective intervention in reducing the incidence and mortality of ALI/ARDS in the general intensive care population⁹⁹. Though expert opinion can be described as strongly being in favour of lung protective / low tidal volume ventilation in order to prevent PLR-ALI in the lung resection population^{115, 122, 124, 141}, the evidence for the efficacy of such a strategy is limited to observational studies^{110, 155, 217} and a single randomised controlled trial examining the efficacy of protective ventilation in preventing pulmonary complications (a composite primary endpoint of which ALI was a part)²¹⁸.

In 2008, Tang et al reported a retrospective review examining the incidence and mortality of ARDS in patients undergoing lung resection in a single centre. Comparison was made between two cohorts of patients; 1139 patients undergoing lung resection between 1991 and 1997, and 1376 undergoing resection between 2000 and 2005. The incidence and mortality from ARDS were observed to fall between the two cohorts from 3.2% and 72% to 1.6% and 45% (incidence and mortality respectively). This the authors attributed to “*more aggressive strategies to avoid pneumonectomy, greater attention to protective ventilation strategies and to the improved ICU management of ARDS*”¹¹⁰. Unfortunately ventilatory parameters during OLV were not recorded though the authors describe that “*ventilatory strategies at this institution have become more protective over the last 5 years*”¹¹⁰.

Licker et al performed a similar observational analysis examining the incidence of PLR-ALI before and after implementation of a ‘protective lung ventilation’ protocol; 558 patients undergoing lung resection from 2003 to 2008 were compared to 553 historical controls undergoing resection from 1998 to 2003¹⁵⁵. The ‘protective lung ventilation’ protocol consisted of small tidal volume, PEEP and recruitment manoeuvres; mean tidal volume was 6.5 ml/kg in the intervention group versus 9.2ml/kg in controls ($p<0.05$), with a correspondingly lower mean inspiratory plateau pressure in the intervention group (12 versus 16cmH₂O; $p<0.05$). Overall the incidence of PLR-ALI was reduced from 3.7 to

0.9% ($p < 0.01$). Importantly, and adding strength to the findings, this difference was maintained after adjustment for baseline characteristics and non-ventilatory peri-operative management (including fluid administration) with the finding that the 'protective lung ventilation' protocol decreased the incidence of ALI, (odds ratio=0.34 (95% CI 0.23-0.75)).

In 2011 Yang et al published the only randomised controlled trial examining the effect of lung protective ventilation on the incidence of post-operative pulmonary complications in the thoracic surgical population²¹⁸. Yang et al compared 'conventional ventilation' ($FiO_2=1$, $V_T=10\text{ml/kg}$, ZEEP, volume controlled ventilation) to a 'protective strategy' ($FiO_2=0.5$, $V_T=6\text{ml/kg}$, $5\text{cmH}_2\text{O}$ PEEP, pressure controlled ventilation) during OLV in 100 patients undergoing lung resection, examining the incidence of the composite endpoint of $PaO_2/FiO_2 < 300\text{mmHg}$ and / or lung infiltration or atelectasis within 72 hours. In the intervention group the incidence of this composite primary outcome was 4% compared to 22% in the conventional ventilation group ($p < 0.05$)²¹⁸. This study also demonstrated a statistically non-significant trend towards a reduced incidence of PLR-ALI (4 cases, 8% incidence in control group, 1 case, 0.5% incidence in the treatment group; $P=0.36$). The remarkable reduction in the incidence of pulmonary complications in the intervention group is striking, but must be interpreted with caution. Firstly, it is worth remembering that studies with small sample sizes commonly overestimate treatment effects^{219, 220}. Secondly, the study design and randomisation techniques utilised in this study have been subject to a multitude of criticisms²²¹. Furthermore, it is noteworthy that the primary endpoint in the final published manuscript of this study²¹⁸ differed from that published initially on the Australian New Zealand Clinical Trials Registry website (ACTRN12609000861257; www.anzctr.org.au; accessed 22nd March 2014). The published manuscript reported the occurrence of $PaO_2/FiO_2 < 300\text{mmHg}$ and / or lung infiltration or atelectasis within 72 hours post-operatively, whilst the published protocol considered the same outcomes but occurring within the first post-operative week.

As Lohser points out, these studies of 'lung protective ventilation' study the composite effect of low V_T ventilation, PEEP and recruitment manoeuvres as a whole, but fail to answer the question of which of V_T reduction, application of PEEP or recruitment manoeuvres are the beneficial intervention¹⁴³. Much of the

purpose of maintaining high V_T during OLV (as historically advocated) was to promote recruitment / prevent development of atelectasis in the dependent, ventilated lung¹⁴⁰. As tidal volumes reduce, the potential for atelectasis increases¹⁴³ and as such, in many protective ventilatory strategies recruitment manoeuvres and the judicious use of PEEP are advocated alongside V_T reduction in order to prevent atelectasis^{124, 128, 143, 222}. It is worthwhile briefly considering the effect of recruitment manoeuvres and PEEP application.

Recruitment manoeuvres during one-lung ventilation

Recruitment manoeuvres have been shown to reduce alveolar dead space and improve arterial oxygenation during OLV^{223, 224}. In this context however it is worth noting the findings of some animal studies conducted in a rat models^{225, 226}. Farias et al demonstrated that a single recruitment manoeuvre of 40cmH₂O for 40 seconds leads to elevation of biomarkers of lung injury in animals with normal lungs²²⁵. Silva et al randomised mechanically ventilated animals (TLV) with ALI into five groups; recruitment by four different protocols and a control group. Whilst all recruitment manoeuvre protocols resulted in improved oxygenation and lung compliance, two of the protocols (those associated with the most rapid increase to maximum airway pressure) resulted in increased mRNA expression in lung tissue of inflammatory, fibrogenetic and apoptotic biomarkers compared to controls ventilated without recruitment²²⁶. Together these studies suggest that firstly, recruitment manoeuvres may not be as benign as first thought and may in themselves cause harm, and secondly that all methods of recruitment may not be equal.

Positive end-expiratory pressure during one-lung ventilation

Application of appropriate levels of PEEP leads to improved oxygenation during OLV²²⁷. In addition there is little doubt that application of PEEP as part of a protective lung ventilation protocol has proved efficacious in decreasing surrogate markers of lung injury in both animal models and human studies^{146, 147, 228}. The effect of PEEP on the development of lung injury during OLV has not however been studied in isolation. In contrast, Schilling et al's comparison of 5ml/kg TV with 10ml/kg and ZEEP in humans demonstrated the independently beneficial effect of low V_T ¹⁴⁸. The effects of PEEP appear to be rather more subtle. Firstly, setting the appropriate level of PEEP during OLV requires careful consideration of the patient's position on the static pulmonary compliance curve

and the cumulative effect of extrinsically applied PEEP and intrinsic ‘auto-PEEP’ (extrinsic-PEEP + intrinsic-PEEP = total-PEEP). Application of PEEP to patients with high levels of auto-PEEP is likely to worsen oxygenation^{143, 227}.

Kozian et al report an elegant study demonstrating the combined effects of low tidal volume ventilation, recruitment manoeuvres and PEEP application during OLV²²⁹. In a porcine model of OLV, the authors used computerised tomography scanning (CT) to determine lung aeration during OLV in animals randomised to a high (10ml/kg) or low (5ml/kg) tidal volume protocol. Use of a recruitment manoeuvre prior to OLV increased the fraction of normally aerated dependent lung, reducing the volume of poorly aerated and atelectatic regions, an effect which (with the addition of 5cmH₂O PEEP) persisted for the duration of OLV. Whilst high TVs lead to marginal further increases in the volume of aerated lung, this only occurred at end-inspiration; at end expiration the volume of aerated lung was equivalent suggesting that during high V_T ventilation the lung was exposed to cyclical recruitment / derecruitment, increasing mechanical stress on the lung²²⁹.

Attempting to ‘unpick’ the impact of the individual components of a lung protective ventilatory strategy is likely to be a futile exercise - low V_T, recruitment manoeuvres, and PEEP application work together to allow reduced airway pressures whilst maintaining oxygenation and preventing harmful atelectotrauma.

1.4.6.2 Peri-operative fluid management

Though numerous studies have implicated the volume of intra-venous fluid administered in the development of PLR-ALI (Tables 1.3 and 1.4), there have been no randomised trials of a fluid restrictive strategy in patients undergoing lung resection. As early as 1984 Zeldin concluded that in order to prevent PLR-ALI, “*the anaesthesiologists must not boldly load the patient up with fluids prior to induction*”¹⁰⁹. It is widely believed in the thoracic anaesthetic and surgical communities that fluid restriction is mandatory in patients undergoing lung resection^{114-116, 200, 230-232}.

Until recently therefore it seemed equipoise did not exist, and that a study of a restrictive versus a liberal fluid strategy would be unlikely, if not ethically unjustifiable. In recent years however, increased attention has been drawn to the potential for renal dysfunction after lung resection; it has been suggested by some that fluid restriction has gone ‘too far’ and that some patients are being subjected to unnecessary risk of renal dysfunction^{49, 233}. As such, studies of alternative fluid management strategies (involving goal directed fluid therapy) have begun^{234, 235}. Unfortunately to date, these studies have been too small to provide any insight into the incidence of PLR-ALI, and have concentrated on extravascular lung water measurement as a surrogate²³⁴. (A detailed review of the application of extravascular lung water measurement in patients undergoing lung resection is provided in Chapter 5).

1.4.6.3 Volatile anaesthesia and lung protection

The question of which mode of anaesthetic delivery (total intravenous anaesthesia (TIVA) with propofol or volatile anaesthesia) for thoracic surgical procedures has been the subject of much debate. A recent update to a 2008 Cochrane review concluded “*that no evidence indicated that the drug used to maintain anaesthesia during one-lung ventilation affected participant outcomes*” and reported that there was a lack of data from randomized controlled trials examining participant outcomes rather than changes in physiological (or immunological) endpoints²³⁶.

Volatile anaesthetic agents undoubtedly have immunomodulatory effects. There has been a great deal of interest in the potential that volatile anaesthetic agents may have a cardioprotective effect^{222, 237}. Laboratory and clinical evidence suggests that volatile anaesthesia during cardiac surgery can lower post-operative Troponin I and Brain Natriuretic Peptide levels, improve left ventricular function, reduce inotrope requirements and shorten critical care and hospital stay. Evidence from studies adequately powered to assess the incidence of major cardiac events and mortality however is still awaited^{237, 238}. More recently, it has been understood that anaesthetic agents can have protective effects on other organs. Several studies in animal models of lung injury have demonstrated both pre- and post-conditioning effects of volatile anaesthetic agents^{239, 240}. The mechanisms of anaesthetic pre- / post-conditioning in the lung

are not completely understood; it appears volatile anaesthetic agents reduce the expression of cytokines and adhesion molecules in alveolar epithelial cells by a number of complex pathways including activation of adenosine, α - and β -adrenergic receptors and increased nitric oxide production^{239, 241}.

There have been several studies directly comparing the pulmonary immune effects of propofol and volatile anaesthesia in patients undergoing thoracic surgery with OLV²⁴¹⁻²⁴³. Both Schilling et al^{148, 241} and de Conno et al²⁴² randomised patients to either propofol or volatile anaesthesia and examined cytokine levels in bronchoalveolar lavage fluid; Schilling et al in BAL from the dependent, ventilated lung^{241, 243}, de Conno et al from the non-dependent, non-ventilated lung²⁴². In all three studies, BAL cytokine levels were significantly lower in patients receiving volatile anaesthesia. In their 2011 study, Schilling et al compared propofol to both sevoflurane and desflurane finding both volatile anaesthetic agents 'suppressed the alveolar inflammatory response' to a similar extent; in keeping with suggestions that immunomodulation is a class effect²⁴¹.

Neither of the studies by Schilling et al sought to make any comparison regarding clinical outcomes, though both were of insufficient size to be powered for this purpose (30²⁴³ and 42²⁴¹ patients respectively). In the study of De Conno et al (sample size 54, 27 patients per group), the incidence of adverse events in the propofol group was significantly higher than in the volatile group (40 vs. 18; $p \leq 0.05$), though there were no patients in either group who developed ARDS as part of this composite end point. In addition, patients in the propofol group had significantly prolonged intensive care unit stay (1.52 vs. 0.87 days; $p \leq 0.05$)²⁴².

1.5 Aims and hypotheses

This thesis presents the rationale, methodology and results of four discrete studies concerning the development of lung injury in the thoracic surgical population undergoing resection of primary lung cancer.

From the literature review presented in this chapter, the author (B.Shelley) offers the following observations:

- ALI/ARDS is reported to occur in four to 11% of patients undergoing lung resection and is the major cause of hospital mortality following lung resection.
- The pathophysiology of lung injury following lung resection is complex and can be broadly conceptualised as occurring secondarily to insults specific to both the ipsilateral (surgical) lung and the contralateral (anaesthetic) lung in addition to those insults common to both lungs.
- Increased recognition of the role of ventilator induced lung injury, and peri-operative fluid prescribing in the pathogenesis of lung injury in this population has brought the prevention of lung injury to the attention of the thoracic anaesthetist. Though high quality evidence is lacking, expert opinion widely favours the adoption of lung protective ventilatory strategies and restriction of peri-operative fluids in patients undergoing lung resection.

From these observations the author (B. Shelley) offers the following two hypotheses examined in Investigations I and II:

Hypothesis I: The use of lung protective ventilatory strategies and restriction of peri-operative fluids is widespread within contemporary UK thoracic anaesthetic practice.

- This is examined in investigation I by conducting an online survey of UK thoracic anaesthetic practice, disseminated by the Association of Cardiothoracic Anaesthetists.

Hypothesis II: Such widespread adoption of strategies aimed at preventing the development of lung injury should result in an overall reduction in both incidence of and mortality from lung injury in patients undergoing thoracic surgery.

- This is examined in investigation II which is a random effects meta-analysis and meta-regression analysis of all published literature since 1994, seeking to define pooled incidence and mortality estimates, and to examine the trends in the incidence of and mortality from PLR-ALI over time.

In the author's reading, and from the studies described in the preceding chapter, it is clear that though the major cause of mortality following lung resection, PLR-ALI remains a rare diagnosis. As such, surrogate end-points are increasingly being used in both laboratory and clinical studies seeking to evaluate the efficacy of preventative strategies. Of these, the use of plasma biomarkers of lung injury, and the trans-pulmonary thermodilution derived measurement of extra-vascular lung water (EVLW) and pulmonary vascular permeability index (PVPI) are prominent. Both measurement of plasma biomarkers and thermodilution measurement of EVLW and PVPI have the potential to provide bedside clinical monitoring of lung injury development in the thoracic surgical population in order to guide clinical decision making, monitor patient progress and serve as a surrogate end points in future clinical studies seeking to prevent, treat, or better understand this important clinical syndrome.

Based on detailed review of the relevant literature (presented in the opening sections of Chapters 4 and 5), the author (B. Shelley) offers the following further observations and hypotheses:

- There is a sound biological plausibility (discussed in detail in Section 4.1), to support the use of Pentraxin 3 as a lung injury biomarker in both the wider critical care environment and in the early post-operative period following lung resection.

Hypothesis III: Pentraxin 3 is a suitable candidate plasma biomarker of lung injury following lung resection.

- This is examined in investigation III. Firstly the properties of the ‘ideal’ lung injury biomarker are defined, against which Pentraxin 3 is compared in an observational cohort of thirty five patients undergoing lung resection for lung cancer.

- Combination of multiple biomarkers (each reflecting different facets of the complex pathophysiology of ALI/ARDS) into panels in order to improve validity has become an increasing focus of biomarker research ²⁴⁴.

Hypothesis IV: A panel of lung injury biomarkers reported in the literature by Freemont et al ⁸³, may be suitable for use in the post-operative thoracic surgical population.

- This is examined in a subset of patients in Investigation III. A total ‘risk of lung injury score’, derived from the simultaneous measurement of 7 biomarkers is compared to the same, pre-defined properties of the ‘ideal’ lung injury biomarker.

- Trans-pulmonary thermodilution measurement of EVLW and PVPI are well validated in the general intensive care population, and are increasingly being used as study endpoints in patients undergoing thoracic surgery ^{235, 245-247}.
- Due to methodological assumptions made in currently clinically available TPTD monitors, there is however significant reason to question the validity of these monitors in the lung resection population.
- Despite increasing use, no such validation has been made. Furthermore, it has been suggested by some, that the methodology of TPTD be amended for use following lung resection ²⁴⁸.

Hypothesis V: Transpulmonary-thermodilution monitoring of EVLW and PVPI are of questionable reliability and validity in the thoracic surgical population. Secondly, adjustment of TPTD methodology to reflect surgical resection of lung tissue will improve reliability and validity following lung resection.

- This is examined in Investigation IV, where the reliability and construct validity of TPTD derived EVLW and PVPI are pursued in an observational cohort of patients undergoing lung resection. Post-operative oxygenation, chest X-ray score and fluid balance are defined as 'constructs' with which association between construct and EVLW / PVPI would be expected.

2 Investigation I: Anaesthesia for lung resection – a survey of UK practice

2.1 Introduction

In recognition of the poor prognosis of patients suffering PLR-ALI and the limited treatment options available, alongside increased understanding of the pathogenesis of the condition, in recent years there has been an increasing interest in methods of preventing PLR-ALI. The conduct of one-lung ventilation (OLV), peri-operative fluid management and mode of anaesthesia are arguably the most easily modifiable risk factors that can be targeted in the prevention of PLR-ALI.

Traditional teaching of thoracic anaesthesia had described one lung ventilation with a target tidal volume of 10 mL/kg, an FiO₂ of 1.0, zero positive end expiratory pressure (ZEEP) and an intention to maintain normocapnia^{124, 139-141}. With increasing understanding of the contribution of ventilator induced lung injury (VILI) to the pathogenesis of PLR-ALI, expert opinion is calling for a revised approach to ventilation during OLV with many advocating the introduction of a ‘lung protective ventilatory strategy’^{115, 122, 124, 141}. The role of VILI in the pathogenesis of PLR-ALI, and the potential role of ‘lung protective ventilation’ in its prevention was reviewed in detail in Chapter 1.

Anecdotally, and with experience limited to West of Scotland practice, in the opinion of the author (Ben Shelley), lung protective ventilation during the period of OLV and a restrictive approach to fluid management are widely practiced. The aim of this investigation was to provide a snapshot of contemporary thoracic anaesthetic practice in the United Kingdom and Ireland, exploring the prevalence of lung protective ventilation, patterns of fluid prescribing and mode of anaesthesia used during lung resection. Facets of lung protective ventilation were defined as use of reduced tidal volume, use of positive end-expiratory pressure and fraction of inspired oxygen administered.

2.2 Methods

An invitation to participate in the survey was e-mailed to all members of the (United Kingdom) Association of Cardiothoracic Anaesthetists (ACTA). Respondents completed an online survey with data collected via the commercially available 'SurveyMonkey' web platform (www.surveymonkey.com) during the months of July to September 2009. Participants were requested to complete questions in the context of their "*current routine 'first choice' practice when anaesthetising for thoracotomy for lobectomy / pneumonectomy with one-lung ventilation... in the absence of any contra-indications or special (patient) considerations... [and where applicable] assuming oxygenation is not a problem... and blood loss is not exceptional*". Questions concerned anaesthetic technique, mode of ventilation during the period of one-lung ventilation, regional analgesic technique (if any), adjunctive analgesia, peri-operative fluid management, management of peri-operative hypotension and choice of lung separation technique.

The survey itself was the product of an iterative design process. Firstly following a review of the relevant literature, a draft survey was prepared by the author (B. Shelley). This was subsequently reviewed, and comment provided, by Professor Stefan Schraag and Dr Alistair Macfie, consultant cardiothoracic anaesthetists at Golden Jubilee National Hospital, Clydebank. The subsequently revised survey was then piloted via the SurveyMonkey online platform by ten thoracic anaesthetists within the Department of Cardiothoracic Anaesthesia at the Golden Jubilee National Hospital; this allowed readability and survey navigation to be checked in addition to providing opportunity for further constructive comment. The further revised survey was then submitted for comment and approval to the committee of the Association of Cardiothoracic Anaesthetists (an elected panel of five consultant cardiothoracic surgeons from throughout the UK). Following further refinement the final survey transcript (reproduced in Appendix One) was resolved.

For this thesis, only the results from questions concerning the practice of one-lung ventilation, peri-operative fluid prescribing and mode of anaesthesia are presented (the published manuscript is provided in Appendix Two).

2.3 Results

2.3.1 Responses received

A total of 132 responses were received; two were excluded as they originated from outwith the UK. This represents at least one reply from 39 of 42 (93%) identified centres performing thoracic surgery in the UK and Ireland with a median response rate of 3 (range 0 - 8) per centre.

2.3.2 Lung protective ventilation

2.3.2.1 Tidal volume during one-lung ventilation

Survey participants were asked - “during the period of one lung ventilation do you... ventilate with a target tidal volume?”

Of the 129 respondents to this question, 53 (41%) respondents report ventilating to a target tidal volume. Of these, 44 (83%) answered the follow-up question “if yes, what target?” The mean (SD) reported ‘target’ tidal volume during the period of one lung ventilation was 6.1 (± 1.5) ml/kg (Figure 2.1).

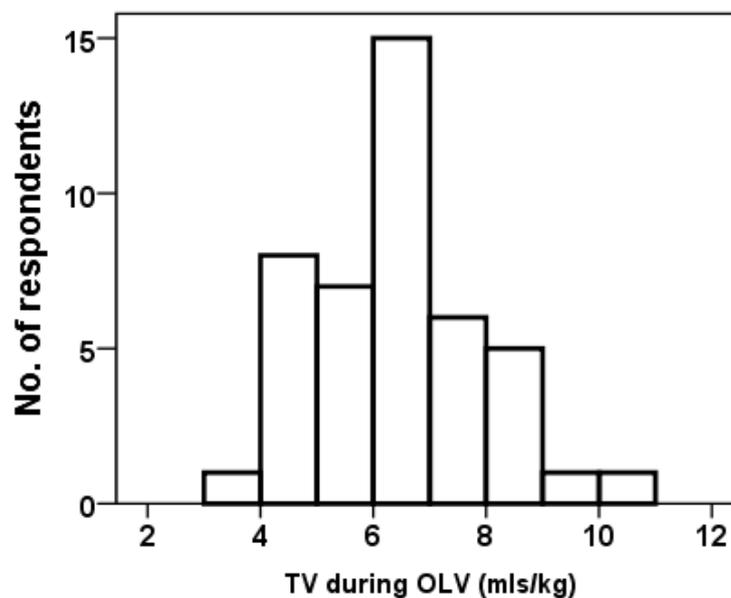


Figure 2.1. Target tidal volume during one-lung ventilation. (N=44)

2.3.2.2 Use of positive end-expiratory pressure during OLV

Survey participants were asked - “during the period of one lung ventilation do you... routinely use positive end expiratory pressure (PEEP)?”

Of the 128 respondents to this question, just under half (57, 45%) report the routine use of positive end expiratory pressure at a median (IQR) level of 5 (4-5) cmH₂O. The distribution of PEEP values reported is demonstrated in Figure 2.2; it should be noted that users of PEEP are relatively underrepresented in Figure 2.2 as 12 of the 57 respondents reporting use of PEEP did not answer the follow up question - “If yes, how much?” Nonetheless it is evident that the distribution of PEEP utilised by UK thoracic anaesthetists is bimodal, with a larger cohort (comprising over half of respondents) not using any PEEP, and another smaller cohort using 4-6 cmH₂O PEEP.

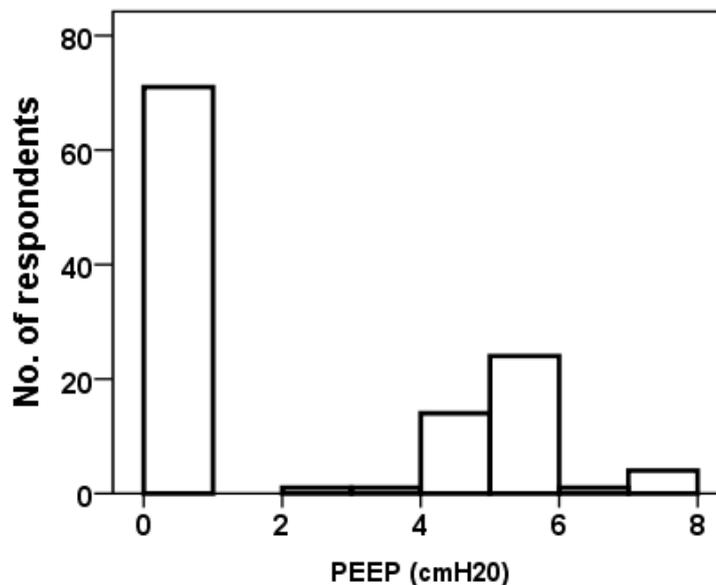


Figure 2.2. Positive end-expiratory pressure values utilised during one-lung ventilation.
(N=116).

2.3.2.3 Fraction of inspired oxygen during OLV

Survey participants were asked - “during the period of one lung ventilation do you...routinely ventilate with a FiO₂=1?”

Of the 128 respondents to this question, the majority (114, 89%) of respondents report routinely ventilating with an FiO₂ less than 1.0 with median FiO₂ of 0.5

(0.5-0.7). The distribution of FiO_2 levels reported is demonstrated in Figure 2.3. It is evident from the figure that the distribution of FiO_2 levels administered by UK thoracic anaesthetists is bimodal, with the majority ventilating with an FiO_2 of between 0.4 and 0.7, whilst a smaller though significant cohort ventilate with $\text{FiO}_2=1.0$.

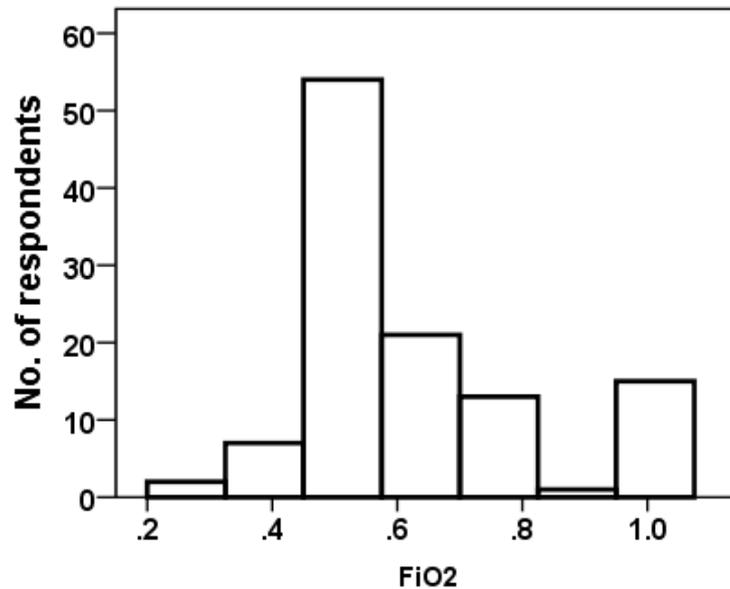


Figure 2.3. Fraction of inspired oxygen administered during one-lung ventilation. (N=113).

2.3.3 Peri-operative fluid management

2.3.3.1 Routine intra-operative fluid administration

Survey participants were asked - “*What is the average volume of fluids you administer intra-operatively? (Assuming blood loss is not exceptional)*”.

In the 117 respondents who answered this question, the mean (SD) volume of fluid administered intra-operatively was 1200 (± 500) ml or 2.7 (± 1.1) ml/kg^G (Figure 2.4).

^G Calculated based on a 2.5 hour operation in a 70kg patient. In fact, the mean duration of surgery in the 34 patients recruited to Investigation III was 2.8 hours.

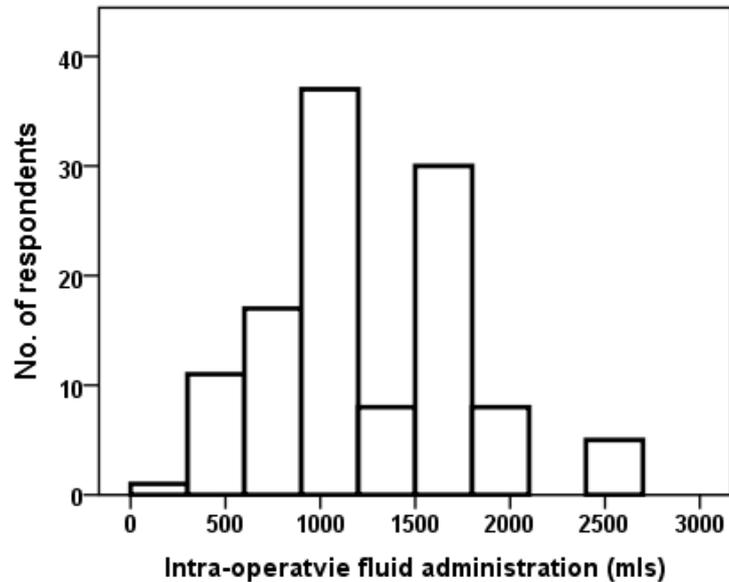


Figure 2.4. Typical intra-operative fluid administration.
(N=118).

2.3.3.2 Routine post-operative fluid prescribing

Survey participants were asked - “*What is your standard fluid prescription? (For the immediate post-operative period / night of surgery - assuming intra-operative losses were not exceptional - in an 'average' 70kg patient)*”.

In the 81 respondents who answered this question, the mean (SD) post-operative fluid prescription was 77 (± 19) mls/hr or 1.1 (± 0.3) mls/kg/hr Figure 2.5).

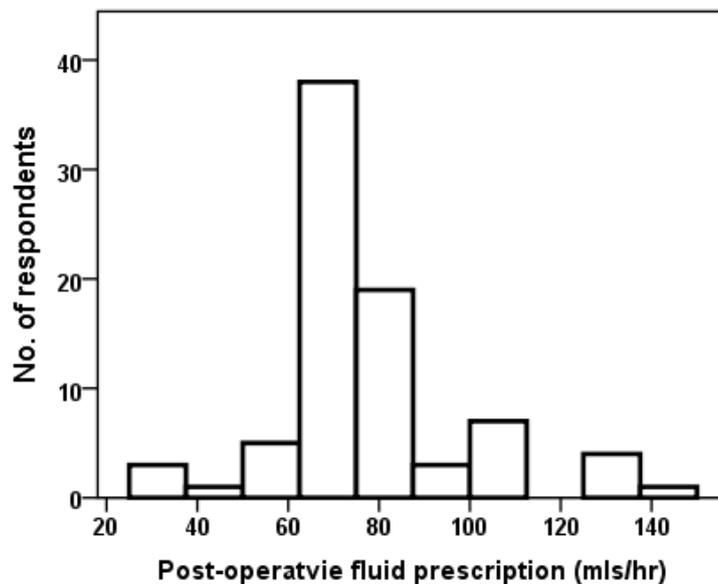


Figure 2.5. Typical post-operative fluid prescribing.
(N=81).

2.3.3.3 Attitudes to fluid restriction

Survey participants were asked to select one of two possible responses:

1. *“I believe it is important to adopt a 'restrictive' approach to post-operative fluids in this patient group [undergoing lung resection].*
2. *I do NOT restrict post-operative fluids in this patient group”.*

Of the 74 respondents to the question, 61 (82%) respondents selected option one, suggesting the majority of UK thoracic anaesthetist do believe it is important to adopt a restrictive approach to post-operative fluid prescribing.

2.3.4 Choice of anaesthetic technique

Survey participants were asked - *“Concerning your typical 'first choice' anaesthetic technique for thoracotomy and one-lung ventilation do you:*

1. *Use target controlled (TCI) Propofol / TIVA?*
2. *Use inhalational anaesthetic agents?”*

Of the 129 respondents answering this question, 109 (84%) respondents indicated that they routinely use inhalational anaesthetic agents for maintenance of anaesthesia during thoracotomy.

2.4 Discussion

The results of the survey reported above suggest that the contemporary practice of one-lung ventilation in the UK has evolved some distance from the classical 'textbook' descriptions. This survey demonstrates that aspects of lung protective ventilation are widespread within UK thoracic anaesthetic practice; nearly half (47%) of UK thoracic anaesthetists report routine use of PEEP during OLV, 40% report ventilating to a target tidal volume (of which the mean observed was 6.1ml/kg) and 89% routinely ventilate with an FiO_2 less than 1.0.

2.4.1 Limitations

Whilst the survey reflects the practice of a large number (130) of thoracic anaesthetists in the United Kingdom and Ireland, unfortunately establishing a denominator for this response rate was impractical because it is unknown what fraction of the 458 Association of Cardiothoracic Anaesthetists (ACTA) members (at the time of survey release, personal communication, ACTA administrator) actively practice thoracic anaesthesia. In addition, by circulating the survey within the membership of ACTA, all thoracic anaesthetists who are not members of ACTA are effectively excluded from participation. Nonetheless, the responses received represent at least one reply from 39 of 42 (93%) identified centres performing thoracic surgery in the UK and Ireland with a median response rate of 3 (range 0 - 8) per centre, suggesting the survey is likely to be broadly representative of practice throughout the UK.

This survey is subject to several potential sources of bias inherent to all survey research. Firstly, distribution of the survey via ACTA results in the risk of introducing selection bias; by definition all UK thoracic anaesthetists who are not members of ACTA were excluded from the study sample. It is plausible to suggest that the two groups of anaesthetists distinguished by ACTA membership may differ systematically in their attitudes and / or practices. Members of ACTA for example, are engaged in the activities of the specialist society, are likely to have more recently attended an ACTA meeting and as such could be hypothesised to have more up to date opinions / practices. Secondly, though difficult to quantify in lieu of the unknown response rate, this survey is also at

risk of non-responder bias. Practitioners with more passionately held opinions concerning their clinical practice are more likely to respond to such a survey; survey results might be more likely therefore to be representative of the extremes of practice, than the practice of the 'average' UK anaesthetist. Similarly, by not mandating an answer to every question in the survey (a conscious decision aimed at improving overall response rate), participants could elect to not answer specific questions. Postulated reasons of not answering include the belief that a questions topic is less 'interesting' or 'important', or the perception that the participants response is the 'wrong answer' for example, where the respondent recognises their practice to be in some way 'unusual' or 'old fashioned'.

A further limitation of the survey is that no attempt was made to establish the respondent's level of experience or the frequency of his/her thoracic anaesthetic practice and as such no assessment can be made concerning the effect experience makes on practice.

2.4.2 UK practice of OLV in comparison to worldwide practice

The findings of the survey were published in 2011 in a manuscript entitled "Anesthesia for thoracic surgery: a survey of practice" (Appendix one)²³⁰. Following its publication, the author (Ben Shelley), was invited to collaborate with several authors from King Saud University, Riyadh, Saudi Arabia, in performing the same survey in the Middle East. Now published as "Anesthesia for thoracic surgery: A survey of middle eastern practice"²⁴⁹, this survey reported similar practices in the Middle East as are seen in the UK. As in the UK, aspects of lung-protective ventilation are practiced by a substantial cohort, though it appears that in general, Middle Eastern practice (with specific reference to conduct of OLV) is more traditional than that seen in the UK. In 2013 Della Rocca et al published a similar survey examining Italian practice; again reduced tidal volume ventilation is common place (with nearly 90% of hospitals reporting tidal volumes of less than 8ml/Kg) and the majority (~80%) routinely applying PEEP²⁵⁰. Whilst there are several other published surveys examining thoracic anaesthetic practice, these largely concern analgesic techniques and make no assessment of the conduct of one-lung ventilation.

2.4.3 Interpretation of the survey's findings

Since its publication, the published manuscript reporting the results of this survey has received 11 citations (source: Google Scholar available at http://scholar.google.co.uk/citations?view_op=view_citation&hl=en&user=yfNVnTUAAAAJ&citation_for_view=yfNVnTUAAAAJ:u5HHmVD_uO8C, accessed 05/10/14). Whilst several of these citations concern analgesic technique or techniques of lung isolation (other topics covered in the manuscript but not reported in this thesis), several have concerned the practice of one-lung ventilation, with differences in opinion regarding whether the findings reflect positively or negatively on current practice:

Qutub et al interpret the survey's findings positively, commenting:

“There is an increasing use of lower VT of 6ml kg⁻¹ or less during OLV among Middle Eastern and British thoracic anaesthesiologists.”

Qutub et al (2014)²⁴⁷

Whilst Licker concludes that despite showing evidence of the adoption of lung protective ventilatory techniques, the survey demonstrates such techniques are not used enough:

“A survey among members of the Association of Cardiothoracic Anaesthetists in the UK revealed that, only 40% of 132 respondents were selecting “low” VT (median 6 ml/kg, interquartile range 5-7 ml/kg), [and] 15% used FiO₂ > 0.8”.

Licker et al (2012)²⁵¹

From the available evidence, it would appear that lung protective ventilatory techniques are part of contemporary thoracic anaesthetic practice worldwide. Without earlier reports with which to make a comparison however, it is impossible to conclude that this represents a shift away from traditional practice, though there is a long and consistent narrative throughout the literature to suggest that such an evolution has taken place^{110, 115, 122, 124}.

The mean target tidal volume reported during OLV was 6.1ml/kg. One remaining question is ‘whether this is low enough?’ Whilst 6ml/kg is substantially less than

traditional recommendations which advocate tidal volumes of 10ml/kg during the period of one-lung ventilation^{124, 139-141}, it must be acknowledged that 6ml/kg is the tidal volume advocated by the US ARDS Network for ventilation of *both* lungs in critically ill patients with ALI/ARDS. It is possible therefore that 6ml/kg whilst an improvement over 10ml/kg, may still represent an excessive tidal volume to apply to one lung. On inspection of Figure 2.1 it can be seen that a substantial proportion of UK thoracic anaesthetists are limiting tidal volume further (to 3-5ml/kg). There are no randomised clinical trials comparing for example 'low' (6ml/kg) to 'very low' (4ml/kg) tidal volume during one lung ventilation, however observational data subjected to multivariate regression by Jeon et al (Table 1.3) suggests that during pneumonectomy, the odds-ratio for development of lung injury increases by 3.37 (CI 1.65-6.86) per ml/kg increase in tidal volume¹⁵². As such, providing tidal volume can be decreased below 6ml/kg safely and without complication, it may be reasonable to do so.

Within the wider critical care environment (where the benefits of lung protective ventilation have been emphatically described), there is some limited evidence to suggest a direct impact of the US ARDS Network's 'lower tidal volume ventilation' study⁹⁹, on the subsequent practice of ventilation, where two before and after studies have demonstrated a fall in tidal volume^{252, 253}. Some years later however, it is clear from several large observational studies that tidal volume reduction to the extent advocated by the ARDS Network study has not been universally adopted^{252, 254}. Rubenfeld et al studied 'barriers' to the provision of lung-protective ventilation in patients with ALI in the intensive care setting identifying:

“physician willingness to relinquish control of ventilator, physician recognition of ALI/ARDS, and physician perceptions of patient contraindications to low tidal volumes as important barriers to initiating lung-protective ventilation. [whilst] Important barriers to continuing patients on lung protective ventilation were concerns over patient discomfort and tachypnea and concerns over hypercapnia, acidosis, and hypoxemia”

Rubenfeld et al (2004)²⁵⁵

In the setting of thoracic anaesthesia, (where arguably the benefits of lung-protective ventilation have been less well established), it is interesting to

speculate that many of the same barriers may be preventing more widespread adoption of lung protective ventilation. In particular, given avoidance of hypoxaemia was the rationale behind the ‘traditional’ recommendations^{140, 142}, it seems likely that concerns regarding hypoxaemia during the finite period of one-lung ventilation may be preventing greater adoption of these techniques. It would be of value to repeat the original survey to firstly observe any trend in the practice of one-lung ventilation, and secondly to identify any barriers to the adoption of lung protective ventilatory techniques.

2.4.4 Peri-operative fluid prescribing

Whilst it is clear from the results of the survey that the majority of UK thoracic anaesthetists believe it is important to adopt a restrictive approach to peri-operative fluid prescribing, what is less clear is what constitutes a restrictive approach? As highlighted by Doherty and Buggy:

“..no common definition of ‘liberal’ or ‘restrictive’ [fluid management] protocols exists in clinical practice. A restrictive regime in one centre may actually be liberal in another”.

Doherty and Buggy (2012)²⁵⁶

This is emphasised by comparison of the following three studies conducted in the lung resection population. Matot et al conducted a randomised controlled trial comparing the effects of ‘high’ volume intra-operative fluid prescribing (8ml/kg/hr) versus ‘low’ volume (2ml/kg/hr) in patients undergoing video-assisted thoracoscopic surgery for lung resection²⁵⁷. Haas et al, studied the effects of goal directed fluid management in patients undergoing lung resection and chose an intentionally “rather fluid aggressive” intra-operative protocol, where intra-operative fluids were administered at 9ml/kg/hr²³⁵; a figure in keeping with the ‘high’ volume group of Matot et al²⁵⁷. Assad et al however, were studying the effects of “liberalized fluid management” and administered intra-operative fluids at 2.5ml/kg/hr²³⁴; a value more in keeping with the ‘low’ volume prescription of Matot et al²⁵⁷.

Whilst many have discussed the topic of restrictive fluid management in thoracic surgery, few have committed themselves to make definable recommendations. Chau and Slinger recommend:

“Crystalloid administration should be limited to <2L intra-operatively [~11ml/kg/hr] and <3 L [125ml/hr / ~1.8ml/kg/hr] in the first 24 hours post-operatively”.

Chau and Slinger (2014)⁴⁸

Evans and Naidu conducted a structured ‘best evidence’ literature review asking the question *“Does a conservative fluid management strategy in the peri-operative management of lung resection patients reduce the risk of acute lung injury?”*²³² They concluded:

“On this best evidence presented, we recommend a conservative strategy of administration of maintenance fluids at 1–2 ml/kg/h in the intra- and post-operative periods”.

Evans and Naidu (2012)²³²

Examined in the context of these recommendations, it can be concluded that peri-operative fluid prescribing by UK thoracic anaesthetists (where typical mean intra- and post-operative infusion rates are 2.7 and 1.1 ml/kg/hr respectively) is well within what may be considered ‘conservative’. It may however be unnecessary to seek a definition for ‘restrictive’ fluid prescribing. In a dynamic situation where an individual’s fluid requirements will depend on a combination of pre-operative deficit, maintenance requirements and ongoing losses, arguably the important factor is that thought is being applied to the issue of peri-operative fluid prescribing, and that there is a general consensus regarding the need to err on the side of restriction.

2.4.5 Maintenance of anaesthesia

The overwhelming majority of UK thoracic anaesthetists use volatile anaesthetic agents for the maintenance of anaesthesia during thoracotomy. Whilst (as discussed in detail in Chapter one (Section 1.4.6.3) there is some evidence to suggest a lung protective immunomodulatory effect of volatile anaesthetic agents, total intravenous anaesthesia (TIVA) with propofol has other theoretical benefits in the thoracic population in terms of maintenance of hypoxic

pulmonary vasoconstriction and separating anaesthesia provision from maintenance of the airway²⁵⁸.

Data from the Royal College of Anaesthetists' Fifth National Audit Project surveying the practice of anaesthesia during a one week period in September 2013 (collecting data from over 20,000 anaesthetics) reports that in 92% of cases, provision of general anaesthesia is via inhalation of volatile anaesthetic agent²⁵⁹. It would appear therefore that TIVA is marginally over-represented in thoracic anaesthetic practice. What this survey unfortunately is unequipped to do is explore the rationale for the anaesthetic choice reported.

2.4.6 Conclusion

In conclusion, UK thoracic anaesthetists appear to be addressing the defined modifiable risk factors for the development of PLR-ALI; aspects of lung protective ventilation are being incorporated into contemporary practice whilst restrictive fluid prescribing and maintenance of anaesthesia using volatile anaesthetic agents is commonplace. It may be reasonable to hypothesise therefore that the incidence of PLR-ALI should be falling as a result. Investigation II of this thesis concerns this question:

“Is the incidence of, and mortality from PLR-ALI falling with time?”

3 Investigation II: Trends in the incidence and mortality of post-lung resection lung injury over time: A meta-regression analysis

3.1 Introduction

Post-lung resection ALI/ARDS is the major cause of early mortality in patients undergoing lung resection^{129, 130}. As described in investigation one, a ‘restrictive’ approach to peri-operative fluid management and aspects of ‘lung protective ventilation’ have been widely incorporated into thoracic anaesthetic practice in the belief they will prevent lung injury.

Reports from single institutions suggest that the incidence of ALI/ARDS following lung resection has fallen over time, with much of this reduction being attributed by the authors to changes in ventilatory practice^{110, 260}. Tang et al reported on ‘a 10-year single institutional experience’ in the Royal Brompton Hospital, London¹¹⁰. By retrospectively comparing a cohort of patients undergoing lung resection between 2000-2005, to a cohort from 1991-1997, Tang et al concluded that “the incidence and mortality from ARDS has fallen significantly over the study period” (incidence from 3.2% to 1.6%, $p=0.01$; mortality from 72% to 45%, $p=0.05$). Though lacking data to make statistical comparison, the authors attributed much of the reduction to the adoption of lung protective ventilatory strategies, reporting that “although the ventilatory parameters on one lung ventilation are not recorded, the ventilatory strategies at this institution have become more protective over the past 5 years”¹¹⁰. In a large observational cohort, Licker et al assessed the impact of the introduction of a “protective lung ventilation protocol”¹⁵⁵ in two affiliated Swiss hospitals. By comparing an ‘historical cohort’ who underwent resection from 1998-2003 (before introduction of the protocol), to a ‘protocol group’ who underwent resection from 2003-2008, Licker et al demonstrated a reduction in the incidence of ALI from 3.8% to 0.9%. Whilst the two groups were relatively evenly matched in terms of baseline patient demographics, the ‘protocol group’ were exposed to significantly lower, tidal volume and inspiratory plateau pressures during one-lung ventilation¹⁵⁵.

In the wider critical care setting, reports concerning trends in ALI/ARDS mortality are conflicting; clinical trialists report mortality is falling^{261, 262}, whilst a larger meta-regression analysis including both randomised and observational studies suggests mortality is stable¹³⁶.

Whilst the two institutional reports cited are encouraging, the potential for confounding is significant, and ultimately they reflect the activity of just three hospitals. The aim of investigation II therefore, is to attempt to extend the evidence base beyond single centre reports and further investigate trends in PLR-ALI incidence and mortality with time. This study is a meta-analysis and meta-regression analysis seeking to answer the following questions from published data:

- *Is the incidence of PLR-ALI falling with time?*
- *Is mortality from PLR-ALI falling with time?*

3.2 Methods

3.2.1 Search strategy

MEDLINE and EMBASE databases were searched for studies reporting the incidence of ALI and/or ARDS in patients undergoing lung resection according to the following search strategy. This search was last updated on 18th March 2013.

1. pneumonectomy.mp. or exp Pneumonectomy/
2. (lung adj resection).tw.
3. (pulmonary adj resection).tw.
4. (thoracic adj surgery).tw.
5. Thoracotomy.mp. or exp Thoracotomy/
6. 1 or 2 or 3 or 4 or 5
7. acute lung injury.mp. or exp Acute Lung Injury/
8. respiratory distress syndrome, adult.mp or exp Respiratory Distress Syndrome, Adult/
9. pulmonary edema.mp. or exp Pulmonary Edema/
10. ALI.mp. or ARDS.tw.
11. 7 or 8 or 9 or 10
12. 6 and 11
13. limit to English language
14. limit 13 to yr="1994-Current"

3.2.2 Inclusion and screening

Studies were included if the incidence of ALI, ARDS or ALI/ARDS (ALI or ARDS) in patients undergoing lung resection surgery was reported, and the time period of study recruitment could be derived from the paper. Inclusion was restricted to studies using the 1994 American-European consensus definition for ALI/ARDS.

Titles and abstracts were screened and review articles, educational pieces, letters and conference proceedings, abstracts or studies not concerning PLR-ALI were excluded. The reference lists of all included articles and all excluded review articles were screened for further relevant studies. The remaining 127 papers were subject to full text review. Papers were subsequently excluded if they concerned pulmonary complications but not PLR-ALI, PLR-ALI was not

defined according to the American-European consensus definition, data was duplicated in another publication or the study concerned a case series of patients with PLR-ALI for which no denominator was provided. Where possible, in situations where PLR-ALI was part of a composite endpoint or no definition was provided authors were contacted for clarification.

3.2.3 Data extraction

Due to inconsistency in reported endpoints, data was extracted on the incidence of, and mortality from ALI, ARDS and ALI/ARDS individually. Where a paper contained a historical control group, these groups were treated as separate patient cohorts within the analysis. Similarly, where data (for example patient demographics) was only provided by study sub-group (e.g. arms of a randomised controlled trial), these study sub-groups were treated as separate cohorts in the meta-regression. Thus, patient 'cohort' rather than 'study' became the unit of analysis. In addition to the incidence of PLR-ALI and the year of study recruitment, data was extracted on the following covariates defined a-priori as being known to influence the incidence of PLR-ALI: age and sex of study subjects, lung resected (pneumonectomy, lobectomy, sub-lobar resection) and laterality of resection, baseline pulmonary function (forced expiratory volume in 1 second (FEV₁) and diffusing capacity for carbon monoxide (DLCO)), prevalence of pre-operative induction chemo- and radio-therapy, duration of one-lung ventilation, intra-operative fluid administration, open or video assisted thoracoscopic technique and analgesic technique.

3.2.4 Meta-analysis

Random effects meta-analysis was performed to generate pooled incidence and mortality estimates along with 95% confidence intervals. Individual analyses were performed for the end points ALI, ARDS and ALI/ARDS for all patients, and where data was available, for subgroups of patients undergoing pneumonectomy or lobectomy. Meta-analysis and meta-regression was performed using 'Comprehensive Meta-Analysis' software (ver. 2.2.064), BioStat, Englewood, New Jersey (www.meta-analysis.com).

3.2.4.1 Effect measure

Incidence and mortality data were extracted as number of events and cohort sample size. The ‘effect measure’ for the purposes of meta-analysis was therefore defined as the logit function of the event rate (Equations 3.1 and 3.2)

$$\text{Event Rate } (p) = \frac{\text{Events}}{\text{Sample size}}$$

Equation 3.1

$$\text{Logit Event Rate} = \text{Log} \left(\frac{p}{1-p} \right)$$

Equation 3.2

3.2.4.2 Weighting

In order to yield a random effects meta-analysis (and meta-regression), cohorts were weighted according to the sum of the within-cohort variance and the residual between-cohort variance²⁶³. Thus the weight assigned to each cohort (W_i^* for the i^{th} cohort, where W^* represent weight under random effects and W represents weight under fixed effects) is computed as:

$$W_i^* = \frac{1}{V_i^*}$$

Equation 3.3

Where, V_i^* is the within-cohort variance of cohort i plus the between-cohort variance, T^2 :

$$V_{Y_i}^* = V_{Y_i} + T^2$$

Equation 3.4

Where Tau-squared (T^2) is defined as the variance of the true effect sizes, i.e. the between-cohorts variance independent of within-cohorts variance. As variance of the true effect size cannot be measured (without studies with infinitely large sample sizes, such that the observed variance is in fact the true variance), T^2 was estimated from the observed effects according to the ‘method

of moments' described by DerSimonian and Laird^{264, 265}. Where T^2 is computed as:

$$T^2 = \frac{Q - df}{C}$$

Equation 3.5

Where,

$$C = \sum W_i - \frac{\sum W_i^2}{\sum W_i}$$

Equation 3.6

Where, as before, W_i is the weight assigned under fixed effects to the i^{th} cohort.

3.2.4.3 Identification of outliers

Screening for potential outliers was performed by visual inspection of forest plots, and examination of standardized residuals²⁶⁶. Standardized residuals (also known as internally studentized residuals) were calculated by the *Comprehensive Meta-analysis* software as the quotient of the raw residual and the sampling variance of the raw residual. A standardized residual of 2.0 or greater was defined as a level at which cohorts would be considered potential outliers and subject to further scrutiny.

3.2.4.4 Heterogeneity

Heterogeneity was explored using Q and I^2 statistics.

The Q statistic

The 'Q-statistic' or 'Q', is derived by determining the deviation of each effect size from its mean, squaring it, weighting this by the inverse of the variance for the given cohort and then summing the values of all of the studies in the analysis to yield the weighted sum of squares or Q :²⁶⁵.

$$Q = \sum_{i=1}^k W_i (Y_i - M)^2$$

Equation 3.7

Where, W_i is the cohort weight (under fixed effects), Y_i is the cohort effect size, M is the summary effect and k is the number of cohorts.

The Q -statistic is then compared with the expected value of Q (were all studies assumed to share a common effect size). This is calculated simply as the degrees of freedom (df):

$$df = k - 1$$

Equation 3.8

Where k is the number of cohorts.

The difference between the observed weighted sum of squares (Q) and the expected (df) reflects the excess variation between cohorts. That is the variation that can be attributed to the difference in true effect between-cohorts, rather than within-cohorts. Comparing Q with a central chi-squared distribution (with $df=k-1$) allows formal statistical testing of heterogeneity yielding a p -value examining the null hypothesis that all cohorts share a common effect size. As the chi-squared test in this context inherently has low power, a p -value of ≤ 0.10 was used to determine statistical significance²⁶⁷.

I-squared

The I-squared (I^2) statistic describes the percentage of total variance across cohorts that is due to heterogeneity rather than chance²⁶⁸, or “*what proportion of the observed variance reflects real differences in effect size?*”²⁶⁹. I^2 was determined as in Equation 3.9:

$$I^2 = \left(\frac{Q - df}{Q} \right) \times 100 (\%)$$

Equation 3.9

Whilst use of specific thresholds for the interpretation of I^2 can be misleading, a rough guide is provided by the Cochrane Collaboration²⁶⁷:

Table 3.1. Interpretation of I^2 .

I^2	Interpretation
0% to 40%	'might not be important'
30% to 60%	'may represent moderate heterogeneity'
50% to 90%	'may represent substantial heterogeneity'
75% to 100%	'considerable heterogeneity'

From the Cochrane Collaboration (2011)²⁶⁷.

3.2.4.5 Subgroup analysis

Where available, data was extracted from each cohort into subgroups of patients undergoing pneumonectomy or lobectomy (including bi-lobectomy). Data was combined within-cohorts according to a random-effects model. In view of the small number of cohorts in each subgroup (less than five on some occasions), within-group estimates of Tau-squared were pooled as advocated by Borenstein et al²⁶⁹. Event rate was compared across subgroups using a Q-test. The proportion of true^H variance explained (R^2) by differences between subgroups was subsequently calculated according to Equation 3.10:

$$R^2 = 1 - \left(\frac{T_{within}^2}{T_{total}^2} \right)$$

Equation 3.10

Where T_{within}^2 is the between-cohorts variance within-subgroups, and T_{total}^2 is the total between-cohorts variance (within-subgroups and between-subgroups).

3.2.4.6 Detection of publication bias

Presence of publication bias was assessed by visual inspection of funnel plots of Logit event rate by standard error. The impact of publication bias on pooled incidence and mortality estimates was assessed by Duval and Tweedie's 'trim and fill' procedure^{270, 271}. This procedure uses an 'iterative' approach,

^H This statistic reflects the proportion of *true* variance explained by the subgroup effect distinct from the *within-cohort* variance. The sum of *true* variance and *within-cohort* variance is the *total* variance observed.

sequentially removing the most extreme small studies from the ‘positive’ side of the funnel plot and re-computing the effect size at each iteration. ‘Trimming’ continues until the funnel plot is symmetrical, providing an ‘adjusted’ effect size estimate. Such a procedure in isolation however would underestimate variability. The ‘fill’ procedure therefore returns the ‘trimmed’ study to the analysis, balanced by an imputed ‘mirror image’ for each, hence maintaining the ‘trimmed’ effect size, but providing a more realistic estimate of variability²⁶⁵.

3.2.5 Meta- regression

Univariate logistic random effects meta-regression analysis was used to explore the association between incidence and mortality of ALI, ARDS and ALI/ARDS and median year of cohort recruitment in addition to other covariates. Between-cohorts variance within the random effects model was computed according to the ‘method of moments’ of DerSimonian and Laird^{264, 265} as described previously.

3.2.5.1 Multivariate meta-regression

One of the initial goals of this investigation was to construct a multivariate logistic meta-regression model to describe the effect of covariates (including median year of study recruitment) on the incidence and mortality of PLR-ALI, and so distil the effect of median year of study conduct from other cofounders. Analogously to recommendations that 10 data points are required per covariate entered into a conventional multivariate regression model²⁷², it is advised that 10 studies are required per covariate entered into a multivariate meta-regression model^{267, 273}. As insufficient studies were available, no multivariate model could be constructed.

3.2.6 Presentation and interpretation

Meta-analyses are presented as ‘forest plots’ of event rate and 95% confidence interval, where the size of the effect size marker is proportional to the weight assigned to that cohort in the random-effects analysis.

Meta-regression analyses are presented as ‘bubble-plots’ where the logit function of the ‘event rate’ (either incidence or mortality) are plotted against the explanatory variable (median year of cohort recruitment or other covariate). The size of the ‘bubble’ reflects any given cohort’s weighting in the analysis (weighted (as with the meta-analysis) according to the sum of the within-trial variance and the residual between-trial variance²⁶³). The exponent of the slope of the regression line yields the odds-ratio for the relationship between event rate and covariate. This is provided as a point estimate and 95% confidence interval. The proportion of true variance explained (R^2) by the addition of a moderator variable (covariate) to the initial meta-analysis was then calculated as below.

$$R^2 = 1 - \left(\frac{T_{unexplained}^2}{T_{total}^2} \right)$$

Equation 3.11

Where $T_{unexplained}^2$ is the residual between-cohorts variance after addition of the moderator and T_{total}^2 is the total between-cohorts variance.

3.3 Results

3.3.1 Studies

Literature searching returned 127 relevant titles of which 35 studies were selected for data extraction (Figure 3.1 documents the flow of studies through the study). After exclusion of duplicated data (resulting from multiple publications from the same centre, or registry reports), data was finally extracted from 21 studies. Data was extracted from several studies in subgroups (either due to the presentation of a historical cohort group or the method by which demographic data was presented); this resulted in 27 patient ‘cohorts’ being available for analysis - 12, 16 and 7 in the ALI, ARDS and ALI/ARDS groups respectively. Of these, the mortality from ALI, ARDS and ALI/ARDS could be determined in 9, 13 and four cohorts respectively. The studies included in the analysis are summarised in Table 3.2. Whilst the median year of cohort recruitment was available for all studies (in lieu of this being an inclusion criterion), data concerning other covariates was not available in a considerable number of studies. Studies with missing data were not included in the respective analyses.

3.3.2 Patient and study demographics

The 27 patient cohorts included in the analysis represent data from 10,647 patients (median 170 (IQR 65-546) patients per cohort), and report incidence and mortality of ALI, ARDS and ALI/ARDS between 1989 and 2009 (median year of cohort recruitment). For the cohorts from which age and sex data could be extracted, median patient age was 62 years, with more males (76%) than females included in the analysis (Table 3.2).

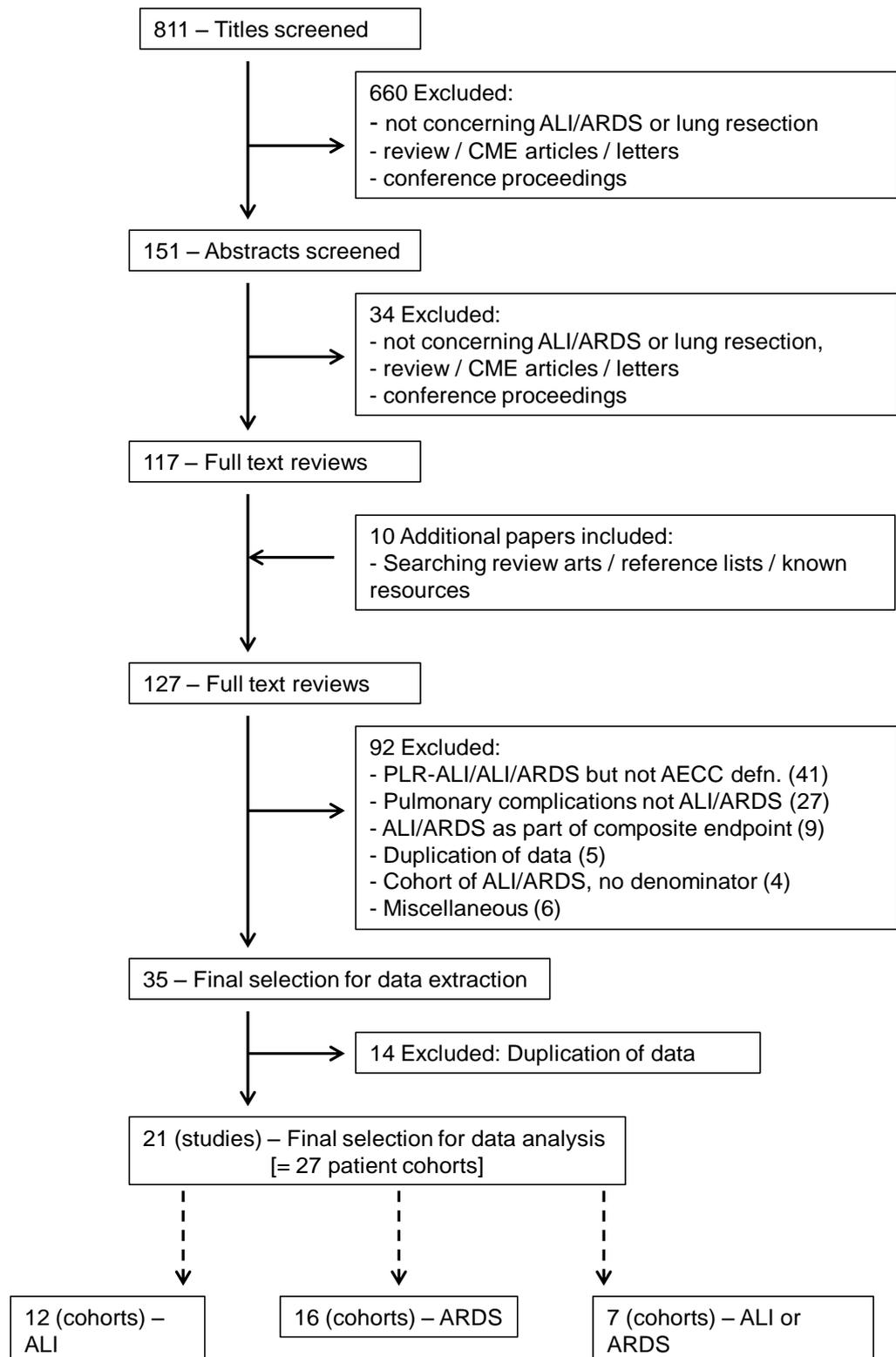


Figure 3.1. Flow diagram depicting study selection.

CME, continuing medical education; AECC defn., American European Consensus Conference definition.

Table 3.2. Summary of included studies.

First author (reference)	Year of publication	Country of origin	Study design	Centres	Cohort	Age	Sex (%male)	Median year of cohort recruitment	N	Nos. of			Endpoints extracted ^A		Follow up	
										P	L	SL	Incidence	Mortality	Incidence	Mortality
Ahn ¹⁶⁶	2012	South Korea	RCT	Single	a	56.0	NA	2009	25	0	25	0	ALI	NA	NS	NA
					b	59.0	NA	2009	25	0	25	0	ALI	NA		
Blank ²⁷⁴	2011	USA	Retro. obs.	Single	-	NA	90	2002	129	129	0	0	ALIorARDS	NA	NS	NA
Brunelli ¹³²	2009	Italy	Pro. obs.	Single	-	66.5	NA	2007	204	27	177	0	ARDS	ARDS	30day or hosp. if longer	30day or hosp. if longer
Dulu ¹¹¹	2006	USA	Retro. obs.	Single	-	NA	45.4	2003	2192	126	1047	1019	ALIorARDS	ALIorARDS	NS	Hospital
Fernandez- Perez ²⁷⁵	2006	USA	Retro. obs.	Single	-	NA	62.9	2000	170	170	0	0	ALI	ALI	60 days	60 days
Gomez- Caro ¹³³	2012	Spain	Pro. obs.	Single	a	63.7	90.5	2008	53	0	53	0	ARDS	ARDS	NS	30 days
					b	64.5	84.6	2008	26	0	26	0	ARDS	ARDS	NS	30 days
Kim ¹³⁴	2010	South Korea	Retro. obs.	Single	-	63.5	90.9	2001	164	164	0	0	ARDS	ARDS	30 days	30 days
Kutlu ¹³⁰	2000	UK	Retro. obs.	Single	-	51.7	57	1994	1139	198	612	329	ALI, ARDS, ALIorARDS	ALI, ARDS, ALIorARDS	NS	Hospital
Langenfeld ²⁷⁶	2012	USA	Retro. obs.	Single	-	NA	NA	2004	625	0	625	0	ALI, ARDS, ALIorARDS	ALI, ARDS, ALIorARDS	NS	Hospital and/or 30 day
Leo ¹⁹⁴	2006	Italy	Retro. obs.	Single	-	62	72.3	2001	202	202	0	0	ARDS	ARDS	NS	90 day
Licker ¹⁵⁵	2009	Switzerland	Retro. obs.	Multi	a	62	64.4	2000	533	114	290	129	ALI	ALI	Resp. distress in first 48h	Hospital
					b	63	63.1	2005	558	98	313	147	ALI	ALI	Resp. distress in first 48h	Hospital

First author (reference)	Year of publication	Country of origin	Study design	Centres	Cohort	Age	Sex (%male)	Median year of cohort recruitment	N	Nos. of			Endpoints extracted ^A		Follow up	
										P	L	SL	Incidence	Mortality	Incidence	Mortality
Marret ²¹⁷	2010	France	Retro. obs.	Single	-	62	86.8	2002	129	129	0	0	ALI	ALI	Hospital	Hospital
Mathisen ¹³⁵	1998	USA	Retro. obs.	Single	a	NA	NA	1989	175	175	0	0	ARDS	ARDS	NS	NS
					b	58.4	NA	1995	412	83	329	0	ARDS	ARDS	NS	Hospital
Ruffini ¹²⁹	2001	Italy	Retro. obs.	Single	-	63	85.0	1996	1142	188	861	93	ALI, ARDS, ALIORARDS	ALI, ARDS, ALIORARDS	NS	Hospital
Song ²⁷⁷	2006	Korea	Retro. obs.	Single	-	61.3	79.3	2002	635	101	505	29	ALI, ARDS, ALIORARDS	NA	30 days	NA
Steger ²⁷⁸	2012	Germany	Retro. obs.	Single	-	NA	80.8	1997	146	78	68	0	ALI, ARDS, ALIORARDS	NA	NS	NA
Stephan ²⁷⁹	2000	France	Retro. obs.	Single	-	59.0	77.0	1995	266	87	142	37	ARDS	NA	30 days	NA
Tang ¹¹⁰	2008	UK	Retro. obs.	Single	-	NA	NA	2002	1376	88	601	687	ARDS	ARDS	NS	NS
Tisdale ²⁸⁰	2009	USA	RCT	Multi	a	61	52.3	2006	65	17	48	0	ARDS	ARDS	NS	NS
					b	63	47.7	2006	65	15	50	0	ARDS	ARDS		
Veen ²⁸¹	2009	Netherlands	Retro. obs.	Multi	-	64	76	2002	91	91	0	0	ARDS	NA	Up to 30 days post discharge	NA
Yang ²¹⁸	2011	Korea	RCT	Single	a	60.0	62	2009	50	0	50	0	ALI	ALI, ARDS	NS	NS
					b	58.0	62	2009	50	0	50	0	ALI	ALI, ARDS		

^AConcerns whether incidence or mortality data was extracted for 'ALI', 'ARDS' or the combined endpoint 'ALI or ARDS'. NS, definition not specified in the paper. NA, result not available from the paper.

3.3.3 Incidence of post-lung resection lung injury

3.3.3.1 Detection of outliers

Screening for potential outliers was performed by visual inspection of forest plots, and examination of standardized residuals (Figures 3.2 and 3.3).

Outlier analysis - ALI incidence

Two potential outliers (PO) were identified (Figure 3.2). PO1 was identified as a potential outlier from the appearance of the forest plot (Figure 3.2a). The standardized residual for this cohort was less than 2.0 (1.47, Figure 3.2b). Further scrutiny of this cohort revealed no reason to suggest that the study population was not generalizable; as such this study was retained in the analysis. PO2 was identified as having a standardized residual greater than -2.0 (-2.1, Figure 3.2b). Inspection of the forest plot suggests this result to be in keeping with several others (for example Kutlu et al and Licker et al (b), Figure 3.2a). Further scrutiny of this cohort revealed no reason to suggest the study population was not generalizable; as such this cohort was retained in the analysis.

Outlier analysis - ARDS incidence

One potential outlier was identified (Figure 3.3). This cohort from Kim et al, 2010¹³⁴, was identified as a potential outlier both from the appearances of the forest plot where it appeared to stand alone (Figure 3.3a), and as having a standardized residual greater than 2.0 (2.37, Figure 3.3b). Further scrutiny of this study revealed the following statement:

“...the patients included in the current study showed higher risk features than those who underwent simple pneumonectomy without lung perfusion scanning, as evidenced by older age, more frequent smoking and poorer pulmonary function test results”.

Kim et al (2010)¹³⁴

As it appears that this study comprised a ‘higher risk’ patient cohort, this study was removed from the analysis. Sensitivity analysis (including this outlying study) was performed throughout.

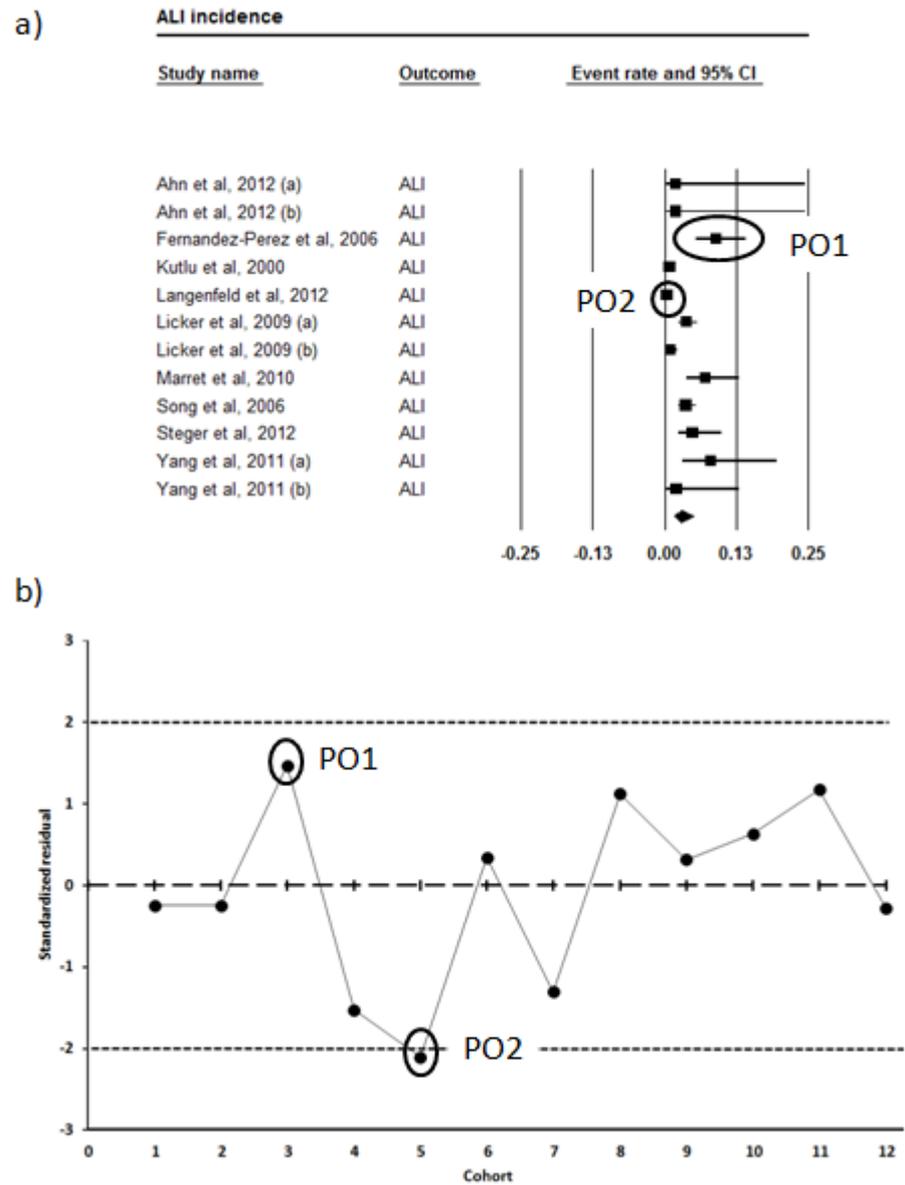


Figure 3.2. Outlier analysis - incidence of ALI following lung resection.

a) forest plot, b) standardized residual plot. Potential outliers (PO) are identified and labelled; labels reflect corresponding cohorts in figures a) and b).

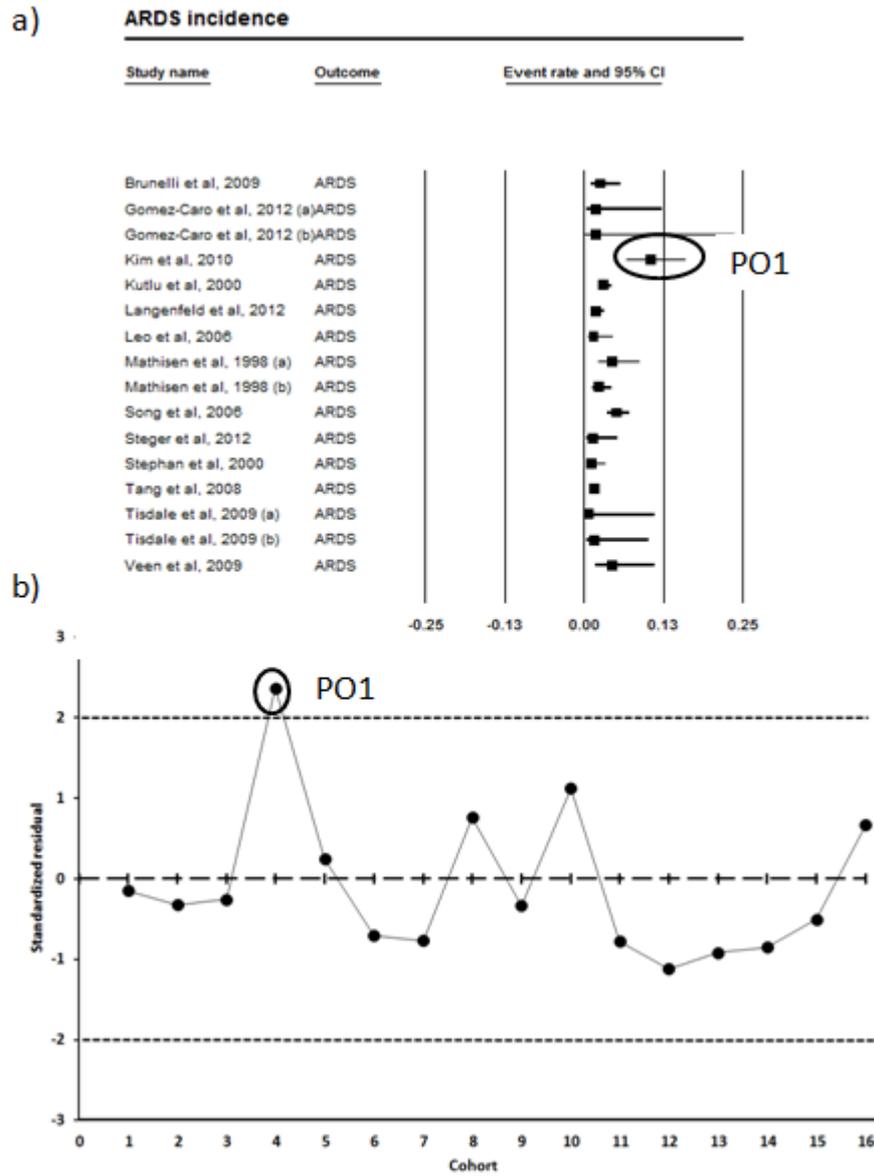


Figure 3.3. Outlier analysis for the incidence of ARDS following lung resection.

a) forest plot, b) standardized residual plot. Potential outliers (PO) are identified and labelled; labels reflect corresponding cohorts in figures a) and b).

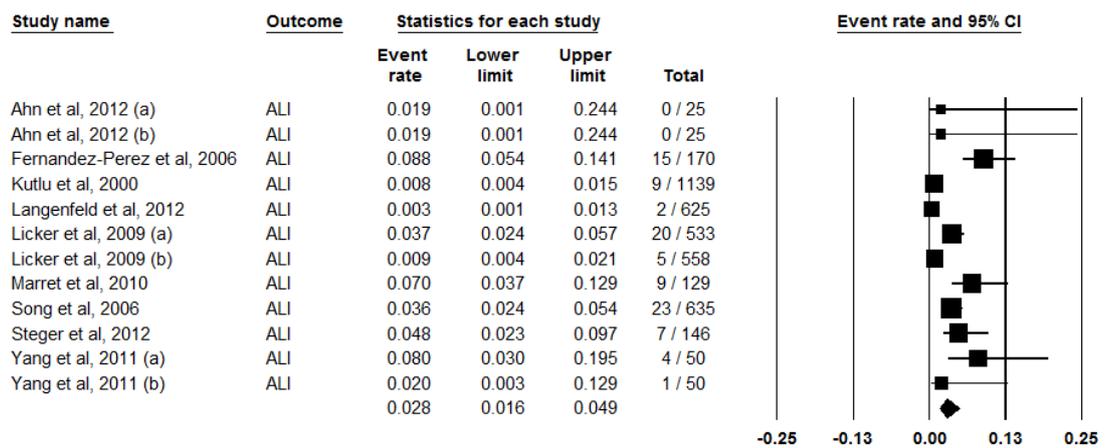
Outlier analysis - ALI/ARDS incidence

No potential outliers were identified (not shown).

3.3.3.2 Meta-analysis

The pooled incidence estimates for ALI, ARDS and ALI/ARDS were 2.8% (1.6-4.9), 2.5% (1.8-3.3) and 3.0% (2.1-4.3) respectively. There was evidence of 'substantial' to 'considerable' heterogeneity in all groups ($I^2=82.7\%$, 54.3% and 78.3% for ALI, ARDS, ALI/ARDS respectively ($p<0.01$ for all, Figures 3.4-6).

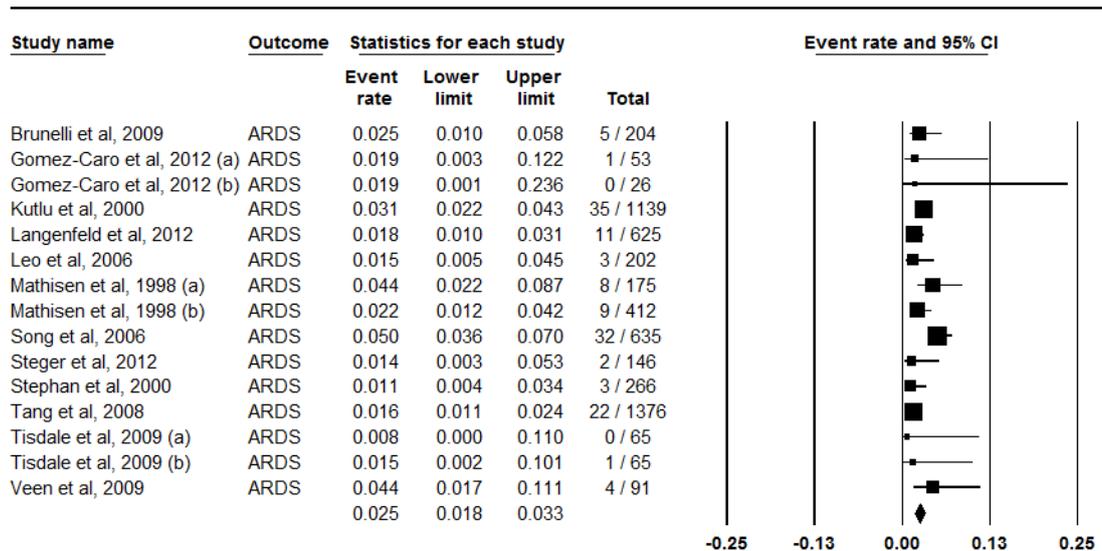
ALI incidence



Tests for heterogeneity: $Q=63.6$, $df=11$, $p<0.01$, $I^2=82.7\%$

Figure 3.4. Random effects meta-analysis of ALI incidence following lung resection.

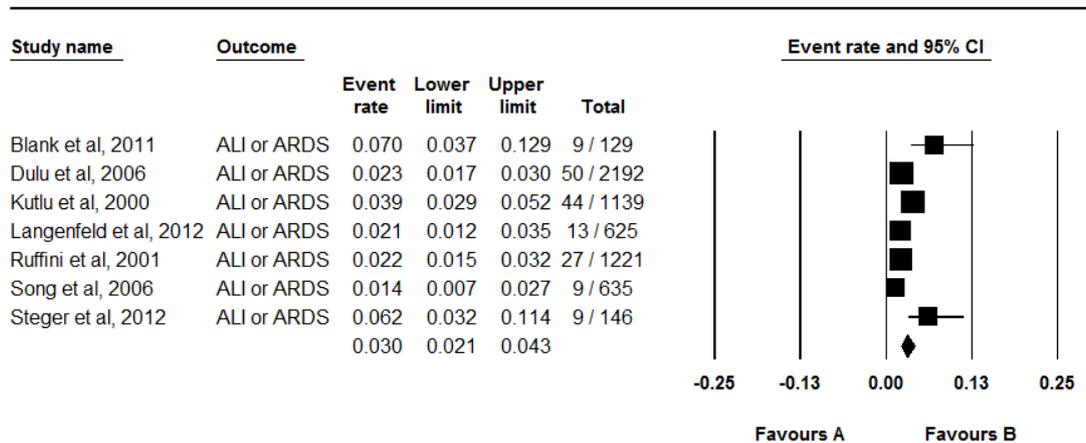
ARDS incidence, outlier excluded



Tests for heterogeneity: $Q=30.6$, $df=14$, $p<0.01$, $I^2=54.3\%$

Figure 3.5. Random effects meta-analysis of ARDS incidence following lung resection (outlier excluded).

ALI or ARDS incidence

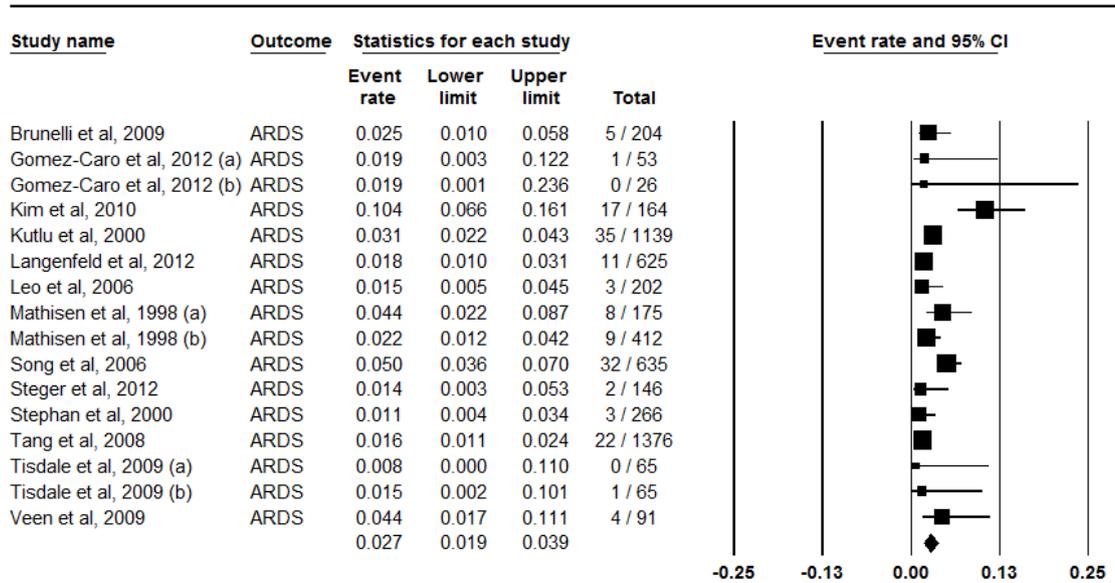


Tests for heterogeneity: $Q=27.6$, $df=6$, $p<0.01$, $I^2=78.3\%$

Figure 3.6. Random effects meta-analysis of ALI/ARDS incidence following lung resection.

Sensitivity analysis: Without exclusion of the outlier, the pooled incidence estimate for ARDS was similar at 2.7% (1.9-3.9), though heterogeneity increased ($I^2=73.9\%$, $p<0.01$, Figure 3.7).

ARDS incidence



Tests for heterogeneity: $Q=57.5$, $df=15$, $p<0.01$, $I^2=73.9\%$

Figure 3.7. Sensitivity analysis: Random effects meta-analysis of ARDS incidence following lung resection (outlier included).

Subgroup analysis

Incidence data specific to subgroups of patients undergoing lobectomy and pneumonectomy was available for 6 lobectomy cohorts and three pneumonectomy cohorts for analysis of ALI incidence, four lobectomy and 7 pneumonectomy cohorts for analysis of ARDS incidence and four lobectomy and four pneumonectomy cohorts for analysis of ALI/ARDS incidence.

The incidence of ARDS and ALI/ARDS but not ALI was significantly higher in patients undergoing pneumonectomy than lobectomy $p= <0.01$, <0.01 and 0.16 respectively (Figures 3.8-10).

ALI incidence by subgroup

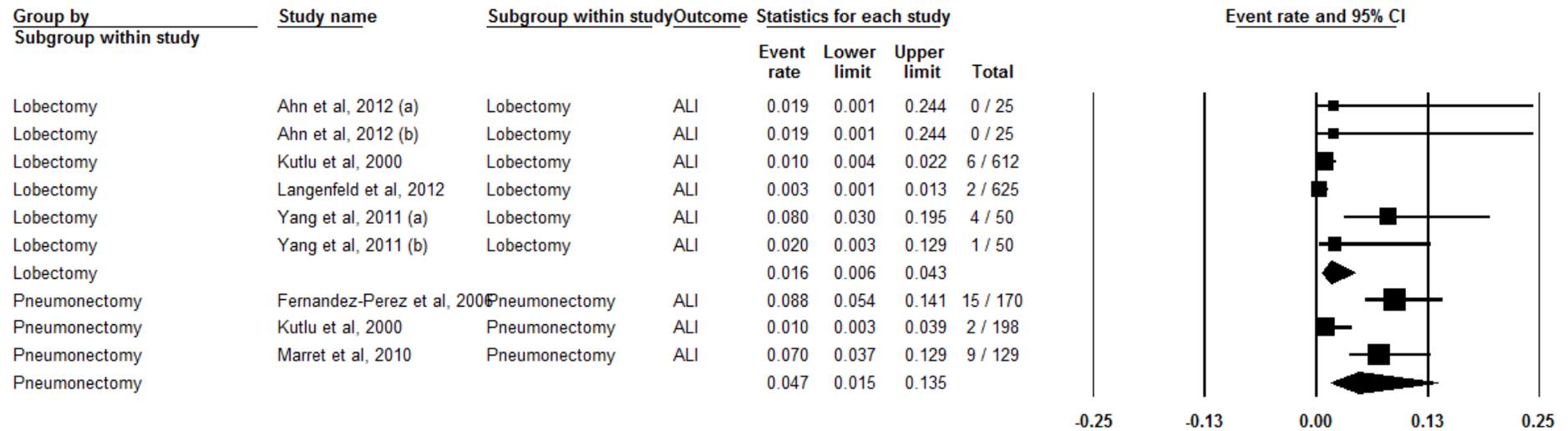


Figure 3.8. Subgroup analysis: Random effects meta-analysis of ALI incidence by type of resection.

ARDS incidence by subgroup, outlier excluded

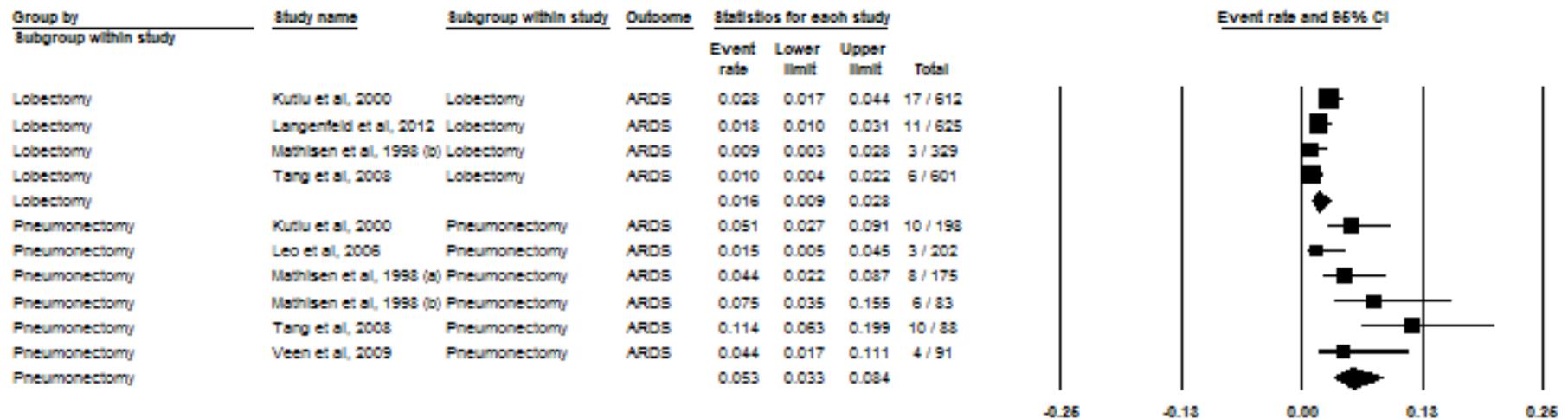


Figure 3.9. Subgroup analysis: Random effects meta-analysis of ARDS incidence by type of resection (outlier excluded).

ALI or ARDS incidence by subgroup

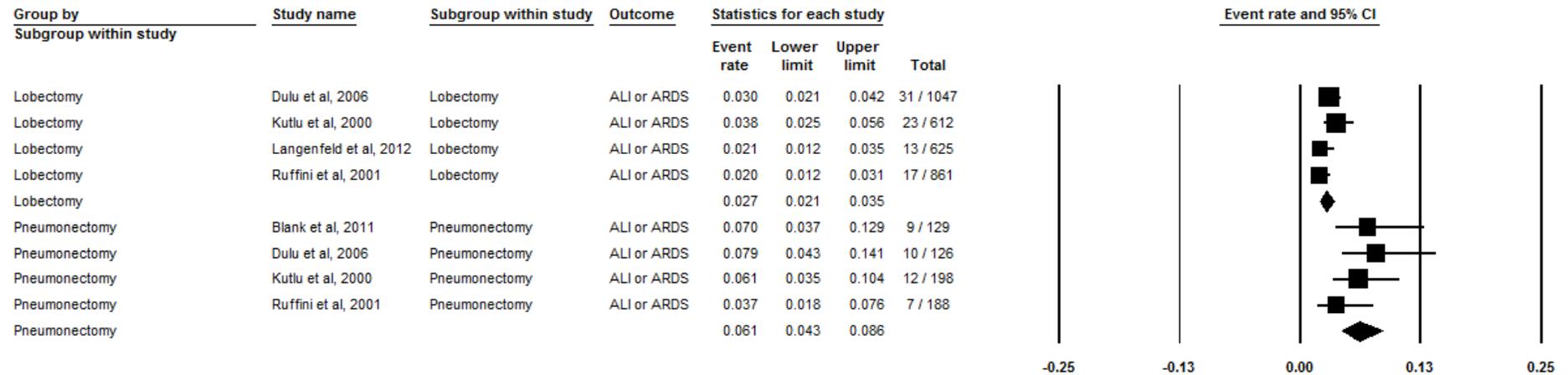


Figure 3.10. Subgroup analysis: Random effects meta-analysis of ALI/ARDS incidence by type of resection.

The proportion of (between-cohorts) variance explained (R^2) by subgroup membership was 34%, 63% and 86% respectively for ALI, ARDS and ALI/ARDS (Figure 3.11).

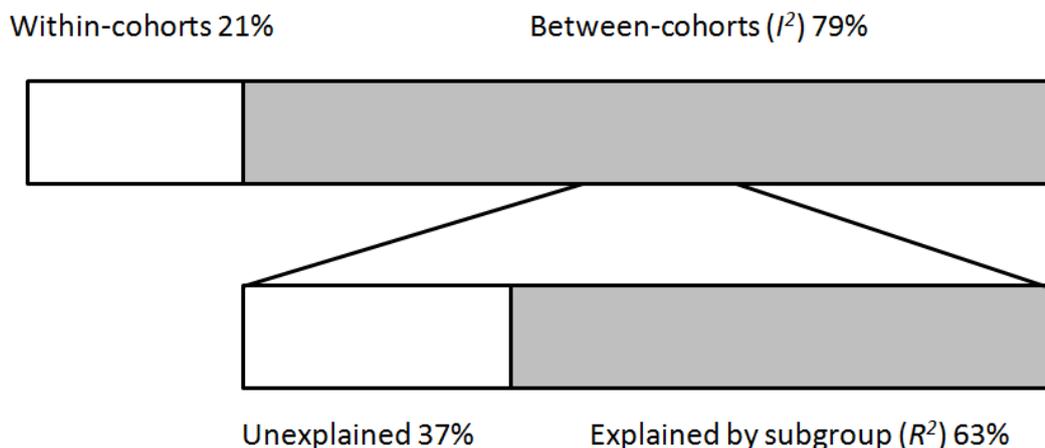


Figure 3.11. Proportion of variance in the incidence of ARDS following lung resection explained by subgroup membership.

Total variance is the sum of within-cohorts and between-cohorts variance. $I^2=79\%$ - i.e. 79% (represented by the shaded area of upper box) of the total variance (total area of upper box) results from between-cohorts variation. Of this between-cohorts variance (total area of lower box), 63% (R^2 , the shaded area of the lower box) is explained by differences between subgroups, whilst 37% of the between-cohorts variance remains unexplained.

Sensitivity analysis: When including the outlier, there remained a significant difference in the incidence of ARDS between patients undergoing lobectomy versus pneumonectomy $p<0.001$, $R^2=68\%$ (Figure 3.12).

ARDS incidence by subgroup

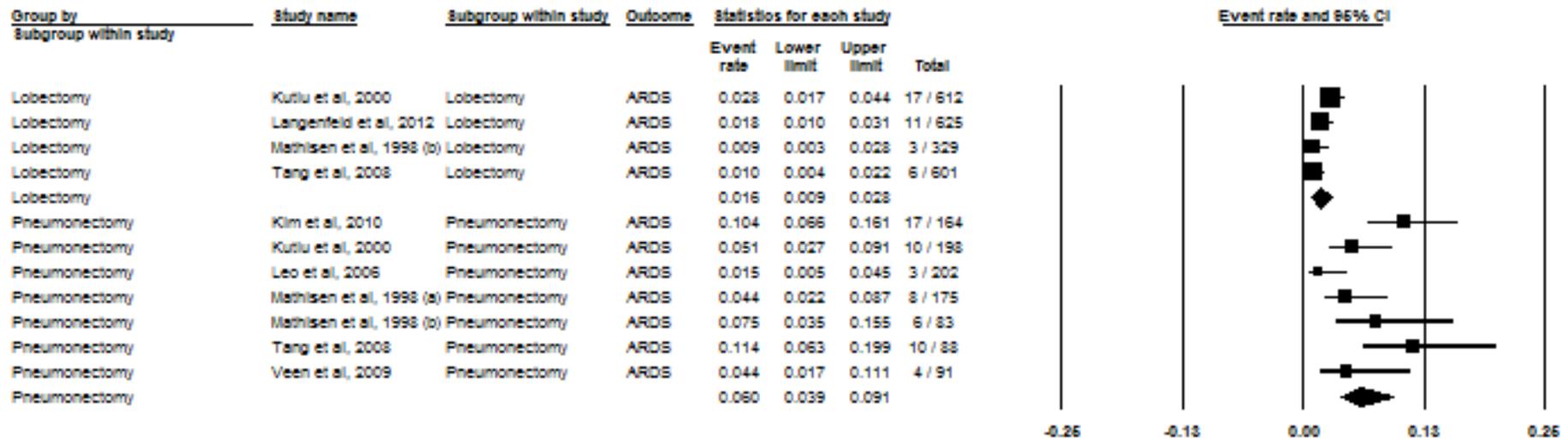


Figure 3.12. Sensitivity analysis: subgroup analysis, random effects meta-analysis of ARDS incidence by type of resection (outlier included)

3.3.3.3 Detection of publication bias

Funnel plots of standard error by Logit event rate are shown in Figures 3.13-16.

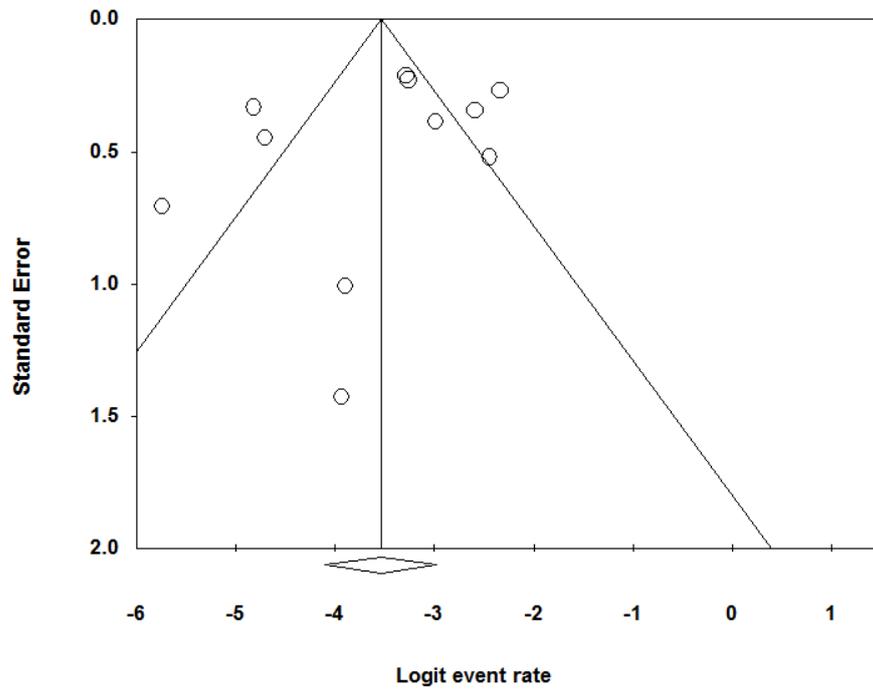


Figure 3.13. Funnel plot of standard error versus Logit event rate for studies reporting incidence of ALL.

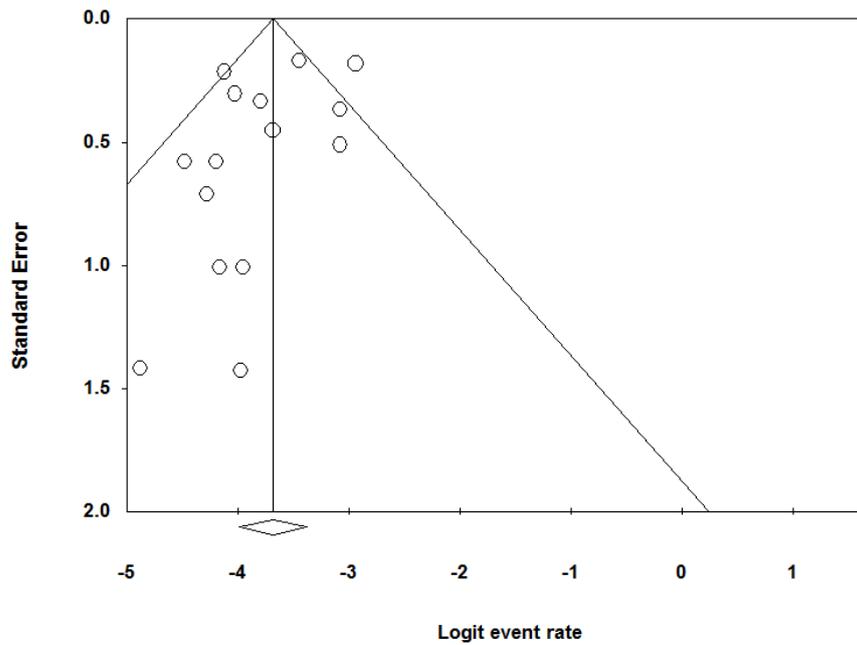


Figure 3.14. Funnel plot of standard error by Logit event rate for studies reporting incidence of ARDS (outlier excluded).

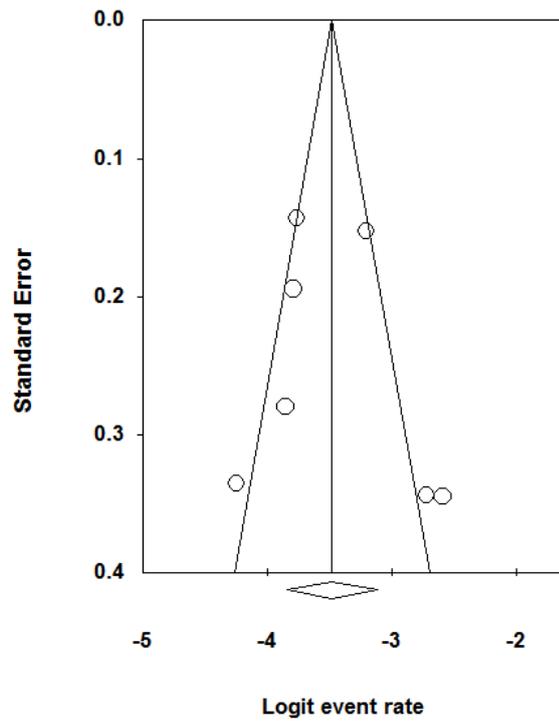


Figure 3.15. Funnel plot of standard error by Logit event rate for studies reporting incidence of ALI or ARDS.

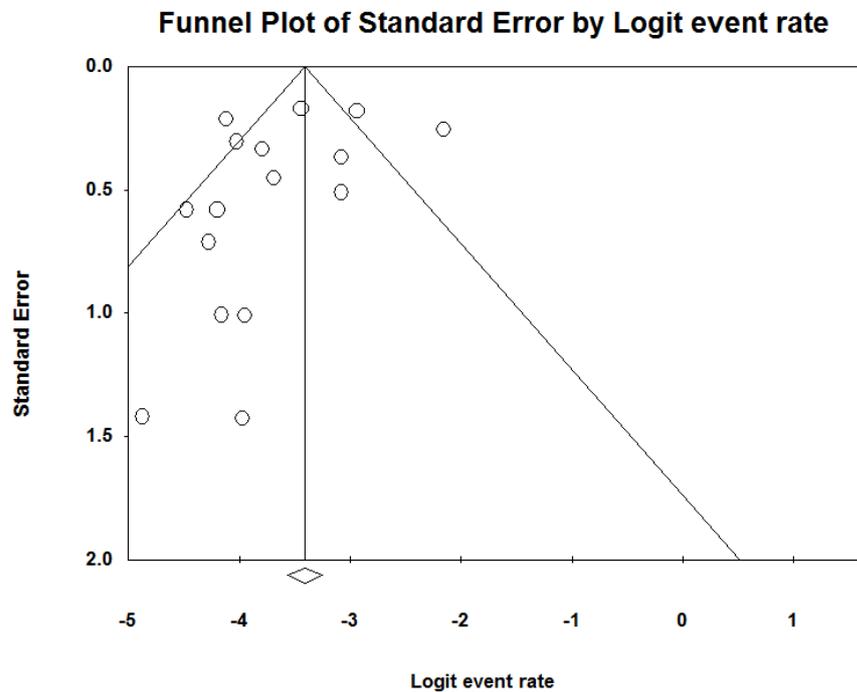


Figure 3.16. Funnel plot of studies reporting incidence of ARDS (including outlier).

Visual inspection of the plots reveals obvious asymmetry of the plots for ARDS and (less so) for ALI incidence, with studies apparently 'missing' to the lower

right hand side of the plots. This suggests publication bias, manifest in the under-reporting of small studies reporting higher incidences (more negative Logit event rates) of ALI and ARDS.

The results of Duval and Tweedie's trim and fill procedure to assess the impact of publication bias are shown in Table 3.3. Adjustment for the effects of publication bias results in increases in the pooled incidence estimates for ALI and ARDS, which are greater for ALI than ARDS. A funnel plot demonstrating the trim and fill adjustment for the incidence of ARDS is shown in Figure 3.17 for illustration.

Table 3.3. Results of Duval and Tweedie's trim and fill procedure on pooled estimates of ALI, ARDS and ALI/ARDS incidence.

Outcome		Studies trimmed	Point estimate	Confidence interval		Q-statistic
				LL	UL	
ALI	<i>Obs.</i>		2.8	1.6	4.8	63.7
	<i>Adj.</i>	4	4.9	2.6	9.0	141.8
ARDS (no outlier)	<i>Obs.</i>		2.4	1.8	3.3	30.6
	<i>Adj.</i>	5	2.8	2.1	3.6	36.4
ALI//ARDS	<i>Obs.</i>		2.9	2.1	4.2	27.6
	<i>Adj.</i>	0	2.9	2.1	4.2	27.6
ARDS (outlier included)	<i>Obs.</i>		2.7	1.9	4.0	57.5
	<i>Adj.</i>	5	3.2	2.3	4.5	65.8

Obs., observed values; *Adj.*, adjusted values following trim and fill. Incidence presented as percent.

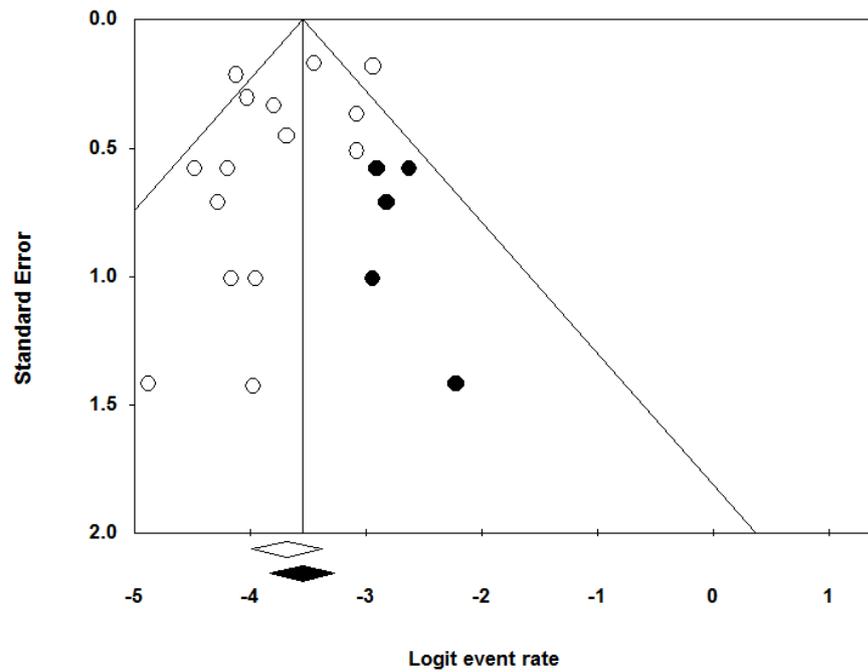


Figure 3.17. Funnel plot of standard error by Logit event rate for studies reporting incidence of ARDS (outlier excluded), demonstrating ‘trim and fill’ procedure.

Open circles are observed studies, open diamond is observed point estimate. Imputed studies are shown as filled circles, and the imputed point estimate as the filled diamond.

3.3.3.4 Meta-regression

Incidence of ALI, ARDS and ALI/ARDS by year

There was no significant relationship between median year of study recruitment and the incidence of ALI, ARD or ALI/ARDS. The odds ratio for ALI, ARDS and ALI/ARDS incidence per year was 1.01 (95% CI 0.89-1.14), 0.98 (0.92-1.04) and 0.95 (0.86-1.05) respectively (Figures 3.18-21 and Table 3.4).

Sensitivity analysis: When including the outlier, there remained no significant relationship between median year of study recruitment and the incidence of ARDS (OR = 0.98 (0.91-1.06)).

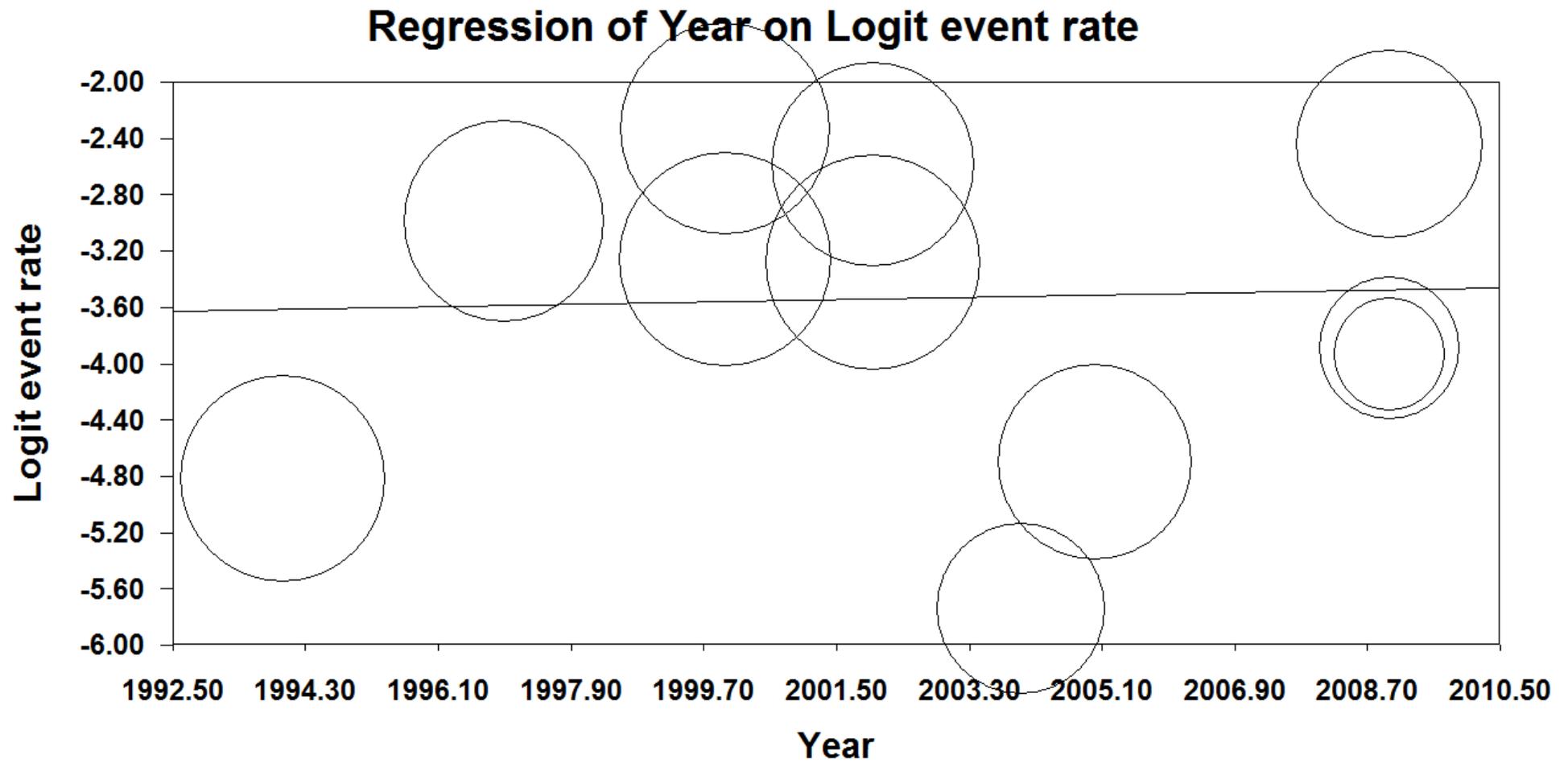


Figure 3.18. Incidence of ALI by median year of cohort recruitment.
Event rate = incidence. Size of 'bubbles' are proportional to cohort weighting.

Regression of Year on Logit event rate

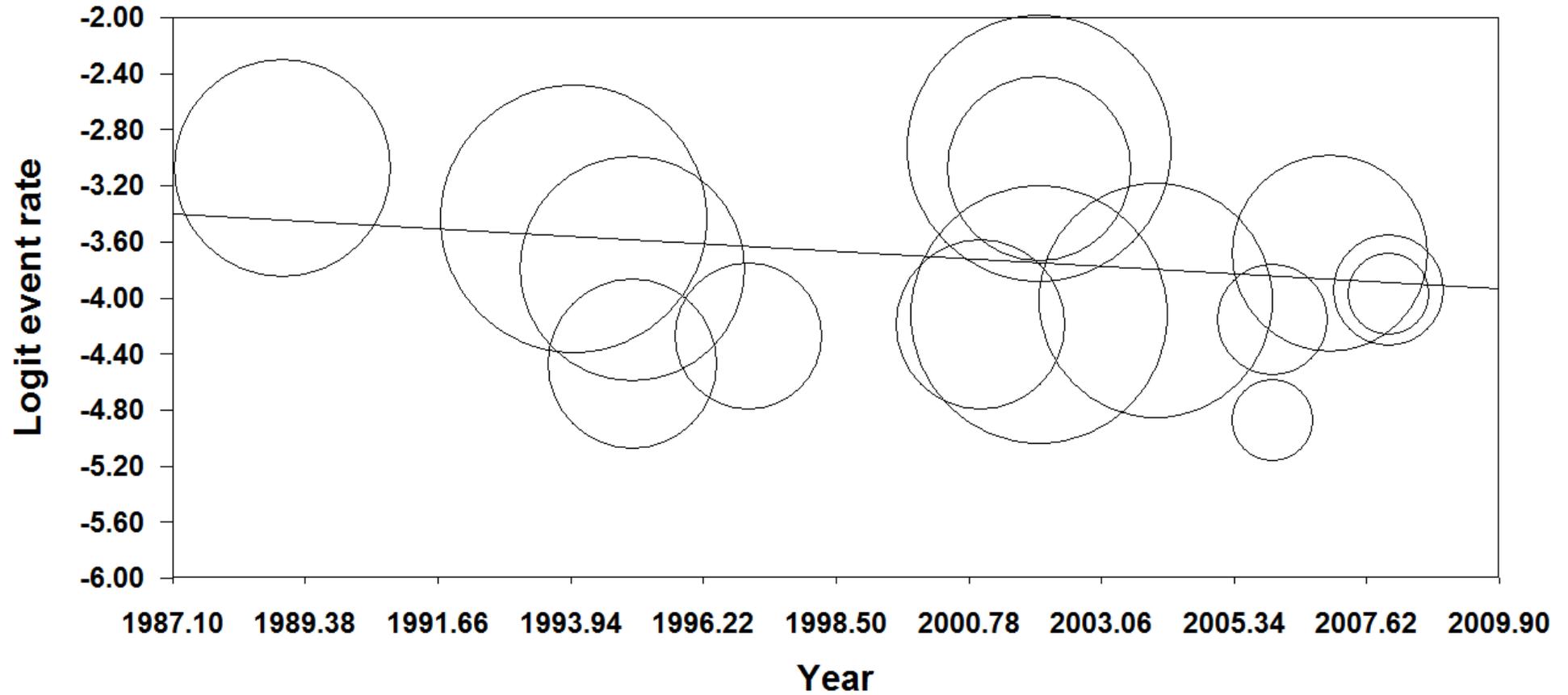


Figure 3.19. Incidence of ARDS (outlier removed) by median year of cohort recruitment. Event rate = incidence. Size of 'bubbles' are proportional to cohort weighting.

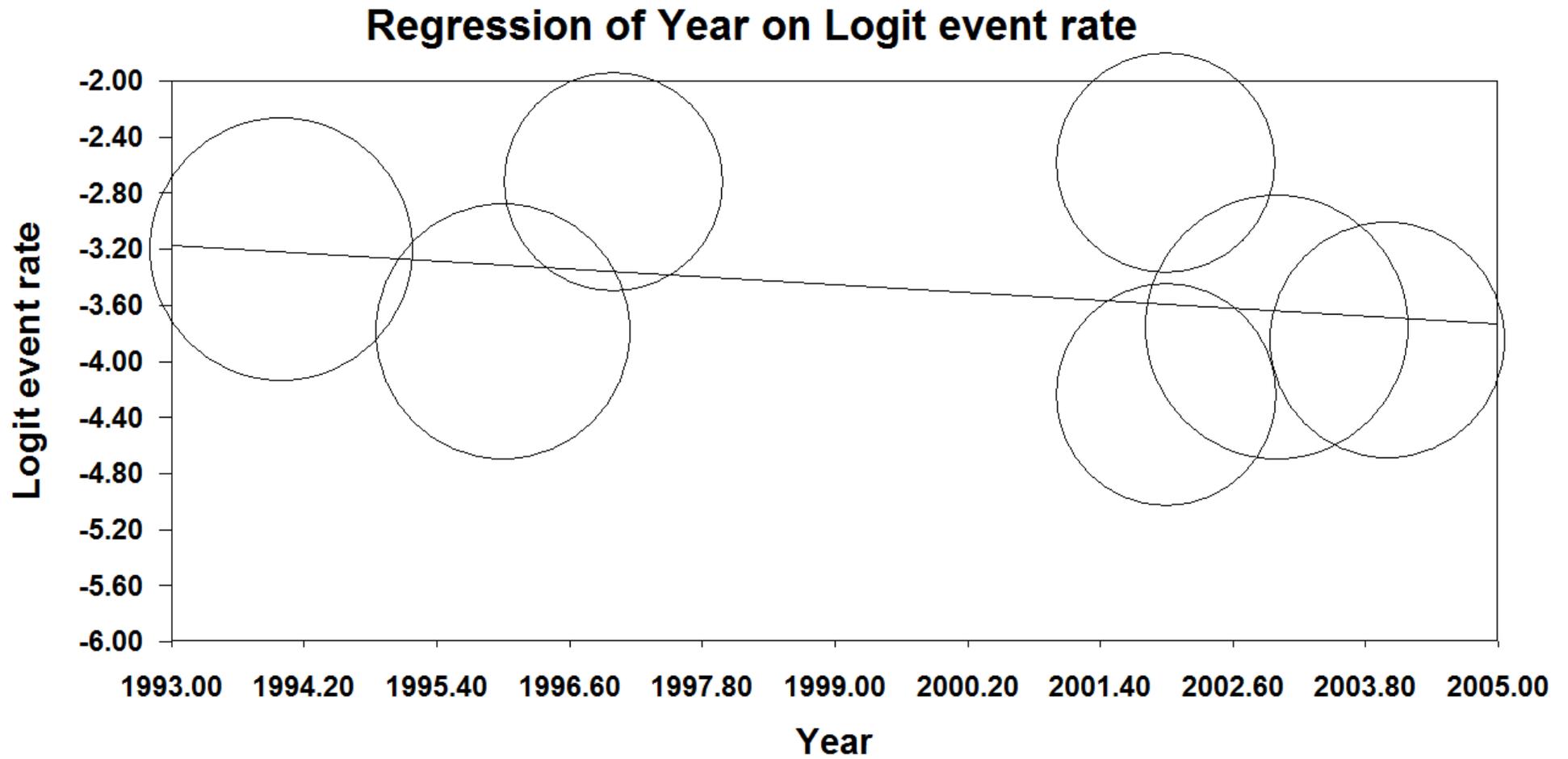


Figure 3.20. Incidence of ALI/ARDS by median year of cohort recruitment.
Event rate = incidence. Size of 'bubbles' are proportional to cohort weighting.

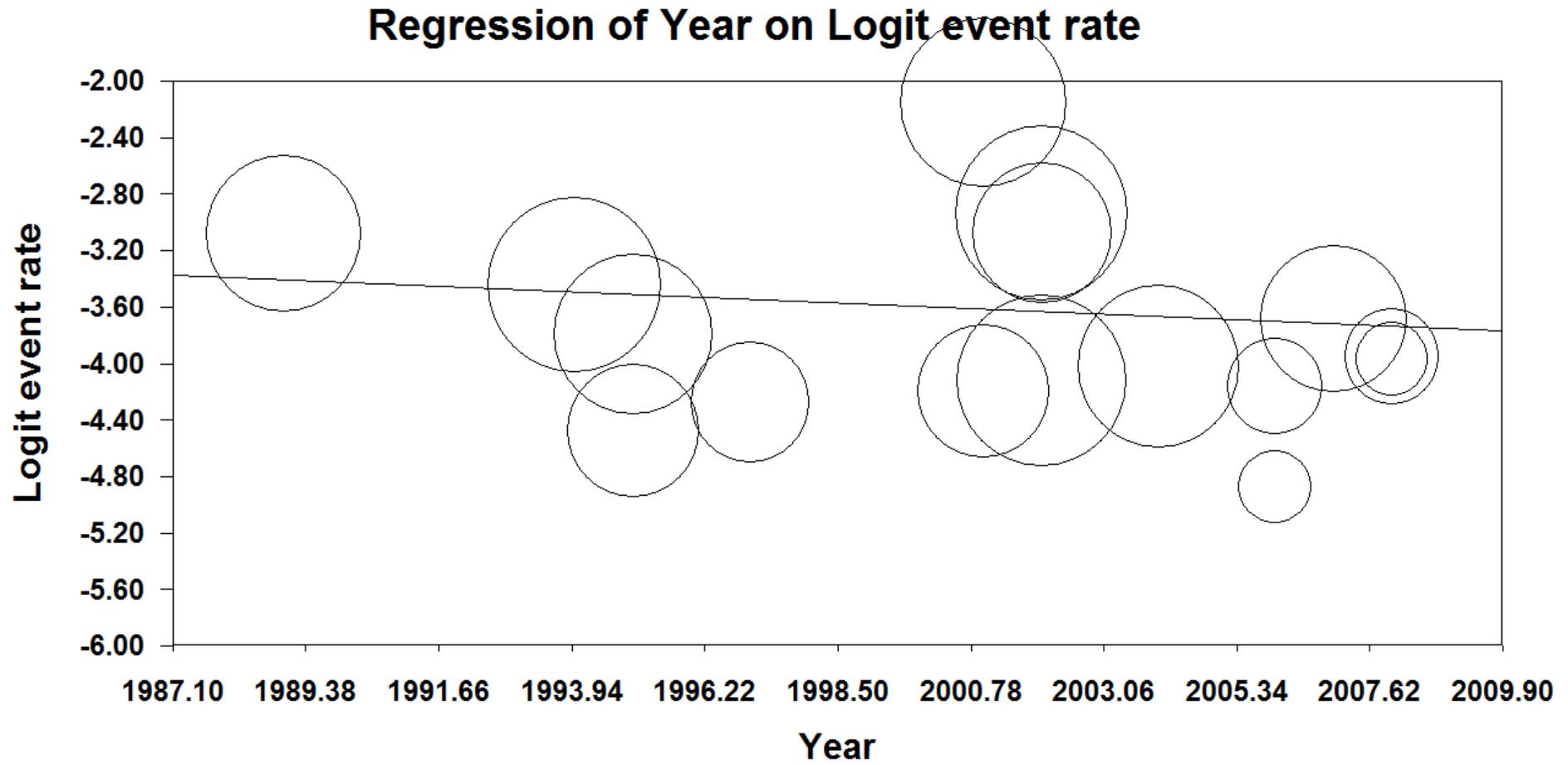


Figure 3.21. Sensitivity analysis: Incidence of ARDS (outlier not removed) by median year of cohort recruitment.
Event rate = incidence. Size of 'bubbles' are proportional to cohort weighting.

Table 3.4. Meta-regression analyses of ALI, ARDS and ALI/ARDS incidence by covariate

Covariate	Outcome	Heterogeneity					Meta-regression						
		n	Q	df	p	I ² (%)	Slope	SE	OR	95% CI		R ² (%)	p
										LL	UL		
Year	ALI	12	63.7	11	<0.01	82.7	0.01	0.03	1.01	0.89	1.15	0	0.88
	ARDS (outlier excluded)	15	30.6	14	<0.01	54.3	-0.02	0.03	0.98	0.92	1.04	0	0.45
	ALI/ARDS	7	27.6	6	<0.01	78.3	-0.05	0.05	0.95	0.86	1.05	0	0.36
	ARDS (including outlier)	16	57.5	15	<0.01	73.9	-0.02	0.04	0.98	0.91	1.06	0	0.66
Age	ALI	9	36.5	8	<0.01	78.1	0.12	0.07	1.13	0.99	1.29	40	0.07
	ARDS (outlier excluded)	11	16.1	10	0.10	38.0	0.00	0.04	1.00	0.92	1.08	0	0.92
	ALI/ARDS	3	10.8	2	<0.01	81.5	-0.07	0.04	0.94	0.86	1.01	56	0.10
	ARDS (including outlier)	12	36.1	11	<0.01	69.5	0.02	0.05	1.02	0.92	1.14	0	0.66
Sex	ALI	9	51.2	8	<0.01	84.4	0.04	0.03	1.04	0.98	1.10	0	0.19
	ARDS (outlier excluded)	10	15.7	9	<0.01	42.5	0.01	0.02	1.01	0.96	1.05	0	0.80
	ALI/ARDS	6	26.2	5	<0.01	80.9	0.01	0.01	1.01	0.98	1.04	0	0.54
	ARDS (including outlier)	11	33.9	10	<0.01	70.5	0.03	0.02	1.03	0.99	1.07	26	0.12
Side	ALI	5	3.7	4	0.45	0.0	-5.32	3.30	0.00	0.00	3.12	0	0.11
	ARDS (outlier excluded)	5	2.9	4	0.57	0.0	-0.04	0.04	0.96	0.89	1.03	0	0.23
	ALI/ARDS	2	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	6	18.7	5	<0.01	73.3	-0.09	0.03	0.92	0.87	0.96	96	<0.01
FEV₁	ALI	6	12.6	5	0.03	60.4	0.02	0.04	1.03	0.95	1.10	0	0.49
	ARDS (outlier excluded)	5	2.3	4	0.69	0.0	0.02	0.11	1.02	0.82	1.27	0	0.85
	ALI/ARDS	0	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	6	18.2	5	<0.01	72.5	-0.06	0.15	0.94	0.70	1.27	12	0.69
DLCO	ALI	2	-	-	-	-	-	-	-	-	-	-	-
	ARDS (outlier excluded)	4	0.5	3	0.92	0.0	0.05	0.07	1.05	0.91	1.21	0	0.50
	ALI/ARDS	0	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	5	17.9	4	<0.01	77.7	0.11	0.03	1.12	1.06	1.18	100	<0.01

Pre-op chemo- therapy	ALI	6	24.3	5	<0.01	79.4	0.01	0.01	1.01	0.99	1.02	0	0.33
	ARDS (outlier excluded)	8	49.5	7	<0.01	49.5	-0.01	0.01	0.99	0.97	1.00	65	0.10
	ALI/ARDS	2	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	9	28.6	8	<0.01	72.0	-0.02	0.01	0.98	0.97	1.00	44	0.06
Pre-op radio- therapy	ALI	2	-	-	-	-	-	-	-	-	-	-	-
	ARDS (outlier excluded)	2	-	-	-	-	-	-	-	-	-	-	-
	ALI/ARDS	1	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	3	16.0	2	0.00	87.5	-0.01	0.02	0.99	0.95	1.03	0	0.57
Duration of OLV	ALI	6	12.6	5	0.03	60.5	0.02	0.02	1.02	0.98	1.05	0	0.41
	ARDS (outlier excluded)	0	-	-	-	-	-	-	-	-	-	-	-
	ALI/ARDS	0	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	0	-	-	-	-	-	-	-	-	-	-	-
Duration of operation	ALI	6	12.6	5	0.03	60.5	0.01	0.01	1.01	0.99	1.04	0	0.32
	ARDS (outlier excluded)	4	0.3	3	0.96	0.0	0.00	0.01	1.00	0.99	1.01	0	0.92
	ALI/ARDS	1	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	N/A	-	-	-	-	-	-	-	-	-	-	-
Intra-op fluids	ALI	4	2.8	3	0.43	0.0	0.00	0.00	1.00	1.00	1.00	0	0.29
	ARDS (outlier excluded)	0	-	-	-	-	-	-	-	-	-	-	-
	ALI/ARDS	1	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	0	-	-	-	-	-	-	-	-	-	-	-
Open vs thoraco- scopic resection	ALI	8	35.4	7	0	80.2	0.00	0.01	1.00	0.97	1.02	0	0.69
	ARDS (outlier excluded)	5	2.3	4	0.685	0	0.28	0.29	1.33	1	2.33	0	0.32
	ALI/ARDS	2	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	6	22.0	5	0.001	77.2	0.34	0.32	1.41	1	2.66	1.10251	0.29

n, number of cohorts in analysis; df, degrees of freedom; SE, standard error; odds ratio per unit of covariate; LL, lower limit; UL, upper limit.

3.3.3.5 Incidence of ALI, ARDS and ALI/ARDS by other covariates

Age

There was a trend towards a significant positive relationship between the incidence of ALI and mean patient age (OR 1.13 (0.99-1.29) per year increase in mean patient age, $R^2=40\%$). There was a trend towards a significant negative relationship between the incidence of ALI/ARDS and mean patient age (OR 0.94 (0.86-1.01) per year increase in mean patient age, $R^2=56\%$), though this finding must be interpreted with caution as data was only available from three cohorts for this comparison (Table 3.4). There was no significant relationship between the incidence of ARDS and mean patient age, with both exclusion and inclusion of the outlier (Table 3.4).

Sex

There was no significant relationship between the incidence of ALI, ARDS nor ALI/ARDS and the percentage of patients of male sex in each cohort (Table 3.4).

Side of resection

There was no significant relationship between the incidence of ALI, ARDS (outlier removed) nor ALI/ARDS and the percentage of patients undergoing right sided resection in each cohort (Table 3.4). Sensitivity analysis where the outlying study was retained, demonstrated a strong negative relationship between the incidence of ARDS and the percentage of patients undergoing right sided resection in each cohort (OR 0.92 (0.87-0.96) per percentage increase in patients undergoing right sided lung resection, $R^2=96\%$).

FEV₁

There was no significant relationship between the incidence of ALI and ARDS (with both exclusion and inclusion of the outlier) and mean FEV₁ (Table 3.4). There was insufficient data available (two cohorts or less) to explore any relationship between mean FEV₁ and the incidence of ALI or ARDS.

DLCO

There was insufficient data available (two cohorts or less) to explore any relationship between mean DLCO and the incidence of ALI or ALI/ARDS. There was no significant relationship between DLCO and the incidence of ARDS (Table 3.4). Sensitivity analysis where the outlying study was retained, demonstrated a

strong positive relationship between DLCO and the incidence of ARDS (OR 1.12 (1.06-1.18) per percentage increase in mean (percent predicted) DLCO, $R^2=100\%$).

Pre-operative chemotherapy

There was no significant relationship between the percentage of patients undergoing induction chemotherapy in each cohort and the incidence of ALI. There was a trend towards a negative association between receipt of induction chemotherapy and the incidence of ARDS (outlier removed), (OR=0.99 (0.97-1.00) per percentage increase in number of patient undergoing induction chemotherapy in each cohort, $R^2=65\%$). Sensitivity analysis where the outlying study was retained, strengthened the observed relationship (OR=0.98 (0.97-1.00), $R^2=44\%$). There was insufficient data available (two cohorts or less) to explore any relationship between receipt of induction chemotherapy and the incidence of ALI/ARDS (Table 3.4).

Induction radiotherapy

There was insufficient data available (two cohorts or less) to explore any relationship between receipt of induction radiotherapy and the incidence of ALI, ARDS (with outlier removed) and ALI/ARDS. There was no significant relationship between the percentage of patients undergoing induction radiotherapy in each cohort and the incidence of ARDS (Table 3.4).

Duration of one-lung ventilation

There was no significant relationship between the mean duration of one-lung ventilation in each cohort and the incidence of ALI. There was insufficient data available (two cohorts or less) to explore any relationship between the mean duration of one-lung ventilation in each cohort and the incidence of ARDS (with or without outlier removed) and ALI/ARDS (Table 3.4).

Duration of surgery

There was no significant relationship between the mean duration of surgery in each cohort and the incidence of ALI nor ARDS (without outlier). There was insufficient data available (two cohorts or less) to explore any relationship between the mean duration of one-lung ventilation in each cohort and the

incidence of ALI/ARDS. Duration of surgery was not recorded in the outlier study (Table 3.4).

Intra-operative fluid administration

There was no significant relationship between the mean volume of intra-operative fluid infused in each cohort and the incidence of ALI. There was insufficient data available (two cohorts or less) to explore any relationship between the mean volume of intra-operative fluid infused in each cohort and the incidence of ARDS (with or without outlier) or ALI/ARDS (Table 3.4).

Analgesic technique

Of the 12 cohorts in which post-operative analgesic technique was reported, 10 studies reported the use of thoracic epidural blockade in excess of 80% of cases, making any meaningful assessment of the relationship between analgesic technique and ALI, ARDS or ALI/ARDS incidence impossible.

Open verses video assisted thoracoscopic resection

There was no significant relationship between the percentage of patients undergoing open resection in each cohort and the incidence of ALI nor ARDS (with or without outlier removed). There was insufficient data available (two cohorts or less) to explore any relationship between the percentage of patients undergoing open resection in each cohort and the incidence of ALI/ARDS (Table 3.4).

3.3.4 Mortality from post-lung resection lung injury

3.3.4.1 Detection of outliers

Screening for potential outliers was performed by visual inspection of forest plots, and examination of standardized residuals (Figure 3.22).

Outlier analysis - ALI mortality

No potential outliers were identified (not shown).

Outlier analysis - ARDS mortality

As with the analysis of ARDS incidence, the study by Kim et al (2010)¹³⁴ was identified as a potential outlier both from the appearances of the forest plot

where it appeared to stand alone (Figure 3.22a) and as having a standardized residual greater than 2.0 (2.11, Figure 3.22b). As this study appears to have been conducted in a ‘higher risk’ patient cohort, this study was removed from the analysis. Sensitivity analysis (including this outlying study) was performed throughout.

ALI/ARDS mortality

No potential outliers were identified (not shown).

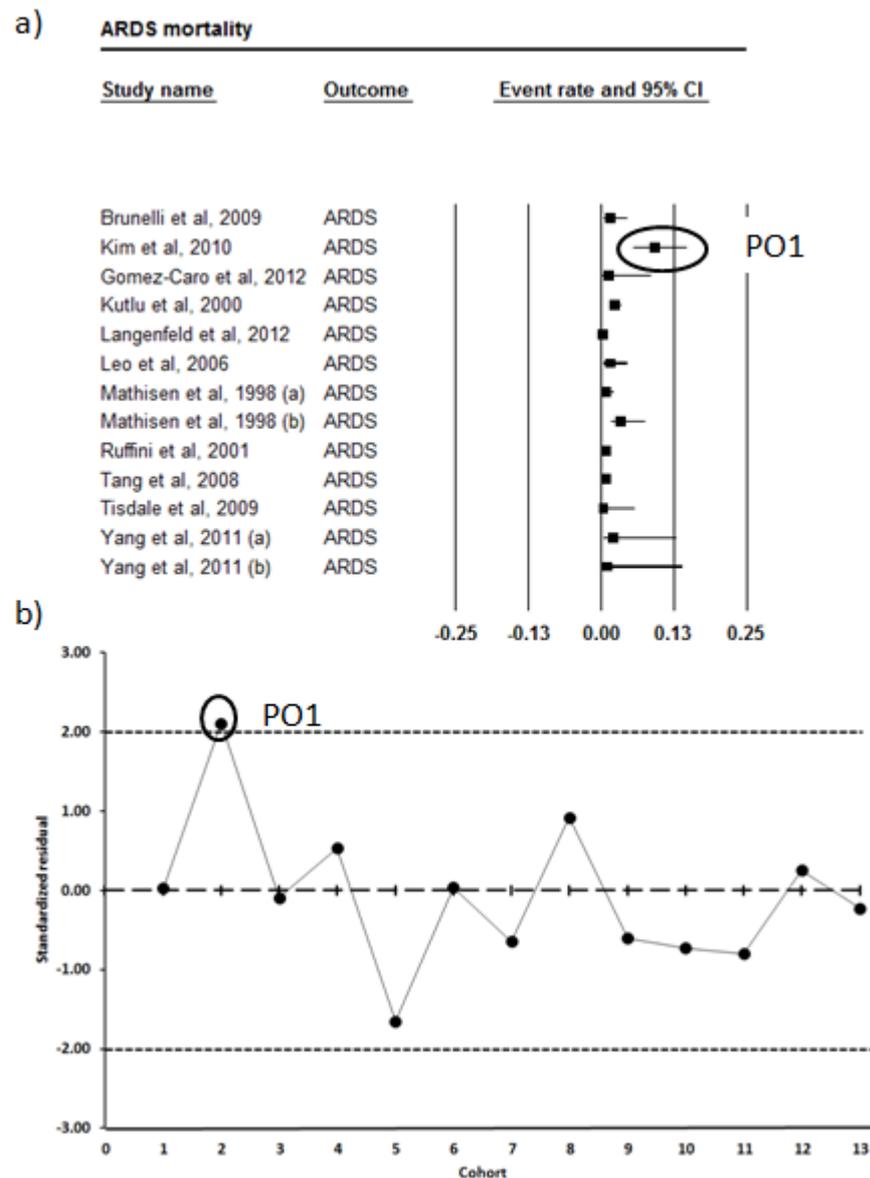


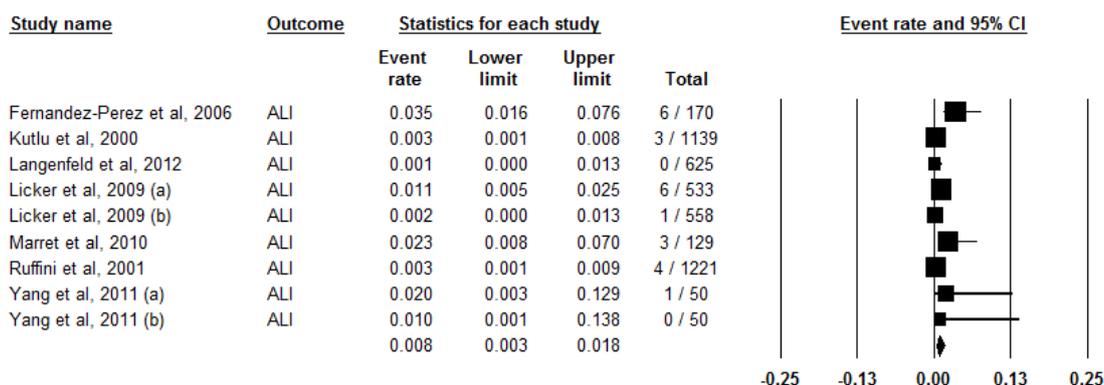
Figure 3.22. Outlier analysis for mortality from ARDS following lung resection.

a) forest plot, b) standardized residual plot. Potential outliers (PO) are identified and labelled, labels reflect corresponding cohorts in figures a) and b).

3.3.4.2 Meta-analysis

The pooled mortality estimates for ALI, ARDS and ALI/ARDS were 0.8% (0.3-1.8), 1.2% (0.8-1.9) and 1.1% (0.6-2.3) respectively (Figures 3.23-5). There was evidence of ‘substantial’ to ‘considerable’ heterogeneity in all groups ($I^2=72.3\%$, 56.9% and 84.5% for ALI, ARDS, ALI or ARDS mortality respectively ($p<0.01$ for all)).

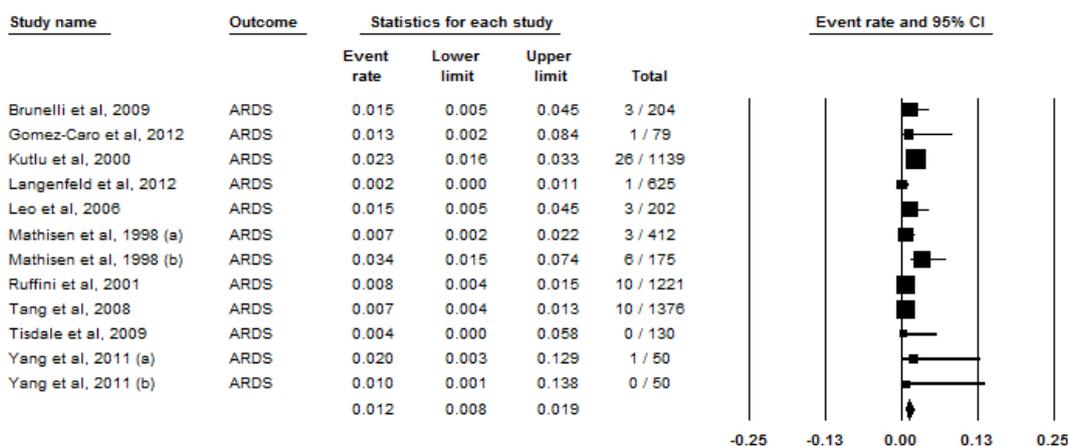
ALI mortality



Tests for heterogeneity: $Q=28.9$, $df=8$, $p<0.01$, $I^2=72.3\%$

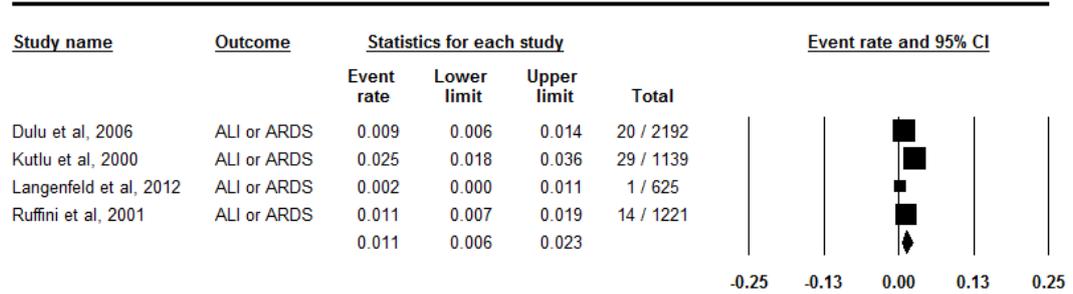
Figure 3.23. Random effects meta-analysis of ALI mortality following lung resection.

ARDS mortality (outlier removed)



Tests for heterogeneity: $Q=25.5$, $df=11$, $p<0.01$, $I^2=56.9\%$

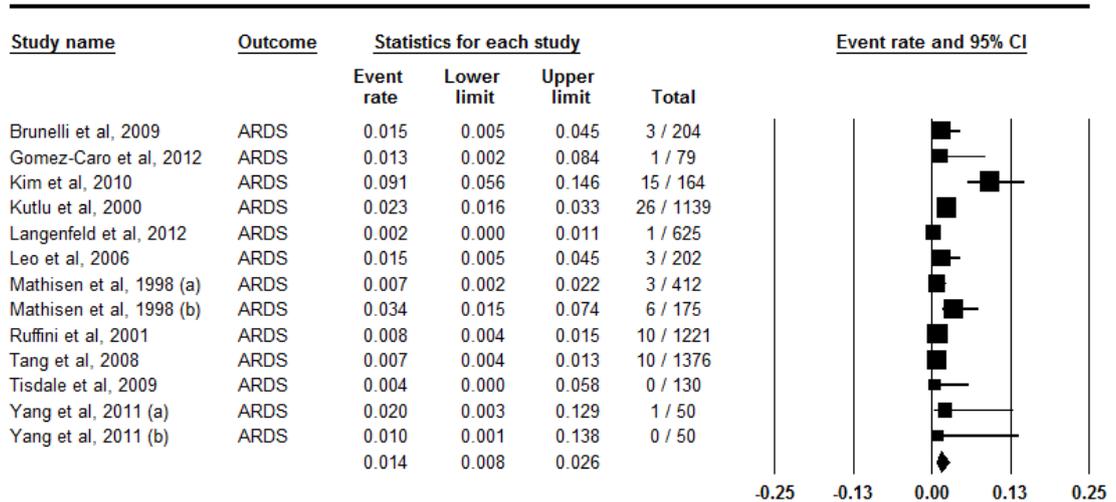
Figure 3.24. Random effects meta-analysis of ARDS mortality following lung resection.

ALI or ARDS mortality

Tests for heterogeneity: $Q=19.3$, $df=3$, $p<0.01$, $I^2=84.5\%$

Figure 3.25. Random effects meta-analysis of ALI/ARDS mortality following lung resection.

Sensitivity analysis: Without exclusion of the outlier, the pooled mortality estimate for ARDS was similar at 1.4% (0.8-2.6), though heterogeneity increased ($I^2=82.2\%$, $p<0.01$, Figure 3.26).

ARDS mortality (including outlier)

Tests for heterogeneity: $Q=67.5$ $df=12$, $p<0.01$, $I^2=82.2\%$

Figure 3.26. Sensitivity analysis: Random effects meta-analysis of ARDS mortality (including outlying study) following lung resection.

Subgroup analysis

Mortality data specific to subgroups of patients undergoing lobectomy and pneumonectomy was available for three lobectomy cohorts and two pneumonectomy cohorts for analysis of ALI mortality, and four lobectomy and two pneumonectomy cohorts for analysis of ARDS mortality. For the endpoint ALI/ARDS mortality, data specific to subgroups was only available for one lobectomy cohort and not available for any pneumonectomy cohorts so no comparative analysis of ALI/ARDS mortality could be performed (Figures 3.27-8).

There was a trend towards reduced mortality in patients undergoing lobectomy compared to pneumonectomy for both ALI and ARDS, $p=0.10$ and $p=0.11$ respectively. The proportion of variance in ALI and ARDS mortality explained (R^2) by subgroup membership was 80.0% and 83.3% respectively.

Sensitivity analysis: Without exclusion of the outlier, there was a stronger trend towards a difference in the mortality from ARDS between patients undergoing lobectomy versus pneumonectomy, $p=0.05$, $R^2=85.7\%$ (Figure 3.29).

ALI mortality by sub-group

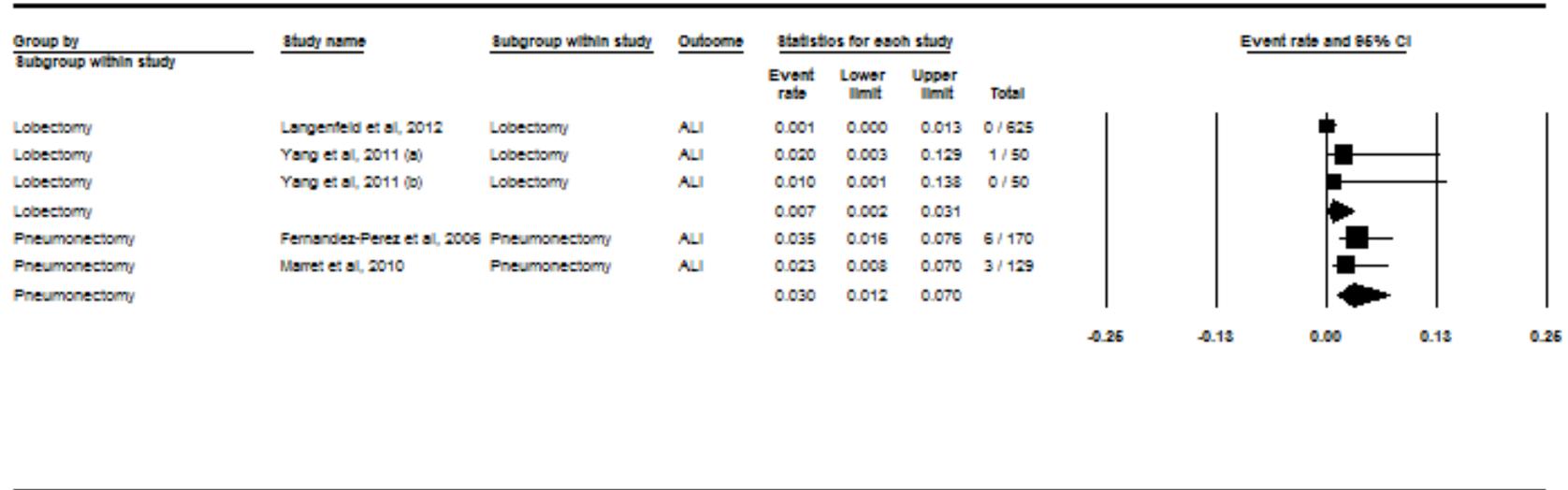


Figure 3.27. Subgroup analysis: Random effects meta-analysis of ALI mortality by type of resection.

ARDS mortality by sub-group (outlier excluded)

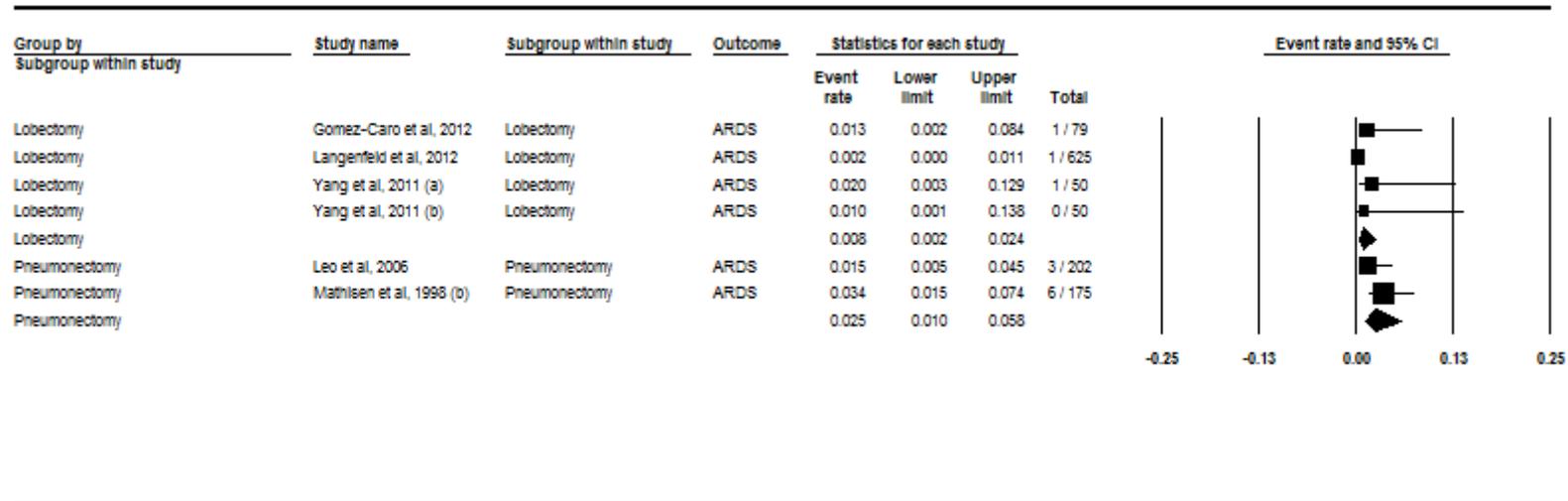


Figure 3.28. Subgroup analysis: Random effects meta-analysis of ALI mortality by type of resection.

ARDS mortality by sub-group (outlier not excluded)

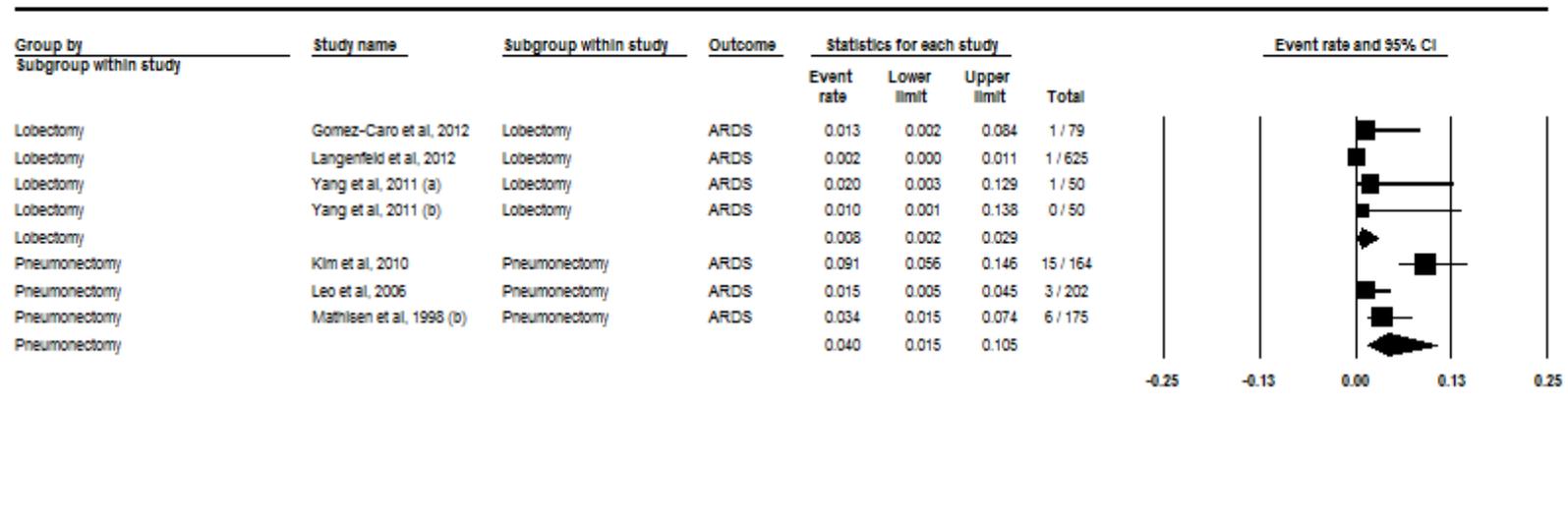


Figure 3.29. Subgroup analysis: Sensitivity analysis. Random effects meta-analysis of ARDS mortality (including outlying study) following lung resection by type of resection.

3.3.4.3 Detection of publication bias

Funnel plots of standard error by Logit event rate are shown in Figures 3.30-3.

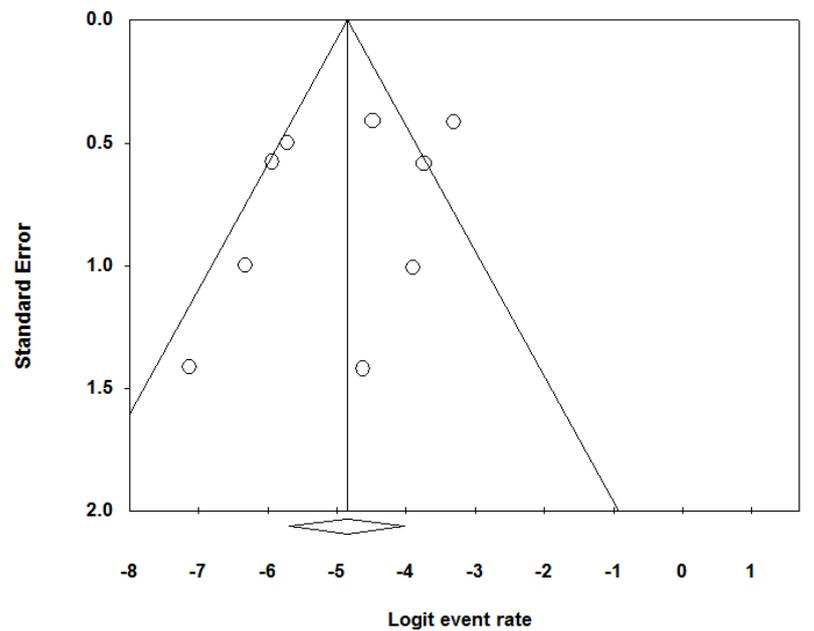


Figure 3.30. Funnel plot of standard error versus Logit event rate for studies reporting ALI mortality.

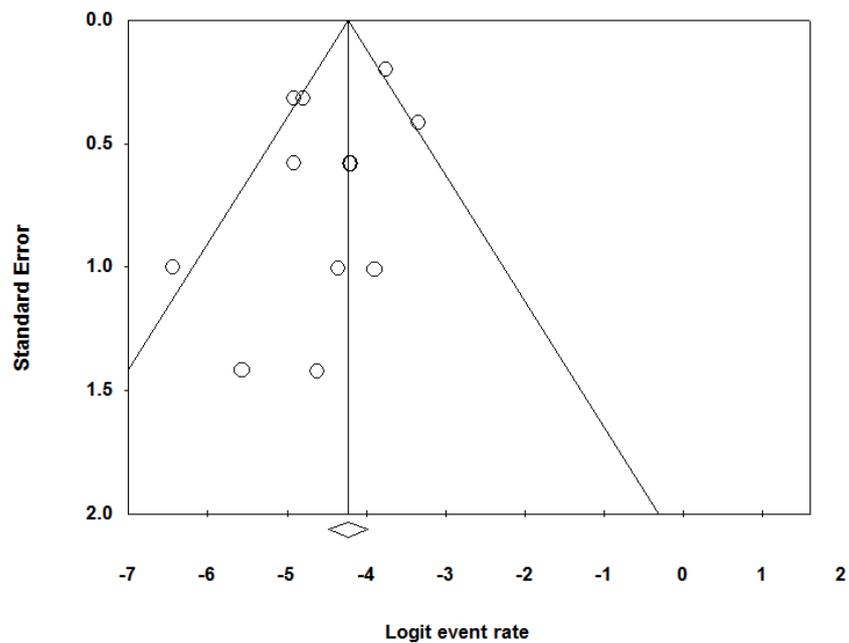


Figure 3.31. Funnel plot of standard error by Logit event rate for studies reporting ARDS mortality (outlier excluded).

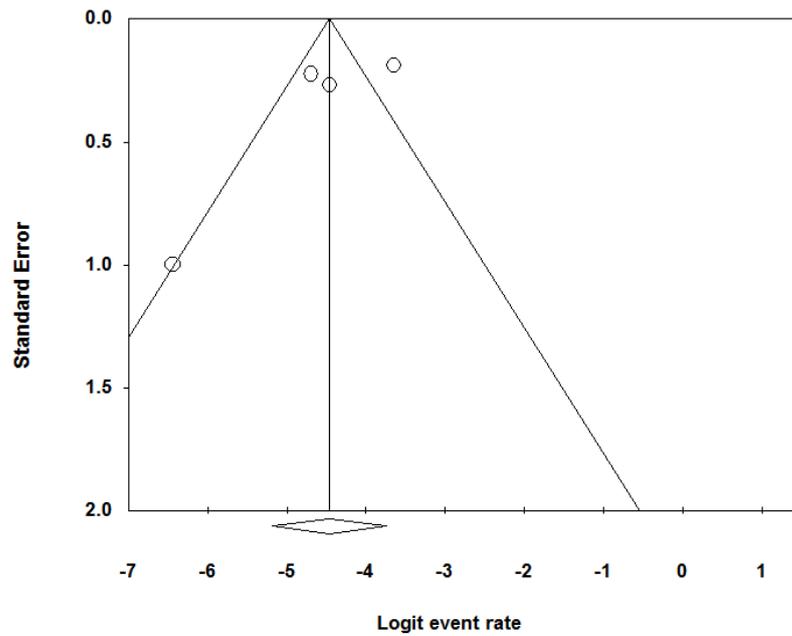


Figure 3.32. Funnel plot of standard error by Logit event rate for studies reporting ALI or ARDS mortality.

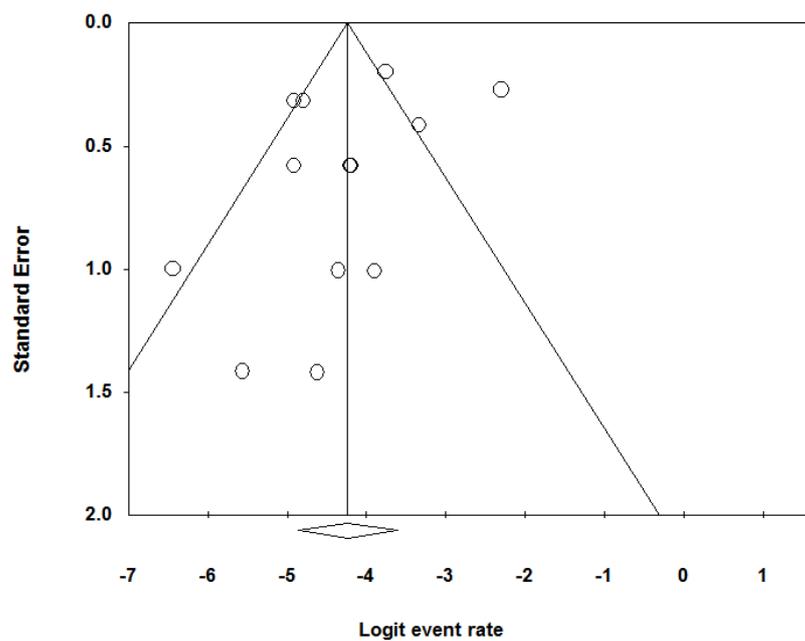


Figure 3.33. Funnel plot of standard error by Logit event rate for studies reporting ARDS mortality (including outlier).

Visual inspection of the plots reveals obvious asymmetry of the plot for ARDS and (less so) for ALI and ALI/ARDS mortality, with studies again apparently ‘missing’ to the lower right hand side of the plots. This suggests publication bias, manifest

in the under-reporting of small studies reporting higher mortality (more negative Logit event rates) from ALI, ARDS and ALI/ARDS.

The results of Duval and Tweedie's trim and fill procedure to assess the impact of publication bias are shown in Table 3.5. Adjustment for the effects of publication bias results in increases in the pooled mortality estimates for ALI, ARD and ALI/ARDS.

Table 3.5. Results of Duval and Tweedie's trim and fill procedure on pooled estimates of ALI, ARDS and ALI/ARDS mortality.

Outcome		Studies trimmed	Point estimate	Confidence interval		Q-statistic
				LL	UL	
ALI	<i>Obs.</i>		0.8	0.3	1.8	28.9
	<i>Adj.</i>	2	1.1	0.5	2.4	36.3
ARDS (no outlier)	<i>Obs.</i>		1.2	0.8	1.9	25.5
	<i>Adj.</i>	2	1.4	0.9	2.1	30.6
ALI//ARDS	<i>Obs.</i>		1.1	0.6	2.3	19.3
	<i>Adj.</i>	2	1.8	0.9	3.8	42.7
ARDS (outlier included)	<i>Obs.</i>		1.4	0.8	2.6	67.5
	<i>Adj.</i>	5	2.5	1.4	4.4	117.4

Obs., observed values; Adj., adjusted values following trim and fill. Mortality presented as percent; LL & UL, lower & upper limit of 95% confidence interval.

3.3.4.4 Meta-regression

ALI, ARDS and ALI/ARDS mortality by year

There was a trend towards reduced mortality over time for ARDS and ALI/ARDS, OR=0.95 (0.89-1.01) and 0.88 (0.77-1.0) per year respectively. There was no significant relationship between median year of study recruitment and ALI mortality, OR=1.06 (0.89-1.27) (Figures 3.36-9 and Table 3.6). The proportion of variance in ARDS and ALI/ARDS mortality explained (R^2) by the median year of study recruitment was 48.2% and 50.2% respectively (Figures 3.34-5).

Sensitivity analysis: where the outlying study was retained, revealed no significant relationship between median year of study recruitment and ARDS mortality, OR=0.96 (0.86-1.07).

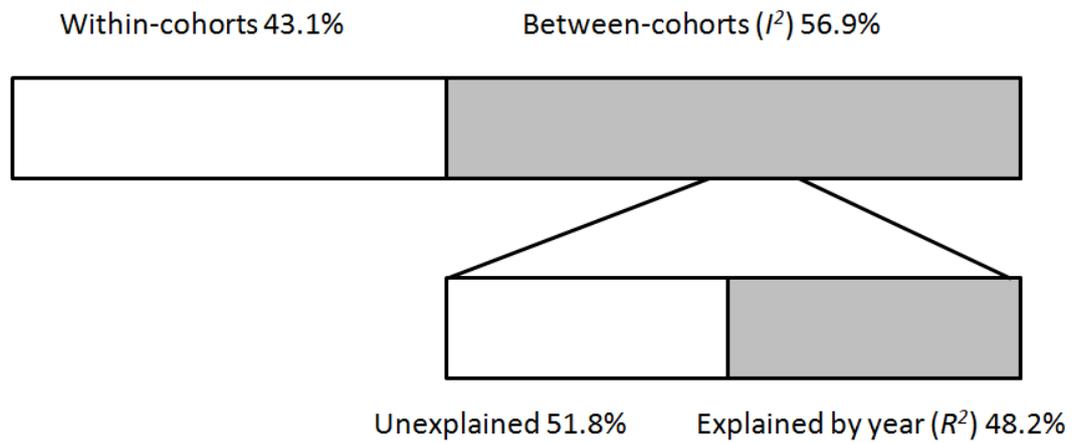


Figure 3.34. Proportion of variance in ARDS mortality explained by median year of study recruitment.

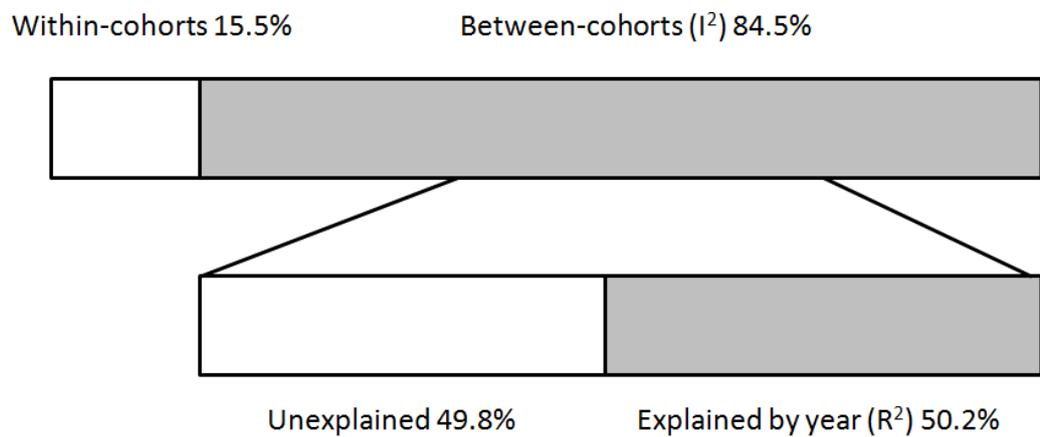


Figure 3.35. Proportion of variance in ALI/ARDS mortality explained by median year of study recruitment.

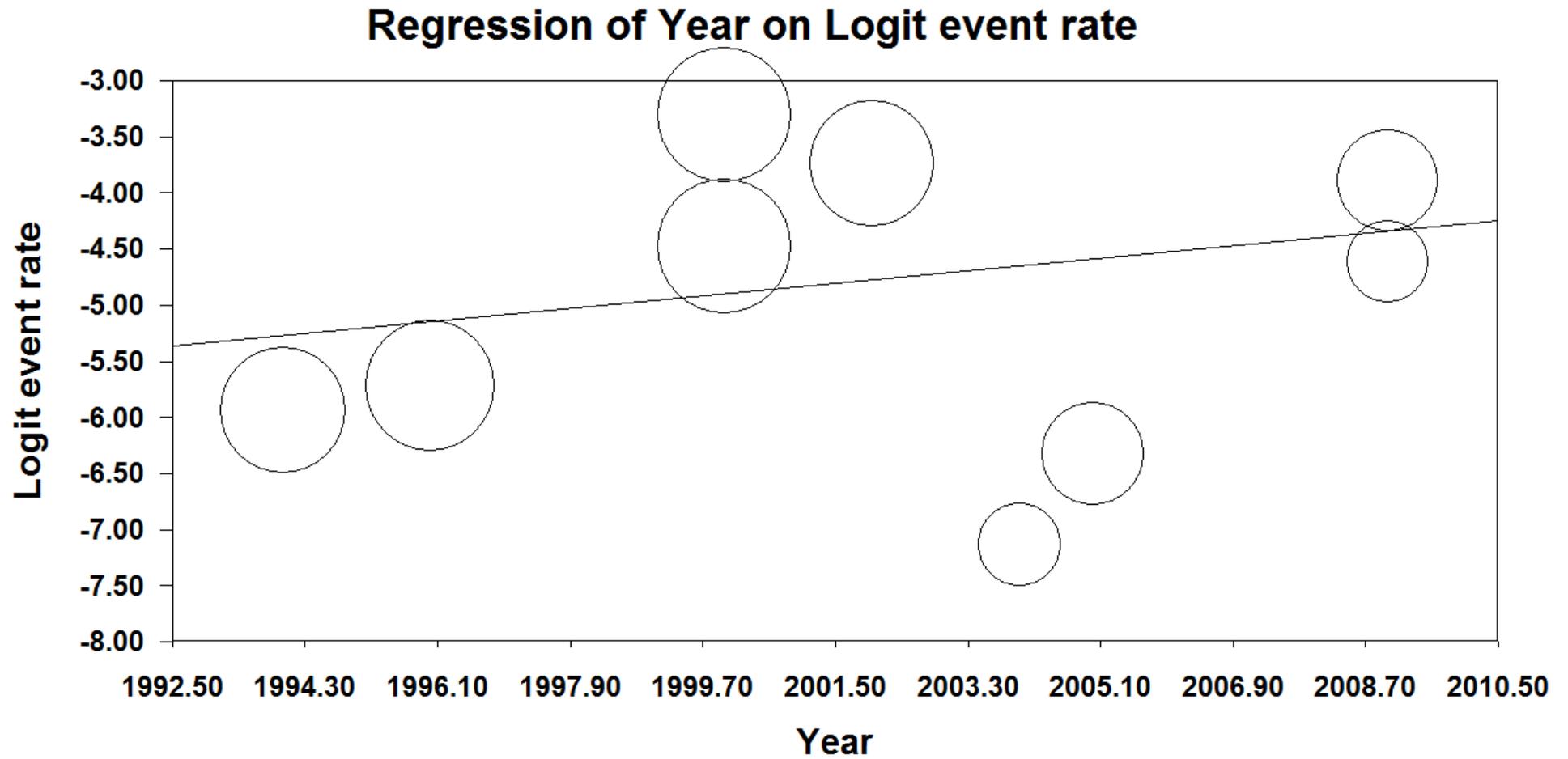


Figure 3.36. ALI mortality by median year of cohort recruitment.
Event rate = mortality. Size of 'bubbles' are proportional to cohort weighting.

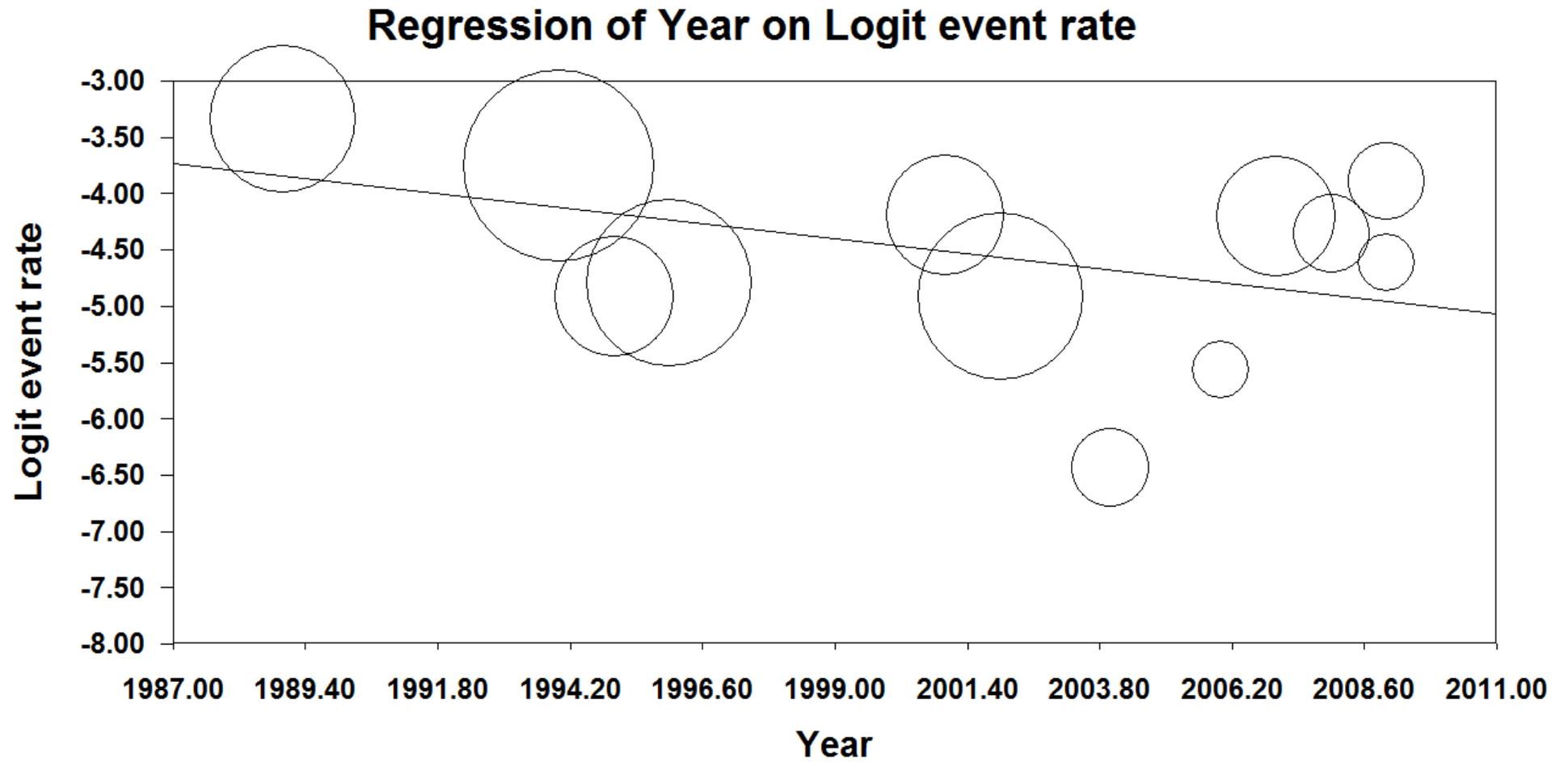


Figure 3.37. ARDS mortality by median year of cohort recruitment.
Event rate = mortality. Size of 'bubbles' are proportional to cohort weighting.

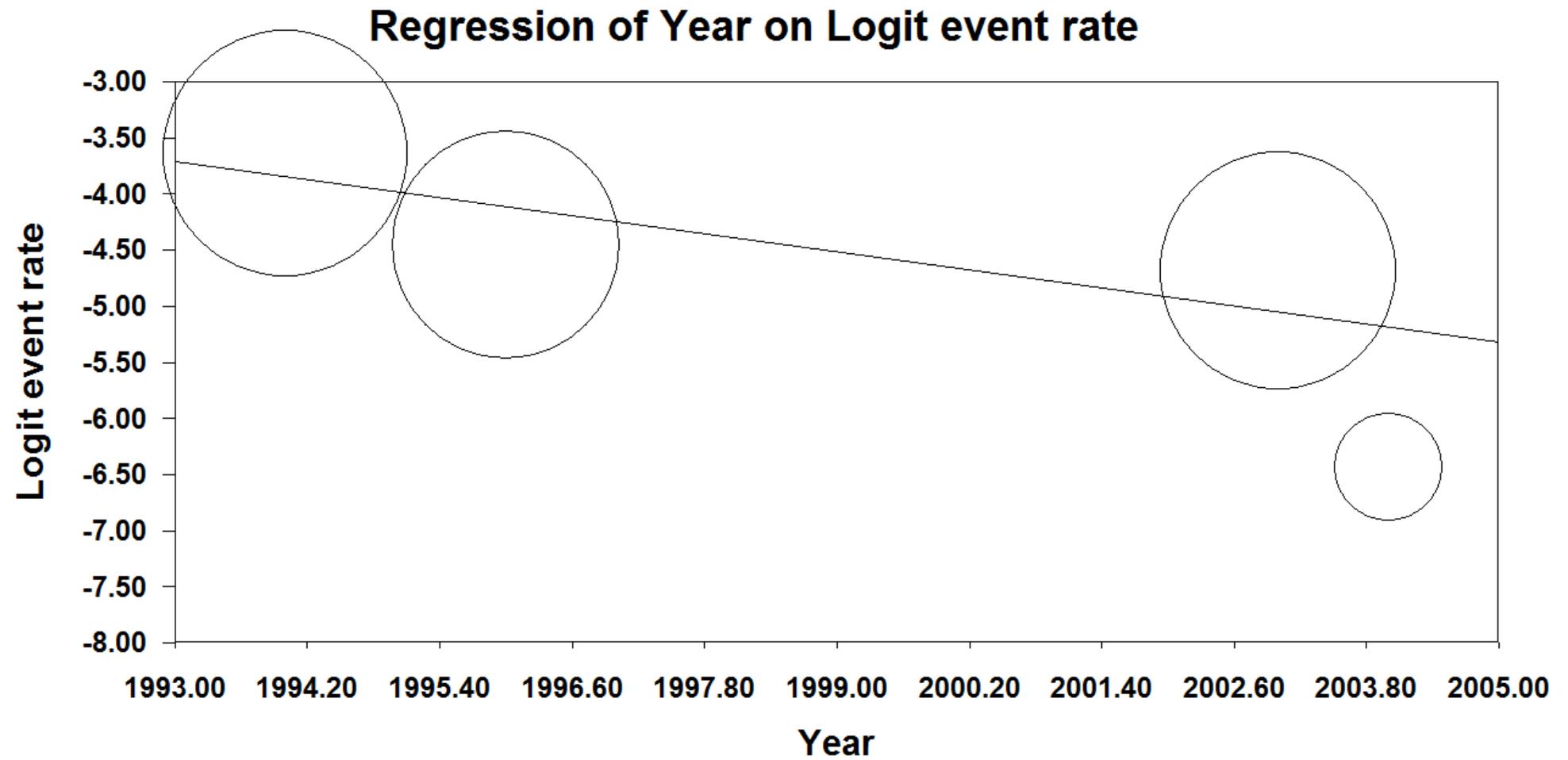


Figure 3.38. ALI/ARDS mortality by median year of cohort recruitment. Event rate = mortality. Size of 'bubbles' are proportional to cohort weighting.

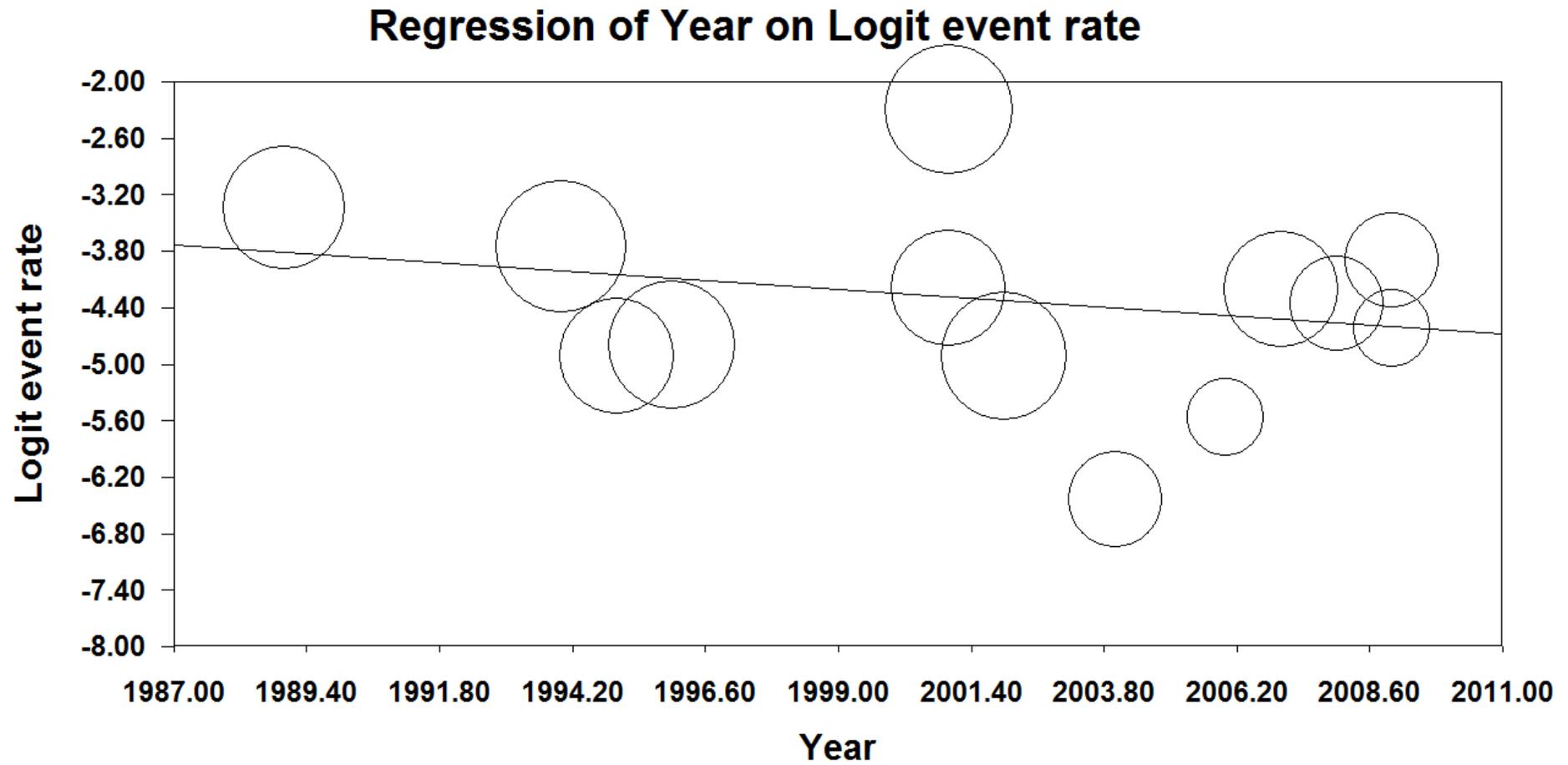


Figure 3.39. Sensitivity analysis: ARDS mortality by median year of cohort recruitment (outlier included).
Event rate = mortality. Size of 'bubbles' are proportional to study weighting

<i>Intra-op fluids</i>	<i>ALI</i>	3	2.871	2	0.24	30.34	-0.13	0.08	0.88	0.75	1.04	100	0.14
	ARDS (outlier excluded)	3	0.735	2	0.69	0.00	-0.07	0.08	0.93	0.80	1.09	0	0.40
	ALI/ARDS	1	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	N/A	-	-	-	-	-	-	-	-	-	-	-
<i>Open vs thoracoscopic resection</i>	ALI	6	10.853	5	0.05	53.93	-0.01	0.02	0.99	0.95	1.03	0.0	0.48
	ARDS (outlier excluded)	6	1.927	5	0.86	0.00	0.01	0.01	1.01	0.98	1.03	0.0	0.71
	ALI/ARDS	2	-	-	-	-	-	-	-	-	-	-	-
	ARDS (including outlier)	7	24.863	6	0.00	75.87	0.01	0.02	1.01	0.97	1.05	0.0	0.62

n, number of cohorts in analysis; df, degrees of freedom; SE, standard error; odds ratio per unit of covariate; LL, lower limit; UL, upper limit. N/A, not applicable – the outlying study did not report the covariate concerned.

3.3.4.5 ALI, ARDS and ALI/ ARDS mortality by other covariates

Age

There was no significant relationship between patient age and ALI mortality (Table 3.6). There was a significant negative relationship between ARDS mortality and mean patient age (OR 0.93 (0.87-0.99) per year increase in mean patient age, $R^2=100\%$). This was not supported by sensitivity analysis where the outlying study was retained, which revealed no significant relationship (Table 3.6). There was insufficient data available (two cohorts or less) to explore any relationship between patient age and ALI/ARDS mortality.

Sex

There was no significant relationship between ALI, ARDS nor ALI/ARDS mortality and the percentage of patients of male sex in each cohort (Table 3.6).

Induction chemotherapy

There was no significant relationship between the percentage of patients undergoing induction chemotherapy in each cohort and ALI mortality (Table 3.6). There was insufficient data available (two cohorts or less) to explore any relationship between receipt of induction chemotherapy and ARDS (outlier removed) or ALI/ARDS mortality. Sensitivity analysis where the outlying study was retained, revealed a trend towards a significant negative relationship between receipt of induction chemotherapy and ARDS mortality (OR=0.00 (0.00-3.20), $R^2=100\%$, Table 3.6).

Duration of one-lung ventilation

There was no significant relationship between the mean duration of one-lung ventilation in each cohort and ALI mortality. There was insufficient data available (two cohorts or less) to explore any relationship between the mean duration of one-lung ventilation in each cohort and ARDS (with or without outlier removed) or ALI/ARDS mortality.

Duration of surgery

There was no significant relationship between the mean duration of surgery in each cohort and ALI or ARDS mortality (without outlier). There was insufficient data available (two cohorts or less) to explore any relationship between the

mean duration of one-lung ventilation in each cohort and ALI/ARDS mortality. Duration of surgery was not recorded in the outlier study (Table 3.6).

Intra-operative fluid administration

There was no significant relationship between the mean volume of intra-operative fluid infused in each cohort and ALI or ARDS mortality (outlier removed). There was insufficient data available (two cohorts or less) to explore any relationship between the mean volume of intra-operative fluid infused in each cohort and ALI/ARDS mortality. Intra-operative fluid administration was not recorded in the outlier study (Table 3.6).

Open verses video assisted thoracoscopic resection

There was no significant relationship between the percentage of patients undergoing open resection in each cohort and ALI nor ARDS (with or without outlier removed) mortality. There was insufficient data available (two cohorts or less) to explore any relationship between the percentage of patients undergoing open resection in each cohort and ALI/ARDS mortality (Table 3.6).

Side of resection, FEV₁, DLCO and induction chemotherapy

There was insufficient data available (two cohorts or less) to explore any relationship between side of resection, mean FEV₁ , mean DLCO, induction chemotherapy and ALI, ARDS or ALI/ARDS mortality.

Analgesic technique

As with the analysis of ALI, ARDS and ALI/ARDS incidence, no meaningful assessment of the relationship between analgesic technique and ALI, ARDS or ALI/ARDS mortality was possible.

3.4 Discussion

The main findings of this study are that whilst there is no evidence to suggest the incidence of ALI and/or ARDS post-lung resection is falling, mortality due to ARDS (but not ALI) does appear to be falling over time.

From the 127 papers identified for full text review, the most common reason for exclusion from the current study was that ALI and ARDS were not defined according to the American-European Consensus Conference definition. Such variability in the definitions used to define PLR-ALI is the major limitation to the current study. By necessarily enforcing strict inclusion criteria, to ensure statistical comparisons made across studies reflect comparisons of patients experiencing the same clinical syndrome, study sample size was limited to just 27 patient cohorts. Within these 27, further inconsistency in reporting the incidence of ALI and/or ARDS led to further reduction of the sample size. Whilst limitation in the number of available studies is a common problem in systematic reviews and meta-analyses (as discussed below), its repercussions are amplified within the methodology of meta-regression^{263, 265, 282}.

3.4.1 Publication bias

Publication bias is more commonly considered in the case of interventional studies where it has been widely described that ‘negative’ results (i.e. demonstrating no benefit from the intervention), are less likely to be published. Such publication bias can result from the design or execution of individual studies, researchers electing not to submit results, journal editorial policies or withholding of results by trial sponsors²⁸³. Irrespective of source, as published results may consequently systematically differ from un-published ones, resultant systematic reviews and meta-analyses based only upon published data may be biased. Publication bias remains a problem in meta-analyses of observational data: The Meta-analysis Of Observational Studies in Epidemiology group (MOOSE), describe that “*publication bias... represents a particular threat to the validity of observational studies*”²⁸⁴. Indeed, observational studies have increased potential for bias (of all aetiologies), tend to show greater effect sizes and exhibit greater heterogeneity²⁸³.

Visual assessment of the funnel plots in Figures 3.13-6 and 3.30-3, suggests there may be significant publication bias in the current study, with studies demonstrating higher incidence and mortality perceived to be 'missing'. It is not difficult to believe that a researcher may be disinclined to publish results which he or she feels reflect negatively on the performance of the institution in which they work. What then would be the implications of such publication bias on the outcomes of the current analyses? It is inherent that underreporting of studies demonstrating high incidence and mortality from ALI and/or ARDS might result in the pooled incidence and mortality estimates being unduly optimistic. This is suggested by the results of Duval and Tweedie's trim and fill procedure, which demonstrates increased incidence and mortality estimates following adjustment for publication bias (Tables 3.3 and 3.5). It is interesting to reflect that following adjustment, the incidence of ALI is 2% higher than for ARDS; such an observation is intuitive given that ALI reflects a 'milder' degree of lung injury and therefore might be expected to occur more often.

There appears to be no reason however, why the potential for publication bias may be different over time period studied; as such one might perceive that publication bias is unlikely to compromise the results of the meta-regression analyses against time. Unfortunately, such an assertion may be misguided. Meta-regression is dependent on their being heterogeneity between studies included in the analysis; without between-study variation, there is no 'proportion of variance' to be explained by meta-regression. Systematic under reporting of studies demonstrating greater incidence and mortality (due to publication bias) will result in there being both a reduced number of studies available for analysis, and reduced between-cohort variability, decreasing the power of the analysis to detect a positive relationship between incidence / mortality and any cofounder. The effect of 'missing' studies on heterogeneity can be appreciated by the increases in the value of the Q-statistic observed when pooled incidence and mortality estimates are adjusted by Duval and Tweedie's trim and fill procedure (Tables 3.3 and 3.5).

3.4.2 Incidence of ALI and/or ARDS following lung resection

Prior to adjustment for publication bias, the pooled incidence estimates for ALI, ARDS and ALI/ARDS were 2.8% (1.6-4.9), 2.5% (1.8-3.3) and 3.0% (2.1-4.3) respectively (Figures 3.4-6). Unsurprisingly, there were high levels of heterogeneity in all groups ($I^2=84.3\%$, 54.3% and 78.3% for ALI, ARDS, ALI/ARDS respectively ($p<0.01$ for all). The remainder of this discussion concerns explanation of the heterogeneity.

In contrast to trends reported in single institutions^{110, 155}, there was no evidence to suggest that the incidence of either ALI, ARDS or ALI/ARDS was falling over time ($p=0.88$, 0.45 and 0.36 for ALI, ARDS and ALI/ARDS). The failure to demonstrate the hypothesised relationship between incidence and time, results in one of two possible conclusions. Firstly, the relationship may not exist (the results of the analyses are ‘true’), or secondly, the relationship does exist, but the study was inadequate to demonstrate it. Both possibilities will be considered in turn.

3.4.2.1 There may be no relationship between ALI/ARDS incidence and time...

Since 2005, there has been a dramatic increase in the number of patients undergoing lung cancer surgery in the UK⁵. Realisation that UK resection rates lagged behind those elsewhere, increased recognition that acceptable levels of peri-operative morbidity and mortality are achievable even in patients previously considered to be ‘very-high risk’, and evidence to suggest resection is efficacious in patients with more advanced disease means surgeons and anaesthetists are increasingly likely to offer surgery to patients previously considered unsuitable for resection¹. As a consequence, one might hypothesise that increased resection rates will be reflected by increasing patient age, co-morbidity and disease stage. Evidence extracted from the ‘English Cancer Repository Dataset’, a database of over 280,000 patients undergoing lung cancer resection in England between 1998 and 2008 confirms the former to be the case; reporting an increase in the proportion of patients in older age groups undergoing surgical resection⁹. Age and co-morbidity (specifically alcohol

¹ For a full explanation of the drivers behind increasing resection rate see the discussion in Section 1.1.2.

consumption, ASA grade and pulmonary function are recognised risk factors for PLR-ALI. It seems likely therefore, that baseline risk of PLR-ALI will have increased during the time analysed by the study. If this is the case, then the finding that ALI and/or ARDS incidence is stable is a significant finding in itself. Stable incidence in the face of increased baseline risk would suggest reduced ‘peri-operative’ risk.

It was intended during this analysis, to perform multivariate logistic meta-regression and so be able to ‘adjust’ for pre-specified confounders influencing ‘baseline risk’. It is disappointing therefore that there was insufficient data available from which to generate a robust analysis. Analogously to recommendations that 10 data points are required per covariate entered into a conventional multivariate regression model²⁷², it is advised that 10 studies are required per covariate entered into a multivariate meta-regression model^{267, 273}. It can be seen from Table 3.4, that there were just 12, 16 and 7 studies reporting the incidence of ALI, ARDS and ALI/ARDS respectively, and that the number of studies reporting many other potential cofounders was much lower. In a paper entitled “Controlling the risk of spurious findings from meta-regression”, Higgins and Thompson comment: “*Advice to systematic reviewers who wish to explore heterogeneity using statistical techniques is often to minimize the number of covariates investigated*”. In this study, and with some regret, the author (B. Shelley) was obliged to heed such advice.

3.4.2.2 A relationship may exist, but may not have been detected...

“One should never use a non-significant finding to conclude that ... a covariate is not related to effect size.”

Borenstein et al (2009)²⁶⁵

It is common misconception, that the statistical power of meta-analysis is high. In meta-analysis, as in an individual study, statistical power depends upon the magnitude of the effect size, and the precision of the estimate of the effect size:

$$Power \propto \frac{Effect\ size}{Precision\ of\ the\ estimate}$$

Equation 3.12

Under fixed-effects, precision of the effect size is largely determined by the total sample size (cumulatively across all studies); as such precision for the summary effect (and hence power) is always greater in meta-analysis than in the individual studies. Under random-effects however, precision is dependent on two sources of error; the within- and between-studies variance. Precision therefore becomes a product of both sample size, and number of studies. If a random-effects meta-analysis contains a large number of studies (and patients), and the effect sizes are relatively consistent, then as in fixed effects, power will be high. If however, few studies are included and/or the effect size varies substantially between studies, the precision of the effect size estimate (and therefore power) will be lower²⁶⁵.

It must be appreciated that the discussion above concerns the power of a meta-analysis to test the ‘main effect’; commonly an assessment of a treatments effect and in the case of the current study the pooled ALI and/or ARDS incidence estimates. The main purpose of the investigation was not however to generate a pooled incidence estimate, but to assess the effect of the moderator variable year (median year of study recruitment) on the incidence of ALI and/or ARDS by meta-regression analysis. For the purpose of power analysis therefore, the ‘effect size’ is not the incidence of acute lung injury (for example), but the difference between the incidence estimates in each cohort, which in many cases is smaller than the overall incidence. Power therefore falls, as the ‘effect size’ reduces with no change to the precision of the estimate²⁶⁵. As described by Hedges and Pigott:

“Moderator analyses are conceptually analyses of interactions (the interaction of treatment and a moderator variable), and tests for interactions are less powerful than tests for main effects in the same designs”

Hedges and Pigott (2004)²⁸⁵

Hedges and Pigott provide a methodology by which the power of a meta-regression analysis may be determined for an individual regression co-efficient

under random effects²⁸⁵. By using this algorithm (the full workings of which are described in Appendix two), it is possible to determine the power of the current study to detect the effect sizes demonstrated by Licker et al (for ALI incidence) and Tang et al (for ARDS incidence) in the single institution reports discussed previously. Licker et al reported a 2.9% decrease in the incidence of ALI, from a baseline of 3.8% over a study period of 5.3 years (OR 0.85)¹⁵⁵. The power of the current study to detect such an effect size was 73%. Tang et al reported a 1.6% reduction in the incidence of ARDS from a baseline of 3.2% over a study period of 5.4 years (OR 0.94)¹¹⁰. The power of the current study to detect such an effect size was 61%.

It can be appreciated from these analyses that the current study, based on the totality of available literature, lacks sufficient power to confidently test for the effect sizes reported by Licker and Tang and colleagues within the pooled incidence estimates. In fact, as shown in Appendix two, the current study was powered to detect an effect size of OR=0.84 per year for ALI and OR=0.92 per year for ARDS. From a baseline incidence of ALI or ARDS of 4% (for example), this corresponds to an absolute risk reduction in ALI or ARDS incidence of 0.64% and 0.32% in the first year respectively.

A recent study by Lopez-Lopez et al offers some further insight into the concept of power analysis for meta-regression²⁸⁶. Lopez-Lopez et al performed a simulation study, examining the effects of the number of studies (k), and the number of patients per study (N), on the precision of the R^2 estimate for a random-effects meta-regression analysis with one covariate, for seven different methods of estimating the variance of the true effect sizes (T^2) (of which the DerSimonian and Laird 'method of moments' used in the current study was one such example^{264, 269}). Where R^2 , is the proportion of true-variance explained by the moderator variable (see Page 116 and Figure 3.11 (Page 130) for explanation). By simulating 10,000 meta-analyses, for each of 325 combinations of k , N , T^2 , and R^2 , Lopez-Lopez et al concluded that the number of studies in the meta-analysis appears to have greatest effect on the predictive power of the model, and that 'accurate' estimation of R^2 can only be expected in meta-analyses containing in excess of 40 studies¹⁹³.

3.4.2.3 Sub-group comparisons

As anticipated, the incidence of ARDS and ALI/ARDS but not ALI was significantly higher in patients undergoing pneumonectomy than lobectomy $p = <0.01$, <0.01 and 0.16 respectively (Figures 3.8-10). The absence of a difference in ALI incidence in patients undergoing lobectomy compared to pneumonectomy is surprising given the high levels of heterogeneity found in this group ($I^2=89\%$). Though there were six cohorts reporting the incidence of ALI after lobectomy, there were only three cohorts reporting the incidence after pneumonectomy. Given that sub-group comparisons within meta-analysis are subject to all of the limitations concerning power discussed above, it is plausible that with so few cohorts reporting ALI incidence after pneumonectomy this comparison lacked power.

The proportion of (between-cohorts) variance explained (R^2) by subgroup membership was 63%, 86% and 34% for ARDS, ALI/ARDS and ALI respectively. Given that over 60% of the between-cohorts variance in (ARDS and ALI/ARDS) incidence is explained by subgroup membership, it is evident that any effect of any other covariate (including year) must be relatively modest. This is likely to have further confounded the meta-regression versus median year of study recruitment analysis. In all situations, the maximum number of cohorts in any individual sub-group was seven (though in many as few as four), making within-subgroup analysis by year unfeasible.

3.4.2.4 Other covariates

There were no consistent relationships demonstrated between any other covariate and the incidence of ALI and/or ARDS. In several comparisons, statistically significant results were returned for relationships between ARDS incidence and covariates when the outlier was included (for example relationships between ARDS incidence and side of resection, DLCO and pre-op chemotherapy; $p = <0.01$, <0.01 and 0.06 respectively (Table 3.4). This can be explained by the composition of the outlying patient cohort. This patient cohort predominantly underwent left sided pneumonectomy, had normal DLCO and did not undergo pre-operative chemotherapy. By visual inspection of the corresponding 'bubble plots' (not shown) reflecting these comparisons, the markedly influential effect of the outlying study on the regression slope was easy to appreciate. As such, these comparisons should be interpreted with caution.

It must be appreciated that all other covariate analyses besides age suffer from limited cohort numbers, due to the lack of availability of data from the primary papers. As such the analysis could only be based on the patient cohorts for which any given covariate was available, reducing the power of any analyses and introducing a further source of bias to the results.

3.4.3 ALI and/or ARDS mortality following lung resection

Prior to adjustment for publication bias, the pooled mortality estimates for ALI, ARDS and ALI/ARDS were 0.8% (0.3-1.8), 1.2% (0.8-1.9) and 1.1% (0.6-2.3) respectively (Figures 3.23-5). Again, there was marked heterogeneity between groups ($I^2=72.3\%$, 56.9% and 84.5% respectively ($p<0.01$ for all)).

In contrast to the analysis between ALI and/or ARDS incidence and year, a statistically significant relationship was observed between ALI/ARDS mortality and median year of study recruitment (OR=0.88 (0.77-1.0)). This was paralleled by a trend towards reduced ARDS, but not ALI mortality (OR=0.95 (0.89-1.01) and 1.06 (0.89-1.27) per year respectively (Figures 3.36-8, Table 3.6). The proportion of variance in ARDS and ALI/ARDS mortality explained (R^2) by the median year of study recruitment was 48.2% and 50.2% respectively.

Such a finding is in keeping with several reports suggesting reduced ALI/ARDS mortality in the wider critical care environment. Spragg et al performed an un-weighted analysis examining ARDS mortality reported in the US Acute Respiratory Distress Syndrome Clinical Trials Network studies²⁶¹. The authors reported that “*studies from the Network permit comparison of mortalities in patients of similar disease severity and source, and in these studies [5 studies, 2,944 patients] mortality has decreased from almost 40% in studies conducted in the mid to late 1990s to approximately 25% in the most recent reports*”²⁶¹. In a study reporting the outcome of 2,451 patients recruited into three of the same five studies, Erikson et al performed an un-weighted logistic regression analysis demonstrating a reduction in crude mortality (from 35% in 1996-7 to 26% in 2004-5²⁶²). By observing the temporal trend to be robust to adjustment for demographic and clinical covariates including receipt of lower tidal volume ventilation, Eirckson et al concluded their “*findings strongly suggest that other advancements in critical care aside from lower tidal volume ventilation,*

accounted for [the] improvement in mortality”²⁶². Zambon et al performed a mixed-effects meta-regression analysis, examining ALI/ARDS mortality reported in 72 studies²⁸⁷. The authors reported significant decrease in overall mortality rates of approximately 1.1%/yr over the period analyzed (1994 to 2006). The mortality reduction was observed for overall and hospital mortality, but not for ICU or 28-day mortality rates²⁸⁷. Zambon et al similarly suggest that (in addition to improvements in ‘respiratory management’, improvements in general ITU care, “*such as improved hygiene, better glucose control, more judicious use of blood transfusions, improved imaging to identify sources of sepsis, and methods to control sepsis*”, were the likely explanation for the reduction in mortality²⁸⁷. It seems likely that the reduction in mortality observed in the current study may be explained by patients requiring critical care for post-operative ARDS benefitting similarly from any such ‘advancements’ in general ITU care.

In contrast to the findings of Spragg²⁶¹, Erickson²⁶², Zambon²⁸⁷ and colleagues, Phua et al observed no reduction in ARDS mortality between 1994 and 2006¹³⁶. Phua et al performed a random-effects meta-regression analysis examining ARDS mortality reported in 42 (randomised and observational) studies. Only sub-type of study (with higher mortality in observational verses randomised studies), and patient age were found on meta-regression analysis to be independently associated with mortality. Phua et al suggest that ARDS mortality may not have improved for several reasons; firstly, the lack of available therapeutic strategies; secondly, failure to adopt therapies proven effective in randomised clinical trials into routine clinical practice (for example low tidal volume ventilation); thirdly because the patient population identified by the AECC definition is “*extremely heterogeneous*”²⁶². The authors cite several methodological explanations for why their finding may be in opposition with those of Zambon et al²⁶².

Though there was no reduction in ALI and/or ARDS incidence in the current study, no assessment of ALI and/or ARDS severity could be made from the study. It is plausible that though the incidence of lung injury is not falling, due to the hypothesised improvements in lung protection, the severity of injury may be less, so explaining reduced mortality.

Following the comments made previously concerning the potential for type-II error in meta-regression analyses, the observation of any statistically significant result appears persuasive. The observed reduction in ALI/ARDS mortality should be nonetheless interpreted with caution. Firstly, all meta-analysis and meta-regression results must be considered ‘observational’²⁶³. Though classically meta-analysis is performed by combining the effects of multiple randomised controlled trials (just three of the 21 studies included in the current analyses were randomised), the meta-regression across studies does not benefit from randomisation. As such, an association between covariate and outcome cannot be considered causal, and may represent an association between cofactor and another un-recorded covariate.

Secondly, observational studies are more variable in design than randomized trials²⁶³. As such some of the marked heterogeneity between studies could result from the heterogeneity in study design, rather than clinical diversity. On this point, there was a large degree of variability between studies in which mortality end-point was reported (Table 3.2). A large number of studies did not report the duration of follow up, whilst others reported hospital, hospital and/or 30 day, 60 or 90 day mortality. Whilst it would have been desirable to restrict data extraction to the same defined mortality end-point, such a course of action would have prohibitively compromised the number of cohorts available for analysis. Analysing ALI and/or ARDS ‘mortality’ measured over a variety of durations could clearly be a further source of bias in the current analysis.

Thirdly, as Higgins and Thompson observe, “*false positive results are more likely in meta-regression than in conventional regression because of the potential presence of heterogeneity*”²⁸². In a simulation study, Higgins and Thomson demonstrate that “*standard meta-regression methods suffer from substantially inflated false-positive rates when heterogeneity is present, when there are few studies and when there are many covariates*”. Further observing that “*these [conditions] are typical of situations in which meta-regressions are routinely employed*”. Though the risk of type-I error will decrease as the number of studies increases, “*it is unclear at what point the risk becomes acceptably small*”²⁸². It should be emphasised that in Higgins and Thompson’s analysis, false positive rates were ‘unacceptably high’ from fixed-effects models conducted in

the presence of heterogeneity; performing analyses by random effects is a distinct strength of the current study.

3.4.3.1 Sub-group analyses

The analyses of ALI and/or ARDS mortality by subgroup were compromised by the limited number of studies available for analysis. There was a trend towards reduced mortality in patients undergoing lobectomy compared to pneumonectomy for both ALI and ARDS, $p=0.10$ and $p=0.11$ respectively.

3.4.3.2 Other covariates

There was a statistically significant negative association between age and ARDS mortality (OR = 0.93 (CI=0.87-0.99) per year increase in mean patient age)). Visual inspection of the relevant bubble plot (not shown) suggests that the regression line was being influenced by the data point representing the cohort of patients from the study by Kutlu et al, whose median age at 51.7 was ~7 years younger than the remaining four studies whose mean age falls in the range 58.4 to 66.5 years. This finding was not robust to sensitivity analyses where the study of Kutlu et al was removed (not shown), or where the outlying study was included (Table 3.6).

There were no other significant associations between any covariate and ALI and/or ARDS mortality.

3.4.4 Conclusion

“The potential for robust conclusions from meta-regression analyses is clearly very limited”²⁶³.

Thompson and Higgins (2002)²⁶³

This statement from Thompson and Higgins above appears very pertinent to the current study. Though this study represents a methodologically robust attempt to describe any trends in ALI and/or ARDS mortality occurring over time from all the currently available published data, the analysis has been thwarted to an extent by inconsistencies in both the definitions used to describe PLR-ALI and the study endpoints reported such that sample sizes were reduced to the point that the potential for ‘robust’ conclusions is limited.

What can be concluded from the current study?

Firstly, the pooled incidence and mortality estimates though subject to publication bias, reflect the ‘best estimate’ of ALI, ARDS and ALI/ARDS incidence and mortality following lung resection available to date. Such estimates could provide useful information against which to bench-mark local practice and inform power analyses for future studies of PLR-ALI. Secondly, though it is possible to conclude that the incidence of ALI, ARDS and ALI/ARDS are not falling with time, the analyses do suggest that any effect of time on incidence is relatively modest. Finally, the mortality from PLR-ALI does appear to be falling, though no conclusion can be made from the current study as to why this is so.

4 Investigation III: Utility of Pentraxin 3 and a multiple biomarker panel as biomarkers informative of lung injury following lung resection

4.1 Introduction

This investigation comprises two discrete studies examining the utility of Pentraxin 3 (PTX3) and a multiple (lung injury) biomarker panel in the early post-operative period following lung resection. Measurement of biomarkers informative of the pathogenesis or clinical progress of lung injury in this population could offer the potential to allow early identification of patients at risk^{80, 261, 288}, guide clinical management^{13, 14, 17}, identify severity of disease, stratify risk^{82, 289} and predict outcome^{80, 82, 261, 288, 290}.

Pentraxin 3 (PTX3) has been described as a potential biomarker of acute lung injury²⁹¹. There is a sound biological plausibility for its use both as a lung injury biomarker in the critical care environment and in the early post-operative period following lung resection (described in detail in the next section). Measurement of PTX3 has not previously been described in the post-operative period following lung resection.

Whilst a great deal of effort has gone into the search for a single ‘ideal’ ALI/ARDS biomarker, combination of biomarkers into panels in order to improve validity has become an increasing focus of biomarker research²⁴⁴. Such panels have been selected from large numbers of potential biomarkers by multivariate regression modelling^{83, 292}, and the resulting panels described include biomarkers representing many different components of the pathogenesis of ALI/ARDS.

The purpose of this investigation is to assess the utility of PTX3, and a multiple biomarker panel described by Fremont et al⁸³ in the early post-operative period following lung resection. Modified Lung Injury Score (mLIS) is defined as a surrogate endpoint of ALI (Table 4.6), and as the aim of this investigation is to examine the use of biomarkers in the identification of *primary* PLR-ALI, the

assessment of oxygenation, CXR-scores and mLIS were restricted to the first 48 hours post-operatively (the time of peak incidence of primary PLR-ALI¹¹⁵). Both PTX3 and the multiple biomarker panel are assessed against the properties of the 'ideal ALI/ARDS biomarker' (as will be defined in Section 4.2.2).

4.2 Literature review: Biomarkers of Acute Lung Injury

This review examines the theoretical principles behind biomarker research, asking ‘what is a biomarker?’ and ‘what are the properties of the ideal biomarker?’, before exploring the limited evidence from studies in the OLV / lung resection population to date. Clearly a comprehensive review of every biomarker would be laborious to collate, tiresome to read and be of little value to the overall goals of this thesis. Attention is focussed on the conceptual framework of biomarker research during which examples are drawn from the ALL biomarker literature. Finally, the case for the candidate biomarkers examined in Investigation III is presented.

4.2.1 What is a biomarker?

A biological marker (biomarker) has been defined by the ‘Biomarker Definitions Working Group’^J as:

“A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.”

Biomarker Definitions Working Group²⁹³

The World Health Organisation (WHO) provides a more general definition suggesting that a biomarker may be:

“any substance, structure or process that can be measured in the body or its products and influence or predict the incidence or outcome of the disease.”

World Health Organisation (2001)⁸⁰

From these definitions at their broadest and most literal interpretation, it can be understood that a biomarker may be a clinical sign, or the result from a monitoring modality (for example extravascular lung water)⁸⁰. For the purposes of this discussion the term biomarker is reserved for the predominantly protein substances⁸⁰ that are measured in plasma or broncho-alveolar lavage /

^J ‘Biomarker Definitions Working Group’ – a group convened by the US National Institute of Health charged to proposed terms, definitions and a conceptual model.

pulmonary oedema fluid discussed widely as being informative in patients with or at risk of ALI.

According to the ‘Biomarker Definitions Working Group’, biomarkers can have a variety of applications²⁹³:

- *As a diagnostic tool*
- *As a tool for staging of disease or classifying the extent of disease*
- *As an indicator of disease prognosis*
- *As a means of prediction and monitoring of response to an intervention*

4.2.2 Properties of the ‘ideal’ lung injury biomarker

Several authors have suggested properties which they consider the ‘ideal’ lung injury biomarker should exhibit (Table 4.1). Whilst many are self-explanatory, each will briefly be considered in turn in the following discussion.

Table 4.1. Properties of the ‘ideal’ lung injury biomarker.

-
- Have ‘biological plausibility’²⁹⁴⁻²⁹⁷
 - Sample easily and safely obtained in the critically ill patient^{82, 294, 296}
 - Highly sensitive and specific in predicting the outcome of interest^{82, 294, 295}
 - Modified by an effective intervention^{82, 295, 296}
 - Vary in proportion to the severity of injury²⁹⁵
 - Associated with clinically important outcomes²⁹⁶
 - Timely, highly reproducible and inexpensively quantified^{82, 294-296}
-

In a similar vein, Shehabi and Seppelt suggest that a biomarker needs to be ‘SMART’^{80, 298} (Table 4.2).

Table 4.2. Properties of a SMART biomarker.

-
- Sensitive (and specific)
 - Measurable (with a high degree of accuracy)
 - Affordable (and safely attainable)
 - Responsive (and re producible)
 - In a Timely fashion
-

From Shehabi and Seppelt^{80, 298}.

4.2.2.1 Biological plausibility

Biological plausibility confers face validity. From a researcher's understanding of the biology of lung injury, if evidence exists that the biomarker measured is implicated in the pathogenesis of injury, then it is plausible to the researcher that changes in the biomarker will reflect changes in the clinical outcome. Biological plausibility is often provided by an accumulation of laboratory and clinical data demonstrating for example, that administration of the biomarker leads to injury in vitro, that manipulation of a biomarker pathway results in increased / decreased mortality in animal models and that in clinical studies patients with ALI/ARDS, more severe ALI/ARDS or suffering mortality have higher biomarker levels. In combination, such evidence provides the researcher with confidence that they are measuring the level of a molecule intimately involved in the pathogenesis of lung injury. The stronger the association demonstrated between biomarker level and clinical outcome, the more likely the causal link, and the greater the plausibility of the biomarker²⁹⁷. In some circumstances potential biomarkers are identified as being so integral to the disease process that the biomarker pathway itself provides a potential target for therapeutic intervention²⁹⁴.

The need for a candidate 'ideal' biomarker to have such 'biological plausibility' is controversial however. Whilst many authors suggest such a defined role is necessary²⁹⁴⁻²⁹⁶, Proudfoot et al argue that it is of little consequence whether the biomarker is involved in the pathogenetic process at all, provided it is suitably prognostic / diagnostic²⁹⁹.

4.2.2.2 Sample easily and safely obtained

For any biomarker to be useful outwith the research setting, it must be feasibly, easily and safely obtained from critically ill patients. Urine is easily and non-invasively obtained, but lacks specificity to the lung. Nonetheless, urinary biomarkers have been associated with improved outcomes in patients with acute lung injury³⁰⁰. Plasma is easily (though marginally more invasively) obtained, but again specificity to the lung is lacking leaving the potential for the pulmonary 'signal' to be swamped by the systemic. Despite this, plasma forms the site of interest of the majority of ALI biomarker research. Pulmonary oedema fluid or

bronchoalveolar lavage specimens collected at bronchoscopy offer the potential of a lung specific sample at the expense of a more invasive test. Bronchoscopy however is impractical as a screening test in large populations of 'at-risk' patients and may be unsafe in patients with more severe disease. Exhaled breath condensate (EBC) offers the potential to non-invasively obtain a lung specific sample in intubated patients but is limited to analysis of small molecules. The role of EBC is yet to be defined with solutions required to the problems of quantifying the degree of dilution, improving reproducibility and avoiding of contamination³⁰¹.

4.2.2.3 Sensitivity and specificity for the outcome of interest

The ALI/ARDS biomarkers literature is beset with scores of publications demonstrating statistically significant increases in a given biomarker in a group of patients with a given outcome. Demonstration of statistical significance however provides no index of how useful the test may be for differentiating between groups³⁰². Assessment of biomarker performance requires assessment of sensitivity (the probability of a positive test given the presence of a disease) and specificity (the probability of a negative test given the absence of a disease) and the calculation of positive and negative predictive values^{80, 302}. Furthermore, these predictive values require interpretation in the context of the clinical problem. A low positive predictive value (high potential for false positives) may not be a problem where a biomarker is to be used as a recruitment criterion in a clinical trial of an innocuous and cheap dietary supplement, but would perhaps be unacceptable for a trial of a novel therapy which carries a significant risk of side effects, and would certainly not be sufficient for use as a diagnostic criterion.

4.2.2.4 Modification by an effective intervention

It would be advantageous if a biomarker were not only capable of identifying the presence of lung injury for example, but that changes in biomarker levels reflected contemporaneous changes in the level of injury. Biomarkers could then be used as an indicator of clinical progression, with resolution of biomarker levels being interpreted as improvement in lung injury.

4.2.2.5 Variation in proportion to the severity of injury

The ability to characterise patients into subgroups according to severity of illness (for example mild, moderate and severe lung injury) could allow early identification of at risk patients and appropriate intervention, prior to the need for critical care unit admission. Similarly, accurate characterisation of severity of illness could facilitate decision making in terms of who to triage to a critical care environment.

4.2.2.6 Association with clinically important outcomes

The need for biomarkers to be associated with clinically important outcomes is self evident. Observation of association with clinical endpoints reflecting severity of the disease process, morbidity and mortality adds validity to a biomarkers selection.

4.2.2.7 Timely, highly reproducible and inexpensively quantified

An ideal biomarker must be timely, both in terms of a timeous change in value in response to intervention, and in result availability. Griffiths et al discuss the potential utility of a biomarker of ALI in guiding titration of ventilator settings in order to minimise ventilator induced lung injury. In order to be clinically useful in such a setting, biomarker levels would need to change in hours rather than days²⁹⁶. Similarly, the result would have to be rapidly analysed and available promptly at the bedside. Biomarker research classically involves batched analysis of frozen samples in a research laboratory setting. After identification of a candidate biomarker, research would be required to provide rapid access to reliable (reproducible) results at the bed side. Undoubtedly, the cost of a biomarker would influence its uptake and the possibility of widespread use; cost-effectiveness analyses would form a necessary part of the biomarkers evaluation.

4.2.3 Classification of biomarkers

Most currently described biomarkers of ALI/ARDS are proteins such as enzymes, receptors, polypeptides, lipoproteins and glycoproteins⁸⁰. Due to the large

number of potential biomarkers, rationale classification is essential. A number of potential classifications have been suggested (Table 4.3).

Table 4.3. Potential classification schemes for ALI/ARD biomarkers

Classification	Sample classes
Clinical	Cause of lung injury: direct, indirect Phase of disease: Early - exudative, late –fibroproliferative
Molecular biologic	Source: Genome, transcriptome, proteome, metabolome
Compartment of origin	Alveolar, vascular, urinary
Pathophysiology	Alveolar-capillary membrane injury, inflammation, activation of coagulation, increased permeability
Cell or tissue of origin	Epithelial, endothelial, extracellular matrix

Table constructed by the author (B Shelley), based on the discussion in Barnett and Ware (2011)⁸⁰.

In practice, a hybrid pathophysiological / cell or tissue of origin classification has been unofficially adopted and is widely used (Figure 4.1)^{80, 289}.

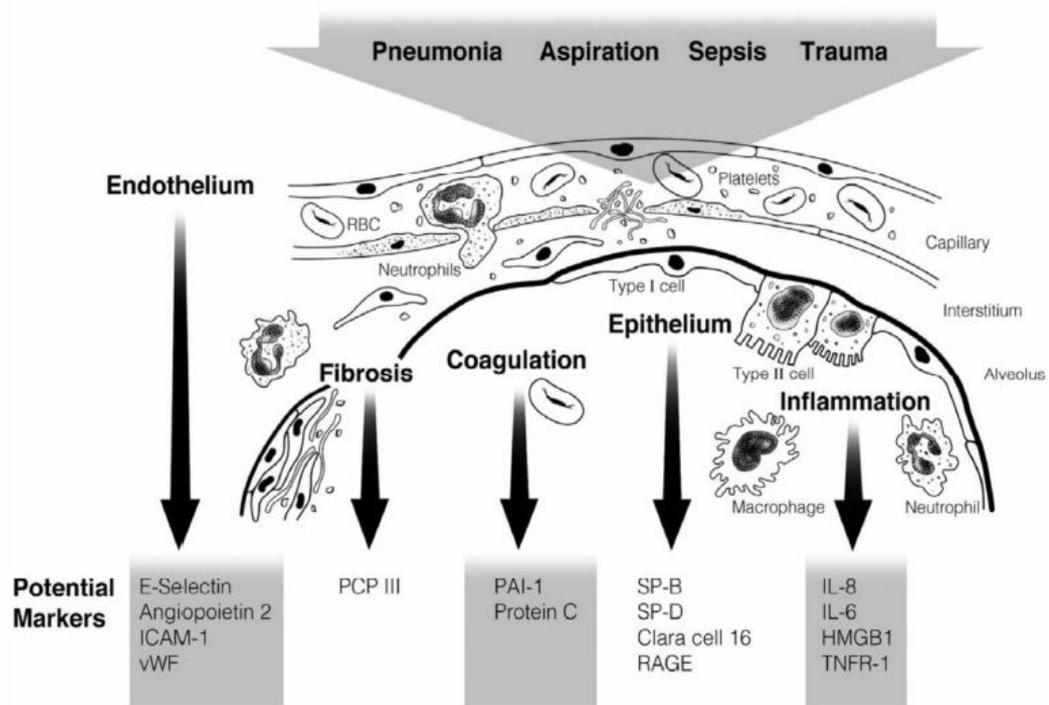


Figure 4.1. Schematic representation of the alveolar capillary interface, demonstrating pathophysiology and source of potential biomarkers of ALI/ARDS.

RBC, red blood cell; T1, type I epithelial cell; T2, type II epithelial cell; ICAM-1, Intercellular adhesion molecule 1; vWF, vonWillebrand factor; PAI-1, Plasminogen activator inhibitor 1; SP, surfactant protein; RAGE, Receptor for Advanced Glycation Products; HMGB1, Human Mobility Group Box 1 protein; TNFR-1, Tumour Necrosis Factor Receptor 1. From Barnett and Ware (2011)⁸⁰.

Such a classification allows identification of biomarkers by their role in the complex pathogenesis of ALI/ARDS. It can easily be understood that a potential therapy for ALI/ARDS which has its mechanism of action through the restoration of epithelial function for example, might be best targeted to a cohort of patients with elevated levels receptor for advanced glycation end-products (RAGE) or surfactant protein D (SP-D).

4.2.4 The role of combining biomarkers into panels

When developing the new 'Berlin' definition of ARDS, the ARDS Definition Task Force considered the potential for biomarkers to be included in the definition concluding that "*despite a large number of candidate biomarkers and genetic markers studied, none has currently demonstrated adequate sensitivity and specificity for use in the diagnosis of ARDS*"⁶⁶. Whilst the taskforce were only considering the use of biomarkers for diagnostic purposes, none have yet been shown to be of value outwith a research setting in order to establish any sort of clinical role. Combination of biomarkers into panels in order to improve validity has become an increasing focus of biomarker research²⁴⁴. To this end, the work of Freemont⁸³, Ware²⁹² and Calfee³⁰³ is worthy of mention.

Fremont et al measured plasma levels of 21 biomarkers in 192 patients admitted to a trauma intensive care unit⁸³. Levels of each biomarker were compared between 107 patients with ALI/ARDS (per the AECC definition) and 74 controls either with no or hydrostatic pulmonary oedema. Following univariate analysis, a backward elimination model was used to select seven biomarkers with the greatest predictive value. A multivariable logistic regression model was then constructed using these seven biomarkers to create a prediction model for the diagnosis of ALI/ARDS. In combination these seven biomarkers predicted ALI/ARDS with an area under the receiving operator characteristic curve(AUROC) of 0.86 (95% CI, 0.82-0.92). Disappointingly the authors provide no estimate of the sensitivity, specificity, nor positive or negative predictive value of the model⁸³.

Ware et al constructed a similar model for prediction of mortality using eight candidate biomarkers in 549 patients with ALI/ARDS recruited to the ARDS Network low tidal volume ventilation trial^{99, 292}. In this study (citing some of the

same authors as Freemont et al's study) the biomarker panel tested included only one biomarker found in the previous panel and had been chosen 'qualitatively' on the basis of previous work. In addition to studying the ability of the biomarker panel to predict ALI/ARDS mortality, Ware et al studied the effect of combining the biomarker panel with known clinical risk factors for ARDS mortality. Six clinical risk factors alone predicted mortality with an AUROCC of 0.82, the eight biomarkers with an AUROCC of 0.76 and a combination of the clinical risk factors and biomarker panel with an AUROCC of 0.85. Relying on the result of a biomarker panel alone to predict outcome in patients with ALI/ARDS, whilst ignoring all existing clinical knowledge appears nonsensical. Combining clinical risk factors and biomarkers in such a fashion appears intuitive and is in keeping with the way biomarkers are used in other conditions, for example diagnosis of acute coronary syndrome where the use of biomarker (Troponin I or T) levels are combined with aspects of the clinical presentation and electrocardiographic changes³⁰⁴.

Both the studies of Freemont et al⁸³ and Ware et al²⁹² have sought to establish biomarker panels without attempt at validation. Calfee et al conducted a more rigorous study examining the prognostic value of a five biomarker panel in combination with clinical risk factors in a derivation cohort derived from the ARDS Network low tidal volume ventilation trial⁹⁹ (n=547) and a replication cohort derived from the ARDS Network higher versus lower positive end-expiratory pressure trial (n=500)^{303, 305}. Biomarkers were selected from a panel of eight biomarkers "previously measured in both cohorts". These eight were reduced to five, using a multivariate logistic regression model and ultimately included five of the same biomarkers used in Ware et al's study²⁹². Clinical risk factors predicted mortality with AUROCC values of 0.68 in the derivation cohort and 0.77 in the replication cohort. Combination of the five biomarker panel with clinical predictors yielded AUROCC values of 0.75 in the derivation cohort and 0.79 in the replication cohort. Once again the authors provide no description of sensitivity or specificity of the models.

It is disappointing that these three studies from high profile research groups, which form the culmination of the current understanding of the role of biomarkers in diagnosis and risk prediction in patients with ALI/ARDS fail to provide a rigorous statistical examination of the utility of the biomarker panels.

4.2.5 Heterogeneity of biomarker expression

As previously highlighted, one of the great barriers to ALI/ARDS research is the significant heterogeneity of the condition of ALI/ARDS. A biomarker or panel of biomarkers which works well for one variant of the disease may lack specificity for another.

Patients with ALI/ARDS of traumatic aetiology are recognised as having lesser mortality than those with non-traumatic aetiology. Though this partially reflects the fact that patients with trauma are often younger with less co-morbid disease, the mortality difference persists even after adjustment for these variables³⁰⁶. Calfee et al examined the biomarker profile of patients with traumatic and non-traumatic ALI/ARDS recruited to the same two US ARDS Network studies³⁰⁶. Biomarkers of acute inflammation (with the exception of Interleukin-6) and of disordered coagulation were no different between the two groups. Biomarkers of endothelial and epithelial cell dysfunction (four in total) however were significantly higher in the non-traumatic group even after adjustment for age, baseline co-morbidities and severity of illness. Furthermore, when the four biomarkers were incorporated as covariates into the multivariate regression model, the mortality difference between groups was attenuated. This, the authors conclude, suggests that patients with non-traumatic ALI are subjected to less endothelial and epithelial cell injury than patients with non-traumatic ALI³⁰⁶.

Septic and non-septic aetiologies for ALI/ARDS form another notable sub-grouping. Moss et al similarly observed differences in biomarkers of endothelial dysfunction between patients with traumatic ALI/ARDS and patients with sepsis and ALI/ARDS; patients with a traumatic aetiology had biomarker levels no higher than controls and significantly lower biomarker levels than patients with ALI/ARDS and sepsis³⁰⁷. Calfee et al observed that Angiopoietin 2 had differential prognostic value for prediction of mortality in septic compared to non-septic patients with ALI/ARDS. Ang-2 levels were similarly elevated in septic survivors and non-survivors but significantly lower in survivors with non-infectious ALI/ARDS than in non-survivors³⁰⁸.

4.3 Literature review: Biomarkers of ALI/ARDS in patients undergoing lung resection

A wide variety of potential ALI biomarkers informative of epithelial injury, endothelial injury, inflammation, oxidative stress and disordered coagulation have been measured in exhaled breath condensate, urine, plasma and bronchoalveolar lavage fluid / epithelial lining fluid in patients undergoing lung resection. The result of these studies are summarised in Tables 7.1-4.

Many of the studies summarised in Tables 7.1-4 were conducted with small sample sizes, often being referred to as 'pilot studies'. Whilst these are informative of what 'signal' might be expected in terms of the trajectory of biomarker levels following lung resection, many make no comparison between biomarker levels and clinical outcomes.

C-reactive protein (CRP)³⁰⁹⁻³¹¹, interleukin (IL)-1 receptor antagonist⁴⁰, interleukin-6^{309, 310, 312}, procalcitonin^{309, 311} and malondialdehyde¹⁶⁵ in plasma were all associated with 'complications', but all of these studies reported an endpoint of composite complications. All of these biomarkers may be considered to be biomarkers of 'systemic inflammation' and it is therefore not surprising that these biomarkers might be associated with 'systemic' complications. Certainly the association between CRP and infectious complications has been well established in a variety of surgical populations³¹³. None of these biomarkers can be considered to be specific to lung injury. With the possible exception of the study of Maeda et al, in which thrombomodulin was associated with post-operative oxygenation (discussed below)³¹⁴, none of the studies reported in Tables 7.1-4 sought nor reported any association between biomarker levels and lung injury.

4.3.1.1 'Lung specific' biomarkers

In the early post-operative period following lung resection, for a biomarker to be informative of pulmonary pathophysiology it is inherently desirable that any biomarker measured is specific to the lung such that any signal from pulmonary inflammation / injury is not over shadowed by the systemic inflammatory

reaction. It would appear however, that measurement of lung specific biomarkers (e.g. biomarkers known to be derived solely from pneumocytes), does not provide a simple solution. Plasma levels of the ‘pulmonary epithelial’ biomarkers surfactant protein D (SP-D)³¹⁵, thrombomodulin³¹⁴, and Krebs von den Lungen-6 (KL-6)³¹⁴⁻³¹⁶ have all been observed to fall post-operatively in proportion to the volume of lung tissue resected.

Bastin et al determined plasma levels of KL-6 and SP-D, pre-operatively, post-operatively and 24 hours post-operatively in 30 patients undergoing lung resection. Both plasma KL-6 and SP-D levels decreased after surgery, with significantly lower levels 24 hours post-operatively than pre-operatively. The ratio of post-operative to pre-operative KL-6 and SP-D was associated with the amount of lung removed, with lower levels after lobectomy compared with lesser resections (Figure 4.2).

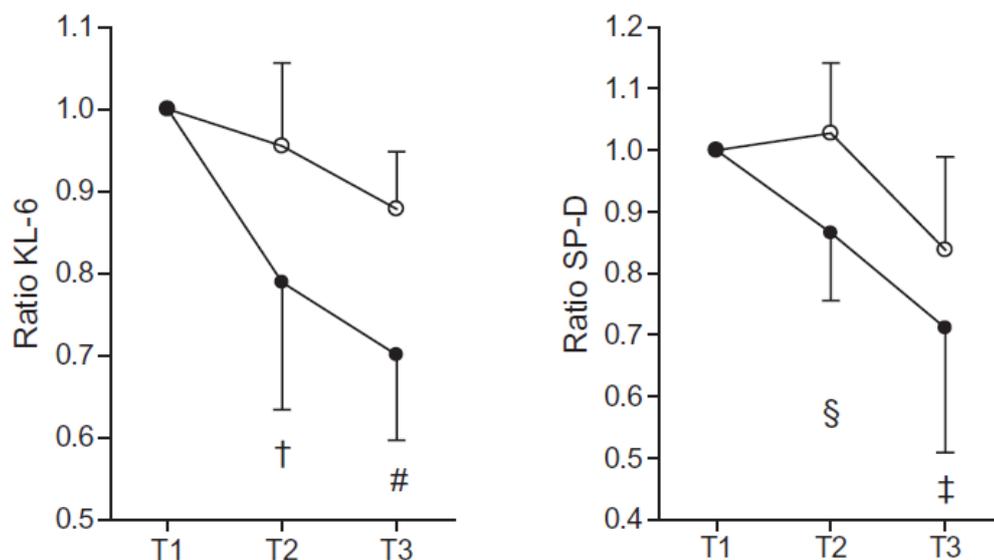


Figure 4.2. Ratio of values of Krebs von den Lungen (KL)-6 and surfactant protein (SP)-D 24 hours and immediately post-operatively versus pre-operatively in patients undergoing lung resection.

Ratio of values at T2 : T1 and T3 : T1 for plasma KL-6 and SP-D after lobectomy (closed circles, $n = 17$) and lesser resection (open circles, $n = 12$); † $p=0.004$, # $p=0.003$, § $p=0.003$, ‡ $P=0.048$ (p-values for lobectomy vs lesser resection). From Bastin et al (2010)³¹⁵.

Similarly observing a fall in KL-6 levels following resection, Sakuma et al introduced the concept that predicted post-operative biomarker levels could be calculated from baseline levels by adjusting post-operative levels according to the residual number of segments remaining post-operatively³¹⁶. By applying this

prediction to KL-6 in 10 patients undergoing lobectomy, the authors observed strong association between the lowest determined post-operative levels and predicted levels ($r=0.89$; $p<0.005$). KL-6 levels were observed to be at their lowest around post-operative day seven and had not returned to baseline by one month post-operatively³¹⁶.

Maeda et al applied the same adjustment to KL-6 and thrombomodulin values determined from 60 patients undergoing lung resection and observed a similar association between predicted and actual post-operative levels ($R^2=0.82$ and 0.62 for KL-6 and thrombomodulin respectively)³¹⁴. Mean actual post-operative thrombomodulin levels were however, higher than predicted ($p=0.0002$). Three of eight patients undergoing lobectomy who had increased (actual and so markedly higher than predicted levels) post-operative thrombomodulin levels had impaired oxygenation post-operatively whilst none of 34 patients demonstrating a fall in actual post-operative levels had impaired oxygenation (no statistical comparison made). The authors conclude that the increased levels of thrombomodulin post-operatively relative to predicted reflect a combination of non-pulmonary thrombomodulin production and pulmonary endothelial injury (thrombomodulin is widely but not exclusively expressed in the pulmonary endothelium and is described as being shed from the endothelium in response to injury), thus patients with markedly increased levels might be considered at risk of clinical sequelae of lung injury³¹⁴.

4.4 Literature review: Pentraxin 3 as a candidate biomarker of lung injury following lung resection

Pentraxin 3 (PTX3) is a novel acute phase protein with structural and familial links to C-reactive protein. In this section I shall discuss the function of PTX3, review its role in the innate immune response (specifically within the lung) and present the case for PTX3 as a candidate biomarker of lung injury following lung resection.

4.4.1.1 Function of Pentraxin 3

Pentraxins are a super-family of conserved proteins which play a key role in innate immunity and regulation of the acute inflammatory response. C-reactive protein (CRP) and serum amyloid P component (SAP) are classical short chain pentraxins and are produced in the liver in response to inflammatory stimuli. In contrast, Pentraxin 3 (PTX3), the first long chain pentraxin to be recognised is rapidly produced and released from a variety of cell and tissue types in response to primary inflammatory signals³¹⁷. As such, in comparison to CRP, PTX3 may be more reflective of localised activation of innate immunity and inflammation than of the systemic host response³¹⁸. Pentraxin 3 behaves as an acute phase protein; normal plasma PTX3 levels are less than 2ng/ml, but can increase to many times this level (up to 200-800ng/ml) during sepsis and other inflammatory conditions^{319, 320}. Pentraxin 3 was originally identified as an immediate early gene; levels peak at six to eight hours following induction, considerably more rapidly than CRP^{320, 321}. Studies measuring CRP and PTX3 in parallel have demonstrated weak or non significant association, suggesting PTX3 measurement may offer further or alternative information rather than simply being ‘another CRP’^{319, 320}.

4.4.1.2 PTX3 expression in the lung

PTX3 expression may be induced by toll-like receptor agonists (e.g. lipopolysaccharide), primary inflammatory cytokines, interleukin-10 and oxidised low-density lipoprotein³²⁰. Several *in-vitro*, animal and clinical studies have demonstrated the increased expression and downstream effects of PTX3

expression in alveolar epithelial cells and endothelial cells in response to stimuli implicated in the pathogenesis of ALI/ARDS.

Alveolar epithelial cells have been identified as a potent source of PTX3. PTX3 gene and protein expression is induced in alveolar epithelial cells directly by tumour necrosis factor- α (TNF- α) and interleukin-1 (IL-1 β) and indirectly by lipopolysaccharide³²². Similar up-regulation of PTX3 expression has been observed in human endothelial cells in response to LPS / PeptidoglycanG, TNF- α and IL-1 β ^{323, 324}. (It should be noted that to date studies examining the interaction between PTX3 and endothelial cells have been carried out in endothelial cell lines of non-pulmonary origin, commonly human umbilical vein endothelial cells).

PTX3 expression is induced by mechanical stretch

Wu et al examined the effects of mechanical stretch on PTX3 release by alveolar epithelial cells *in vitro*³²⁵. Seeking to mimic the effects of ventilator-induced lung injury, cells were subject to 'tightly controlled and physiologically relevant cyclic mechanical stretch'. Cells subjected to cyclic elongation of 20% demonstrated increased PTX3 gene expression, release of PTX3 protein and induced apoptosis. In addition there was strong correlation between PTX3 expression and the magnitude of apoptosis³²⁵.

PTX3 expression and oxidative stress

The observation of a binding site for the redox-sensitive transcription factor nuclear factor $\kappa\beta$ (NF- $\kappa\beta$) and the recognition that oxidative stress has consistently been demonstrated in patients with sepsis (as it also has in patients with ALI/ARDS¹⁶⁰), led Galley et al to examine the effects of antioxidants on PTX3 expression³²⁶. In line with previous reports, PTX3 expression from endothelial cells was increased in response to stimulation with lipopolysaccharide / peptidoglycan G, TNF- α and IL-1 β . Co-administration of the antioxidants N-Acetylcysteine and Trolox led to reduced PTX3 expression suggesting that in-vitro at least, PTX3 expression is regulated by antioxidants³²⁶.

PTX3 and endothelial dysfunction

Binding of P-selectin to P-selectin glycoprotein-1 expressed on the surface of neutrophils, facilitates rolling and tethering of neutrophils and transendothelial

migration to extravascular sites of inflammation. PTX3 binding to endothelial P-selectin causes relocation of this molecule to the cell surface³²⁷.

PTX3 expression in response to ischaemia-reperfusion

Increased serum levels of PTX3 have been recorded in patients and animals undergoing an ischaemic event, in the heart³²⁸, kidney³²⁹ or intra-abdominally³³⁰. In the lung, increased PTX3 levels have been associated with increased risk of primary graft dysfunction (a process in which ischaemia-reperfusion injury is implicated) after lung transplantation³³¹.

4.4.1.3 PTX3 in ALI/ARDS

PTX3 in animal models of ALI/ARDS

PTX3 is highly conserved in evolutionary terms, meaning results obtained from rodent studies are likely to be reflective of PTX3 activity in humans^{317, 319}. Okutani et al determined PTX3 expression in the lung in a variety of rat models of ALI³³². ALI was induced by either intravenous administration of lipopolysaccharide (LPS) or haemorrhage followed by resuscitation (HR) and rats were then subjected to high volume (12ml/kg, no PEEP) or low volume ventilation (6ml/kg, 5cmH₂O PEEP). High volume ventilation enhanced PTX3 expression in sham animals (no ALI) and in both models of ALI. Furthermore, the expression of PTX3 correlated with the severity of lung injury as determined by oxygenation, lung elastance and wet to dry ratio ($r \geq 0.6$; $p < 0.0001$ for all). Importantly, the same signal was seen when PTX3 activity was quantified by measurement of PTX3 mRNA expression in the lung or PTX3 protein expression in lung or serum. In a second set of experiments, in order to evaluate the effect of injurious ventilation on PTX3 expression in isolation, ALI was induced by injurious ventilation (25ml/kg) or by LPS administration or by HR alone (i.e. no mechanical ventilation in LPS/HR groups). Whilst LPS and HR induced little lung injury, injurious ventilation was associated with significant histological evidence of lung injury, increased wet to dry lung ratio and enhanced expression of PTX3³³².

He et al observed that PTX3 protein levels in bronchoalveolar lavage (BAL) fluid were associated with the severity of lung injury in a murine model³³³. Mice were subjected to increasing doses of intratracheal LPS administration. He et al

additionally explored the interaction between PTX3 and tissue factor (TF) activity. TF activity was positively correlated with PTX3 expression. The authors then administered human anti-TF antibody to 'humanised' transgenic mice, subjected to the same LPS model. Lung injury was reduced as evidenced by reduced lung injury score and BAL cell count in antibody treated animals. Importantly antibody administration also reduced plasma TF activity and the expression of PTX3 in BAL fluid and lung tissue³³³.

Real et al subjected genetically modified mice, both deficient and over expressing the murine PTX3 gene to lung injury induced by high tidal volume ventilation³³⁴. PTX3 deficient and wild type mice developed a similar degree of lung injury whilst PTX3 over expression led to more rapid development of lung injury³³⁴. These findings are in keeping with the hypothesised role of PTX3 in excess being responsible for causing enhanced injury.

Pentraxin 3 in human ALI/ARDS

Mauri et al measured plasma PTX3 levels in 21 patients with ALI/ARDS²⁹¹. Pentraxin 3 levels on day one were observed to be significantly different between survivors and non-survivors (median 65 versus 100 ng/ml respectively; $p=0.04$), where SOFA and SAPS-II score were not. In addition, PTX3 levels correlated positively with lung injury score, number of organ failures and SOFA score. BAL was performed 'when clinically indicated'; BAL PTX3 levels were correlated with plasma PTX3 levels in 13 patients ($r^2=0.368$; $p<0.01$)²⁹¹.

Pentraxin 3 as a candidate ALI/ARDS biomarker

Though the literature surrounding PTX3 is in its infancy, there is much to suggest that PTX3 may have a role as a biomarker of lung injury. PTX3 has a biologically plausible role in the pathogenesis of ALI and being produced locally (though not exclusively) in the lung in response to tissue injury and inflammation has the potential to provide a more lung specific signal. Studies measuring PTX3 in pulmonary tissue (and mRNA), bronchoalveolar lavage specimens and plasma have provided similar results suggesting serum may be a safe and easily obtainable source for the sample^{291, 332}. PTX3 has been associated with severity of illness in animal models of ALI^{332, 333} and in a single human study of patients with the condition²⁹¹. In addition in humans PTX3 appears to be associated with ALI/ARDS mortality²⁹¹. Furthermore, in animal models at least, PTX3 levels

appear to respond to therapeutic interventions (both low tidal-volume ventilation³³² and anti-tissue factor antibody³³³). Though further testing is required, the early data suggests that PTX3 satisfies many of the requirements of the 'ideal' ALI biomarker.

PTX as a biomarker of ALI after lung resection

The pathogenesis of post-lung resection acute lung injury is discussed in detail in Chapter 1. There have been no reported studies of PTX3 as a biomarker of ALI in one-lung ventilation models of lung injury or in humans undergoing lung resection. Nonetheless, there are several features of the above discussion that make PTX3 an attractive candidate as a biomarker following lung resection. Firstly, the observation that PTX3 is induced by mechanical stretch and appears to behave as a biomarker of ALI in ventilator induced lung injury (VILI) models is suggestive, as VILI is implicated in the injury to the dependent, ventilated lung during lung resection. Secondly, the observation that PTX3 expression is regulated by red-ox balance; oxidative stress has been implicated in injury to both lungs during lung resection. Finally, the observation of increased PTX3 expression following ischaemia and reperfusion, a mechanism responsible for injury to the non-ventilated, operative lung in all sub-total lung resections is suggestive of a potential role for PTX3 as a biomarker of lung injury following lung resection.

4.5 Methods

This investigation comprises two discrete studies examining the utility of Pentraxin 3 (PTX3) and a multiple (lung injury) biomarker panel following lung resection. The PTX3 study was conducted in a cohort of 35 patients undergoing lung resection of presumed primary lung cancer, whilst the multiple biomarker panel study was conducted in a subset of 22 patients from the same cohort. The methods are presented as ‘generic methods’, which are common to both studies and then individually for each study. Comparison is made to the properties of the ‘ideal’ lung injury biomarker defined in Section 4.2.2 (Table 4.1).

4.5.1 Generic Methods

4.5.1.1 Ethical approval

This study received ethical approval from the West of Scotland Research Ethics Committee 2 (REC reference 10/S0709/43). The additional measurement of the multiple biomarker panel was approved as a substantial amendment (AM-01).

4.5.1.2 Patient population

Thirty five patients undergoing elective lung resection at the Golden Jubilee National Hospital were recruited to the study by the author (Ben Shelley). Inclusion criteria were the provision of informed consent, age greater than 16 years and planned elective open lung resection (by lobectomy or pneumonectomy) for presumed primary lung cancer. Patients were excluded if they were pregnant, had ongoing participation in any investigational research which could undermine the scientific basis of this study, were undergoing lung resection for non malignant disease or secondary malignancy, were planned to undergo a wedge / segmental lung resection, or a resection via a thoracoscopic / minimal access technique or were taking over the counter ‘vitamin’ / ‘antioxidant’ medication.

All patients underwent a postero-lateral muscle sparing thoracotomy, lung resection and mediastinal lymph node sampling as appropriate carried out by a single surgeon. Anaesthetic technique was standardised to total intravenous

anaesthesia with Propofol and Remifentanyl; post-operative analgesia was provided by thoracic epidural blockade. Lung separation was achieved by double lumen endotracheal intubation (Mallinckrodt™, Medtronic, Dublin); the choice of left or right sided double-lumen endotracheal tube was left to the discretion of the anaesthetist responsible for the case.

A smaller cohort of 22 patients were subsequently selected from the 35 patient cohort for the measurement of the multiple biomarker panel. This was a sample of convenience, with selection based simply on the availability of sufficient plasma samples to allow biomarker measurement.

4.5.1.3 Laboratory sampling

Biomarker sample handling

Twenty millilitres of arterial blood was collected immediately prior to induction of anaesthesia (referred to hereafter as ‘pre-operatively’), immediately post-operatively following admission to the post-operative care unit (‘post-operatively’), and approximately 24 hours post-operatively in the high dependency unit (‘24 hours post-operatively’). All blood samples were taken from a radial arterial cannula where possible (routinely inserted prior to induction of anaesthesia), to avoid unnecessary venepuncture. Where an arterial cannula was no longer in situ at twenty-four hours post-operatively, a venous sample was obtained. Samples were collected in vacuum filled containers which were filled to the marked line. Samples were collected as follows:

- 1 x 4ml “LH Lithium Heparin” (Green top) tube (BD Vacutainer®)
- 2 x 4ml “Serum Sep Clot Activator (Yellow top) tube (BD Vacutainer®)

All samples were transported to the clinical biochemistry laboratories at GJNH immediately after collection. One ‘yellow top’ (SST) bottle was processed as a routine clinical sample for the measurement of C-reactive protein (below). All other samples were centrifuged at room temperature immediately upon receipt, before plasma was manually separated and aliquoted into 0.5ml aliquots. Aliquots were frozen immediately and stored at -70°C prior to analysis.

4.5.1.4 Pre-operative data collection

Patient demographics

Patient demographics were collected prospectively at the time of recruitment by face to face questioning and by extraction of data from the patients' 'paper' and 'electronic' medical notes. Data was collected on a dedicated case report form.

Smoking history

Smoking history was explored with the patient at recruitment. Patients were categorised into 'current smokers' (smoking regularly sometime in the last month), 'ex-smokers' (complete abstinence for greater than one month), and 'never smokers'. Smoking history was quantified in pack years according to standard formulae³³⁵.

Pre-operative pulmonary function

Data was extracted from routinely performed pulmonary function test (PFTs) results available within the paper or electronic medical record. When carried out at Golden Jubilee National Hospital, tests were performed by trained respiratory physiologists according to standard guidelines³³⁶⁻³³⁹. Occasionally where PFTs were performed in the referring hospital, complete results were not available and results were extracted from referral letters. Where more than one set of PFTs had been performed, data was extracted from the most recent. The following parameters were collected: forced expiratory volume in one second (FEV₁ - absolute value and as percent predicted), forced vital capacity (FVC), the ratio of FEV₁ to FVC and diffusing capacity for carbon monoxide (DLCO - absolute and percent predicted). Oxygen saturation (on air at rest) was routinely recorded by nursing staff on admission to the ward pre-operatively.

Pre-operative functional status

At recruitment, a detailed history was taken from each patient concerning pre-operative functional status. Patients were 'talked through' the World Health Organisation / Zubrod Performance status scale³⁴⁰, the New York Heart Association (NYHA) functional classification³⁴¹ and the Medical Research Council (MRC) breathlessness scale³⁴² in order to classify functional status. Detail was sought concerning limitation in exercise function or the performance of

activities of daily living in order to quantify pre-operative functional status in metabolic equivalents (METS) according to standard definitions³⁴³.

Co-morbidities

In the derivation of the Thoracoscore (below), number of co-morbidities was an independent predictor of hospital mortality³⁴⁴. In this study, 95% of the co-morbidity was related to 10 major diagnoses; data was therefore sought on these 10 co-morbidities. Co-morbidities were defined either as described by Falcoz et al, or from accepted definitions as follows:

1. **Smoking addiction** - defined as *'currently smoking regularly or abstinent for less than one month'*³⁴⁵³⁴⁶³⁴⁶³⁴⁶.
2. **History of cancer** - *history of diagnosis or treatment for any previous cancer (not including current presentation).*
3. **Chronic obstructive pulmonary disease** - defined as per the *'Global Strategy for Management and Prevention of COPD (GOLD)'* (updated 2010)³⁴⁶.
4. **Arterial hypertension** - *'either documented history of hypertension diagnosed and treated with medication, diet and/or exercise or currently on pharmacologic therapy to control hypertension'*³⁴⁷.
5. **Heart disease** - *'history of either coronary artery disease (CAD) or congestive cardiac failure (CCF).*
 - *CAD as evidenced by one of the following:*
 - *Currently receiving medical treatment for CAD,*
 - *History of Myocardial Infarction,*
 - *Prior cardiovascular intervention including, but not limited to, CABG and/or PCI'*³⁴⁷.
 - *CCF - physician documentation or report of clinical symptoms of heart failure'*³⁴⁷.
6. **Diabetes mellitus** - *'history of diabetes diagnosed and/or treated by a physician'*³⁴⁷.
7. **Peripheral vascular disease** - indicated by *'claudication either with exertion or rest; amputation for arterial insufficiency; aorto-iliac occlusive disease reconstruction; peripheral vascular bypass surgery, angioplasty, or stent; documented AAA, AAA repair, or stent; positive non-invasive testing documented'*³⁴⁷.
8. **Obesity** - *'Body mass index greater than 30'*³⁴⁸.
9. **Alcoholism** - *'alcohol abuse was recorded if there was a documented (recent or ongoing) history of alcoholism or alcohol related medical diagnoses'.*

10. **Hyperlipidaemia** - *'history of hyperlipidaemia diagnosed and/or treated by a physician'*.

Pre-operative risk scoring

American Society of Anaesthesiologists grade

Following collection of demographic data, exploration of functional status and recording of patient co-morbidities, a subjective assessment of American Society of Anaesthesiologists (ASA) grade was made by the author (Ben Shelley).

Thoracoscore

The Thoracoscore was described by Falcoz et al, who derived the score in 15,183 patients undergoing thoracic surgery³⁴⁹. The score is derived by calculation from a regression equation where coefficients are awarded based on parameters found to be significantly associated with in-hospital death on multivariate analysis: patient age, sex, MRC dyspnea score, American Society of Anesthesiologists score, WHO/Zubrod performance status classification, priority of surgery (elective / urgent or emergency), diagnosis group (benign/malignant), procedure class (pneumonectomy / other), and the presence of comorbid disease (number of co-morbidities from the ten described above)³⁴⁹.

Surgical Lung Injury Prediction score

The Surgical Lung Injury Prediction score (SLIP), was described by Kor et al, who derived the score in 4366 patients undergoing surgery with anaesthesia lasting greater than three hours¹⁰⁵. The score is easily calculated from readily available pre-operative risk factors spread over three domains: high risk surgical procedures (cardiac, vascular and thoracic), co-morbidities (diabetes mellitus, COPD or gastro-oesophageal reflux disease) and modifying conditions (alcohol abuse). In the derivation cohort (which included 646 thoracic surgical patients, 19 of whom developed lung injury), the SLIP was able to predict patients who developed early post-operative ALI with an area under the receiver operating characteristic curve of 0.82 (95% CI 0.78-0.86)¹⁰⁵.

SLIP was determined per the description of Kor et al¹⁰⁵ with the following exceptions:

1. Alcohol abuse was recorded if there was a documented (recent or ongoing) history of alcoholism or alcohol related medical diagnoses, or the

patient reported drinking greater than 28 units of alcohol per week (in keeping with Kor et al's definition of "more than 14 alcohol-containing drinks per week")¹⁰⁵.

2. The incidence of gastro-oesophageal reflux disease (GORD) was not collected prospectively; as such the regular prescription of an H₂ receptor antagonist or proton pump inhibitor was recorded as a surrogate of GORD.

4.5.1.5 Intra-operative data collection

Intra-operative ventilatory parameters

Ventilatory parameters were collected continuously (and automatically) for the duration of the anaesthetic by the Recall AIMS electronic anaesthetic charting system (Informatics Clinical Information Systems Limited, Glasgow); data is recorded by the system at approximately 20 second intervals. Data concerning tidal volume (V_T), peak airway pressure (P_{peak}) and fraction of inspired oxygen (FiO_2), were extracted against time, both for the duration of the operation, and during the period of one-lung ventilation (OLV). The area under the parameter vs time curve was determined in Microsoft Excel by the trapezoidal method in order to provide an index of cumulative exposure to each variable.

Duration of surgery and one-lung ventilation

Time of commencing anaesthesia was determined as the onset of consistent, stable end-tidal carbon dioxide (ETCO₂) recording (representing intubation). Similarly extubation (anaesthetic end) was determined as the time that ETCO₂ recording was lost. In most cases the start and end of the period of one-lung ventilation were prospectively recorded in the RECALL system by the primary anaesthetist. Where this was not recorded, the onset and offset of OLV were determined by manual inspection of the V_T , P_{peak} and ETCO₂ vs time curves.

4.5.1.6 Post-operative data collection

Clinical endpoints

Oxygenation

The ratio of partial pressure of arterial oxygen (PaO_2) to fraction of inspired oxygen (FiO_2) ($\text{PaO}_2/\text{FiO}_2$ ratio) was derived from routinely measured arterial blood gas analyses (Siemens RAPIDLAB[®], Siemens, Munich, Germany). PaO_2 was not adjusted for patient temperature. The timing and frequency of arterial blood gas analysis was not protocolised.

The fraction of inspired oxygen (FiO_2) was recorded at the time of arterial blood gas analysis by the HDU nurses. Oxygen therapy was either provided by facemask via a humidified Kendall[™] Nebulizer Adapter (Covidien, Dublin, Ireland), where FiO_2 was estimated according to the set FiO_2 on the venture device or via nasal cannulae (Intersurgical, Wokingham, Berkshire) where FiO_2 was estimated according to Table 4.4.

Table 4.4. Estimation of FiO_2 based on nasal cannula flow.

Oxygen flow rate (L/min)	Estimate FiO_2 (%)
1	24
2	28
3	32
4	36

In order to examine trends in oxygenation over time, and determine oxygenation contemporaneously with chest x-ray and biomarker sampling, mean $\text{PaO}_2/\text{FiO}_2$ ratio was determined in 6 hourly segments; $\text{PaO}_2/\text{FiO}_2$ ratio at 6 hours post-operatively was determined as mean $\text{PaO}_2/\text{FiO}_2$ ratio of all ABGs obtained from 3:00-8:59 (hours:minutes) post-operatively. Mean $\text{PaO}_2/\text{FiO}_2$ ratios at 12, 18, 24, 30, 36 and 42 hours post-operatively were determined in the same manner.

In order to allow assessment of oxygenation in patients where ABG results were not available, or at time points where ABG sampling was not performed, the ratio of saturation of oxy-haemoglobin (SaO_2) to FiO_2 was determined ($\text{SaO}_2/\text{FiO}_2$). SaO_2 and FiO_2 are collected routinely in the hospital critical care electronic records system (Centricity CIS, GE Healthcare, Wilmington, Massachusetts), and so were available at the vast majority of time points. $\text{SaO}_2/\text{FiO}_2$ has been advocated as a surrogate for $\text{PaO}_2/\text{FiO}_2$ ratio in the diagnosis

of ALI/ARDS when $\text{PaO}_2/\text{FiO}_2$ ratio is unavailable^{350, 351}. Rice et al compared $\text{SaO}_2/\text{FiO}_2$ to $\text{PaO}_2/\text{FiO}_2$ ratio in 1074 patients with ARDS, demonstrating (in their derivation cohort; 672 patients, 2673 data points) that there was a strong linear relationship between $\text{PaO}_2/\text{FiO}_2$ and $\text{SaO}_2/\text{FiO}_2$ ratio ($\text{SaO}_2/\text{FiO}_2$ ratio = $64 + 0.84 \times [\text{PaO}_2/\text{FiO}_2]$; $r=0.89$; $p<0.0001$)³⁵⁰. Prior to use of $\text{SaO}_2/\text{FiO}_2$ as a study endpoint, the validity of $\text{SaO}_2/\text{FiO}_2$ as a surrogate for $\text{PaO}_2/\text{FiO}_2$ in this cohort was explored. $\text{PaO}_2/\text{FiO}_{2\text{calculated}}$ was calculated from $\text{SaO}_2/\text{FiO}_2$ and compared to paired measured values of $\text{PaO}_2/\text{FiO}_2$ ($\text{PaO}_2/\text{FiO}_{2\text{measured}}$) by testing of linear association and Bland-Altman analysis.

$\text{PaO}_2/\text{FiO}_2$ ratio and $\text{SaO}_2/\text{FiO}_2$ ratio were analysed both as continuous variables, and dichotomised into groups with $\text{PaO}_2/\text{FiO}_2$ ratio <300 and $\geq 300\text{mmHg}$ (equivalent $\text{SaO}_2/\text{FiO}_2$ ratio <316 or ≥ 316) in order to identify cohorts of patients with 'good' and 'poor' post-operative oxygenation.

Chest X-ray scoring

Chest X-ray scores were dual reported by the author (Ben Shelley, BS), and Dr Oona Tanner (OT - specialist trainee in anaesthesia, West of Scotland School of Anaesthesia and advanced intensive care medicine trainee). Images were provided to the reviewers in electronic format, anonymised and randomised by the hospital Picture Archiving and Communication System (PACS) administrator who had no other role in the conduct of the study. Image analysis took place in one sitting. Images were viewed electronically on the Centricity DICOM viewer v3.1.2 (GE Healthcare, Wilmington, Massachusetts). As neither the author nor Dr Tanner had any direct clinical role in caring for the study patients, neither reviewer had seen any of the images in any context prior to analysis.

In line with the findings of Meade et al³⁵² and the recommendations of the ARDS Definition Task Force⁶⁶, a 'consensus process' was undertaken prior to X-ray scoring. Following agreement on a scoring system, BS and OT worked through the training guide "*Chest X-Ray Interpretation for the Diagnosis of ARDS*" provided by the ARDS Definition Task Force⁶⁶, initially scoring films independently, and then together allowing discussion of the reasons for discrepancy and consensus to be reached concerning the application of the scoring proforma.

Chest X-rays were reported according to a novel scoring system devised by the author (Ben Shelley). Reporters were first asked to confirm the technical acceptability of the CXR film and to count the number of ‘scoreable’ quadrants of the X-ray. In this way, quadrants where lung had been excised (e.g. the two quadrants of a hemi-thorax where pneumonectomy had taken place) or where there was residual pneumothorax (e.g. incomplete re-expansion of residual lung tissue following lobectomy) were excluded from scoring.

Reporters were then asked to score the presence of opacities in each ‘scoreable’ quadrant. Opacities were defined as “*opacities consistent with pulmonary edema (may be very mild, patchy, and asymmetric) that are not fully explained by pleural effusions, pulmonary nodules or masses, or lobar/lung collapse (i.e., radiographically consistent with the diagnosis of ARDS)*” as per the guidance provided by the ARDS Definition Task Force⁶⁶.

Rather than simply scoring presence or absence of opacity, in order to provide more sensitive discrimination between images, scores (per quadrant) were then awarded according to the intensity of infiltrate as previously advocated by Yang et al and Ahn et al^{166, 218} (Table 4.5).

Table 4.5. Awarding of scores during chest X-ray scoring.

	Score
No opacities	0
Presence of opacities occupying:	
<1/3 of the quadrant	1
1/3 to 2/3 of the quadrant	2
>2/3 of the quadrant	3

Quadrant scores were added to provide an overall score. This score was then corrected according to the number of ‘scoreable’ quadrants to provide a total CXR score (minimum score = 0, maximum score = 12). The mean of the reviewers overall scores was then calculated to provide a ‘combined’ score which was used for analysis.

Modified Lung injury score

Lung injury score (LIS) was calculated in line with the principals of the original LIS described by Murray et al⁵⁴. This was modified by the author (Ben Shelley) to allow use in the early post-operative period following lung resection as follows:

Oxygenation

Oxygenation was determined as described above. Where $\text{PaO}_2/\text{FiO}_2$ was not available, $\text{SaO}_2/\text{FiO}_2$ was substituted according to the cut points described in Table 4.6.

Chest radiography score

Chest X-ray interpretation for the derivation of the lung injury score as originally described is derived as a simple count of the number of CXR quadrants containing 'alveolar consolidation'. CXR scoring for derivation of the LIS was performed alongside scoring for CXR score detailed above. The number of quadrants in which opacities of any extent (consistent with the definitions of the ARDS Definition Taskforce) were observed was counted, and similarly corrected based on the number of 'scoreable' quadrants. Again a mean 'combined' score calculated from each review's score was used for analysis. Use of a combined score for analysis allowed for non integer values of the overall modified LIS (mLIS).

Table 4.6. Derivation of the 'modified' Lung Injury Score (mLIS)

Chest X-ray score		Quadrant score
No opacities consistent with pulmonary oedema		0
Opacities consistent with pulmonary oedema confined to 1 quadrant		1
Opacities consistent with pulmonary oedema confined to 2 quadrants		2
Opacities consistent with pulmonary oedema confined to 3 quadrants		3
Opacities consistent with pulmonary oedema confined to 4 quadrants		4
Overall CXR score derived as (quadrant score / number of 'scoreable' quadrants) x 4		
Hypoxaemia score		Score
(based on $\text{SaO}_2/\text{FiO}_2$ only if $\text{PaO}_2/\text{FiO}_2$ not available)		
$\text{PaO}_2/\text{FiO}_2 \geq 300$	(or $\text{SaO}_2/\text{FiO}_2 \geq 316$)	0
$\text{PaO}_2/\text{FiO}_2$ 225-299	(or $\text{SaO}_2/\text{FiO}_2$ 253-315)	1
$\text{PaO}_2/\text{FiO}_2$ 175-224	(or $\text{SaO}_2/\text{FiO}_2$ 211-252)	2
$\text{PaO}_2/\text{FiO}_2$ 100-174	(or $\text{SaO}_2/\text{FiO}_2$ 148-210)	3
$\text{PaO}_2/\text{FiO}_2 < 100$	(or $\text{SaO}_2/\text{FiO}_2 < 148$)	4
Total score = (Overall CXR score + Hypoxaemia score) / 2		

PEEP and Compliance

No patients in the cohort required positive pressure ventilation in the first 48 hours post-operatively and so no 'PEEP score' nor 'Compliance score' would be available.

Ventilatory support

Ventilatory support was defined as any of intubation and mechanical ventilation, non-invasive ventilation or high flow nasal oxygen during the first 48 hours post-operatively. The airway interface is routinely recorded in the CIS critical care unit electronic charting system and so need for ventilatory support could be deduced by interrogation of the database.

Length of stay

High dependency unit stay

High dependency unit (HDU) admission was defined as the time of the first recorded oxygen saturation recorded in the CIS critical care unit electronic charting system. As the timing of actual discharge from the HDU can vary considerably from the time of fitness for discharge for logistical reasons (such as time of day and availability of ward beds), for the purposes of this study, HDU discharge was defined as the time continuous oxygen saturation recording ceased. This is believed to reflect the timing of 'step-down' of perceived patient dependency (e.g. level 1 patients nursed in the HDU are often stepped down to four hourly intermittent vital signs observations rather than the continuous monitoring that is provided to level 2 patients). One patient was admitted from HDU to the intensive care unit and subsequently died. HDU stay for this patient was right censored and the patient given a value equivalent to the longest recorded length of stay.

Hospital stay

Whilst it is routine for patients to be admitted to our institution on the day before major lung resection, again for logistical reasons some patients are admitted earlier (e.g. some patients travel a considerable distance to access care at Golden Jubilee National Hospital and as such are often admitted for several days pre-operatively to allow time for clinical investigations and pre-operative assessment). 'Hospital stay' for the purposes of the study was therefore defined as 'post-operative' hospital stay, that is the number of days elapsed between the day of surgery and the day of discharge. One patient died in hospital; hospital stay for this patient was right censored and the patient given a value equivalent to the longest recorded length of stay.

4.5.2 Utility of Pentraxin 3 as a biomarker of post-lung resection lung injury

4.5.2.1 Biomarker measurement

Pentraxin 3 measurement

Pentraxin 3 was measured in duplicate using the commercially available 'Duo Set' enzyme immunoassay system (R and D Systems Europe Ltd, Abington, Oxon, UK) within the laboratories of Aberdeen University Academic Unit of Anaesthesia and Intensive Care under the supervision of Professor Helen Galley.

Briefly, 96 well plates were prepared by coating with anti-PTX3 monoclonal antibody (the 'capture' antibody) and incubating overnight. Following washing, 100µL recombinant human PTX3 (as a calibration standard) or plasma was added to each well. After incubation for 2 hours, plates were then washed again and then incubated with a biotinylated anti-PTX3 polyclonal antibody (the 'detection' antibody) for 40 minutes. Following a further wash, samples were incubated for an hour with streptavidin-horseradish peroxidase, then a chromagen substrate, before the reaction was stopped by administration of hydrochloric acid. The reaction was then quantified spectrophotometrically at a wavelength of 450nm. A standard curve was generated from the averaged calibration standards, from which sample results were determined by comparing the optical density of the samples to the standard curve. The coefficient of variation of this assay was 5.7%.

C-reactive protein measurement

Samples for measurement of CRP were processed as routine clinical samples in the clinical biochemistry laboratories at Golden Jubilee National Hospital under the supervision of Dr Frank Findlay. CRP was determined by enhanced immunoturbidimetric assay run on a Roche Cobas 6000 analyser. The reference range is <10mg/L, with a lower limit of detection of 1.0 mg/L and a coefficient of variation (CV) of 1.72% at a CRP level of 18.9mg/L and CV of 1.75% at CRP level of 42.6 mg/L.

4.5.2.2 Comparison with the properties of the ideal ALI biomarker

Sensitivity and specificity in predicting outcomes of interest

The sensitivity and specificity of PTX3 in predicting increased modified lung injury score (mLIS), oxygenation and CXR score was assessed both contemporaneously (PTX3 measurement simultaneously with oxygenation, CXR score and mLIS measurement), reflecting 'diagnostic' value of PTX3 and in respect to future values (biomarker levels 24 hours post-operatively and oxygenation, CXR score and mLIS measurement between 24 and 48 hours post-operatively) reflecting 'prognostic' value of the biomarker.

Specifically, in order to examine the diagnostic value of PTX3, comparison was made between plasma PTX3 concentration 24 hours post-operatively and PaO₂/FiO₂ 24 hours post-operatively and CXR score and mLIS at approximately 24 hours postoperatively (on post-operative day one). In order to examine the prognostic value of PTX3, plasma PTX3 concentration 24 hours post-operatively was compared with 'worst recorded' oxygenation from 24-48 hours post-operatively and CXR score and mLIS recorded on post-operative day two.

Variation in proportion to the severity of injury

To examine whether PTX3 or CRP levels vary in proportion to the severity of oxygenation impairment, association was sought between PaO₂/FiO₂ ratios at 24 hours post-operatively (as a continuous variable) with biomarker levels determined 24 hours post-operatively. To examine whether PTX3 or CRP levels vary in proportion to the severity of chest x-ray score, post-operative day one CXR-scores (as a continuous variable) were compared with biomarker levels determined 24 hours post-operatively.

Modification by an effective intervention

The volume of lung tissue resected (or 'size' of the resection)^{127, 128}, and the duration and conduct of one-lung ventilation^{128, 151, 152} have all been associated with increased incidence of PLR-ALI. As such a lesser lung resection, shorter duration of one-lung ventilation and a lung protective ventilatory strategy might be considered 'effective interventions' to which association might be expected of the 'ideal' ALI biomarker. Association was therefore sought between biomarker levels and the volume of lung resected, the duration of one-lung

ventilation and ventilatory parameters (peak airway pressure (P_{peak}), tidal volume (V_T) and fraction of inspired oxygen (FiO_2) during the period of one lung ventilation).

Volume of lung resected was characterised in two ways. Firstly, resections were divided into sub-lobar resection^K, lobectomy (including bilobectomy), and pneumonectomy. Secondly, to allow distinction between the anatomically larger lobar resections (e.g. right lower or left upper lobe - 5 segments) and smaller resections (isolated right middle lobectomy - 2 segments), the number of segments resected was calculated from the operation note based on a 19 segment model of pulmonary anatomy. The number of segments resected was not amenable to division into quartiles (as the nine patients having 5 segments resected would necessitate an arbitrary quartile division in the middle of this group), therefore patients were divided into tertiles by number of pulmonary segments resected. For this analysis patients undergoing sub-lobar resection were excluded.

The duration of one-lung ventilation (in minutes) and the areas under the P_{peak} , V_T and FiO_2 verses time curves (section 4.5.1.5 - endpoints, intra-operative data) were divided into quartiles and association sought between quartiles and biomarker levels.

Association with clinically important outcomes

Association was sought between biomarker levels and 'clinically important outcomes', defined as the need for ventilatory support, and the duration of high dependency unit (HDU) and hospital stay. An HDU stay of greater than 48 hours was defined as 'prolonged' and biomarker levels compared between patients with an HDU stay of 48 hour or less, or greater than 48 hours.

^K Though the inclusion / exclusion criteria were explicit in recruiting patients planned to undergo lobectomy or pneumonectomy, on five occasions sub-lobar resection was performed. These patients were not excluded from the analyses in order not to compromise what was already a modest sample size. They do however constitute a cohort in which the surgical insult might be considered less severe.

4.5.2.3 Statistical handling

All groups containing less than 10 patients were assumed to be non-parametric in distribution. Otherwise, data was tested for normality and the presence of outliers by visual inspection of box plots and by Shapiro-Wilk testing.

Comparisons of parametrically distributed biomarker levels between groups were made using an independent-samples t-test using Levene's test for equality of variances, or one way analysis of variance as appropriate. Comparisons of non-parametrically distributed biomarker levels between groups were made with the Mann-Whitney U test or Kruskal-Wallis test as appropriate. Post-hoc testing was performed by least significant difference or Dunn's procedure as appropriate. Linear association between continuous variables were assessed using Pearson correlation or Spearman's rho as appropriate.

Predictive value of the biomarkers for outcomes of interest was determined by the generation of receiver operating characteristic (ROC) curves, and the corresponding area under the curve (AUC). The optimal cut-off biomarker level was defined by calculation of the point at which the sum of sensitivity and specificity was maximal (Youden's index). Positive and negative predictive values (PPV and NPV respectively) were then calculated as follows:

$$PPV = \frac{\textit{number of true positives}}{\textit{number of true positives} + \textit{number of false positives}}$$

Equation 4.1

$$NPV = \frac{\textit{number of true negatives}}{\textit{number of true negatives} + \textit{number of false negatives}}$$

Equation 4.2

Statistical analysis was performed using SPSS Statistics v21 (IBM Corporation, Armonk, New York). No adjustments were made for multiple comparisons.

4.5.2.4 Power

There have been no previous reports of the measurement of PTX3 in patients undergoing lung resection. The sample size of 35 constitutes a sample of

convenience for what must be considered a ‘hypothesis generating’ pilot study. On discussion between the author (Ben Shelley), the project supervisor (Professor John Kinsella) and statistical advisors from the Robertson Centre for Biostatistics (University of Glasgow), it was concluded this sample size should be adequate to detect ‘a signal’. Without conducting a pilot study in a modest sample size such as this, and at least confirming the presence of a detectable increase in PTX3 level following lung resection, it was felt there was insufficient grounds for recruitment of a larger cohort.

4.5.3 Utility of a multiple lung injury biomarker panel following lung resection

4.5.3.1 Composition of the panel

Fremont et al described a 7 biomarker panel derived from 192 patients admitted to a trauma intensive care unit⁸³. The composition of the 7 biomarker panel described by Fremont et al is detailed in Table 4.7:

Table 4.7. Multiple biomarker panel as defined by Fremont et al (2010)⁸³

<i>Biomarker</i>	<i>Source / Pathobiology</i>
Receptor for Advanced Glycation End Products (RAGE)	Epithelium (type 1 pneumocyte)
Procollagen Peptide III (PCP-III)	Fibroblast (collagen deposition)
Angiopoietin 2 (Ang 2)	Endothelium
Interleukin 6 (IL-6)	Pro-inflammatory cytokine
Tumour Necrosis factor α (TNFa)	Pro-inflammatory cytokine
Interleukin 10 (IL-10)	Anti-inflammatory cytokine
B-type Natriuretic Peptide (BNP)	Ventricular myocyte

4.5.3.2 Multiple biomarker panel measurement

Single measurements for each marker in the multiple biomarker panel were performed by trained post-doctoral research assistants in the laboratories of Glasgow Biomedical Research Centre, University of Glasgow under the supervision of Dr Charles McSharry (Principal Clinical Scientist). All 7 biomarkers were measured using commercially available enzyme immunoassays.

Receptor for advanced glycation end product (RAGE), interleukins 8 and 10 (IL-8 & -10), Angiopoietin 2 (Ang-2) and Tumour Necrosis Factor alpha (TNF- α) were measured using the commercially available 'Quantikine' enzyme immunoassay system (R and D Systems Europe Ltd, Abington, Oxon, UK). Pro-collagen peptide III (PCP-III) and B-type Natriuretic Peptide (BNP) were measured using commercially available enzyme-linked immunosorbent assays (Caltag Medsystems Ltd, Buckingham, UK). Both manufacturers employ a quantitative sandwich enzyme immunoassay technique. Briefly, a microplate is provided by the manufacturer, pre-coated with monoclonal antibody specific for the biomarker of interest. In a series of wash / incubation cycles, samples or calibration standards were added to the plate, incubated with an enzyme linked monoclonal antibody specific for the marker of interest, and incubated with a substrate solution. The reactions were then quantified spectrophotometrically at a wavelength of 450nm. A standard curve was generated from the averaged calibration standards, from which sample results were determined by comparing the optical density of the samples to the standard curve. The precision of these assays were^L: RAGE 3.6%, IL-8 4.5%, Ang-2 7.7%, TNF- α 6.9%, PCP-III 15.6%, BNP 14.3%.

Generation of a risk of lung injury score

Fremont et al derived this seven biomarker panel in a cohort of 192 patients admitted to a trauma intensive care unit⁸³. Plasma levels of 21 acute lung injury biomarkers were compared between 107 patients with ALI/ARDS (per the AECC definition) and 74 controls either with no or hydrostatic pulmonary oedema. Following univariate analysis, a backward elimination model was used to select

^L As the biomarkers were only determined by singular measurement, the CV described represents a CV determined from the duplicate measurement of the calibration controls, not the entire data set.

the seven biomarkers with the greatest predictive value. A multivariable logistic regression model was then constructed using these seven biomarkers to create a prediction model for ALI/ARDS (Figure 4.3).

In this model, a value of each biomarker corresponds to a points scale (at the top of Figure 4.3). The sum of the individual biomarker points scores is then calculated, providing a total points score, which then corresponds to a probability for the diagnosis of ALI (at the bottom of Figure 4.3). In the current investigation, the prediction model provided by Fremont et al (Figure 4.3) was blown-up, and printed onto graphing paper allowing scores to be derived from individual patient biomarker levels.

The total point score, derived in this way was then treated as a continuous variable in subsequent analyses. As the pre-test probability for the diagnosis of ALI in the study population (elective thoracic surgical patients) differs from that studied by Fremont et al (trauma intensive care patients), the 'probability of ALI' (bottom section of the model in Figure 4.3) for individual subjects was not calculated.

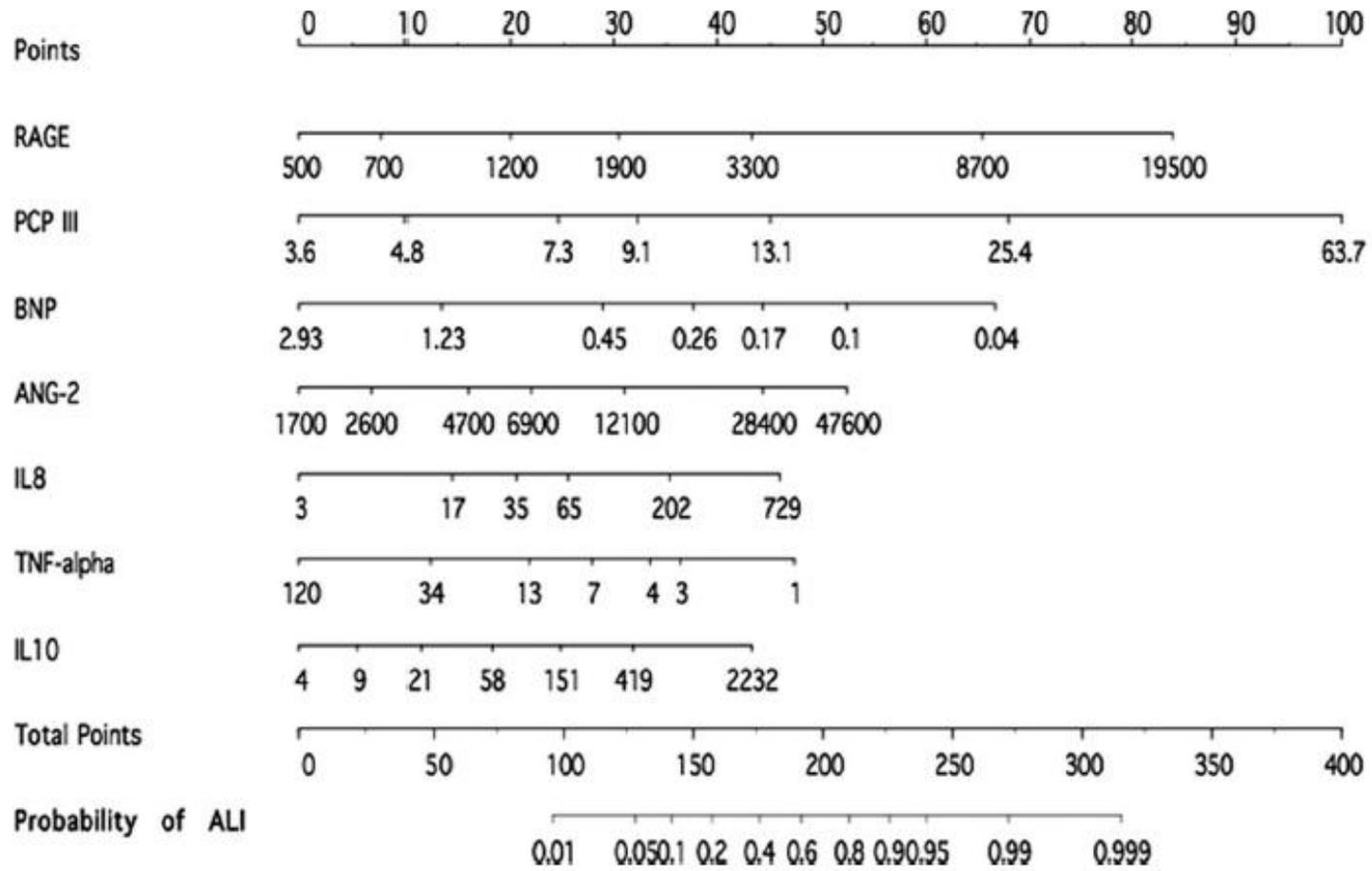


Figure 4.3. A prediction model for the probability of ALI.
From Freemont et al (2010)⁸³.

4.5.4 Comparison with the properties of the ideal ALI biomarker

As in the investigation of Pentraxin 3, scores derived from the multiple biomarker panel were compared with the properties of the ideal ALI biomarker. Briefly, sensitivity and specificity of post-operative biomarker scores in predicting mLIS were sought and quantified as the area under the receiver operating characteristic curve. Association was determined between panel scores and the severity of post-operative oxygenation impairment and CXR score. The hypothesis that post-operative changes in biomarker panel score would be reduced in proportion to the volume of lung tissue resected, the duration of one-lung ventilation and indices of lung protective ventilation (factor previously described as being independent predictors of the severity of lung injury) was tested. Finally association was sought between biomarker panel scores and the length of HDU and hospital stay. Statistical handling was as described in the PTX3 investigation.

4.6 Results – Biomarkers of ALI following lung resection

4.6.1 Patient demographics

Thirty five patients undergoing lung resection were recruited to the study. One patient was excluded as their tumour was found not to be resectable at thoracotomy and so no lung resection was performed, and one patient was excluded because (contrary to the surgical plan at the time of recruitment), lung was resected via a video assisted thoracoscopic technique. Demographic and pre-operative data for the remaining 33 patients are shown in Table 4.8. Surgical, length of stay and mortality data are shown in Table 4.9.

4.6.2 Clinical outcomes

4.6.2.1 Oxygenation following lung resection

Arterial blood gas acquisition was not protocolised; arterial blood gas results displayed are therefore those taken when clinically indicated. Similarly, whilst placement of an arterial cannula is routine practice in patients undergoing thoracic surgery in our institution (and therefore all patients had an arterial cannula placed immediately pre-operatively), the maintenance of the cannula, the decision to remove it, and the decision whether to replace it if dislodged was that of the clinical team. Arterial blood gas data (and therefore $\text{PaO}_2/\text{FiO}_2$) was not available at any post-operative time point in 6 patients. In the remaining 27 patients, data was available with variable frequency. Median $\text{PaO}_2/\text{FiO}_2$ recorded for the study group as a whole is displayed over time in Figure 4.4. Each time point represents $\text{PaO}_2/\text{FiO}_2$ results obtained over the 6 hour window three hours either side of the recorded value (or from 0 to 3 hours post-operatively for the '<3' time point). Where more than one result was available in a given time-point, the mean value was computed and used for analysis.

Table 4.8. Demographic and pre-operative data for the study population

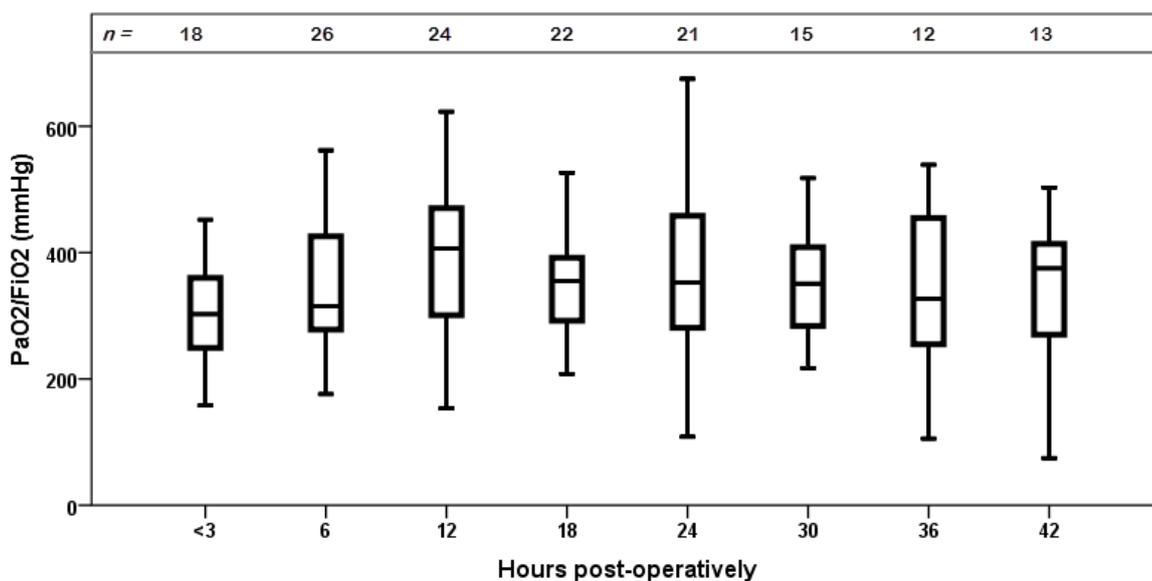
Characteristics	n	Frequency
Patient demographics		
Age (years)	33	69.7 (61.1-69.7 [35.3-81.9])
Male sex	33	17 (51.5%)
Height (cm)	33	165.4 (9.8)
Weight (kg)	33	71.8 (14.8)
Body mass index	33	26.1 (4.6)
Smoking history		
Current smoker	33	11 (33.3%)
Pack years history	32	38.0 (20.5-76.0 [0-158.0])
Pre-operative pulmonary function		
Forced expiratory volume ₁ (FEV ₁) (L)	33	2.0 (1.7-2.7 [0.9-3.9])
FEV ₁ /Forced vital capacity (FVC) (%)	31	67.0 (60.0-71.0 [37.5-86.0])
FEV ₁ % predicted	33	83.0 (74.5-98.5 [30.0-117.0])
PPO FEV ₁ % predicted	33	69.1 (54.2-78.2 [23.7-94.8])
DLCO (mmol/min/kPa)	27	5.6 (4.5-8.0 [3.1-9.5])
DLCO % predicted	29	66.0 (57.5-91.5 [38.0-109.0])
PPO DLCO % predicted	29	54.7 (50.0-64.1 [33.7-106])
Oxygen saturation on air (%)	33	97 (96-98 [93-100])
Pre-operative functional status		
Zubrod Performance Status (0-4)	33	14 (42.4%) / 16 (48.5%) / 3 (9.1%) / 0 / 0
NYHA (1-4)	33	15 (45.5%) / 14 (42.4%) / 4 (12.1%) / 0
MRC dyspnoea scale (0-5)	33	16 (48.5%) / 7 (21.2%) / 9 (27.3%) / 1 (3%) / 0 / 0
Estimated exercise tolerance (<4, 4-6, >6 metabolic equivalents)	33	9 (27%) / 16 (49%) / 8 (24%)
Co-morbidities		
History of cancer	33	12 (36%)
COPD	33	21 (64%)
Essential hypertension	33	11 (33%)
Heart disease	33	7 (21%)
Diabetes mellitus	33	1 (3%)
Peripheral vascular disease	33	0 (0%)
Obesity	33	6 (18%)
Alcoholism	33	6 (18%)
Hyperlipidaemia	33	14 (42%)
Total no. of co-morbidities per patient ^A	33	3.0 (1.5-4.0 [0-6])
Risk scores		
ASA (≥III)	33	15 (46%)
Thoracoscore (% predicted mortality)	33	1.9 (1.5-3.7 [0.5-16.2])
Surgical lung injury prediction score (SLIP)	33	26 (16-33 [10-37])

Values are number (%) mean (SD) or median (IQR [range]). n - number of patients from which result is derived^AOf the ten co-morbidities described in the derivation of Thoracoscore³⁴⁴

Table 4.9. Surgical, length of stay and mortality data for the study population

Characteristic	n	Frequency
Resection type	33	
Pneumonectomy		5 (15%)
Extended lobectomy		2 (6%)
Simple lobectomy		22 (67%)
Sub-lobar		4 (12%)
No of pulmonary segments resected	33	4 (3-5 [0-10])
Right sided procedure	33	15 (45.5%)
Duration of surgery (minutes)	33	162.9 (29.7)
Duration of one-lung ventilation (minutes)	32	75.1 (24.2)
Pathology	33	
Primary lung cancer		30 (91%)
Other (benign / malignant)		1 (3%) / 2 (6%)
Lung cancer staging	30	
IA		7 (21%)
IB		9 (27%)
IIA		1 (3%)
IIB		2 (6%)
IIIA		10 (29%)
IIIB		1 (3%)
IV		0
Length of stay		
Length of HDU stay (hours)	33	44.5 (40.9-46.5 [39.0-493.5])
Length of hospital stay (days)	33	7 (5.8-9.3 [4-16])
Mortality		
Hospital mortality	33	1 (3.0%)

Values are number (%), mean (SD) or median (IQR [range]). n - number of patients from which result is derived.

**Figure 4.4 Median PaO₂/FiO₂ (mmHg) ratio recorded post-operatively.**

Values in the accompanying table represent the number of patients results (n) from which the median is derived.

'Poor' post-operative oxygenation was defined as $\text{PaO}_2/\text{FiO}_2$ less than 300mmHg. The number of patients with $\text{PaO}_2/\text{FiO}_2$ values recorded as 'poor' at any given time point are displayed in Figure 4.5.

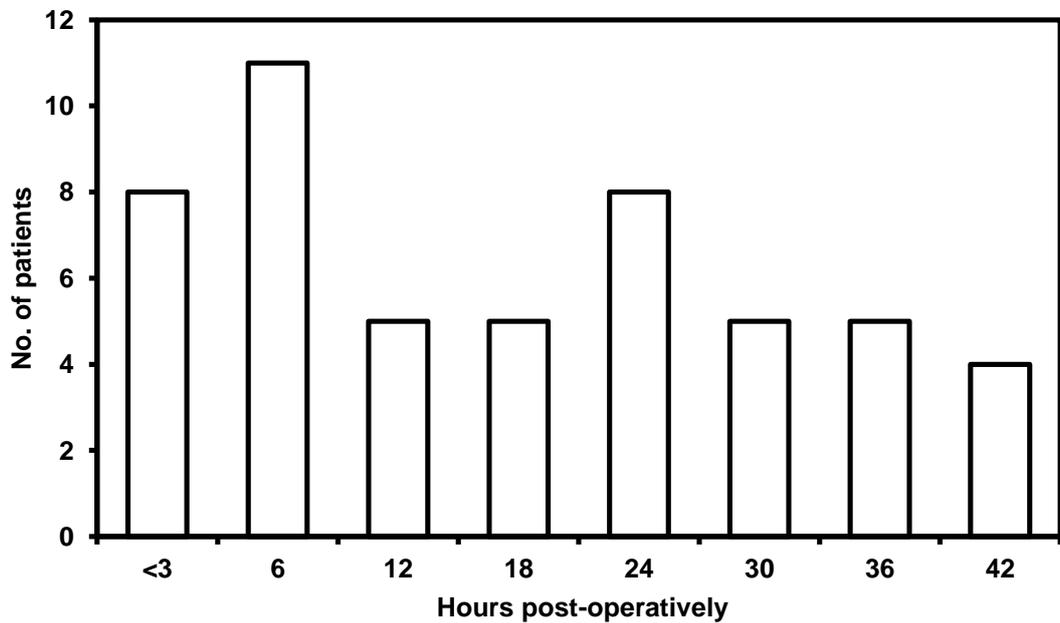


Figure 4.5. Number of patients with 'poor' oxygenation at any given time point post-operatively.

'Poor' post-operative oxygenation defined as $\text{PaO}_2/\text{FiO}_2 < 300\text{mmHg}$.

Visual inspection of Figures 4.4 and 4.5 suggests a similar trend. Patients appear to have relatively low $\text{PaO}_2/\text{FiO}_2$ ratio in the early post-operative period (and accordingly more patients have 'poor oxygenation'), which improves by 12 hours post-operatively where $\text{PaO}_2/\text{FiO}_2$ appears to peak (low numbers of patients with 'poor' oxygenation). Subsequently oxygenation appears to fall, with a nadir mean $\text{PaO}_2/\text{FiO}_2$ ratio recorded 18-24 hours post-operatively and a further peak in the number of patients recorded as having 'poor' oxygenation.

Validity of $\text{SaO}_2/\text{FiO}_2$ ratio as a surrogate for $\text{PaO}_2/\text{FiO}_2$ ratio following lung resection

Two hundred and fifteen paired $\text{PaO}_2/\text{FiO}_2$ and $\text{SaO}_2/\text{FiO}_2$ data points were available for analysis (representing data from 29 individual patients) over a range of $\text{PaO}_2/\text{FiO}_2$ from 70 to 940 mmHg and $\text{SaO}_2/\text{FiO}_2$ from 97 to 459. SaO_2 varied from 85 to 100%. There was a highly significant positive association between $\text{PaO}_2/\text{FiO}_{2\text{measured}}$ and $\text{PaO}_2/\text{FiO}_{2\text{calculated}}$ (where $\text{PaO}_2/\text{FiO}_{2\text{calculated}}$ is calculated from $\text{SaO}_2/\text{FiO}_2$ according to the relationship described by Todd et

al³⁵⁰), but this was only moderate in strength ($r=0.68$, $p<0.0001$; $n=215$; Figure 4.6).

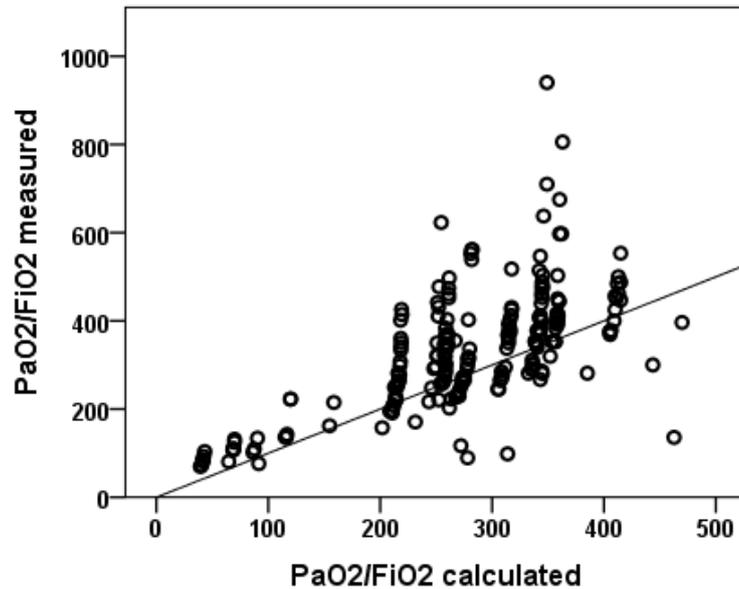


Figure 4.6. Association between measured and calculated values of $\text{PaO}_2/\text{FiO}_2$ following lung resection.

Line represents line of identity. $r=0.68$; $p<0.0001$; Spearman's rho. $n=215$.

Bland-Altman analysis revealed a mean bias ($\text{PaO}_2/\text{FiO}_{2\text{measured}} - \text{PaO}_2/\text{FiO}_{2\text{calculated}}$) of +55.0mmHg with limits of agreement of between +256.9 and -146.9mmHg (Figure 4.7).

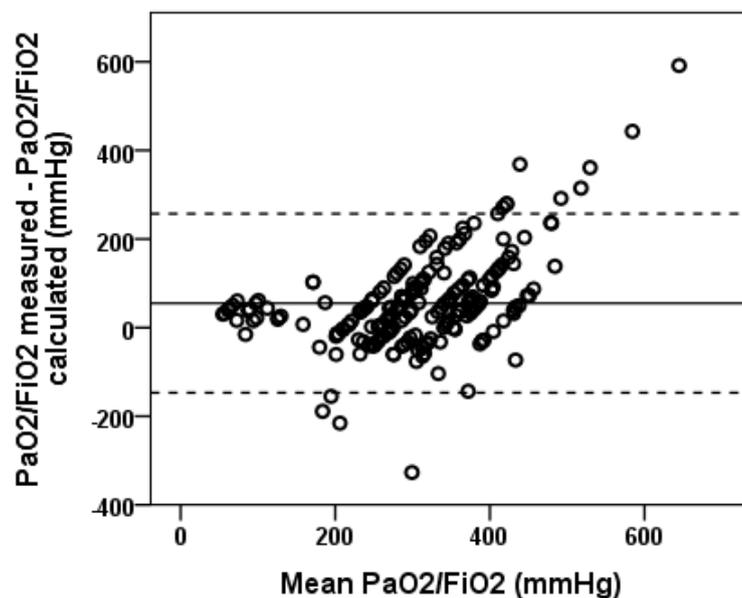


Figure 4.7. Bland-Altman analysis of the agreement between measured and calculated values of $\text{PaO}_2/\text{FiO}_2$.

Solid line represents mean bias, dashed lines represent limits of agreement ($\pm 2\text{SD}$).

Due to the (clinically) unacceptable level of bias between $\text{PaO}_2/\text{FiO}_2$ measured and $\text{PaO}_2/\text{FiO}_2$ calculated in this population, subsequent analysis of $\text{SaO}_2/\text{FiO}_2$ as a surrogate of post-operative oxygenation was abandoned. Visual inspection of Figure 4.6 suggests the linear relationship between $\text{PaO}_2/\text{FiO}_2$ and $\text{SaO}_2/\text{FiO}_2$ is compromised as oxygenation improves. Rice et al's original validation of the relationship between $\text{PaO}_2/\text{FiO}_2$ and $\text{SaO}_2/\text{FiO}_2$ ratio was performed in patients with ARDS and $\text{SaO}_2 \leq 97\%$ ³⁵⁰. Restricting the comparison of $\text{PaO}_2/\text{FiO}_2$ measured and $\text{PaO}_2/\text{FiO}_2$ calculated in the current study to values where SaO_2 levels were $\leq 97\%$ improved the strength of the association between variables markedly ($r=0.96$; $p<0.0001$; Spearman's rho; Figure 4.8).

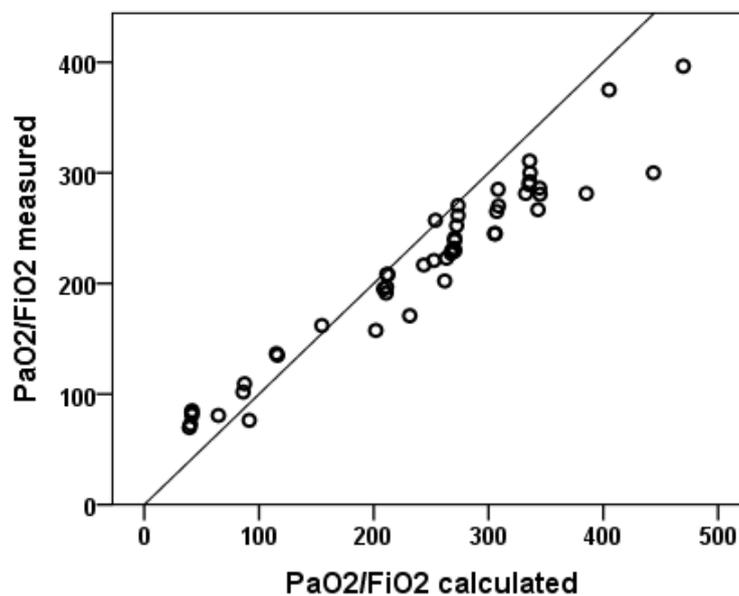


Figure 4.8. Association between measured and calculated values of $\text{PaO}_2/\text{FiO}_2$ following lung resection when $\text{SaO}_2 \leq 97\%$.

Solid line represents line of identity. $r=0.96$; $p<0.0001$; Spearman's rho. $n=68$.

4.6.2.2 Chest X-ray score following lung resection

Chest X-ray (CXR) acquisition was not protocolised; CXR results displayed are therefore those taken when clinically indicated. There were CXRs available for analysis for 28 patients on post-operative day 1 and 27 patients on post-operative day 2. These 55 CXRs were dual reported and the mean CXR score used for subsequent analysis. Inter-rater reliability was explored by determining Type 3 Intraclass Correlation Coefficient (two-way mixed model for agreement, average measures). This revealed 'substantial' agreement between raters ($\text{ICC}=0.61$).

In patients for whom paired CXR scores were available on post-operative days one and two, median CXR score was higher on day two post-operatively compared to day one (Figure 4.9), though this difference was not statistically significant ($p=0.21$, Wilcoxon Signed Ranks Test, $n=22$).

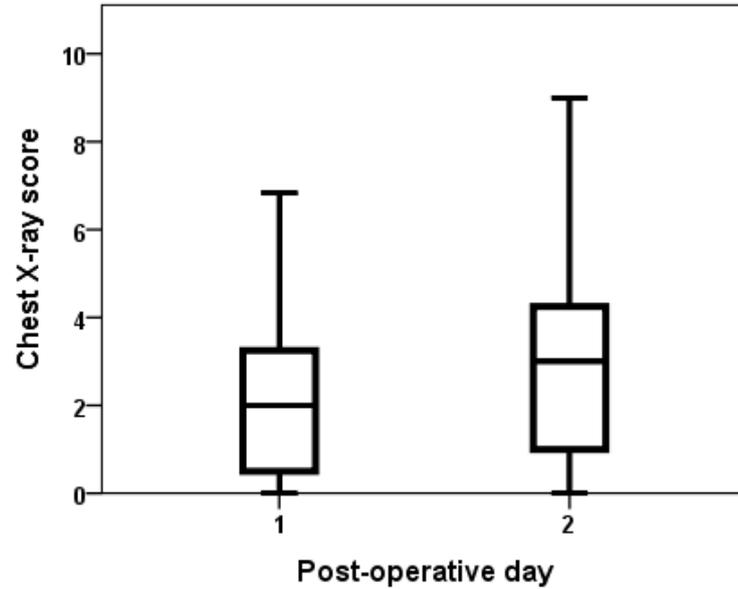


Figure 4.9. Post-operative chest X-ray scores by day.
 $p=0.21$, Wilcoxon Signed Ranks Test, $n=22$.

Representative chest X-rays demonstrating the derivation of the CXR score are shown in Figure 4.10.

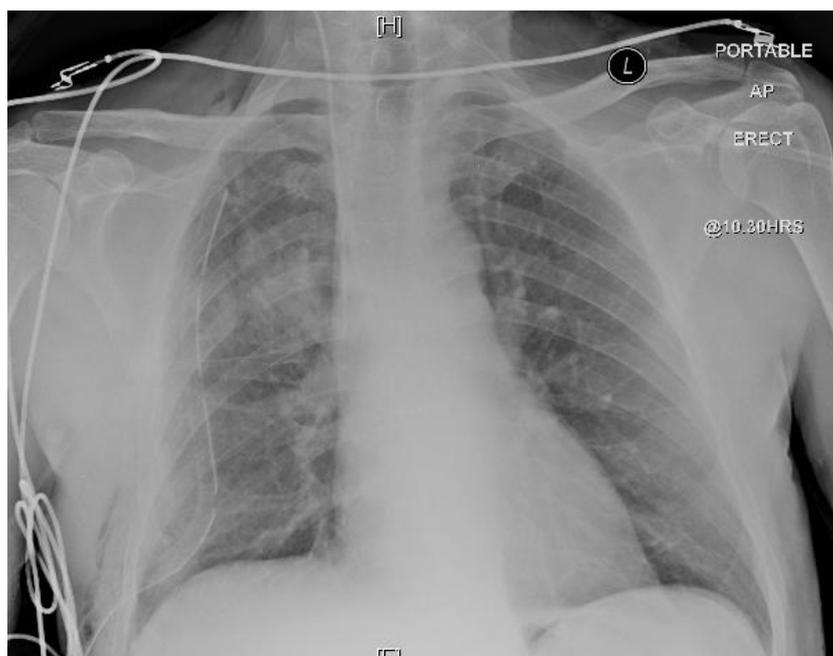
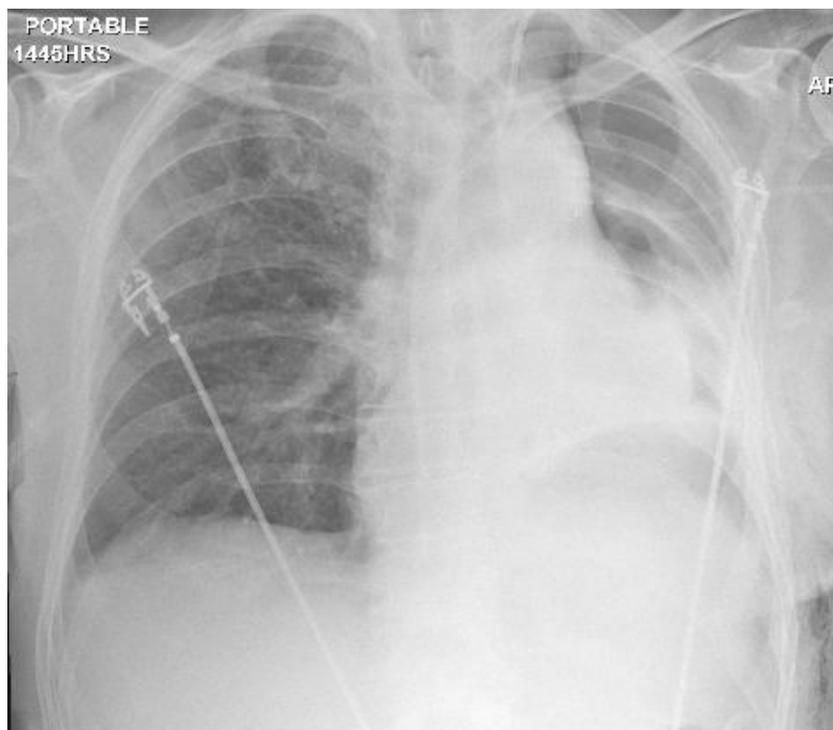


Figure 4.10. Sample chest X-rays illustrating derivation of the chest x-ray score.

Top panel: 72 year old female on post-operative day one following left pneumonectomy. Both left sided quadrants contain no lung, therefore the number of scoreable quadrants = 2. Both right sided quadrants contain no 'opacities not fully explained by effusions, lobar / lung collapse, or nodules', yielding a quadrant score of 0 for each. The overall CXR-score therefore = $(0/2) \times 4 = 0$ [(quadrant score / number of 'scoreable' quadrants) \times 4]. *Bottom panel:* 68 year old male on post-operative day two following multiple wedge resection on right lung. All quadrants are deemed scoreable. 'Opacities' can be seen occupying 1/3 to 2/3 of the RUQ (score=2), <1/3 of the RLQ (score=1) and 1/3 to 2/3 of the LLQ (score=2). There are no opacities in the LUQ. Total quadrant score therefore = 5 and overall CXR-score therefore = $(2+1+2/4) \times 4 = 5$.

4.6.2.3 Modified lung injury score following lung resection

Paired post-operative CXRs were available for all 21 patients for whom PaO₂/FiO₂ data was available at 24 hours post-operatively, allowing calculation of modified lung injury score (mLIS) 24 hours post-operatively in all 21 patients. The distribution of mLIS 24 hours post-operatively is demonstrated in Figure 4.11. It is evident from this figure, that the distribution of mLIS 24 hours post-operatively is bimodal. As might be expected, the majority of patients had low mLIS scores, however there is second peak in LIS representing those with LIS greater than 1.5. It is hypothesised that this group of patients with mLIS over 1.5 are a distinct group of patients (comprising 6 of the 21 patient cohort), who demonstrate evidence of post-operative lung injury. Only one patient had a mLIS greater than 2.5, classified (according to Murray et al's original derivation⁵⁴) as 'severe' lung injury. A mLIS of greater than 1.5 was therefore defined as a 'positive' clinical outcome against which the sensitivity and specificity of the candidate lung injury biomarkers could be examined.

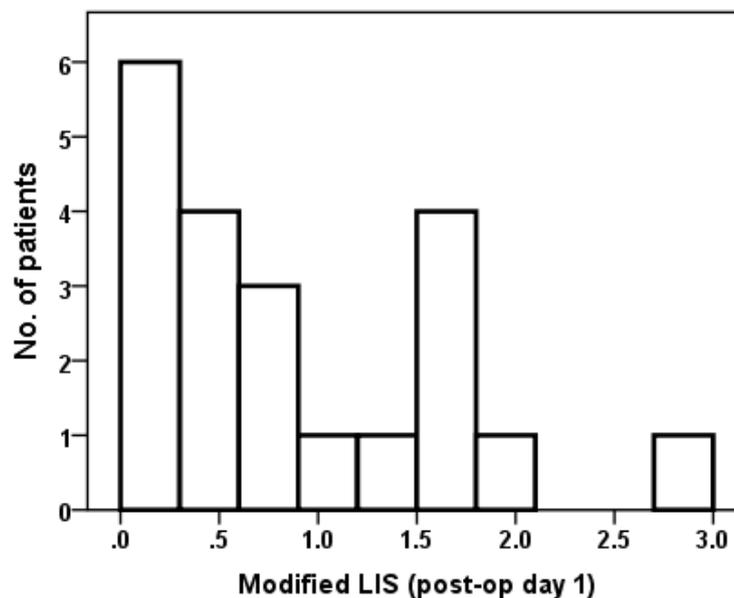


Figure 4.11. Distribution of modified LIS on post-operative day one. (n=21).

Paired arterial blood gas results and chest x-ray scores, allowing calculation of mLIS were available in 14 patients on post-operative day two (POD-2). As on POD-1, whilst the majority of patients had low mLIS on POD-2, there remained a population of patients (4 of the 14 patients) who had mLIS greater than 1.5 (Figure 4.12).

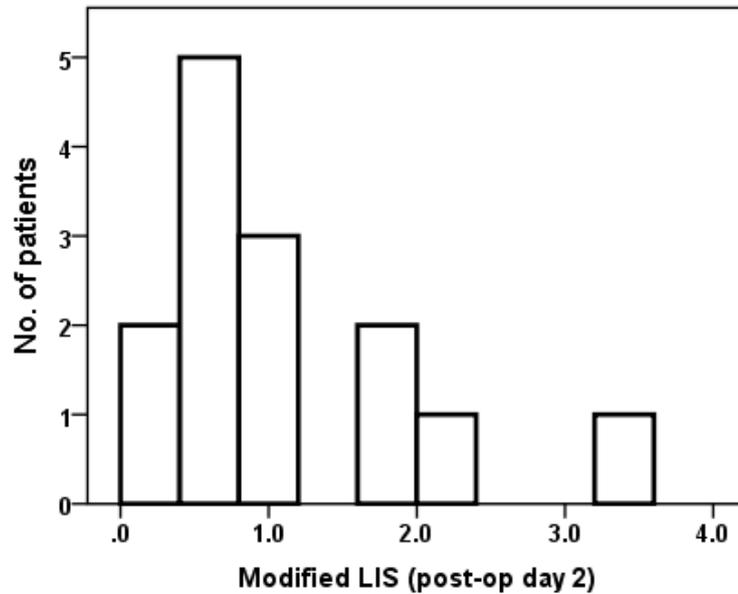


Figure 4.12. Distribution of modified LIS on post-operative day two. (n=14).

4.6.3 Pentraxin 3 as a biomarker of lung injury following lung resection

4.6.3.1 Changes in Pentraxin 3 and C-reactive protein following lung resection

PTX3 levels were not available immediately post-operatively for one patient, and CRP levels were not available for one patient immediately post-operatively and one patient 24 hours post-operatively. These patients were excluded from the longitudinal analysis of biomarker levels.

There were no significant increases in PTX3 and CRP levels immediately post-operatively, however by 24 hours post-operatively, both biomarkers demonstrated a marked increase ($p < 0.01$ for both; Friedman test; Figures 4.13 and 4.14).

Three patients demonstrated no post-operative PTX3 rise. These patients were retained in the analysis, but for each comparison a sensitivity analysis was performed excluding these three patients from the analysis.

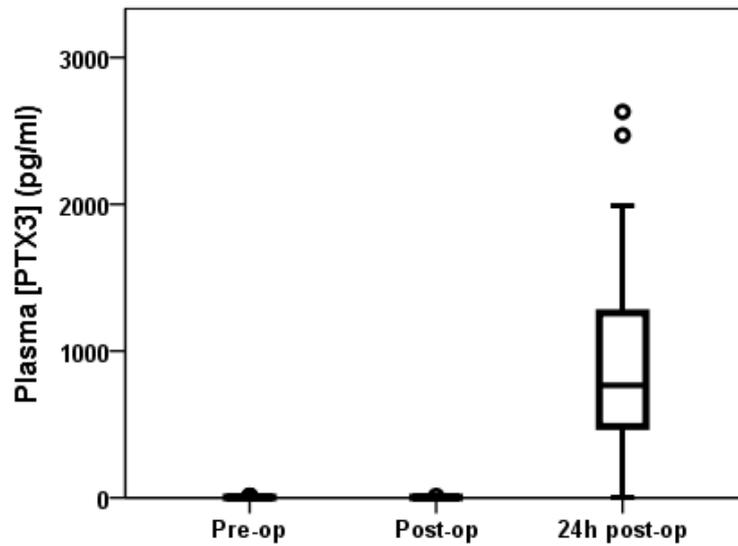


Figure 4.13. Changes in plasma PTX3 following lung resection.
 $p < 0.01$; Friedman test. $n = 32$.

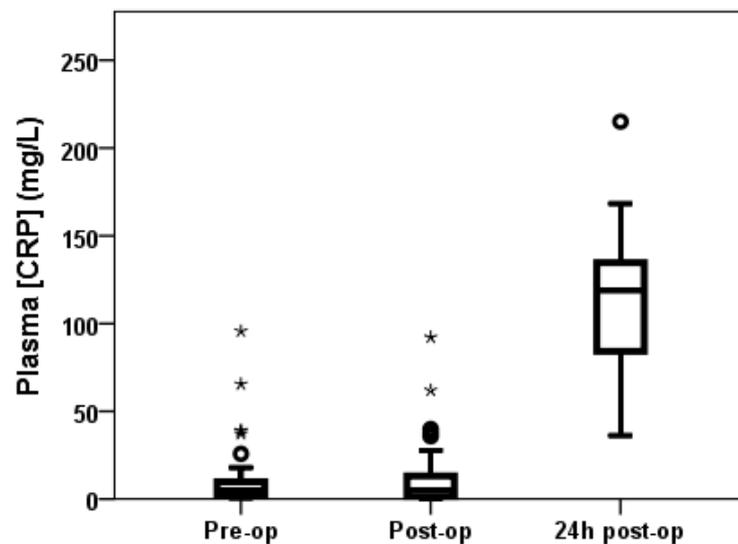


Figure 4.14. Changes in plasma CRP following lung resection.
 $p < 0.01$; Friedman test. $n = 32$.

4.6.3.2 Sensitivity and specificity of PTX3 and CRP for predicting clinical outcomes of interest

Diagnostic utility of PTX3 and CRP

Modified lung injury score on post-operative day one was available in 21 patients. Dichotomising these patients into groups of patients with mLIS > 1.5

and those with $mLIS \leq 1.5$ left 6 and 15 patients per group respectively. Median PTX3 level 24 hours post-operatively was significantly higher in patients with $mLIS > 1.5$ on post-operative day 1 ($p=0.03$, Mann-Whitney U test; Figure 4.15).

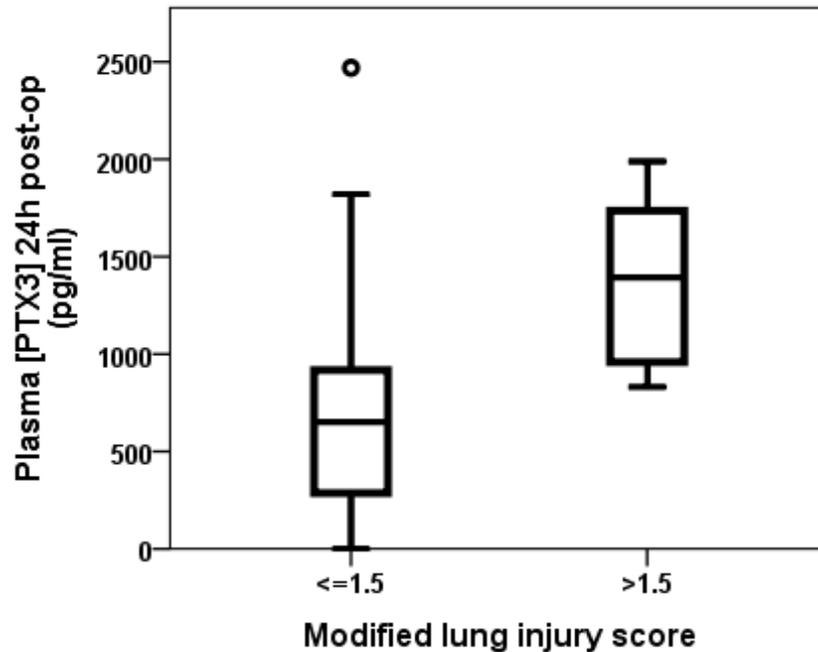


Figure 4.15. Modified lung injury score on post-operative day one versus PTX3 concentration.

$p=0.03$; Mann-Whitney U test. $n=21$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX3 response to surgery, there remained a trend towards increased PTX3 levels in patients with $LIS > 1.5$ ($p=0.05$, Mann-Whitney U test; $n=19$, not shown).

There were no significant differences in CRP levels 24 hours post-operatively in patients with $mLIS \leq 1.5$ compared to > 1.5 ($p=0.35$; Mann-Whitney U test; not shown).

Sensitivity and specificity

A receiver operating characteristic curve was constructed to determine the predictive value of 24 hour post-operative PTX3 levels for $mLIS > 1.5$ (Figure 4.16).

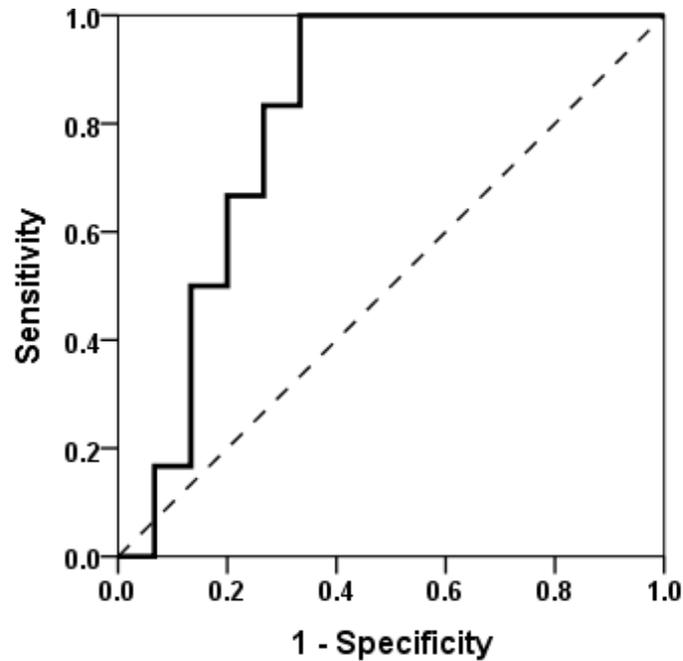


Figure 4.16. ROC curve describing the predictive value of PTX3 24h post-operatively for modified lung injury score greater than 1.5 on post-operative day one. AUC=0.81 (95% CI=0.62-0.99).

The optimal cut off value of PTX3 (defined as the point of maximal summative sensitivity and specificity from the ROC curve analysis) was 767.2pg/ml. The performance of this cut-off level for predicting mLIS>1.5 24 hours post-operatively is described in Table 4.10.

Table 4.10. Predictive performance of a cut-off of 24 hour post-operative PTX3 of 767.2 pg/ml for modified lung injury score > 1.5 on post-operative day one.

<i>Parameter</i>	<i>Value</i>
Area under the ROC Curve (95% Confidence interval)	0.81 (0.62-0.99)
Sensitivity	100%
Specificity	67%
Positive predictive value	55%
Negative predictive value	100%

As there was no significant difference in CRP levels in patients with elevated mLIS, ROC curve analysis of CRP data was not performed.

Prognostic utility of PTX3 and CRP

Modified lung injury score on post-operative day two was available in 14 patients. Dichotomising these patients into groups of patients with mLIS >1.5 and

those with mLIS ≤ 1.5 left 4 and 10 patients per group respectively. Median PTX3 level 24 hours post-operatively was significantly higher in patients with mLIS > 1.5 ($p=0.05$; Mann-Whitney U test; Figure 4.17).

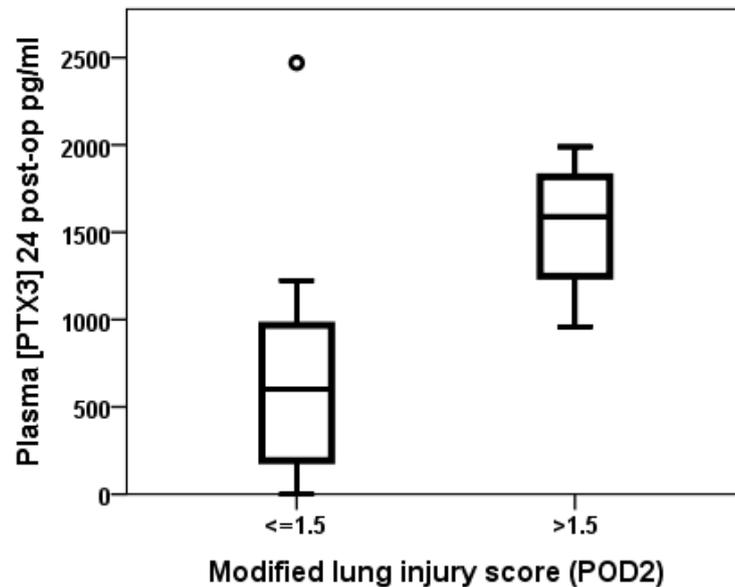


Figure 4.17. Modified lung injury score on post-operative day two versus PTX3 concentration.
 $p=0.05$; Mann-Whitney U test. $n=14$.

Sensitivity analysis: After exclusion of a single patient who demonstrated no PTX3 response to surgery (and for whom a mLIS could be calculated on POD2), there remained a non-significant trend towards increased PTX3 levels in patients with LIS > 1.5 ($p=0.06$, Mann-Whitney U test; $n=13$; not shown).

There were no significant differences in CRP levels 24 hours post-operatively in patients with mLIS \leq or $>$ than 1.5 ($p=0.77$, Mann-Whitney U test; $n=14$; not shown).

Sensitivity and specificity

A receiver operating characteristic curve was constructed to determine the predictive value of 24 hour post-operative PTX3 levels for mLIS greater than 1.5 on post-operative day 2 (Figure 4.18).

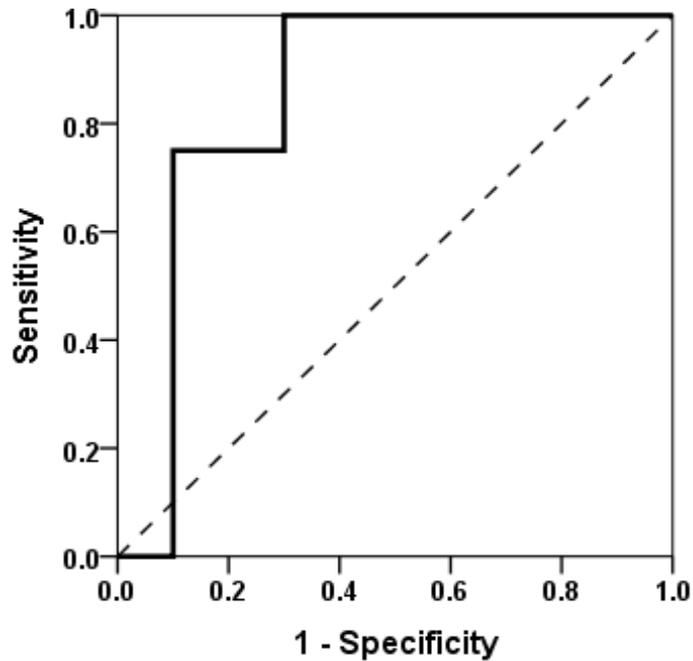


Figure 4.18. ROC curve describing the predictive value of PTX3 24h post-operatively for modified lung injury score greater than 1.5 on post-operative day two. AUC=0.85 (95% CI=0.64-1.00).

The optimal cut off value of PTX3 (defined as the point of maximal summative sensitivity and specificity from the ROC curve analysis) was 914.2pg/ml. The performance of this cut-off in predicting mLIS>1.5 on POD-2 is described in Table 4.11.

Table 4.11. Predictive performance of a cut-off of 24 hour post-operative PTX3 of 914.2 pg/ml for modified lung injury score > 1.5 on post-operative day two.

<i>Parameter</i>	<i>Value</i>
Area under the ROC Curve (95% CI)	0.85 (0.64-1.0)
Sensitivity	100%
Specificity	70%
Positive predictive value	57%
Negative predictive value	100%

As there was no significant difference in CRP levels in patients with elevated mLIS on POD-2, ROC curve analysis of CRP data was not performed.

Predictive value of PTX3 for post-operative oxygenation and chest X-ray score

Similar ROC curve analysis was performed to examine the predictive value of PTX3 level 24 hours post-operatively for post-operative oxygenation (positive

end-point defined as PaO₂/FiO₂ less than 300mmHg) and post-operative CXR score (positive endpoint defined as CXR score greater than 3). These comparisons were made of both the diagnostic and prognostic value of PTX3 for poor oxygenation and elevated CXR score (Table 4.12).

In view of the lack of association between CRP and post-operative modified lung injury score, these analyses were not performed for C-reactive protein.

Table 4.12. Predictive value of PTX3 for poor post-operative oxygenation and elevated chest X-ray score

<i>Parameter</i>	<i>PaO₂/FiO₂</i>		<i>CXR score</i>	
	Diagnostic	Prognostic	Diagnostic	Prognostic
Time point	24h post-op	'Worst' recorded 24-48h post-op	POD1	POD2
Cut off	914.2 pg/ml	914.2 pg/ml	937.8 pg/ml	914.2 pg/ml
Area under the Receiving Operating Characteristic Curve (95% Confidence interval)	0.76 (0.55-0.97)	0.76 (0.52-1.0)	0.87 (0.73-1.00)	0.64 (0.40-0.87)
Sensitivity	75%	75%	86%	60%
Specificity	77%	78%	76%	71%
Positive predictive value	66%	75%	86%	54%
Negative predictive value	83%	78%	76%	75%
No of patients in analysis (n)	21	16	28	27

4.6.3.3 Variation in proportion to the severity of injury

Relationship between PTX3 level and severity of oxygenation impairment

There was a trend towards a linear relationship between PTX3 levels and $\text{PaO}_2/\text{FiO}_2$ 24 hours post-operatively ($r=-0.40$, $p=0.08$, Spearman's rho; Figure 4.19).

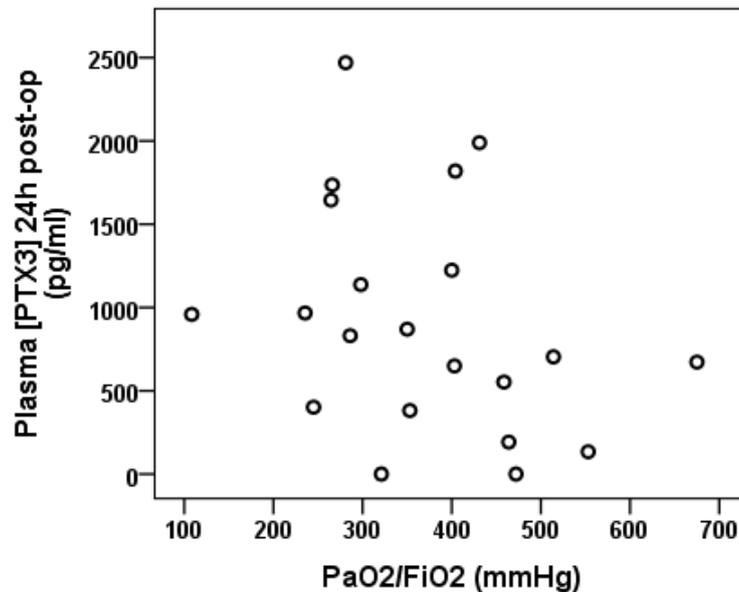


Figure 4.19. Association between $\text{PaO}_2/\text{FiO}_2$ and PTX3 concentration 24 hours post-operatively.

$r=-0.40$, $p=0.08$; Spearman's rho. $n=21$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained a trend towards a linear relationship between PTX3 levels and $\text{PaO}_2/\text{FiO}_2$ 24 hours post-operatively ($r=-0.38$, $p=0.10$, Spearman's rho; not shown).

Paired CRP levels and ABG data 24 hours post-operatively was available in 21 patients. There was no linear relationship between CRP level and $\text{PaO}_2/\text{FiO}_2$ 24 hours post-operatively ($r=-0.09$, $p=0.70$, Spearman's rho; not shown).

Relationship between PTX3 level and chest X-ray scores

On post-operative day one, chest X-rays were performed a median of 4.1 (3.0-5.6) hours before '24 hour' plasma samples were obtained. There was a linear relationship between PTX3 levels 24 hours post-operatively and CXR score on post-operative day one ($r=0.38$, $p=0.04$, Spearman's rho; $n=28$; Figure 4.20).

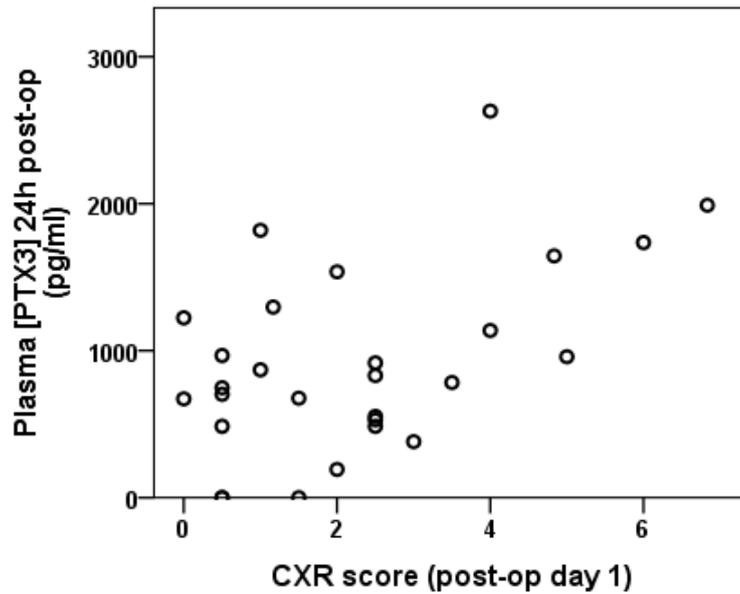


Figure 4.20. Plasma PTX3 level 24 hours post-operatively versus post-operative day 1 CXR score.

$r=0.38$, $p=0.04$, Spearman's rho, $n=28$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, the statistically significant relationship between PTX3 level and CXR was lost ($r=0.31$, $p=0.14$, Spearman's rho; $n=25$; not shown).

There was a similar (but stronger) linear relationship between CRP levels 24 hours post-operatively and CXR score on post-operative day one ($r=0.46$, $p=0.02$, Spearman's rho; $n=27$; Figure 4.21).

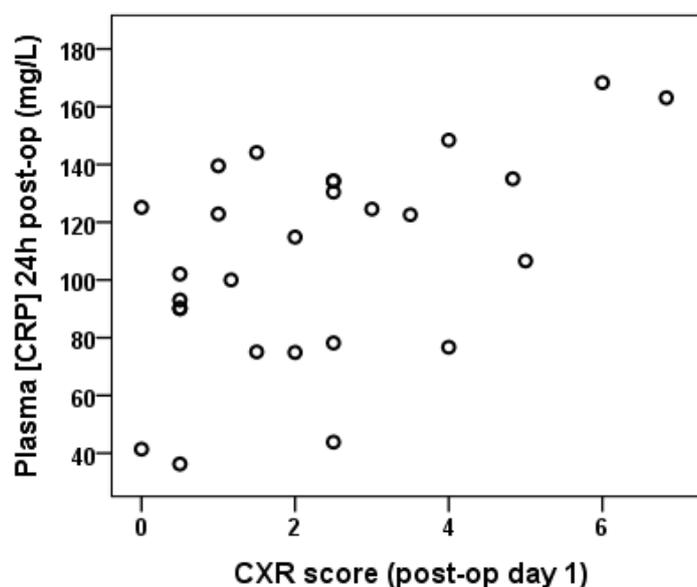


Figure 4.21. Plasma CRP level 24 hours post-operatively versus CXR score on post-operative day one.

$r=0.46$, $p=0.02$, Spearman's rho; $n=27$.

4.6.3.4 Modification by an effective intervention

Lesser resection

Type of resection

Of the 33 patient cohort, 4 patients underwent sub-lobar resection, 24 patients underwent lobectomy and 5 patients underwent pneumonectomy. There was no difference in PTX3 level across the three resection types ($p=0.33$, Kruskal-Wallis test; $n=33$; not shown).

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained no significant differences in PTX3 level across the three resection types ($p=0.56$, Kruskal Wallis test; $n=30$; not shown).

Of the 32 patients in whom a CRP sample 24 hours post-operatively was available, there was no significant difference in CRP level across the three resection types ($p=0.59$, Kruskal-Wallis test; $n=32$, not shown).

Volume of lung resected

Four of the 33 patient cohort underwent sub-lobar resection and so were excluded from this analysis. The remaining 29 were divided into tertiles by the number of pulmonary segments resected. There were no significant differences in PTX3 level across the tertiles ($p=0.25$, Kruskal-Wallis test; Figure 4.22).

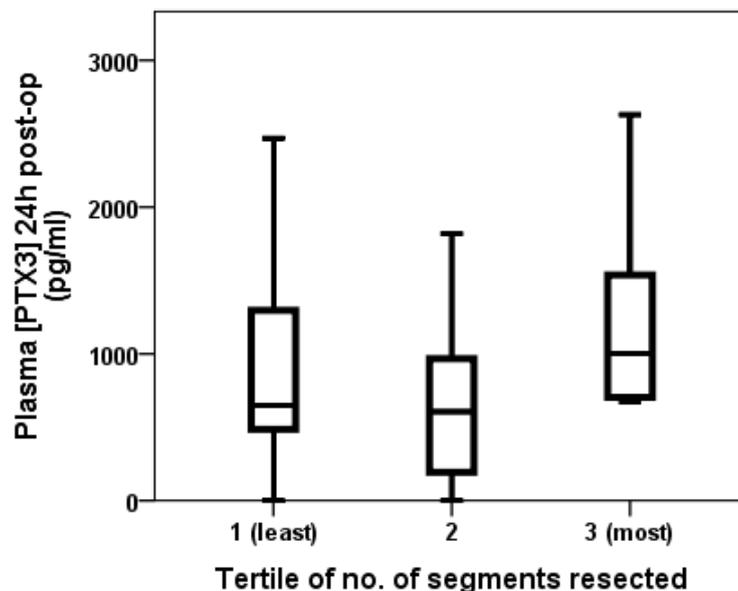


Figure 4.22. No of pulmonary segments resected versus PTX3 level 24 hours post-operatively.

$p=0.25$, Kruskal-Wallis test. $n=29$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX3 response to surgery, there remained no significant differences in PTX3 level across the three resection types ($p=0.33$, Kruskal-Wallis test; $n=26$; not shown).

Of the 32 patients in whom a CRP sample 24 hours post-operatively was available, 4 patients underwent sub-lobar resection and so were excluded from the analysis. The remaining 28 patients were divided into tertiles by the number of pulmonary segments resected. There were no differences in PTX3 level across the tertiles ($p=0.93$, Kruskal-Wallis test; $n=28$; not shown).

Duration of one-lung ventilation

The duration of one-lung ventilation (OLV) could be determined for 32 patients. Mean duration of OLV was 75.1 ± 24.2 minutes. There was no significant difference in PTX3 level across quartiles of OLV time ($p=0.28$, Kruskal-Wallis test; $n=32$; Figure 4.23). Visual inspection of figure 4.23 suggests the possibility of a threshold effect in the relationship between PTX3 and OLV time; the median PTX3 level in the quartile of patients undergoing the longest duration of OLV appears markedly higher than in the other three quartiles (no statistical comparison made).

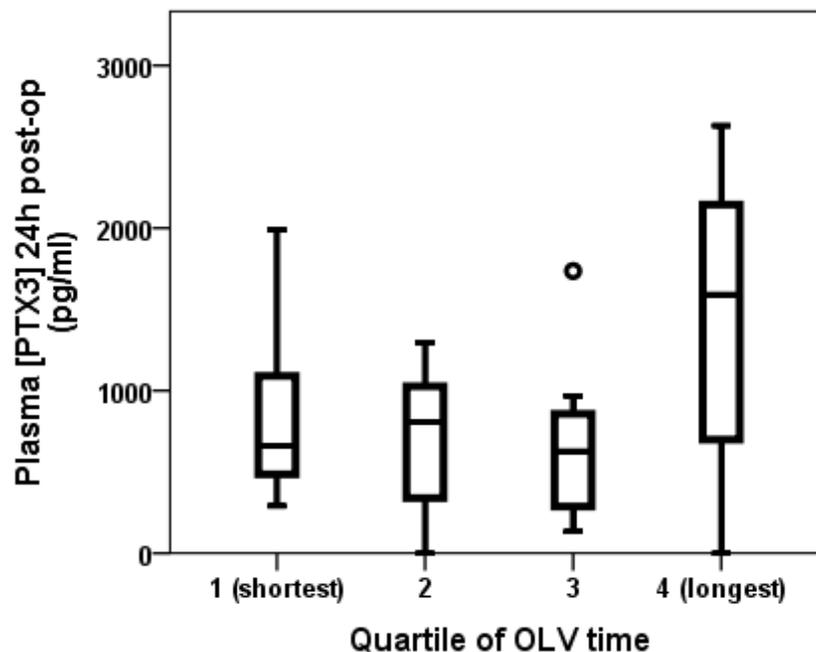


Figure 4.23. Plasma PTX3 24 hours post-operatively versus quartile of one-lung ventilation time.
 $p=0.28$, Kruskal-Wallis test. $n=32$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained no significant difference in PTX3 levels across quartiles of OLV time ($p=0.11$, Kruskal-Wallis test; $n=29$; not shown).

There was no significant difference in CRP level across quartiles of OLV time ($p=0.70$, Kruskal-Wallis test; $n=31$; not shown).

Lung protective ventilation

Ventilatory parameters during the period of one-lung ventilation were available in 31 patients. Figure 4.24 shows a representative series of P_{peak} , V_T and FiO_2 versus time curves for a single patient.

PTX3 versus peak airway pressure

There were no significant differences in PTX3 level across all quartiles of area under the P_{peak} versus time curve ($P_{\text{peak(AUC)}}$) ($p=0.20$ Kruskal-Wallis test, $n=31$, Figure 4.25).

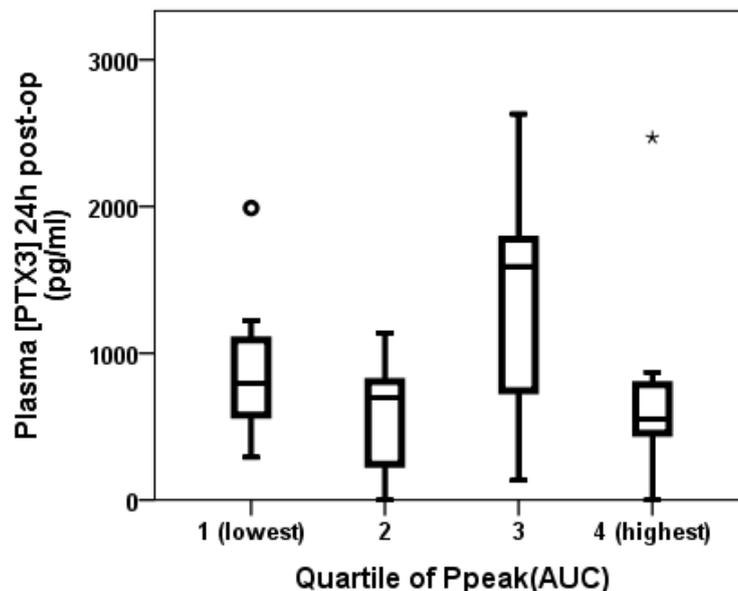


Figure 4.24. Plasma PTX3 24 hours post-operatively versus quartile of area under the peak airway pressure versus time curve.

$P_{\text{peak(AUC)}}$ area under the peak airway pressure versus time curve. $p=0.20$, Kruskal-Wallis test; $n=31$.

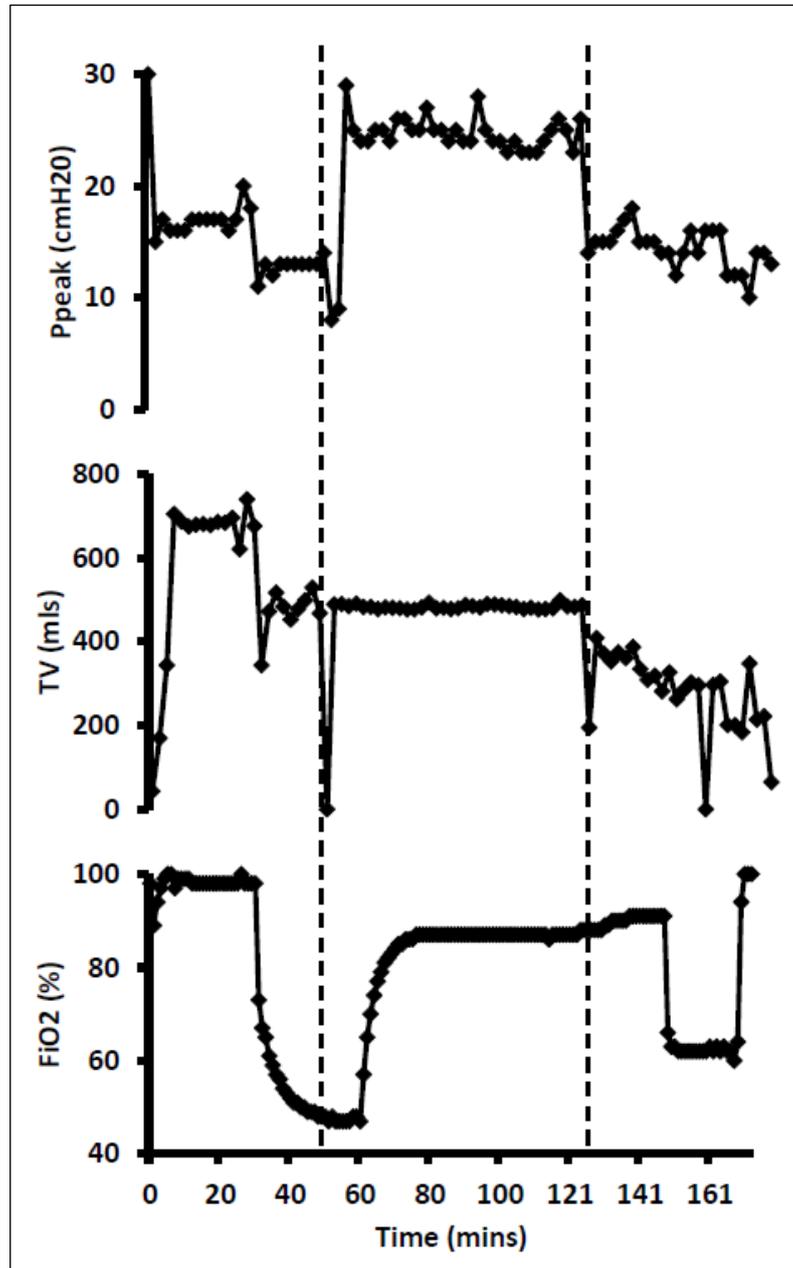


Figure 4.25. Example peak airway pressure (Ppeak), tidal volume (V_T) and fraction of inspired oxygen (FiO_2) versus time curves for a patient undergoing lung resection.

The period of one-lung ventilation (recorded on this occasion on the anaesthetic record) is marked between the dashed lines and is clearly discernible from the characteristic changes in Ppeak and V_T curves.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained no significant difference in PTX3 level across all groups of $Ppeak_{(AUC)}$ ($p=0.67$, Kruskal-Wallis test; $n=28$; not shown).

PTX3 versus tidal volume

There were no significant differences in PTX3 level across all quartiles of area under the tidal volume versus time curve ($V_{T(AUC)}$; $p=0.15$, Kruskal-Wallis test; Figure 4.26).

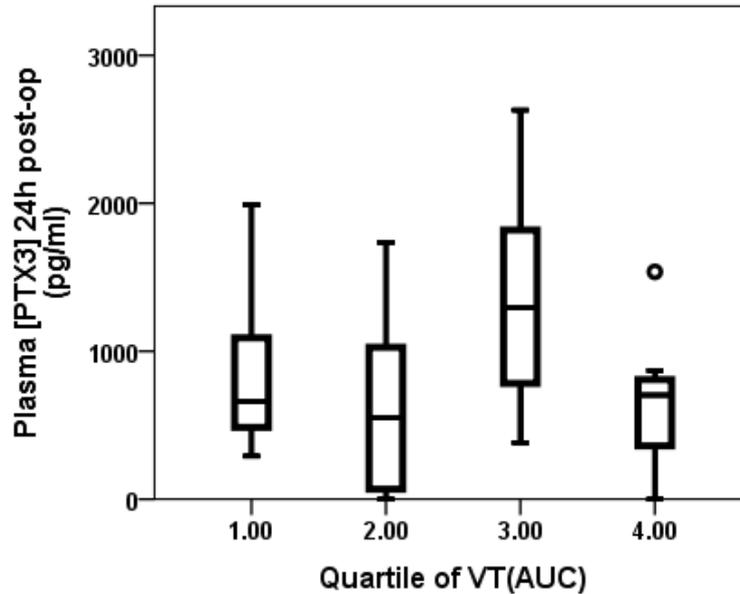


Figure 4.26. Plasma PTX3 24 hours post-operatively versus quartile of area under the tidal volume versus time curve,

$V_{T(AUC)}$, area under the tidal volume versus time curve. $p=0.15$, Kruskal-Wallis test; $n=31$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained no significant difference in PTX3 level across all across all quartiles of $V_{T(AUC)}$ ($p=0.56$, Kruskal-Wallis test; $n=28$; not shown).

PTX3 versus fraction of inspired oxygen

There were no significant differences in PTX3 level across quartiles of area under the FiO_2 versus time curve during the period of OLV ($FiO_{2(AUC)}$; $p=0.34$, Kruskal-Wallis test; $n=31$; Figure 4.27).

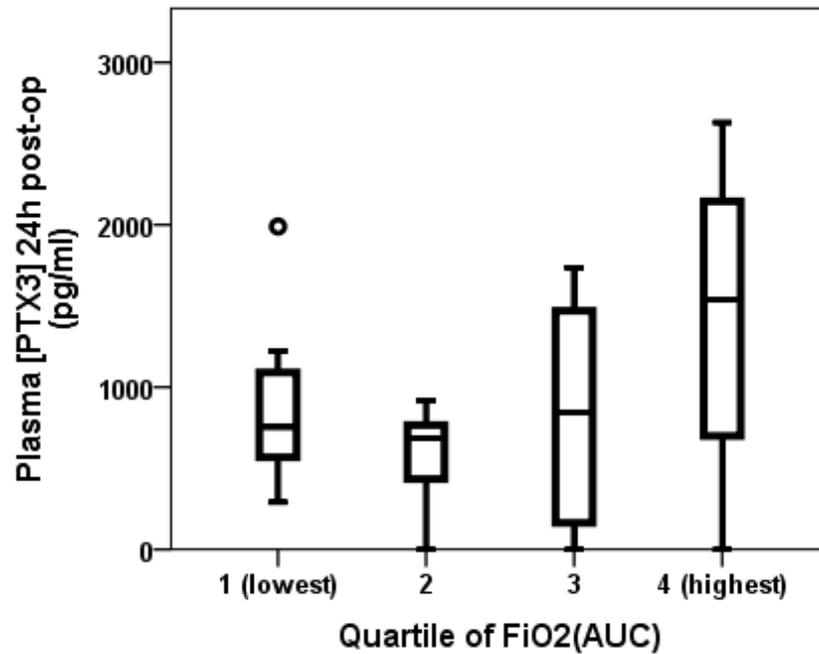


Figure 4.27. Plasma PTX3 24 hours post-operatively versus quartile of area under the FiO₂ versus time curve.

FiO₂(AUC), area under the FiO₂ versus time curve. $p=0.34$, Kruskal-Wallis test, $n=31$.

Visual inspection of Figure 4.27, is again suggestive of a threshold effect in the relationship between FiO₂ and PTX3 level; median PTX3 level in the quartile of patients exposed to the highest FiO₂ appears markedly higher than in the other three quartiles (no statistical comparison made).

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained no significant differences in PTX3 level across quartiles of FiO₂(AUC) ($p=0.45$, Kruskal Wallis test; $n=28$; not shown).

CRP versus ventilatory parameters

There were no significant differences in CRP level across quartiles of area under the P_{peak} , V_T nor FiO₂ verses time curves (Table 4.13).

Table 4.13. Relationship between CRP level and lung protective ventilation.

<i>Parameter</i>	<i>CRP level mg/L</i>				<i>p</i>	<i>n</i>
	1	2	3	4		
P_{peak}(AUC)	130.4 (101.2-144.2)	91.6 (84.1-123.9)	128.9 (107.4-142.6)	124.6 (43.8-139.6)	0.42	30
V_T(AUC)	125.1 (101.2-132.6)	113.6 (83.5-152.5)	123.7 (111.3-139.6)	90.1 (74.8-134.3)	0.52	30
FiO₂(AUC)	130.4 (101.2-144.2)	106.4 (84.1-124.9)	107.4 (84.9-135.9)	134.3 (75.1-148.4)	0.51	30

p by Kruskal Wallis test. n, no of patients from which result derived.

4.6.3.5 Association with clinically important outcomes

Need for ventilatory support

One patient required nasal CPAP, and one patient required mechanical ventilation post-operatively. As ventilatory support was only required in two patients, no further statistical comparison regarding association between PTX3 and CRP levels and need for ventilatory support was made.

High dependency unit stay

Median high dependency unit stay in the 33 patient cohort was 44.5 (42.8-46.6) hours. (As might be expected) visual inspection of the distribution of HDU stay duration revealed a strongly positively skewed distribution (Figure 4.28).

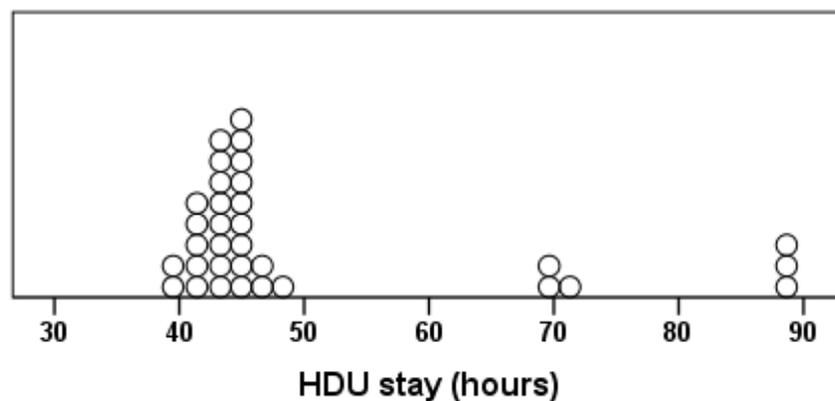


Figure 4.28. Distribution of HDU stay.
Data points represent individual patients, n=33.

Eighty per cent of HDU stay values lay in the range 40-48 hours post-operatively, reflecting standard practice in our institution where patients are routinely 'stepped-down' to ward care on the morning of post-operative day two. As such, an HDU stay of greater than 48 hours was defined as 'prolonged'.

There was a trend towards higher PTX3 levels in patients with prolonged HDU stay ($p=0.08$, Mann-Whitney U test; $n=33$; Figure 4.29).

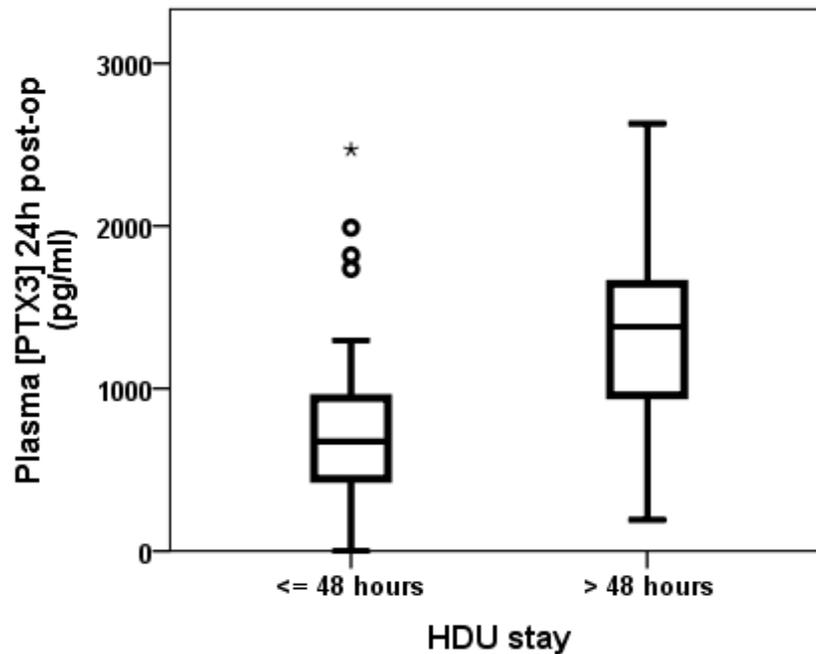


Figure 4.29. PTX3 level 24 hours post-operatively value versus duration of HDU stay. $p=0.08$, Mann-Whitney U test, $n=33$.

Sensitivity analysis: Exclusion of the three patients who demonstrated no PTX3 response to surgery, weakened the relationship between PTX3 level and prolonged hospital stay ($p=0.13$, Mann-Whitney U test; $n=30$; not shown).

There was no significant difference in CRP values between patients with normal and prolonged HDU stay ($p=0.61$, independent samples t-test; $n=32$; not shown).

Hospital stay

Median hospital stay in the 33 patient cohort was 7 (6-9.5) days. There was a modest positive correlation between LOS and PTX3 level ($r=0.44$, $p=0.01$, Spearman's rho; Figure 4.30).

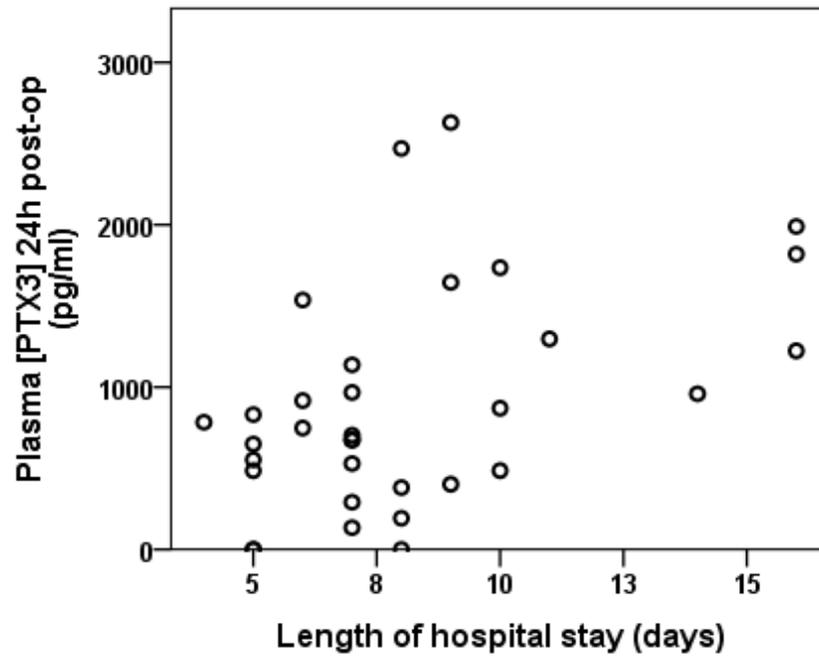


Figure 4.30. Association between length of hospital stay and PTX3 level 24 hours post-operatively.

$r=0.44$, $p=0.01$, Spearman's rho; $n=33$.

Sensitivity analysis: After exclusion of the three patients who demonstrated no PTX response to surgery, there remained a modest positive correlation between LOS and PTX3 level ($r=0.40$, $p=0.03$; $n=30$; not shown).

There was a trend towards a statistically significant association between LOS and CRP level 24 hours post-operatively ($r=0.30$, $p=0.10$, Spearman's rho; Figure 4.31).

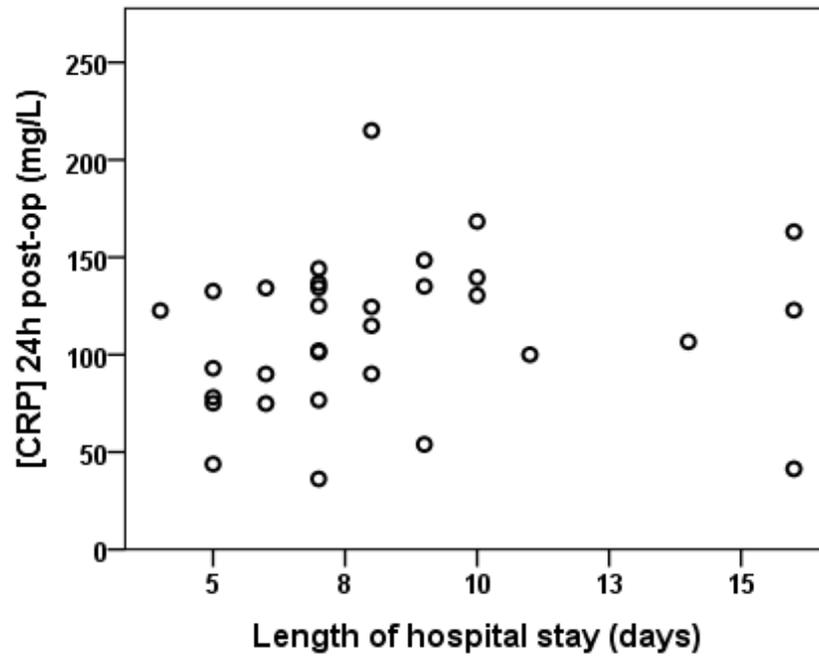


Figure 4.31. Association between length of hospital stay and CRP level 24 hours post-operatively.
 $r=0.30$, $p=0.10$ Spearman's rho; $n=32$.

4.6.4 Utility of a multiple (lung injury) biomarker panel following lung resection

4.6.4.1 Changes in individual biomarkers following lung resection

Receptor for advanced glycation end product

There were significant changes in plasma receptor for advanced glycation end products (RAGE) levels across the three time points ($p < 0.01$, Friedman test; $n = 22$, Figure 4.32). Post-hoc testing by pairwise comparisons (adjusted for multiple comparisons) revealed median RAGE was increased immediately post-operatively, but then fell significantly 24h post-operatively ($p \leq 0.01$ for both). There was no significant difference between RAGE levels pre-operatively and 24h post-operatively.

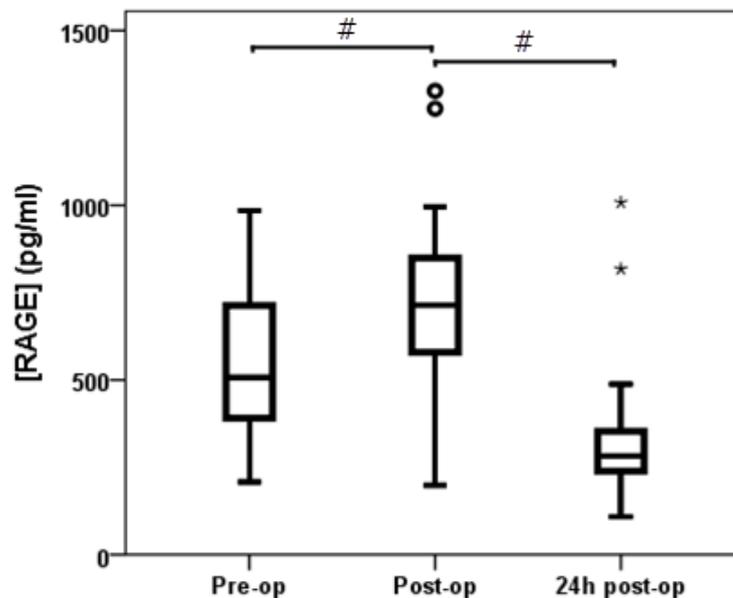


Figure 4.32. Changes in plasma RAGE following lung resection. # $p < 0.01$, Wilcoxon signed ranks test. $n = 22$.

Post-hoc analysis: Relationship of changes in plasma RAGE to volume of lung resected

Volume of resected lung tissue was quantified as number of pulmonary segments resected and was then compared with change in plasma RAGE level between pre-operative levels and those observed immediately and 24 hour post-operatively (Δ RAGE). There was a trend towards a negative association between volume of lung resected and Δ RAGE_{24h post-op - pre-op} ($r = -0.40$, $p = 0.07$; Spearman's rho; $n = 22$; not shown) and a significant negative association between volume of

lung resected and $\Delta\text{RAGE}_{\text{post-op} - \text{pre-op}}$ ($r=-0.43$, $p=0.046$; Spearman's rho; $n=22$; Figure 4.33).

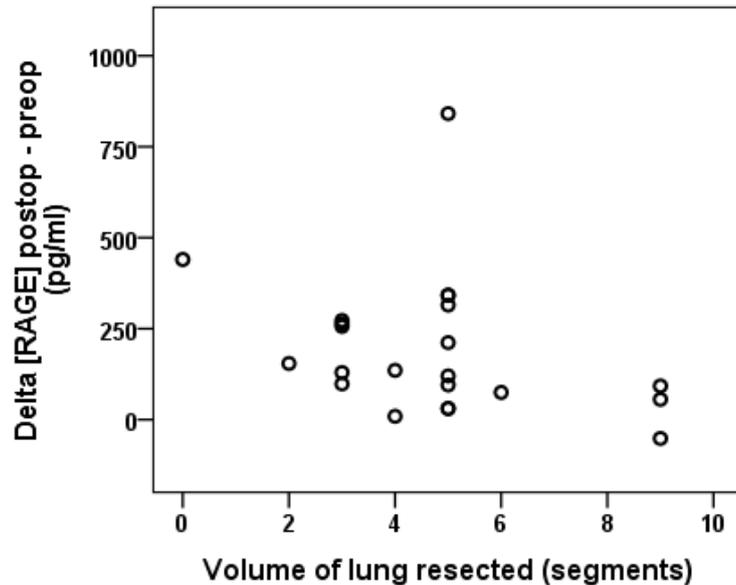


Figure 4.33. Relationship between change in plasma RAGE level immediately post-operatively and volume of lung tissue resected. $r=-0.43$, $p=0.046$; Spearman's rho. $n=22$.

In view of this relationship, post-operative plasma RAGE levels were then adjusted for the number of pulmonary segments present at the time of sample draw, to provide a value of RAGE per lung segment (RAGE_{adj}). Analysis of changes in plasma RAGE_{adj} , revealed a similar significant change in plasma RAGE_{adj} level across all three time-points ($p<0.01$, Friedman test with pairwise comparisons), but appeared to magnify the immediate post-operative increase ($p<0.01$ for immediately post-operatively vs pre-operatively and 24 hours post-operatively), however following adjustment, RAGE_{adj} levels 24 hours post-operatively remained non-significantly different from pre-operatively ($p=0.50$, Wilcoxon signed ranks, $n=22$, Figure 4.34).

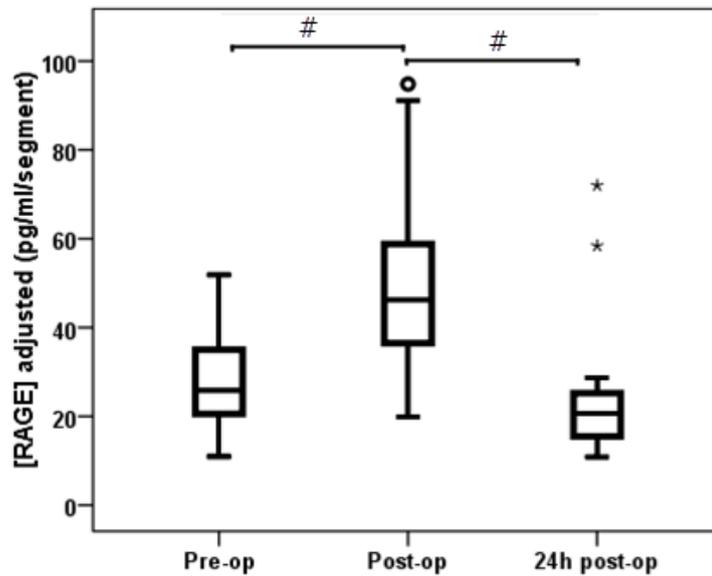


Figure 4.34. Plasma RAGE level adjusted for volume of lung resected following lung resection.

$p < 0.01$, Wilcoxon signed ranks test; $n = 22$.

Procollagen Peptide III

There were significant changes in PCPIII level across the three time points ($p < 0.01$, Friedman test; $n = 22$; Figure 4.35). Post-hoc testing by pairwise comparisons (adjusted for multiple comparisons) revealed median PCPIII level fell immediately post-operatively ($p < 0.01$, pre-operatively vs immediately post-operatively), before returning to baseline 24h post-operatively ($p = 1.0$, pre-operatively vs 24 hours post-operatively).

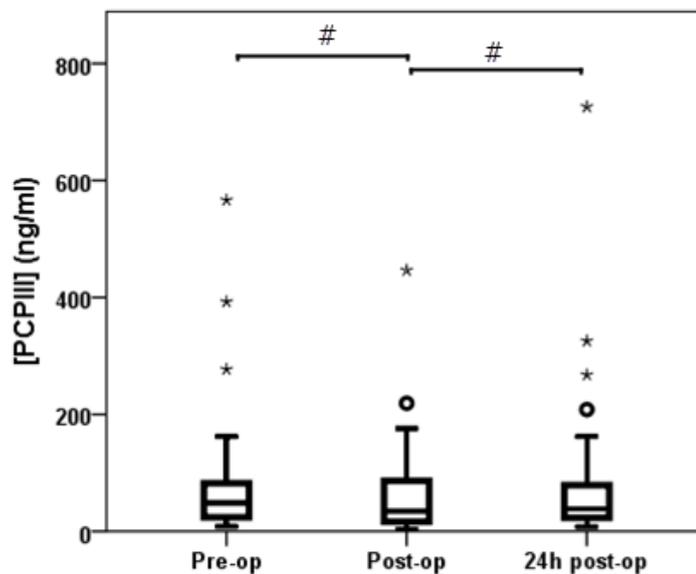


Figure 4.35. Changes in plasma PCPIII following lung resection.

$p < 0.01$, Wilcoxon signed ranks test; $n = 22$.

Post-hoc analysis: Relationship between changes in plasma PCPIII and volume of lung resected

In view of the observed post-operative fall in plasma PCPIII, volume of resected lung tissue in segments was compared with change in plasma PCPIII level between pre-operative levels and those observed immediately post-operatively (Δ PCPIII). There was no association between volume of lung resected and Δ PCPIII_{post-op - pre-op} ($r=-0.007$, $p=0.98$, Spearman's rho; not shown). No analysis of PCPIII values adjusted for the volume of lung resected was therefore performed.

B-type Natriuretic Peptide

Results for B-type natriuretic peptide (BNP) are summarised in Figure 4.36. Returned BNP levels were considerably larger than expected.

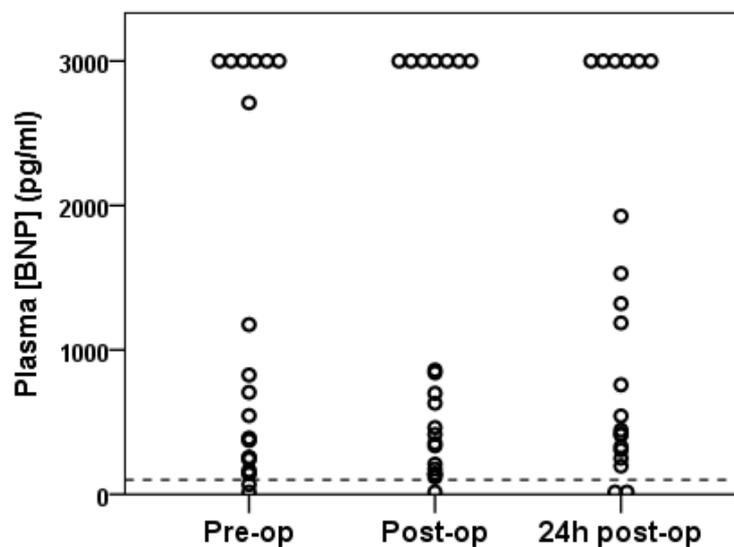


Figure 4.36. Plasma BNP level following lung resection.
Horizontal dashed line at 100pg/ml.

As can be seen in Figure 4.36 for the majority of patients (in fact all but two), pre-operative BNP levels were greater than 100pg/ml, a cut off which has been recommended as being diagnostic for congestive cardiac failure³⁵³. Furthermore BNP levels were greater than 1.5 times the upper reference range of the assay (i.e. greater than 3000pg/ml), in six patients pre-operatively, seven patients immediately post-operatively and six patients 24 hours post-operatively. Such levels of BNP are far in excess of what might be expected in patients with NYHA grade IV heart failure³⁵³, findings clearly at odds with the clinical condition of the patients in this cohort. In view of these obvious uncertainties surrounding

the validity of the measured BNP levels, no further analysis of peri-operative BNP levels was performed.

Angiotensin-2

There were significant changes in Angiotensin 2 (Ang-2) level across the three time points ($p < 0.01$, Friedman test; $n = 22$, Figure 4.37). Post-hoc testing by pairwise comparisons (adjusted for multiple comparisons) revealed a significantly increased Ang-2 level 24h post-operatively ($p < 0.01$ and $p = 0.06$ for Ang-2 24 hours post-operatively versus pre-operatively and immediately post-operatively respectively).

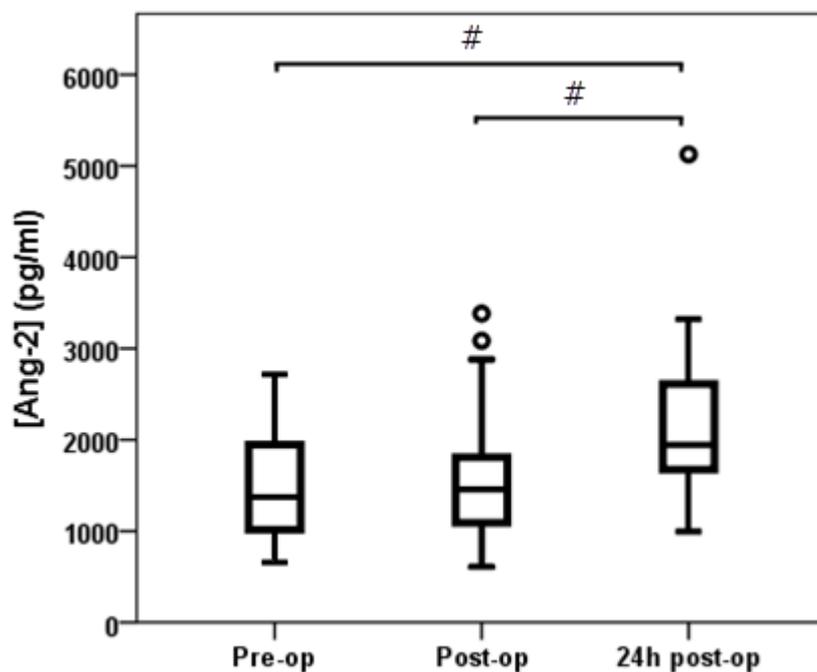


Figure 4.37. Changes in plasma Ang-2 following lung resection.
$p < 0.01$, Wilcoxon signed ranks. $n = 22$.

Interleukin-8

Observation of the data over the three time points reveals IL-8 levels are essentially constant at < 0.10 pg/ml across all three time points in the majority of patients (Figure 4.38). There were no significant changes in IL-8 levels across the three time points ($p = 0.23$, Friedman test; $n = 22$).

Tumour necrosis factor alpha

Tumour necrosis factor alpha (TNF- α) was not detected in any sample.

4.6.4.2 Application of a risk of lung injury score

Following the observations above concerning the changes in individual biomarker levels following lung resection, the risk of lung injury score described by Freemont et al, was modified to allow its use in this cohort. Firstly, data for BNP (where the results were of dubious validity) and for TNF- α (where TNF- α levels were not recorded in any sample) were excluded. Scores were then obtained for the remaining five biomarkers using the prediction tool described by Freemont et al (see Figure 4.3, Section 4.5.3.2). Observed levels of plasma RAGE were adjusted prior to scoring to reflect the volume of lung resected:

$$RAGE_{adj} = RAGE \times \frac{\text{No. of segments resected}}{19} \times 19$$

Equation 4.3

This provided a cumulative ‘risk of lung injury score’ for a five biomarker panel. Figures 4.40 and 4.41 show the results of the five biomarker panel, scored immediately post- and 24 hours post-operatively in each individual patient.

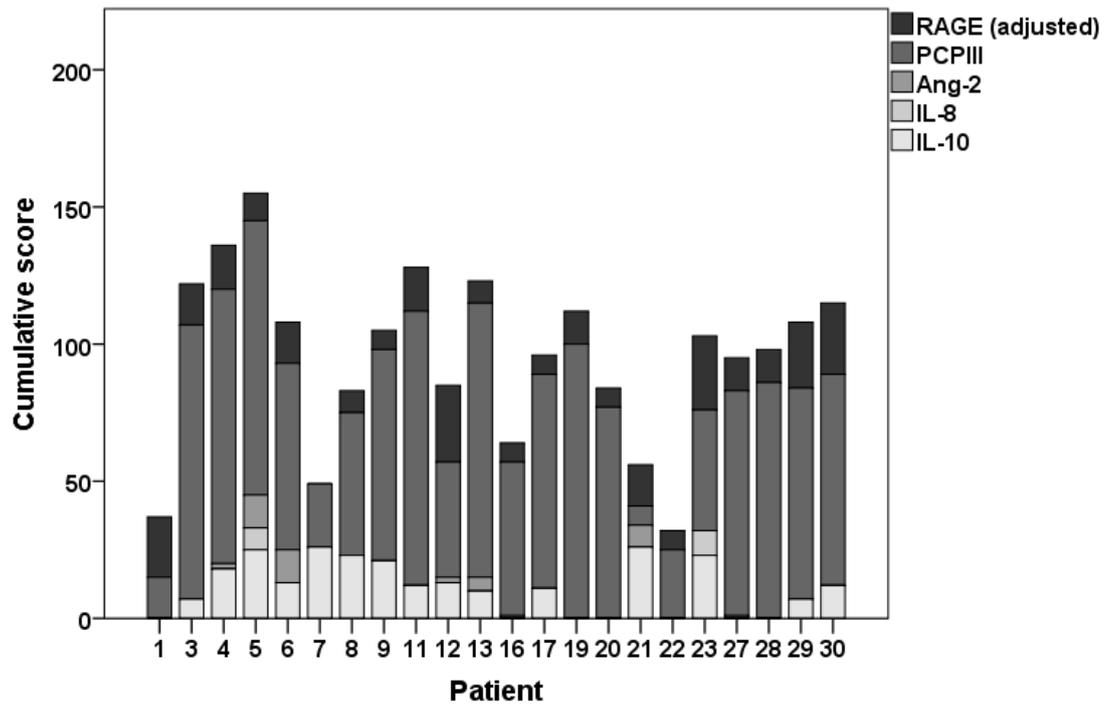


Figure 4.40. Cumulative 'risk of lung injury' scores per patient, immediately post-operatively for a five biomarker panel.
n=22.

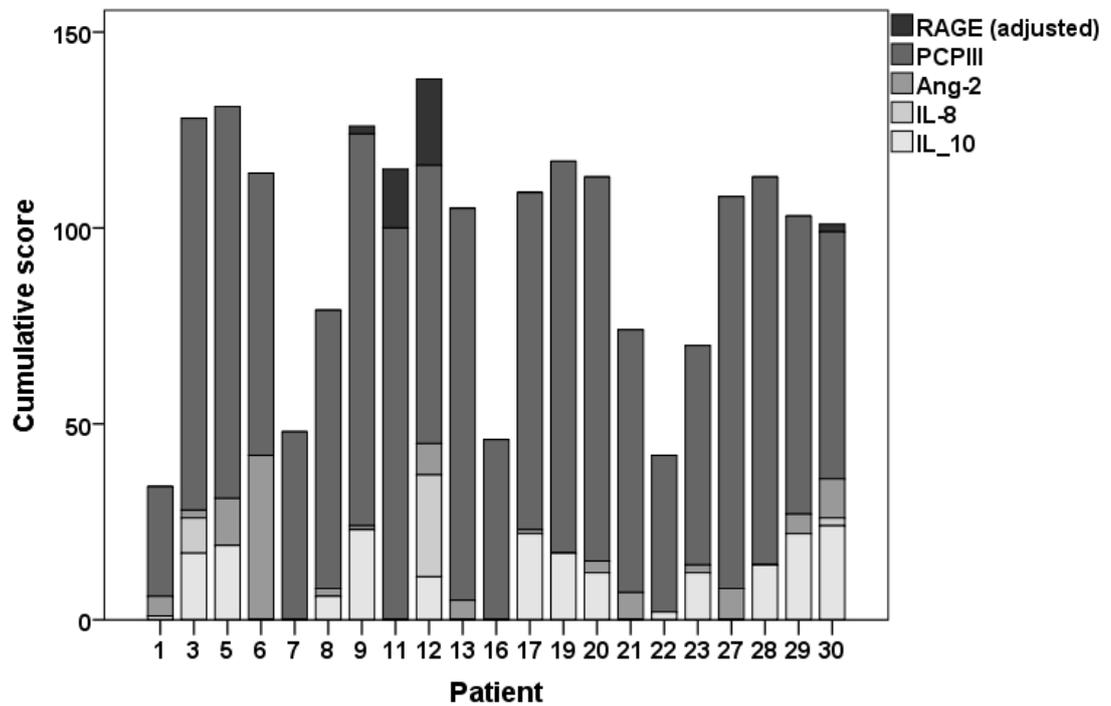


Figure 4.41 Cumulative 'risk of lung injury' scores per patient, 24 hours post-operatively for a five biomarker panel.
n=22.

Visual inspection of Figures 4.40 and 4.41 reveals that the overwhelming majority of the cumulative score is attributable to PCPIII, such that changes in the other biomarkers are to a degree 'swamped' by the PCPIII score. In view of this, and the observation that PCPIII scores in fact fell immediately post-operatively, and were no different from baseline 24 hours post-operatively, results for PCPIII were excluded and a further score was thus obtained for a four biomarker panel (comprising RAGE (adjusted for the volume of lung resected), Ang-2, IL-8 and IL-10). Figures 4.42 and 4.43 demonstrate the individual patient scores for the four biomarker panel.

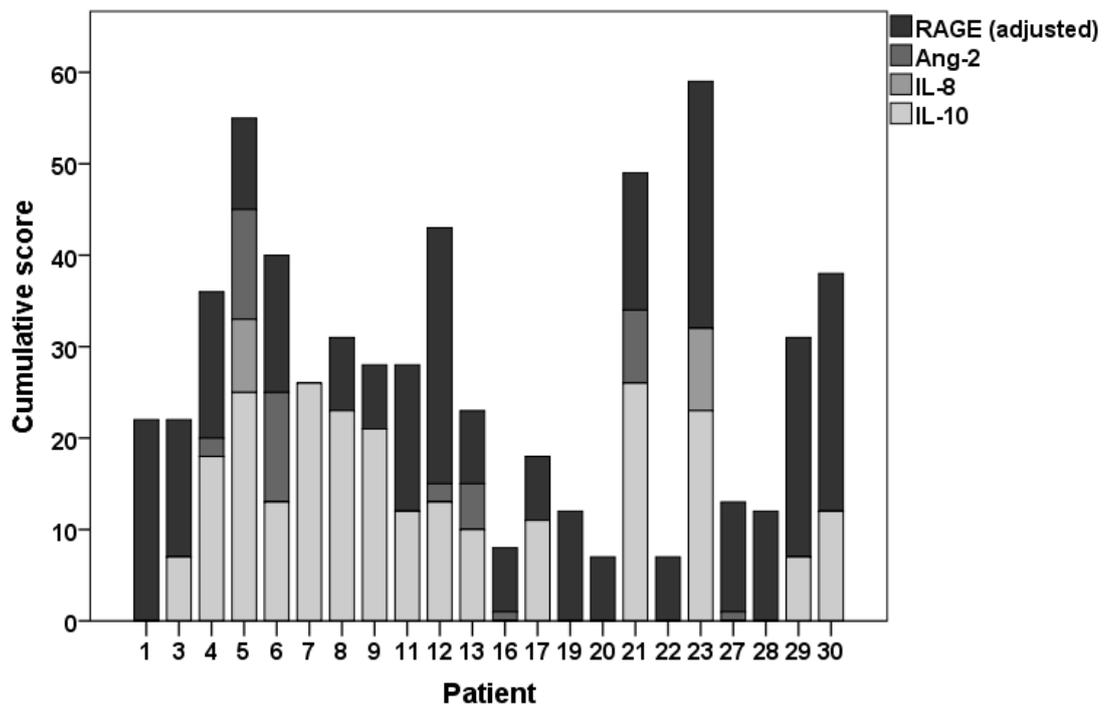


Figure 4.42. Cumulative 'risk of lung injury' scores per patient, immediately post-operatively for a four biomarker panel.

n=22.

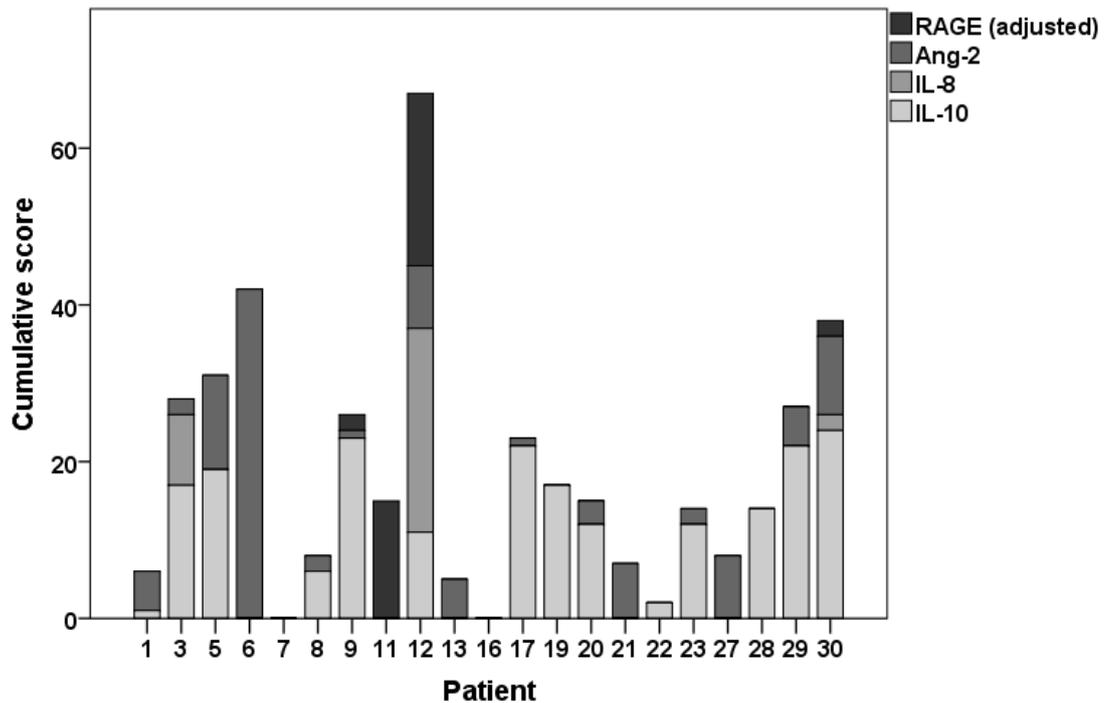


Figure 4.43. Cumulative 'risk of lung injury' scores per patient, 24 hours post-operatively for a four biomarker panel.
n=22.

Sensitivity and specificity of a multi-biomarker 'risk of lung injury score' for predicting clinical outcomes

Assessment was made of the relationship between post-operative multiple biomarker panel scores 24 hours post-operatively (for both a four and five biomarker panel) and modified Lung Injury Score (mLIS) on post-operative day one, to assess the diagnostic value of the panels. To assess the prognostic value of the panels, biomarker panel scores immediately post-operatively were compared with mLIS on post-operative day one and biomarker panel scores 24 hours post-operatively were compared with mLIS on post-operative day two.

Diagnostic value of biomarker panel score for predicting modified lung injury score on post-operative day one

Modified lung injury score on post-operative day one was available for 14 patients in whom the multi-biomarker panel was measured. Dichotomising these patients into groups of patients with mLIS on post-operative day one > 1.5 and those with mLIS ≤ 1.5 left 5 and 9 patients per group respectively.

There was no difference in either median five or four biomarker panel score 24 hours post-operatively between the two groups of mLIS ($p=0.46$ and 0.18 respectively, Mann-Whitney U test; $n=22$; not shown). In the absence of any significant difference in four or five biomarker panel score 24 hours post-

operatively and mLIS on post-operative day one, receiver operating characteristic curve analysis was not performed.

Prognostic value of biomarker panel score for predicting modified lung injury score on post-operative days one and two

There was no difference in either median five or four biomarker panel score immediately post-operatively between the two groups of mLIS ($p=0.54$ and 0.12 for a five and four biomarker panel respectively, Mann-Whitney U test; $n=22$; not shown).

Modified lung injury score on post-operative day two was available for 9 patients in whom the multi-biomarker panel was measured. Dichotomising these patients into groups of patients with mLIS on post-operative day one greater than 1.5 and those with mLIS less than or equal to 1.5 left 2 and 7 patients per group respectively. There was no significant difference in either median five or four biomarker panel 24 hours post-operatively and between the two groups of mLIS on post-operative day two ($p=0.38$ and 0.19 respectively, Mann-Whitney U test; $n=22$; not shown).

In the absence of any significant difference in four or five biomarker panel score at any time point between groups of mLIS on post-operative days one and two, receiver operating characteristic curve analysis was not performed.

Variation in proportion to the severity of injury

To examine whether multiple biomarker panel scores vary in proportion to the severity of oxygenation impairment, 'worst' recorded $\text{PaO}_2:\text{FiO}_2$ ratios at 6 and 24 hours post-operatively were compared with multiple biomarker panel scores immediately and 24 hours post-operatively respectively. There were no significant associations between biomarker panel score for either a five or four biomarker panel and oxygenation at any time point (Table 4.14).

To examine whether multiple biomarker panel scores vary in proportion to the severity of chest x-ray score, post-operative day one CXR scores were compared with biomarker panel scores. There were no significant associations between biomarker panel score for either or five or four biomarker panel and CXR score (Table 4.14).

Table 4.14. Association between five and four biomarker panel score and severity of post-operative oxygenation impairment and chest X-ray score.

		PaO ₂ /FiO ₂ 6h post-op	PaO ₂ /FiO ₂ 24h post-op	CXR Score 24h post-op
5 biomarker score				
- immediately	<i>r</i>	-0.3		
post-op	<i>p</i>	0.28		
	<i>n</i>	15		
- 24h post-op	<i>r</i>		-0.1	-0.27
	<i>p</i>		0.72	0.32
	<i>n</i>		14	15
4 biomarker score				
- immediately	<i>r</i>	0.23		
post-op	<i>p</i>	0.40		
	<i>n</i>	15		
- 24h post-op	<i>r</i>		0.06	-0.49
	<i>p</i>		0.83	0.06
	<i>n</i>		14	15

r and *p*, association by Pearson correlation or Spearman's rho as appropriate; *n*, no of patients on which analysis is based.

Modification by an effective intervention

Lesser resection

Type of resection

Of the 22 patient cohort for whom the multiple biomarker panel was measured, one patient underwent sub-lobar resection, 18 patients underwent anatomic lobectomy, and three patients underwent pneumonectomy. Due to the small numbers in the sub-lobar resection and pneumonectomy group, no statistical comparison was made between these groups.

Volume of lung resected

One of the 22 patient cohort underwent sub-lobar resection and so was excluded from this analysis. The remaining 21 were divided into tertiles by the number of pulmonary segments resected. There were no differences in multiple biomarker panel score between tertiles of number of pulmonary segments resected (for either a four or five biomarker panel, immediately or 24 hours post-operatively, Table 4.15)

Table 4.15. Multiple biomarker panel scores immediately and 24 hours post-operatively by number of pulmonary segments resected.

Tertile of 'number of pulmonary segments resected'	Time-point	Median multiple biomarker panel score				
		1 (least)	2	3 (most)	p	n
Five biomarker panel	Post-op	101.5 (73.5-122.0)	95.0 (84.0-112.0)	111.5 (78.5-119.0)	0.74	21
	24h	113.0 (74.0-126.0)	113.0 (70.0-117.0)	102.0 (74.5-104.0)	0.58	20
Four biomarker panel	Post-op	33.5 (20.0-44.5)	22.0 (12.0-28.0)	28.5 (24.5-34.5)	0.36	21
	24h	14.0 (7.0-31.0)	15.0 (8.0-17.0)	16.0 (2.5-32.5)	0.95	20

Biomarker panel scores are median (IQR). p values by Kruskal-Wallis test for all. n, no of patients on which analysis is based.

Lung protective ventilation and duration of one-lung ventilation

Of the 22 patients for whom results of the multi-biomarker panel were available, ventilatory parameters during the period of one-lung ventilation were available in 21 patients; the duration of one-lung ventilation could be deduced in all patients. As with the analysis of PTX3 and CRP, to explore the association between ventilatory parameters during the period of OLV and total risk of lung injury scores, the areas under the curve (AUC) from the peak airway pressure (P_{peak}), tidal volume (V_T) and FiO_2 versus time curves and the duration of one-lung ventilation were divided into quartiles. 'Risk of lung injury' scores determined immediately and 24 hours post-operatively for a five and four biomarker panel were compared with ventilatory parameters using one way analysis of variance or Kruskal Wallis test as appropriate (Table 4.16).

Table 4.16. Risk of lung injury scores across quartiles of peak air way pressure, tidal volume, fraction of inspired oxygen and one-lung ventilation time for a five biomarker panel.

<i>Time point</i>		<i>Multiple biomarker panel score</i>					
<i>Quartile of parameter:</i>		1	2	3	4	p	n
P_{peak}(AUC)	Post-op	85.0 (84.0- 98.0)	102.0 (95.0- 123.0)	112.0 (103.0- 115.0)	83.0 (49.0- 108.0)	0.35 ^{KW}	21
	24h	113.5 (113.0- 126.0)	108.5 (105.0- 114.0)	101.0 (70.0- 117.0)	63.5 (41.0- 91.0)		
V_T(AUC)	Post-op	75.8 (31.3)	100.5 (15.9)	95.8 (47.5)	102.2 (32.3)	0.57 ^{AN}	21
	24h	113.0 (74.0- 114.0)	111.0 (105.0- 117.0)	108.0 (46.0- 115.0)	85.5 (59.0- 102.0)		
FiO₂(AUC)	Post-op	98.0 (85.0- 108.0)	70.0 (49.0- 96.0)	103.0 (83.0- 105.0)	112.0 (108.0- 115.0)	0.26 ^{KW}	21
	24h	114.0 (113.0- 115.0)	91.5 (48.0- 113.0)	79.0 (70.0- 105.0)	105.5 (102.0- 112.5)		
OLV time (mins)	Post-op	85.0 (56.0- 98.0)	117.5 (105.0- 128.0)	89.5 (49.0- 103.0)	108 (95.0- 115.0)	0.09 ^{KW}	21
	24h	96.2 (38.0)	117.8 (9.4)	78.0 (35.7)	89.5 (29.2)		

Values are mean (SD) or median (IQR). p by ^{KW}Kruskall Wallis test or ^{AN}analysis of one-way variance as appropriate. n, no of patients on which analysis is based.

There was no difference in total lung injury score for a four or five biomarker panel, immediately- and 24 hours post-operatively across quartiles of any ventilatory parameter (Tables 4.16 and 4.17) with the possible exception of OLV time. Immediate post-operative total risk of lung injury score (for a five biomarker panel) demonstrated a non-significant trend towards a difference across the quartiles of OLV time (p=0.09, Kruskal-wallis test, Table 4.16). Pairwise comparisons were not performed as the result was not statistically significant; visual inspection of the data (Figure 4.44) does not suggest any linear trend between total risk of lung injury score and OLV time. This trend was not supported by the results of the four biomarker panel (where p=0.95 (Kruskal-wallis test), for the same comparison, Table 4.17).

Table 4.17. Risk of lung injury scores across quartiles of peak air way pressure, tidal volume, fraction of inspired oxygen and one-lung ventilation time for a four biomarker panel.

<i>Time point</i>		<i>Multiple biomarker panel score</i>					
		1	2	3	4	p	n
P_{peak}(AUC)	Post-op	12.0 (7.0-28.0)	25.5 (18.0-40.0)	38.0 (12.0-55.00)	31.0 (26.0-31.0)	0.51 ^{KW}	21
	24h	15.0 (14.0-26.0)	11.5 (7.0-23.0)	17.0 (14.0-31.0)	7.0 (2.0-17.5)	0.68 ^{KW}	21
V_T(AUC)	Post-op	30.2 (19.3)	19.8 (9.3)	25.2 (18.4)	38.0 (12.6)	0.28 ^{AN}	21
	24h	26.4 (27.5)	15.7 (8.2)	12.0 (11.9)	19.8 (16.4)	0.60 ^{AN}	21
FiO₂(AUC)	Post-op	26.0 (16.2)	29.5 (18.6)	29.8 (18.6)	26.0 (12.6)	0.97 ^{AN}	21
	24h	28.0 (26.3)	13.7 (11.7)	10.6 (10.0)	22.5 (12.9)	0.35 ^{AN}	21
OLV time (mins)	Post-op	113.0 (74.0-114.0)	116.0 (113.0-126.0)	74.5 (48.0-109.0)	102.0 (73.5-105.5)	0.95 ^{KW}	21
	24h	26.4 (27.5)	18.2 (9.2)	13.2 (10.7)	18.3 (17.4)	0.65 ^{AN}	21

Values are mean (SD) or median (IQR). p by ^{KW}Kruskall Wallis test or ^{AN}analysis of one-way variance as appropriate. n, no of patients on which analysis is based.

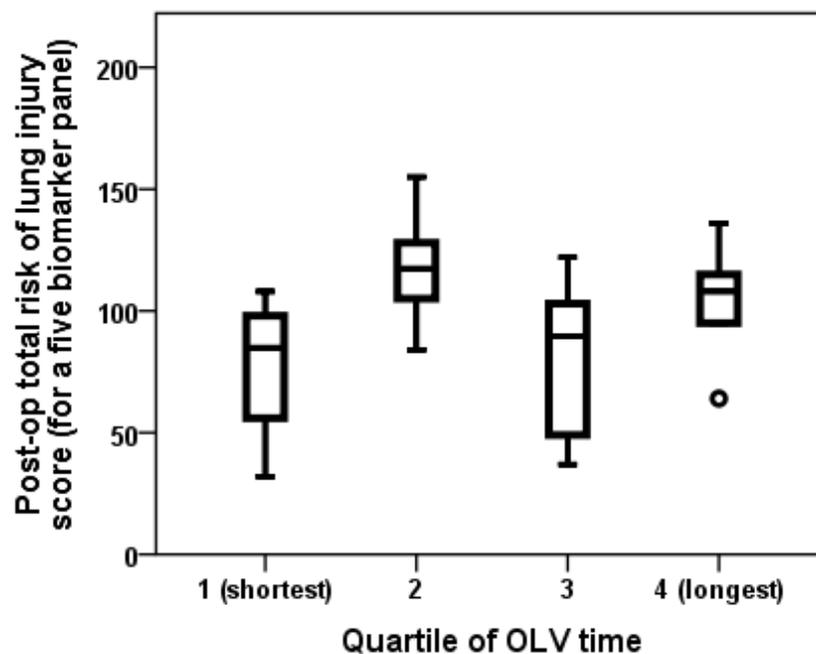


Figure 4.44. Risk of lung injury score immediately post-operatively for a five biomarker panel versus duration of one-lung ventilation.
p=0.09, Kruskal-wallis test. n=21.

Association with clinically important outcomes

High dependency unit stay

High dependency unit (HDU) stay of greater than 48 hours was defined as prolonged. In the 22 patient cohort in which the multiple biomarker panel was measured, HDU was 'prolonged' in four patients. There were no significant differences in multiple biomarker panel score between patients with 'normal' or 'prolonged' HDU stay (for either a four or five biomarker panel, immediately or 24 hours post-operatively, Table 4.18)

Table 4.18. Multiple biomarker panel score by duration of HDU stay.

	Time point	Multiple biomarker panel score			
		'Normal'	'Prolonged'	<i>p</i>	<i>n</i>
<i>HDU stay:</i>					
Five biomarker panel	Post-op	101.5 (83.0-122.0)	94.0 (74.5-109.0)	0.77	22
	24h	109.0 (79.0-115.0)	85.5 (58.0-119.5)	0.57	21
Four biomarker panel	Post-op	24.5 (13.0-31.0)	40.5 (23.0-51.0)	0.23	22
	24h	15.0 (7.0-26.0)	26.0 (7.0-52.5)	0.57	21

p by Mann Whitney U test for all. *n*, no of patients on which analysis is based.

Hospital stay

There was no association between multiple biomarker panel score and length of hospital stay (for either a four or five biomarker panel, immediately or 24 hours post-operatively, Table 4.19)

Table 4.19 Association between multiple biomarker panel score and length of hospital stay.

	<i>Time point</i>	<i>r</i>	<i>p</i>	<i>n</i>
Five biomarker panel	Post-op	-0.05	0.82	22
	24h	0.06	0.79	21
Four biomarker panel	Post-op	-0.15	0.50	22
	24h	0.03	0.91	21

r and *p* by Spearman's rho for all. *n*, no of patients on which analysis is based.

4.6.5 Summary of results

In order to summarise how well PTX3, CRP and both the four and five biomarker panels compare to the properties of the ‘ideal’ lung injury biomarker, the strength of evidence provided for each property has been graded as follows:

- ++** Consistent evidence provided, in agreement with hypotheses, sensitivity analyses support main analysis.
- +** Some evidence provided, in agreement with hypotheses, but is either inconsistent, or results of sensitivity analyses and main analysis are not consistent.
- No supportive evidence provided

Table 4.20. Summary of results – Utility of PTX3, CRP and a multiple biomarker panel as biomarkers of lung injury following lung resection.

	<i>PTX3</i>	<i>CRP</i>	<i>Five biomarker panel</i>	<i>Four biomarker panel</i>
Sensitivity and specificity for the outcome of interest	++	—	—	—
Variation in proportion to the severity of illness	++	—	—	—
Modification by an effective intervention	+	—	—	—
Association with clinically important outcomes	++	+	—	—

4.7 Discussion

4.7.1 Pentraxin 3 as a biomarker of post-lung resection lung injury

There have been no previous reports of the measurement of PTX3 following lung resection. Others however have reported elevated PTX3 levels following other types of surgery. Akerfeldt et al measured PTX3 on days four and 30 post-operatively in patients undergoing orthopaedic and cardiac surgery, observing a peak in PTX3 on post-operative day four, and concluding that PTX3 “shows a much smaller increment in humans in comparison with CRP”³⁵⁴. Part of the rationale for selecting PTX3 as a candidate lung injury biomarker for this study were the reports of previous observations suggesting that PTX3 levels peak more rapidly than CRP following an inflammatory insult^{321, 355}. Peri et al (for example) measured PTX3 levels in patients admitted to the coronary care unit (CCU) with symptoms of acute myocardial infarction, observing that PTX3 levels peaked at 7.5 hours following CCU admission whilst C-reactive protein did not peak until 24 hours after admission³⁵⁶. It is plausible that Akerfeldt et al’s conclusion reflects CRP measurement at close to peak values whilst PTX3 levels may have attained peak levels some time previously and begun to wane. Kunes et al determined PTX3 levels in patients undergoing both ‘on-pump’ and ‘off-pump’ coronary artery bypass grafting³⁵⁷. In patients undergoing cardiopulmonary bypass PTX3 levels were significantly higher than baseline by then end of the operation; peak levels were observed on the first post-operative day. Mirroring the findings of the current study in thoracic surgical patients, in patients undergoing ‘off-pump’ surgery (without use of cardiopulmonary bypass), PTX3 levels were unchanged from baseline immediately post-operatively, but again appeared to peak on post-operative day one, returning to baseline by day three (Kunes et al made no assessment of interval PTX3 level between the end of the operation and 24 hours post-operatively)³⁵⁷. Having detected an elevated PTX3 level at a single time point following lung resection, it is impossible on the basis of the current study to conclude whether PTX3 levels were detected before or after their peak. It would be of value to better determine the trajectory of PTX3 levels following lung resection, the observation of an elevated level at 6 or 12 hours post-

operatively (for example) could greatly increase the potential prognostic utility of the biomarker.

Pre-operative PTX3 levels were <20 pg/ml in all patients whilst 24 hours post-operatively PTX3 values varied from <2 pg/ml to 2630 pg/ml reflecting in excess of a thousand fold variation in PTX response. In three patients, 24 hours post-operative PTX3 level remained below the level of detection of the assay (<2 pg/ml). Whilst these results reflect samples which were measured in duplicate, in an assay with an acceptable coefficient of variation of approximately 5% and so are presented with some confidence, a sensitivity analysis excluding these three patients was performed throughout. In almost all cases, this was supportive of the primary analysis. It is evident that there is a large degree of heterogeneity in the PTX3 response - a finding which could prove an asset in a potential biomarker. To serve as illustration, Muller et al observed mean PTX3 levels to vary 1000-fold between patients with septic shock and controls, whilst variation in excess of a 100-fold was evident within the septic shock group³⁵⁵. Whilst such heterogeneity may represent variation in the severity of inflammatory stimulus, it will also be to some extent determined by the host response.

The results of the current study suggest that PTX3 offers potential as a biomarker informative of lung injury in the thoracic surgical population. PTX3 compared favourably to properties of the 'ideal' lung injury biomarker, and appeared to identify a population of patients with elevated post-operative lung injury score with high sensitivity. Johnson has suggested that in the research field of clinical risk prediction, an area under the receiver operating characteristic curve of *"0.75 is good and greater than 0.8 is exciting"*³⁵⁸. In this context the values obtained in the current study of 0.81 for diagnostic predictive value and 0.85 for prognostic predictive value are encouraging, though it must be acknowledged the confidence intervals for these estimates are wide, reflecting the modesty of the sample size.

At the cut-offs described, the sensitivity (and consequently negative predictive value) of PTX3 for predicting elevated lung injury score was high, whilst specificity was (67-70%) with PPV of 55-7%. Given these values, it is interesting to speculate what clinical role PTX3 levels might have. Putting concerns

regarding the confidence of the estimates observed aside temporarily, from the negative and positive predictive values observed in this study one could be confident that a patient with a 'negative' PTX3 value will not develop 'lung injury' (i.e. an elevated mLIS), whilst a 'positive' value suggests that a patient has an approximately 55% chance of developing the outcome. Such values are arguably satisfactory grounds on which to base clinical decisions such as whether to discharge from high dependency early (if 'negative'), or whether to maintain a watchful eye, re-site an arterial line (!), induce a diuresis, start inhaled broncho-dilators or commence a trial of high flow nasal oxygenation if 'positive'.

It is an ambition of the author that PTX3 may serve as a suitable surrogate endpoint for use in clinical research in this population. In addition to providing benefit to the thoracic surgical population, one-lung ventilation has been described as a 'human model' of lung injury²⁹⁹. Such a model therefore, if validated, could facilitate early phase clinical investigation of therapeutic or preventative therapies before translation to wider critical care environment³⁵⁹. To this end, the lack of association between PTX3 level and indices of lung protective ventilation require further consideration. The 'ideal' lung injury biomarker must be modifiable by an 'effective intervention'; a property that would clearly be pre-requisite if the biomarker was the endpoint of a clinical study of a novel therapy. Whilst there was some suggestion from the data that PTX3 levels were highest in the quartiles of patients exposed to the longest duration of one-lung ventilation and the highest fractional inhaled oxygen concentrations (signified by the visual appearances of Figures 4.23 and 4.27), there was no association between PTX3 and the area under either the peak airway pressure or tidal volume versus time curves.

Quantifying 'exposure' to ventilatory parameters in this way (by area under the parameter versus time curve), was a novel initiative introduced by the author (Ben Shelley), seeking to take advantage of the rich data source provided by the Recall AIMS charting system (as illustrated in Figure 4.24) Such a method has not been described previously though is analogous to the "ventilator hyperpressure index" described by Licker et al¹²⁸. Licker et al determined airway hyperpressure index as the "product of inspiratory plateau pressure >10 cmH₂O and the duration of OLV", observing the index to be an independent predictor of the development of ALI (OR=3.53 (CI1.7-8.6, p<0.01))¹²⁸. Licker's observations were

however made over ten years ago, during which time (as described in chapters one and two) the practice of one-lung ventilation has 'evolved'. Though ventilator settings were not protocolised in this study, it is the author's anecdotal opinion that lung protective ventilation is widely practiced locally (mean V_T during the period of OLV was 6.9 ml/kg in the study cohort (data not shown)).

Bastin et al reported significant elevations in the established lung injury biomarkers receptor for advanced glycation products (RAGE), von-Willebrand factor and interleukin (IL)-6 following lung resection but similarly were unable to demonstrate any association between biomarker levels and OLV duration, plateau pressure or tidal volume³¹⁵. It seems reasonable to hypothesise that the lack of association observed between PTX3 levels and ventilatory parameters may be because the practice of lung protective ventilation has yielded any variation in ventilator settings insufficient to influence an injury, which is only in part 'ventilator induced'. This may be especially so if the relationship between tidal volume / peak airway pressure and 'injury' is non-linear. Evidence from animal models has suggested that ventilator induced lung injury may occur in such a non-linear fashion⁹⁰. Using a scintigraphic technique to simultaneously determine epithelial and endothelial permeability in a rat model of VILI, de Prost et al demonstrated the existence of a 'threshold effect' with 'dramatic' changes in both epithelial and endothelial permeability occurring as end-inspiratory pressure was increased between 20 and 25 cmH₂O³⁶⁰.

PTX3 consistently outperformed C-reactive protein as a lung injury biomarker. This is supportive of previous findings suggesting heterogeneity between the PTX3 and CRP response. C-reactive protein and PTX3 levels 24 hours post-operatively were not significantly associated in the current study ($r=0.32$, $p=0.08$; Spearman's rho; $n=32$; data not shown)^{69, 70}. Though PTX3 is an acute phase protein from the same pentraxin 'super-family' as CRP, it would appear conclusively that measurement of PTX3 offers additional information than that obtained from CRP measurement.

4.7.2 Utility of a multiple lung injury biomarker panel following lung resection

There is no evidence from the results presented that the ‘risk of lung injury score’ as presented by Fremont et al⁸³ has any utility in the lung resection population. Fremont et al derived this score in a population of patients with ALI/ARDS of traumatic aetiology and its adoption for this study was made on the basis that lung injury after lung resection may more closely resemble sterile, traumatic lung injury than that of septic, atraumatic origin. Furthermore, this panel constitutes an aesthetic combination of epithelial, endothelial, fibrotic, pro-inflammatory, anti-inflammatory and heart failure markers, leading one to believe that multiple aspects of the complex pathophysiology of ALI/ARDS are well represented. There are a number of potential explanations for the ‘negative’ findings observed.

Firstly, the score was devised in a population of critically ill patients with established ALI/ARDS; a degree of lung injury of markedly greater severity than that seen in the post-operative population studied. Given the dramatic difference in pre-test probability for the diagnosis of ALI/ARDS, simply applying the same scores (based on the same individual biomarker cut-off levels) was likely naive and represents a significant methodological flaw. Given a much larger patient cohort, it would have been more appropriate to construct a new multivariate regression model (either with the same or alternative biomarkers).

Secondly the seven biomarker panel was reduced to five due to the necessity to exclude the BNP results (discussed below), and the failure to detect TNF- α in any sample. Unknown to the author (B. Shelley) at the time of planning this study, Bastin et al, collecting plasma samples at the same time points, were similarly unable to detect any TNF- α response in patients undergoing lung resection³¹⁵. Yim et al reported the generation of TNF- α to be ‘minimal’ in all patients (undergoing lung resection by either thoracotomy or video assisted thoracoscopic technique) both during and after surgery³⁶¹. The mean values of observed TNF- α were ≤ 10 pg/ml at all time points³⁶¹, values below the lower level of detection of the assay kit used in this study.

Time from onset of surgery to attaining peak levels of any potential biomarker is a further challenge to studies of this sort in the post-operative population. Whilst in a critically ill patient with trauma or sepsis a sustained elevation of biomarker levels might be expected in most cases, the trajectory of many biomarkers in surgical models is of a discrete intra- or post-operative peak followed by resolution. Receptor for advance glycation end products (RAGE) peaked immediately post-operatively, whilst angiopoietin-2 and interleukin (IL)-10 peaked on post-operative day one. Mismatch between sample timing and that of biomarker peak is also a likely explanation for elevated IL-8 levels being detected in so few patients in the current study. Yim et al documented a peak in IL-8 levels occurring four hours post-operatively with virtual resolution by 24 hours post-operatively³⁶¹. Komatsu et al reported similar findings³⁶²; it seems likely that if an IL-8 peak did occur in the current patient cohort, it was simply not detected due to it having occurred between the sample time points selected. Such differences between the timing of peak biomarker levels add further complication when considering combination of biomarkers into a score such as that described by Fremont et al⁸³. Either a panel of biomarkers must be selected where peak levels can be anticipated to occur simultaneously (for example IL-10, Ang-2 and PTX3), or biomarker assays must be made at different time points and combined once all results available; a strategy that is likely to reduce any practical clinical utility of the panel.

It is inherently desirable for a lung injury biomarker to be sufficiently 'lung specific' so as to be able to distinguish between pulmonary injury and systemic inflammation. To this end, biomarkers of pulmonary epithelial function are an obvious candidate (there are no markers truly specific to the *pulmonary* endothelium). In the specific context of patients undergoing lung resection however, lung specificity conveys a further complication. As observed previously for Krebs von den Lungen (KL)-6 and Surfactant protein (SP)-D, both pulmonary epithelial biomarkers^{315, 316}, in this study post-operative RAGE levels were observed to vary in proportion to the volume of pulmonary tissue resected ($r=0.43$, $p=0.046$; Figure 4.33). Whilst adjustment of biomarker values to account for their anticipated fall (as subsequently performed for RAGE in the current study and previously advocated by both Maeda³¹⁴ and Sukama³¹⁶ and colleagues)

is an attractive solution, such models need further validation before they can be recommended and add further complexity to any investigation.

4.7.3 Limitations

Not protocolising the timing of arterial blood gas analysis nor maintenance of intra-arterial cannula has proved a significant limitation of this study and can only be described as a learning experience for the author.

It must be recognized that the FiO_2 values used in the calculation of PaO_2/FiO_2 were estimates, based on the set FiO_2 on the venturi device (which are held generally to be very reliable²⁷³) or on the flow rate through nasal cannulae. No direct measurement of FiO_2 was made; this was felt to be impractical in the clinical context of study (where PaO_2/FiO_2 was calculated over multiple time points in each individual patient over a prolonged period of time). It is widely recognized however that the performance (in terms of delivered FiO_2) of oxygen delivery devices varies substantially in relation to tidal volume⁷² and pattern of respiration (in the case of nasal cannulae, nose versus mouth breathing²⁸²). Inaccuracy in estimation of FiO_2 is therefore a limitation of this study, though one shared by the majority of the literature on the topic, where for pragmatic reasons it is not routine practice to measure FiO_2 in spontaneously breathing patients.

Whilst duration of HDU stay was taken as the duration of continuous pulse oximetry monitoring in attempt to avoid artefactual extension of the duration of HDU stay by non-clinical reasons (such as time of day and availability of ward beds), hospital stay can also be artefactually extended by similar 'non-clinical' reasons. It is a limitation of this study therefore that no assessment was made to distinguish between 'fitness for discharge' and 'actual' hospital discharge.

Whilst no formal power analysis was performed for this pilot study, the further reduction in sample size from the original 35 to just 14 patients for whom modified lung injury score could be determined on post-operative day two is disappointing. As a result the potential for negative findings to be the result of type-I error is considerable. Given the multiple comparisons made in a small cohort of patients, the potential for type-I error must also be appreciated; all

positive results must be considered 'hypothesis generating' rather than evidential. It must be emphasised however, in the study of PTX3, no single positive result is being championed in isolation and the results form a consistent, coherent argument in favour of PTX3 as a lung injury biomarker.

The lack of results for IL-8 and TNF- α are discussed above. The implausible results from the B-type Natriuretic Peptide (BNP) analysis, necessitating their exclusion from analyses was a further limitation. Due to constraints of plasma availability and funding, these analyses were made as singulate assays with no possibility to rerun the assay. Inspection of the optical density verses BNP concentration 'standard curve' suggested no problems with the assay procedure. 'Standards' were measured in duplicate; the coefficient of variation of the standard results was 14.3%, a value which, though high, is inadequate to explain yielded results being hundred times greater than anticipated.

4.7.4 Conclusions

The results of the current investigation reveal that PTX3 appears to conform to many of the properties of the 'ideal' lung injury biomarker in patients undergoing lung resection suggesting therefore that post-operative PTX3 measurement may have a role in this population. Though encouraging, there remains however much further work to be done before PTX3 measurement could be routinely advocated. Firstly, the current study needs to be replicated in a larger cohort in order to confirm the predictive values observed. Secondly, the predictive value of PTX3 needs to be confirmed against the 'hard' end-points of ARDS diagnosis (as defined by the 'Berlin' definition), need for post-operative mechanical ventilation and mortality, rather than the surrogate endpoints of modified Lung Injury Score, oxygenation and chest X-ray score as studied in the current investigation. Thirdly, the post-operative kinetics of PTX3 require further exploration in order to more accurately characterise the optimal timing of blood sampling.

In contrast, the current investigation demonstrates no role for the multiple biomarker panel studied. Much was learnt however of the challenges of biomarker measurement in the thoracic surgical population; such knowledge will be useful in the planning of future studies. Firstly, the importance of

appropriately timing blood sampling to biomarker kinetics was illustrated. In all future studies the author (B. Shelley) would advocate a preliminary pilot study of lesser sample size but measuring biomarker levels at more frequent time points in order to establish kinetics before seeking validation of a biomarkers utility. Secondly, due to the subtleties of differing pathogenesis, and a lower grade (sub-clinical) lung injury seen in post-operative patients compared to critical care patients, caution must be exercised in the translation of findings from the wider critical care environment to post-operative populations. Thirdly, whilst 'pulmonary specificity' may be desirable in distinguishing pulmonary inflammation from systemic, measurement of pulmonary epithelial biomarker levels following lung resection brings the additional challenge of the need to adjust post-operative values; such adjustments require further validation. Angiotensin-2, RAGE and IL-10 were all elevated in the majority of patients following lung resection. These three biomarkers may therefore be suitable for use individually or in combination with others following lung resection and the significance of the post-operative increases observed requires further exploration.

5 Investigation IV: Reproducibility and construct validity of transpulmonary thermodilution derived extravascular lung water and pulmonary vascular permeability index following lung resection

5.1 Introduction

Measurement of extravascular lung water (EVLW) and pulmonary vascular permeability index (PVPI) by transpulmonary thermodilution (TPTD) has the potential to be a useful monitoring modality in the early post-operative period following lung resection^{115, 363}. EVLW and PVPI have the potential to aid clinical decision making in this population and may in addition provide a useful surrogate endpoint for clinical research. Though yet to find an established role in clinical practice, EVLW and PVPI have been widely measured in the general critical care and post-operative populations. The validity of TPTD derived indices has been established in the critical care population (discussed in detail in the following literature search), but reproducibility and validity have not been established in post-operative patients who have undergone lung resection, where anatomical disruption of the pulmonary vascular bed and post-operative changes in pulmonary-vascular interaction might be hypothesised to compromise validity.

In this study, the reproducibility and construct validity (cross-sectional and longitudinal) of TPTD derived EVLW and PVPI is examined in patients undergoing lung resection. Reproducibility is derived from replicate TPTD measurements according to standard equations. Post-operative oxygenation ($\text{PaO}_2/\text{FiO}_2$ ratio), chest X-ray score and fluid balance are defined as 'constructs' with which association between construct and EVLW / PVPI would be expected. Specifically, the premise of this investigation is that construct validity of TPTD derived EVLW and PVPI could be inferred by the observation of negative association between EVLW / PVPI and $\text{PaO}_2/\text{FiO}_2$, positive association between EVLW / PVPI and CXR score and positive association between EVLW and cumulative fluid balance.

As will be discussed in detail in the following literature search, current clinically available monitors for the measurement of TPTD derived indices rely on there being a fixed and linear relationship between pulmonary blood volume (PBV) and intra-thoracic blood volume (ITBV)^M. It is intuitive however that following lung resection, where the volume of the pulmonary vascular bed is by definition reduced, that this relationship is unlikely to be maintained. It has been suggested therefore that the methodology of TPTD be amended for use following lung resection²⁴⁸ (discussed in detail in Appendix 5). It is the secondary hypothesis of this investigation that such adjustment of TPTD methodology will improve construct validity of EVLW and PVPI measurement following lung resection.

^M ITBV – the volume of blood within the thorax encompassing great veins, cardiac chambers, pulmonary vasculature and aorta. A fuller explanation follows in Section 5.3.2.1.

5.2 Literature review: Assessing utility of a diagnostic test

Investigation IV of this thesis concerns the utility of extravascular lung water (EVLW) and pulmonary vascular permeability indices (PVPs) measurement using the single indicator transpulmonary thermodilution technique as a clinical monitor in the post-operative period following lung resection. Prior to discussion of the specifics of the technique, I will firstly consider the purpose of collecting diagnostic information and secondly examine the criteria by which the usefulness of a diagnostic test may be assessed. For example purposes, reference is made to cardiac output measurement because the measurement of cardiac output is inherent to the measurement of EVLW/PVPs, and a large evidence base already exists concerning the reproducibility and validity of cardiac output measurement.

Whilst diagnostic tests are commonly performed in order to make a diagnosis, Sackett et al³⁶⁴ describe four further purposes for the data obtained:

- *“To judge severity of illness”.*
- *“To predict subsequent clinical course and prognosis of the illness and the patient”.*
- *“To estimate the likely responsiveness to therapy in the future”.*
- *“To determine the actual response to therapy in the present”.*

Sackett et al go on to describe that whatever the reason for applying the diagnostic test the *“conscious clinician”* is mandated to make some assessment of the usefulness of the diagnostic criteria. Whilst Sackett et al are discussing the use of commonly applied diagnostic tests in clinical practice, the introduction of a novel test (such as EVLW measurement) be it for clinical or research purposes requires (at least) the same consideration. To this end, Sackett et al provide eight criteria which can be applied *“for deciding the usefulness of a diagnostic test”*³⁶⁴.

1. *“Has there been an independent, ‘blind’ comparison with a ‘gold standard’ of diagnosis?”*

2. *Has the diagnostic test been evaluated in a patient sample that included an appropriate spectrum of mild and severe, treated and untreated, disease?*
3. *Was the setting for this evaluation, as well the filter through which study patients passed, adequately described?*
4. *Have the reproducibility of the test result (precision) and its interpretation (observer variation) been determined?*
5. *Has the term normal been defined sensibly as it applies to this test?*
6. *If the test is advocated as part of a cluster or sequence of tests, has its individual contribution to the overall validity of the cluster or sequence been determined?*
7. *Have the tactics for carrying out the test been described in sufficient detail to permit their exact replication?*
8. *Has the utility of the test been determined?"*

Whilst some of the criteria are more applicable in the context of EVLW and PVPI measurement than others, in reviewing the background and evidence to date concerning measurement of EVLW and PVPI in the diagnosis and quantification of lung injury (Sections 5.4 and 5.6 respectively), attempt has been made to apply many of these criteria. Criterion 1 and 4, 'validity' and 'reproducibility' merit special consideration.

5.2.1 Validity

The 'Oxford Dictionary of Epidemiology' defines validity of a clinical measurement as "*an expression of the degree to which a measurement measures what it purports to measure*"³⁶⁵. Most commonly in medicine this is ascertained by comparison of the performance of a novel diagnostic test with that of a 'gold standard' (Sackett et al's first criterion³⁶⁴). The gold standard represents a 'definitive diagnosis' and may be established in a variety of ways in different circumstances; the key point is that the gold standard is taken to represent the 'truth'. Such an approach for example is commonly used in the field of cardiac output monitoring in which novel cardiac output monitors are compared to the pulmonary artery catheter³⁶⁶. Difficulty occurs however, in cases (such as the diagnosis of ALI/ARDS) where there is no gold standard. Though both the 'American-European Consensus Conference'⁵⁵ and 'Berlin'⁵⁰

definitions of ALI/ARDS attempt to provide criteria by which ALI/ARDS may be diagnosed, fundamentally ALI/ARDS is a clinical syndrome which lacks a gold standard diagnostic test.

Several discrete types of validity have been described. These are discussed below, and summarised, with reference to examples reflecting the diagnosis of ALI/ARDS in Table 5.1.

5.2.1.1 Criterion validity

Criterion validity concerns the “*extent to which a measurement correlates with an external criterion of the phenomenon under study*”³⁶⁵. In the case of correspondence to a gold standard (discussed above), the gold standard is the criterion to which the novel measurement is compared, often referred to as the reference measurement. Two further types of criterion validity are recognised:

Concurrent validity^N

Concurrent validity concerns the assessment of association between criterion and the measurement when both refer to the same point in time; in assessing agreement between a novel cardiac output monitor (measurement) and the pulmonary artery catheter (criterion) both measurements are taken simultaneously. Concurrent validity is also interpreted to reflect the ability of the measurement to appropriately distinguish between groups of patients determined by an alternative criterion; for example the ability of the measurement to identify presence of disease (where the criterion maybe an alternative or gold-standard diagnostic test) or to identify patients who have increased severity of disease (where the criterion may be a measure of disease severity).

Predictive validity^N

Predictive validity concerns the assessment of association between criterion and the measurement when they are separated in time; the ability of the measurement to predict the criterion is assessed. Predictive validity may be

^N There is some discrepancy in reported definitions for types of validity. Some authors consider concurrent and predictive validity to be subsets of criterion validity, whilst others recognise them to be types of validity in their own right. In either situation the broad meanings of the terms as they are applied appears to be consistent.

Table 5.1. Types of validity.

Validity measure	Explanation	Example as applied to ALI/ARDS
Face validity	Test appears 'on its face' to represent lung injury	Patients identified by test to have lung injury appear to clinicians to do so. Test "feels right" to clinicians.
Criterion validity	Test corresponds to a gold standard measure of lung injury	Not available. Diffuse alveolar damage on histology represents closest parallel.
- Concurrent ^A validity	Test is able to distinguish between groups that it theoretically should be able to distinguish between.	Test is able to identify groups of patients with or without ALI/ARDS or distinguish between mild, moderate or severe lung injury. Test is able to distinguish patients with cardiogenic and non-cardiogenic pulmonary oedema.
- Predictive ^A validity	Test is able to predict something it theoretically should be able to predict.	Test is able to identify a group of patients with poor outcome e.g. prolonged duration of mechanical ventilation or death.
Construct validity	Extent to which the test corresponds to other measurements that would theoretically support the concept (or construct) being measured.	Association observed between test and (for example) PaO ₂ /FiO ₂ , CXR score, lung injury score or pulmonary compliance.
Content validity	Extent to which the test reflects all of the aspects of the phenomena being studied.	Patients identified by the test to have lung injury must demonstrate clinical, physiological and pathological components of the syndrome.

PaO₂ - partial pressure of oxygen; FiO₂ - fraction of inspired oxygen; CXR – chest X-ray.

^A - There is some discrepancy in reported definitions for type of validity. Some authors consider concurrent and predictive validity to be subsets of criterion validity, whilst others recognise them to be types of validity in their own right. In either situation the broad meanings of the terms as they are applied appears to be consistent.

Based on, though modified from Rubenfeld (2003)³⁶⁷ and Ferguson et al (2012)⁶⁶.

observed if the measurement is able to identify patients who go on to develop a specific outcome (where the criterion may be mortality or prolonged hospital stay for example), or to identify patients who will respond to therapy (where the criterion reflects an alternative measurement of treatment response).

Quantitative evaluation of criterion validity

As discussed above, criterion validity concerns the “*extent to which a measurement correlates with an external criterion*”³⁶⁵; it is perhaps unsurprising therefore that traditionally criterion validity has been assessed by the use of correlation and regression analysis. In their 1986 landmark paper, Bland and Altman challenged such a practice highlighting the subtle but important difference between association (as determined by calculation of a correlation coefficient) and agreement. Agreement they argue should be assessed by the use of bias and precision statistics, providing mean bias between the measurements and upper and lower limits of agreement, within which 95% of bias measurements should lie^{368, 369}.

What is less certain however is what constitutes an acceptable level of bias, in the face of which a monitoring technique can be considered valid. In 1999, Critchley and Critchley performed a meta-analysis of studies using bias and precision statistics to compare cardiac output measurement techniques³⁶⁹. They reported a lack of clearly defined criteria for acceptance of a new technique. Using graphical methodology, Critchley and Critchley illustrate that consideration must be made of the individual limits of precision of both the comparator and reference technique. As such where limits of precision of individual techniques are commonly in the range of 10-20%, then limits of agreement between the techniques of up to $\pm 30\%$ may be considered acceptable³⁶⁹.

5.2.1.2 Validity in the absence of a gold standard

Whilst the observance of correspondence to a gold standard is undoubtedly a proof of validity and classically the method by which validity of a clinical monitor is tested, such ‘criterion validity’ represents one of a number of different measures of validity^{365, 367, 370} (Table 5.1). In such circumstances (as in ALI/ARDS), where a gold standard is not available, recourse to one of the other described forms of validity may be useful.

Construct validity

Construct validity concerns the selection of one or more “logical consequences” of the disease. Observation of these consequences is taken to represent the gold standard; in such cases the “logical consequences” are called constructs and the validity test is referred to as construct validity³⁶⁴.

The concept of construct validity is demonstrated by Ely et al, who in a study of 38 intensive care patients sought to assess the reliability and validity of the Richmond Agitation-Sedation Scale (RASS)³⁷¹. No gold standard for level of sedation exists, and whilst there are a number of sedation scales in common use there is no single objective assessment to which they could compare the RASS. Consequently Ely et al explored the construct validity of the RASS concluding that construct validity would be provided by observation of association with five characteristics: 1. Attention screening examination, 2. Glasgow Coma Scale (GCS) score, 3. Quantity of psychoactive medication administered, 4. Successful extubation and 5. Bispectral electroencephalography. Conceptually, the RASS scale would have construct validity if lower RASS score (more sedated) is associated with patients who have poorer attention, lower GCS, are receiving greater doses of sedative medication, are less likely to be successfully extubated and have lower bispectral index (and vice versa).

Construct validity and ARDS

In the process of deriving an updated definition of ARDS, the ‘ARDS Definition Task Force’ defined a ‘conceptual model’ of ARDS⁵⁰:

“The panel agreed that ARDS is a type of acute diffuse, inflammatory lung injury, leading to increased pulmonary vascular permeability, increased lung weight, and loss of aerated lung tissue. The clinical hallmarks are hypoxemia and bilateral radiographic opacities, associated with increased venous admixture, increased physiological dead space, and decreased lung compliance. The morphological hallmark of the acute phase is diffuse alveolar damage (i.e., oedema, inflammation, hyaline membrane, or haemorrhage)”.

‘ARDS Definition Task Force’ (2012)⁵⁰

Whilst such a model does not provide a definition nor diagnostic criteria for ALI/ARDS it can be appreciated that from this, a number of ‘constructs’ can be

derived, against which a novel definition, diagnostic test or modality could be assessed. For example, if association is found between test *X* and the *clinical* findings of decreased oxygenation and typical radiological appearances; *physiological* evidence of increased shunt or decreased lung compliance and; the presence of diffuse alveolar damage in *pathological specimens* (in animal models or ex vivo human specimens), then it seems likely that test *X* is a measure of ALI/ARDS; ergo test *X* has construct validity as a measure of ALI/ARDS. Clearly the strength of the relationship observed and the finding of a quantitative relationship between test *X* and the associated variables would increase confidence in such validity.

There has been no explicit discussion of what constitutes ‘construct validity’ for ALI/ARDS in the literature to date, however a multitude of studies have looked at clinical, physiological and pathological endpoints when studying lung injury for a variety of reasons.

Content validity

Primarily a tool of the social scientist, content validity concerns the extent to which a measurement reflects all of the components of the phenomena being studied^{365, 367, 370}. The Oxford Dictionary of Epidemiology provides the following example: in order to have content validity a measurement of functional health status must incorporate all of the components of functional health: occupational, family and social functioning etc³⁶⁵. If ALI/ARDS is taken to be represented by the conceptual model of the ARDS Definition Task Force reproduced above⁵⁰, then any new definition of ALI/ARDS would have to encompass all of the elements described. Similarly for any measure of lung injury to exhibit content validity as a measure of ARDS (on the assumption ARDS is represented by the above model), it would be required to demonstrate the ability of the measure to identify patients with clinical, physiological and pathological features of ARDS.

5.2.2 Reproducibility

Where validity is taken to be “an expression of the degree to which a measurement measures what it purports to measure”³⁶⁵, ‘reproducibility’ (also referred to as ‘repeatability’ or ‘reliability’) reflects the ability of a test to consistently make a measurement and obtain the same results on repeated

measurements³⁷⁰. Any measurement is to some extent liable to error, and as such repeated measurements (of a constant) will rarely yield exactly the same result but rather will tend towards consistency. Reliability is the assessment of this ‘tendency towards consistency’ observed across repeated measurements.

Unsurprisingly therefore “*the best way to examine repeatability is to take repeated measurements on a series of subjects*”³⁶⁸. Importantly to ensure assessment of reliability, such repeated measurements or ‘replicates’ require to be taken on the same individual in identical conditions and are commonly therefore performed in quick succession³⁷². Where physiological measurements are prone to errors, such as in the measurement of cardiac output (where for example, respiration induces cyclical changes in cardiac output), the precision of a measurement can be improved by performing repeated measurements and averaging their results³⁶⁹; the averaged result is then taken to be the ‘true’ result for clinical interpretation.

5.2.2.1 Quantitative evaluation of reliability

Whilst the mean of repeated measurements can be used to provide an individual summary value for clinical use, the same mean value could be obtained from dramatically different data sets. For illustration, though the triplicate cardiac output values 4.8, 4.9, 5.3 L/min and 2.9, 4.5, 7.6 L/min both yield the same mean cardiac output (5.0L/min), the clinician can be more confident in the mean result of the former dataset than the latter due to the narrower range over which the data is spread. Quantitative evaluation of such ‘confidence’ in the mean value obtained can be determined by the use of precision statistics:

Coefficient of variation (CV) is first determined as

$$CV = \frac{SD}{Mean}$$

Equation 5.1

From which coefficient of error (CE) and precision can be derived:

$$CE = \frac{CV}{\sqrt{n}}$$

Equation 5.2

Where n is the number of repeated measurements. Precision is then calculated as:

$$Precision = 2 \times CE$$

Equation 5.3

As in the discussion above, concerning what can be considered to be an acceptable level of bias, there is no clearly defined limit at which precision may be considered acceptable. Holm et al describe that “*according to usual practice*” a coefficient of variation of less than 10% may be considered ‘good’; between 10 and 15% considered ‘acceptable’ and greater than 15% considered ‘poor’³⁷³. Whilst no reference is provided for the derivation of such ‘usual practice’, the use of the cut-offs described are supported by others³⁷⁴⁻³⁷⁷.

Pragmatically, the importance of the precision of any monitoring technique is dependent upon the magnitude of the change in the variable the clinician wishes to be able to detect. Even the most imprecise of monitors is likely to be able to detect a change of 50% whilst a change of 5% (if perceived to be of clinical significance) would require an implausibly precise monitor. The minimum change that can be reliably recognised by a device may be determined by calculation of the least significant change (LSC)^{366, 374}, where:

$$LSC = Precision \times \sqrt{2}$$

Equation 5.4

Whilst no reference is provided for the derivation of the precision statistics described, these definitions have found widespread acceptance within the literature^{366, 374, 376, 378, 379}.

5.3 Literature review: Principles of Trans-pulmonary Thermodilution

This section describes the principles of indicator dilution measurement of blood flow, and explains how from these measurements, volumes may be determined. A detailed discussion is made of the assumptions underlying TPTD, and their potential for error in various states of normal and abnormal physiology.

5.3.1 Measurement of flow

5.3.1.1 The principle of indicator dilution

As described by Reuter et al, indicator dilution techniques can be conceptualised in three discrete stages^{380, 381}:

- 1) *A known amount of an exogenous substance (the 'indicator') is injected into the circulation;*
- 2) *The circulation carries the indicator through the heart where it is mixed and diluted;*
- 3) *A detector positioned downstream measures and records the concentration of indicator over time.*

When an indicator is injected into a blood vessel, its concentration is promptly diluted by flowing blood. The speed at which this dilution takes place is a function of the magnitude of flow. If flow between injection point and detector location is high, then due to rapid dilution, the concentration of the indicator will fall quickly and the change in indicator concentration detected downstream will be small. Conversely, if flow is low, the indicator concentration will be diluted less and the concentration detected downstream will be greater.

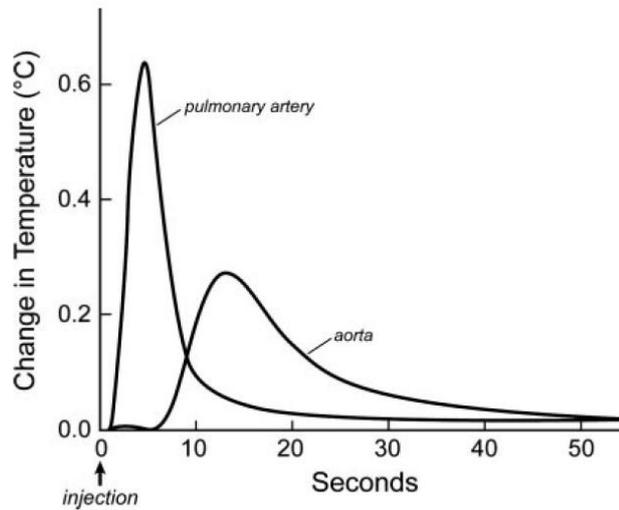


Figure 5.1. Sample change in blood temperature verses time curves during thermodilution measurement of cardiac output.

As measured by thermistors in the pulmonary artery or distal aorta. From Isakow and Schuster (2006)³⁸².

In practice, to determine cardiac output (CO) the indicator is injected into the central circulation via a venous cannula located in a central vein and its passage is detected at a point downstream either in the pulmonary artery (trans-cardiac thermodilution, TCTD), or in the distal aorta (trans-pulmonary thermodilution, TPTD). The passage of the indicator is determined against time with the generation of a thermodilution (concentration vs time) curve (Figure 5.1) from which flow can be derived (Equation 5.5 - known as the 'Stewart-Hamilton' equation after the two (independent) researchers responsible for the initial description and subsequent refinement of the technique³⁸³).

$$Q = A / \int_0^{\infty} c(t) dt$$

Equation 5.5.

Where Q is flow, A is the quantity of the indicator injected, and the integral represents the area under the concentration-time curve.

Though a number of indicators have been used over the years for the clinical measurement of cardiac output (CO), the indicator most commonly used is temperature; either through the injection of cold saline or through the heating of blood via a thermal filament incorporated into the structure of the catheter.

Equation 5.5 has consequently been modified so that temperature can be used as the indicator:

$$CO = \frac{(T_b - T_i) \times V_i \times K}{\int_0^{\infty} \Delta T_b dt}$$

Equation 5.6.

Where T_b is blood temperature at the time of injection, T_i is the injectate temperature, V_i is injectate volume^o, K is a catheter specific correction factor^p, and the integral represents the area under the thermodilution curve.

Thus CO is proportional to the duration of transit of warmed or cooled blood, and inversely proportional to the mean change in blood temperature.

5.3.1.2 Assumptions / differences between trans-cardiac and trans-pulmonary thermodilution

The accuracy and reproducibility of thermodilution measurements depend on a multitude of physical (relating to the injectate and injection), physiological (relating to the monitored patient) and numerical factors. Discussion will be made of those factors which relate specifically to TPTD measurements in clinical use.

Shape of the thermodilution curve

The temperature vs. time curves obtained during TPTD are broader and flatter when compared to those obtained from TCTD; consequently the observed change in blood temperature (ΔT) is of lesser magnitude (Figure 5.1). TPTD measurements are therefore more vulnerable to errors caused by baseline drift and recirculation artefact (below).

^o T_i : injectate temperature after taking into account factors such as the intra-corporeal fraction of the injection catheter dead space, dead space of the injection catheter, injector set, any extension tubing, and temperature of both the blood before injection and of the extracorporeal dead space before injection.

^p K : correction factor accounting for the differences in the specific gravity and specific heat capacity of blood vs. saline, and dead space of the intravascular portion of the injecting catheter.

Recirculation, loss, and detainment of indicator

Indicator recirculation

Whilst in theory the decay in the temperature verses time curve is assumed to be represented by a mono-exponential decay (Figure 5.2), in practice, recirculation of indicator occurs. To overcome this 'recirculation artefact', in calculation of the integral of the thermodilution curve in modern equipment, the thermodilution curve is anticipated to be monoexponential and free of recirculation artefact from 80 to 50% of peak temperature change³⁸², after which point the curve is truncated. The monitor then fits a straight line to the assumed mono-exponential decay, extrapolating the curve beyond the point of recirculation (Figure 5.2).

Given the broader shape of the curve in TPTD compared to TCTD (reflecting the greater distance between superior vena cava and distal aorta), it is possible that recirculation artefact (stemming from the recirculation of indicator via fast pathways (e.g. cardiac and renal) being superimposed on the primary aortic thermodilution curve³⁸⁴) may be present before mono-exponential decay becomes established³⁸². Such an occurrence would lead to an artefactual over-recovery of indicator reducing estimated cardiac output.

By simultaneous assessment of pulmonary arterial and aortic thermodilution curves in a canine model, Bock et al demonstrated that during trans-pulmonary thermodilution, mono-exponential extrapolation of the thermodilution curve downslope leads to a calculated return of indicator of in excess of 100%, a physically impossible finding implying 'recirculation artefact' is routinely present³⁸⁵. The same authors went on to quantify the effect of indicator recirculation on the TPTD determination of CO reporting that indicator recirculation typically led to an underestimation of CO by TPTD of 2-3% when compared to TCTD.

Indicator loss

The properties of the ideal indicator have been defined by a number of authors³⁸². Amongst these is the necessity that the indicator is confined to (and therefore not lost from) the intravascular compartment between injection and detection sites. If indicator is lost from the circulation, cardiac output will be over-estimated as less indicator will be detected downstream. Where the indicator is cold saline the potential exists for conductive re-warming of indicator by surrounding tissue. This

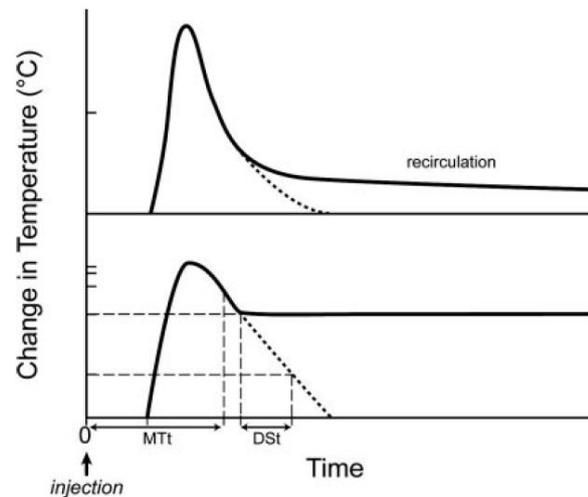


Figure 5.2. Diagrammatic representation of temperature-time curve during a thermodilution measurement.

Change in temperature vs. time (upper curve) and on a semi-log scale (lower curve). Solid lines represent recorded values showing effects of indicator recirculation. The dotted lines represent the monoexponential decay 'fit' by the monitor in order to overcome the effects of recirculation. Note that the decay of the thermal curve becomes linear when graphed on the semi-log scale (bottom). Also shown are typical points used to measure the mean transit time (MTt) and the downslope time (DSt). From Isakow and Schuster (2006)³⁸².

might be expected to present more of an issue during low flow states or when the indicator travels a longer distance en route to the detector as is the case in TPTD. The presence of extra-vascular heat sinks as might occur in pericardial or pleural effusion might be expected to further compromise TPTD measurements.

By simultaneously measuring cardiac output using TCTD and TPTD in 48 intensive care patients, Bock et al were able to calculate the amount of thermal indicator loss, demonstrating a 7% thermal loss between pulmonary artery and distal aorta and resulting in a systematic overestimation of CO by TPTD³⁸⁶.

Clearly, there is some loss of cold indicator from the circulation during TPTD, an occurrence which leads to errors in the estimation of CO by TPTD, but which is capitalised upon in the measurement of intrathoracic volumes (below).

Indicator detainment

Whilst some indicator is irrecoverably 'lost' during thermodilution, much of the cold indicator that is 'lost' into the pulmonary extravascular space eventually returns to the intravascular space. Although preserved, this 'detained' indicator arrives late, abnormally prolonging the TPTD curve. Furthermore the potential exists for detained indicator to arrive at the detection point after the downslope of

the thermodilution curve has been truncated (in order to avoid recirculation artefact) and so be methodologically 'lost'.

The effects of indicator loss (leading to an artifactual increase in the estimated cardiac output) and the effects of recirculation artefact and indicator detainment (leading to an artifactual reduction in the estimated cardiac output) will to an extent cancel one another out; as pointed out by Reuter however "*which mechanism is of greater quantitative significance remains unclear*"³⁸⁰.

Changes in CO during measurement

During spontaneous or mechanical ventilation, stroke volume varies with respiration by 10 to 50%³⁸⁷. Reproducibility of thermodilution readings can be improved by ensuring injection takes place at the same point in the respiratory cycle³⁸⁸. Conventionally reproducibility is further improved by averaging multiple consecutive measurements though it is important to ensure serial measurements are made at a time of relative stability as CO can of course change between measurements. Harris et al reported that during measurement, as cold injectate traverses the pulmonary circulation an approximately 10% reduction in heart rate is observed in 20% of patients; an effect postulated to be a direct effect of temperature on the sinus node³⁸⁹.

Tricuspid regurgitation

TCTD cardiac output measurements are generally considered to be unreliable in the presence of significant tricuspid regurgitation. Reverse flow of indicator from right ventricle to right atrium can result in indicator detainment, where regurgitated indicator leads to prolongation of the thermodilution curve or 'loss' of the indicator, as the regurgitated indicator arrives at the PA thermister too late, after the thermodilution curve has been truncated. Consequently tricuspid regurgitation can lead to an increase or decrease in the estimated CO. In TPTD, where the thermodilution curve is broader and measurement takes place over several cardio-respiratory cycles, it might be hypothesised that TPTD derived estimates of CO might be more resilient to inaccuracies induced by tricuspid regurgitation. There are no reported studies of the effect of tricuspid regurgitation on TPTD.

5.3.1.3 Direct comparisons between trans-cardiac and trans-pulmonary thermodilution cardiac output measurement

In general it can be seen that good correlation is observed between the TCPD and TPTD measurements whilst CO is consistently overestimated (bias < 10%) by TPTD techniques³⁸⁰. The overestimation of CO by TPTD has been variously attributed to the transient reduction in HR (and therefore cardiac output) resulting from administration of a cold bolus³⁹⁰⁻³⁹², unaccounted for loss of thermal indicator during pulmonary transit^{386, 390, 391, 393-396} and early recirculation artefact^{391, 397}. Most authors agree however that the modest difference between CO_{TCTD} and CO_{TPTD} is within the realms of what could be considered acceptable in the clinical context. It should be appreciated however that errors in determination of CO will be compounded during thermodilution measurement of volumes (discussed in the next section) which utilise CO (often more than once) in their calculation.

5.3.2 Measurement of volumes

As a result of observations that the shape of the thermodilution curve depends both on the flow and the volume of blood into which dye is distributed, methodologies have been derived to enable simultaneous measurement of blood volumes using indicator dilution techniques.

5.3.2.1 'Mean transit time' method for the measurement of volume of distribution

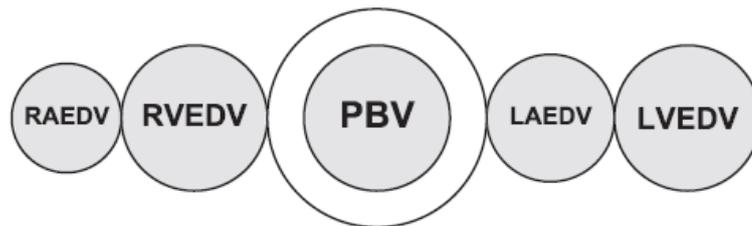
The volume of distribution of an indicator during TPTD measurement consists of the blood volume between the site at which the bolus is delivered (the tip of the central venous cannula in the superior vena cava) to the site at which passage of indicator is detected (the tip of a femoral arterial catheter in the distal aorta) and so includes the volume of a portion of the SVC, all four cardiac chambers, the pulmonary blood volumes and the aorta. As such this volume is conventionally referred to as the intrathoracic blood volume (ITBV, Figure 5.3). Where temperature is the indicator concerned, thermal indicator is not restricted to the vascular space, but may be lost into the vessel walls and the surrounding lung parenchyma. This volume of distribution for a thermal indicator (which is

significantly greater than the ITBV) is referred to as the intrathoracic thermal volume (ITTV, Figure 5.3).

The volume of distribution (V_d) of an indicator can be calculated as the product of flow (cardiac output) and the average or mean transit time for the indicator from the site of injection to the site of detection (MTt).

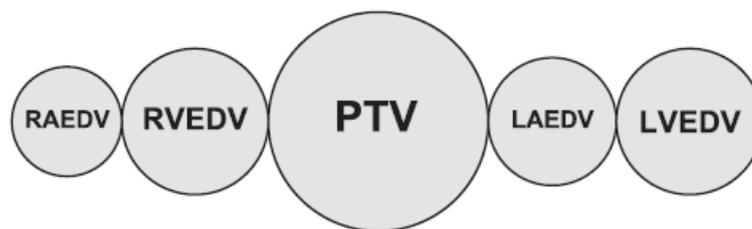
$$V_d = CO \times MTt$$

Equation 5.7



Intrathoracic blood volume, ITBV.

Shaded area represents ITBV. RA, RV, LA & LV-EDV, right and left atrial and ventricular end-diastolic volumes. PBV, pulmonary blood volume.



Intrathoracic thermal volume, ITTV.

Shaded area represents ITTV. PTV, pulmonary thermal volume.

Figure 5.3. Schematic representation of volumes measured during trans-pulmonary thermodilution techniques.

Adapted from Brown et al (2009)³⁶³.

MTt is determined from the thermodilution curve as the ratio of two integrals:

$$MTt = AT + \frac{\int_0^{\infty} c(t) \times (t - AT) dt}{\int_0^{\infty} c(t) dt}$$

Equation 5.8.

Where AT (appearance time) is the elapsed time between injection of the indicator and its appearance at the detection site and c is the concentration of the indicator.

Consequently, in the case of transpulmonary thermodilution, the total volume of distribution of the thermal indicator the intra-thoracic thermal volume (ITTV) can be determined from the mean transit time of the indicator.

$$ITTV = CO \times MTt_{thermal}$$

Equation 5.9.

It is evident from equations 5.8 & 9 that measurement of volumes derived from the transit time method are the function of three integrals (one for measurement of CO and two for measurement of MTt), and so the potential exists for errors in measurement of CO and MTt to be compounded in the final measurement result ^{378, 382}.

5.3.2.2 'Slope volume' methods for measurement of chamber volumes

The 'slope volume' method represents a second method by which a volume can be derived during thermodilution. If an amount of indicator (A) is injected into a chamber of static volume (V) (Figure 5.4), then the concentration of the indicator in the chamber (C) can be represented as:

$$C = \frac{A}{V}$$

Equation 5.10.

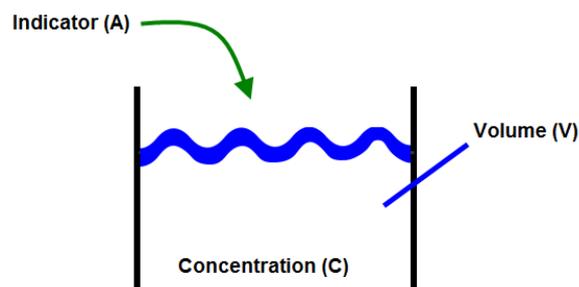


Figure 5.4. Schematic representation of an indicator in a closed chamber.
 Drawn by the author (B Shelley), 2014.

If the indicator is delivered into a chamber of constant volume, but with a constant flow (Q) through the chamber (Figure 5.5) then assuming mixing is instantaneous and complete, the initial concentration of the indicator at the time of injection (t_0) would also be represented by equation 5.10.

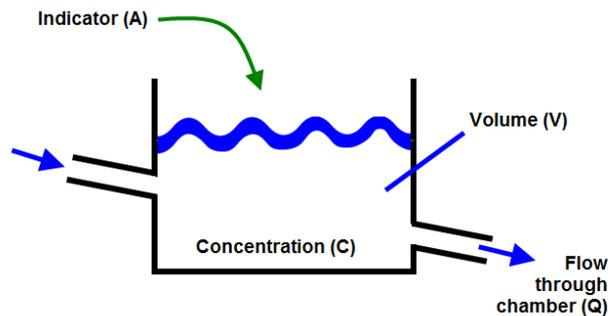


Figure 5.5. Schematic representation of an indicator in an open chamber with constant flow. Drawn by the author (B Shelley), 2014.

The subsequent rate of change in the amount of indicator in the chamber (dA/dt) would then be determined by the flow through the chamber, the volume of the chamber and the amount of indicator introduced such that:

$$\frac{dA}{dt} = -\frac{QA}{V} = -QC$$

Equation 5.11.

Substitution Equation 5.10 into Equation 5.11 and dividing both sides of this equation by V gives:

$$\frac{dC}{dt} = -\frac{QC}{V}$$

Equation 5.12.

Solution of Equation 5.12 by integration yields:

$$C = ke^{-\left(\frac{Q}{V}\right)(t)}$$

Equation 5.13.

Where k is the constant of integration. To evaluate k , the known solution for the equation at t_0 is used i.e. that at t_0 the moment of injection of indicator, $C_0 = A/V$. Substituting these values into Equation 5.13 yields:

$$C = \frac{A}{V} e^{-\left(\frac{Q}{V}\right)(t-t_0)}$$

Equation 5.14.

A semi logarithmic plot of this function with concentration on the Y axis and t on the x axis is a straight line with a negative slope of Q/V.

Q can be calculated simultaneously by estimation of the area under the thermodilution curve as above. As such by interrogation of the linear down-slope of the logarithmic thermodilution curve in addition to the AUC to determine Q, V can be estimated.

What volumes are measured?

Whilst cardiac output (or total flow in L/min) remains relatively constant as blood passes from the superior vena cava to distal aorta, the circulation cannot of course be represented by a single chamber of volume (V) as in the simplified model above, rather it is composed of a number of chambers of varying volume connected in series (superior vena cava, right atrium, right ventricle, pulmonary blood volume, left atrium, left ventricle, aorta). The question then is which if any of these volumes does $V_{\text{slope-volume}}$ represent?

Insight is provided by the frequently cited work of Newman et al³⁹⁸. Newman et al demonstrated in a series of experiments (both in laboratory models and in humans), that for a system of chambers connected in series, whilst the time to peak concentration (the peak of the thermodilution curve) is effected by the volumes of the smaller chambers, the subsequent downslope of the curve is determined solely by the volume of the largest chamber in the series. As the flow through each chamber is assumed to be uniform, then for each chamber the linear slope of the logarithmic thermodilution curve would be the chamber flow divided by the chamber volume. With smaller chambers therefore, the slope Q/V will be greater; indicator is described as rapidly 'washing out' of smaller chambers. As such, the downslope observed at the downstream detection site must represent the decay of the concentration-time curve from the largest volume chamber.

During TPTD, the pulmonary circulation constitutes the largest chamber such that where dye is the indicator used, pulmonary blood volume (PBV) is determined from

the downslope of the dye-dilution curve (Equation 5.15, Figure 5.3). In the case of thermal indicator, pulmonary thermal volume (PTV) is determined (Equation 5.16, Figure 5.3).

$$PBV = CO \times DSt_{\text{dye}}$$

Equation 5.15.

$$PTV = CO \times DSt_{\text{thermal}}$$

Equation 5.16.

In the clinical practice of TPTD, it must be emphasised that rather than the slope of the logarithmic thermodilution curve, the ‘down-slope time’ (DSt) is used in the calculation of volumes. The DSt is derived from the thermodilution curve (as indicated in Figure 5.2), and is time taken for the temperature decay to fall from 85% to 45% of its maximum response³⁹⁹. Whilst DSt is therefore linearly related to the down-slope of the curve, all volumes derived will be ‘virtual’ volumes rather than accurate representations of the chamber volumes.

Assumptions

Before proceeding to discuss the role of these volumes in the derivation of EVLW, it is worth pausing to consider some of the assumptions inherent to the ‘down-slope time technique’ of volume measurement. In the narrative above, it is inherently assumed that the chamber volume in which mixing takes place is of constant volume, that mixing is complete and instantaneous, that no recirculation occurs and that flow is constant³⁹⁸.

Constant volume

Though the mixing volume of any indicator during trans-pulmonary thermodilution comprises the volumes of the cardiac chambers and the pulmonary blood volume, the volume derived from the DSt technique is that of the pulmonary blood (or thermal) volume alone. Though the volumes of the cardiac chambers change during the cardiac cycle, as pulmonary blood volume is assumed to remain constant, Newman concluded that modification of the theory to account for the contractile nature of the heart is not necessary³⁹⁸.

Complete mixing

Mixing is an important issue; incomplete mixing of indicator would lead to erroneously low results - the volume derived from the slope volume method represents the apparent volume into which the indicator is mixed³⁹⁸. In Newman’s

laboratory validation of the slope-volume technique, the right sided cardiac chambers, the pulmonary blood volume and the left sided cardiac chambers were each represented by a single chamber. Though it is likely that complete mixing takes place in the chambers of the heart (it is an inherent assumption of the Fick method of CO measurement that mixing is complete), it is less likely that the pulmonary vasculature can be considered a single mixing chamber. As such, it is being assumed that complete mixing has taken place in the heart and main pulmonary artery prior to reaching the pulmonary circulation.

Recirculation

Whilst inaccuracies in the measurement of cardiac output resulting from indicator recirculation (where CO determination relies on the area under the thermodilution curve) are relatively modest, the estimation of mean transit time is more sensitive to the changes in the slope of the monoexponential decay function³⁸⁴. Using a deconvolution technique (requiring simultaneous assessment of pulmonary arterial and aortic thermodilution curves) Bock et al, explored the quantitative effect of indicator recirculation on TPTD estimates of EVLW, reporting that the overestimation of the mean transit time of heat is approximately 10%³⁸⁴. Such an overestimation resulted in an overestimation in EVLW of approximately 2ml/kg (20%) in baseline conditions and in excess of 4ml/kg (13%) in conditions of raised EVLW. Though initially suggesting that monoexponential extrapolation (as commonly employed) was therefore “*unsuited for the determination of thermal recovery*”³⁸⁵, in favour of the more complex deconvolution technique, Bock et al later concluded that the deconvolution technique appeared less practical for clinical practice and that “*overall the gain in accuracy appears to be small compared to the more invasive procedure*”⁴⁰⁰.

Constant flow

Any variation in flow during the measurement period could potentially change the shape of the thermodilution curve. The prolonged duration of TPTD means that a subject is required to breathe during the measurement and as such, flow (cardiac output) will vary through the respiratory cycle by between 10 and 50%³⁸⁷. The effects of variations in flow with respiration on the validity of the down-slope time technique have not been explored.

5.4 Literature review: Single indicator trans-pulmonary thermodilution measurement of extra-vascular lung water

Extravascular lung water (EVLW) is a theoretical construct representing the fluid volume of the lung, and encompasses all of the fluid within the lung but outside of the vascular compartment, including extravasated plasma in addition to intracellular water, lymphatic fluid and surfactant³⁶³. Conceptually increased EVLW is taken to represent the clinical syndrome of pulmonary oedema and as such EVLW has been described as “*the morphologic correlate of pulmonary oedema*”²⁹. Measurement of EVLW relies on the assumption that a proportion of thermal indicator delivered into the pulmonary vascular compartment (as part of a TPTD measurement) is able to rapidly diffuse across the vascular wall and equilibrate within the lungs extravascular water content. In this section, the methodology, reproducibility and validity ‘single’ indicator trans-pulmonary thermodilution are explored.

Whilst its validity has been well demonstrated, the technique of ‘double indicator’ thermo-dye dilution (where simultaneous injection of both cold and dye indicators is required) is time consuming, cumbersome and expensive, and despite promise has failed to become established in routine clinical practice^{396, 401, 402}. An alternative to the thermo-dye dilution approach is provided by the ‘single’ (thermal / cold) indicator thermodilution (STD) such that by a series of calculations and assumptions EVLW may be derived from an injection of a thermal indicator alone.

5.4.1 Methodology of STD measurement of EVLW

Recall from Equations 5.9 and 5.16 above, that intra-thoracic thermal volume (ITTV) can be determined as the volume of distribution of cold indicator, and that pulmonary thermal volume (PTV) may determined from the same injection of cold indicator by the down-slope time technique. As the name suggests, ITTV is greater than PTV by an amount which is approximately equivalent to the thermal volume of the non-pulmonary chambers in series, i.e. the blood volumes of the cardiac chambers. As the blood volumes of the cardiac chambers are largest at end-

diastole this volume has by convention become known as the global end-diastolic volume (GEDV, Figure 5.6) where:

$$GEDV = ITTV - PTV$$

Equation 5.17.

It is evident from Figure 5.6 that EVLW may be determined by subtraction of ITBV from ITTV.

$$EVLW_{TDD} = ITTV - ITBV$$

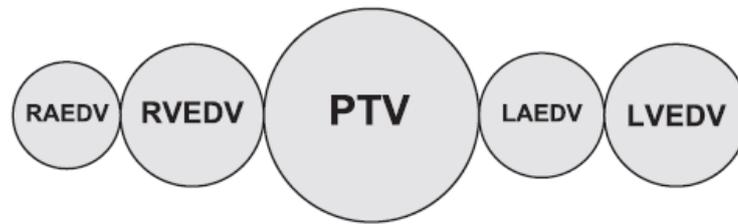
Equation 5.18

This is the methodology of ‘double’ indicator thermo-dye dilution (TDD) where ITBV may be determined from the volume of distribution of an indicator dye, and ITTV from the volume of distribution of a cold indicator (Equation 5.9). Whilst ITTV may be measured in the same way, ITBV however cannot be directly measured by STD but must be derived.

The observations of Sakka et al³⁹⁶ are fundamental to the determination of EVLW by STD. Sakka et al demonstrated in a population of 57 critically ill patients (with the diagnoses of sepsis and subsequent multiple organ dysfunction (n = 23), ARDS (n = 17), polytrauma (n = 6), and after major surgery (n = 11)) that there is a constant and linear relationship between ITBV and GEDV (Figure 5.7) such that:

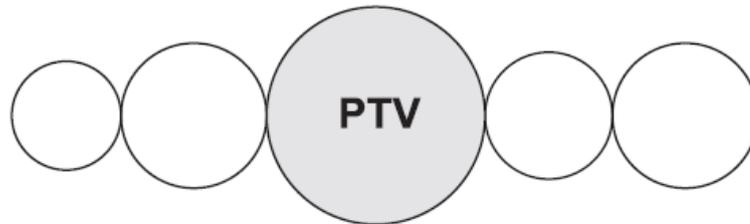
$$ITBV = (1.25 \times GEDV) - 28.4 \text{ (ml)}$$

Equation 5.19.

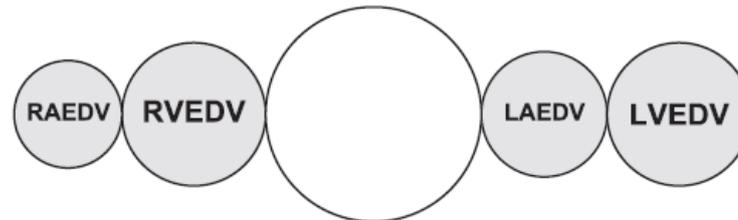


Intrathoracic thermal volume, ITTV.

Shaded area represents ITTV. PTV, pulmonary thermal volume. RA, RV, LA & LV-EDV, right and left atrial and ventricular end-diastolic volumes.

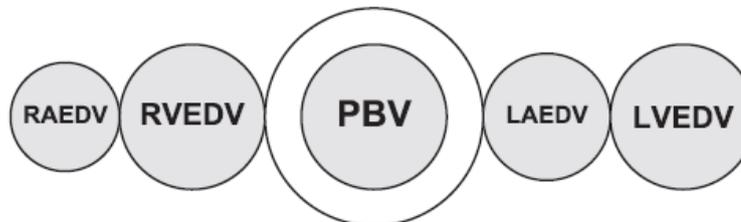


Pulmonary thermal volume, PTV.



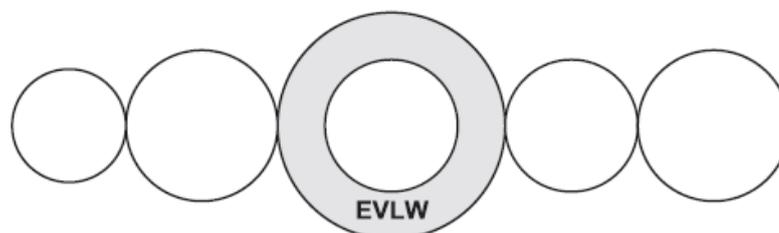
Global end-diastolic volume, GEDV.

Shaded area represents GEDV. $GEDV = ITTV - PTV$.



Intrathoracic blood volume, ITBV.

Shaded area represents ITBV. $ITBV = 1.25 \times GEDV$.



Extravascular lung water, EVLW.

$EVLW = ITTV - ITBV$.

Figure 5.6. Schematic representation of volumes from which EVLW is derived during 'single indicator' trans-pulmonary thermodilution.

Adapted from Brown et al (2009)³⁶³.

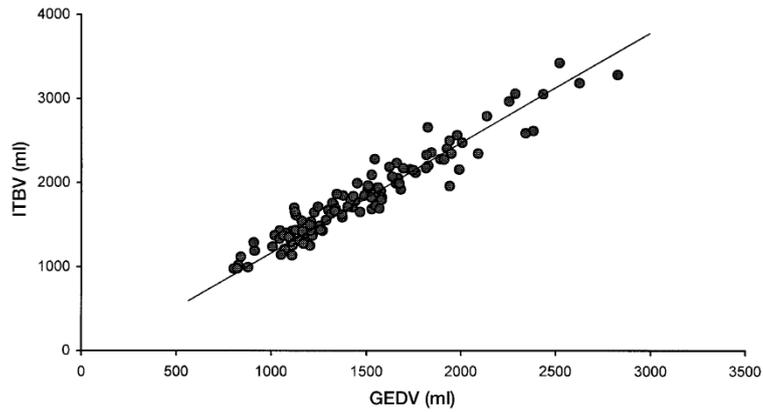


Figure 5.7. Relationship between ITBV and GEDV in 57 critically ill patients.
 $ITBV = (1.25 \times GEDV) - 28.4$ (ml). From Sakka et al (2000)⁴⁰³.

Sakka et al went on to validate this relationship in a further cohort of 209 patients (with diagnoses of sepsis (n = 99), ARDS (n = 31), severe head injury (n = 38), haemorrhagic shock (n = 19), intracranial haemorrhage (n = 19), and cerebral infarction (n = 3)) by comparing ITBV index (ITBVI, indexed to body surface area) derived from STD according to Equation 5.19 and ITBVI measured by TDD. They observed good correlation $r=0.97$ ($p<0.0001$) and very little bias between the two observations.

The relationship observed by Sakka et al (Equation 5.19) has since been simplified to:

$$ITBV = (1.25 \times GEDV)$$

Equation 5.20.

From which, once ITBV is known, it is a simple step to derive EVLW:

$$EVLW_{STD} = ITTV - ITBV_{STD}$$

Equation 5.21.

By substitution of Equations 5.9, 5.16, 5.17 and 5.20 into 5.21, EVLW can thus be derived from a STD injection:

$$\begin{aligned} EVLW_{STD} &= (CO \times MTt_{thermo}) - 1.25(CO \times MTt_{thermo} - CO \times DSt_{thermo}) \\ &= (CO \times MTt_{thermo}) - 1.25 CO(MTt_{thermo} - DSt_{thermo}) \end{aligned}$$

Equation 5.22.

5.4.2 Reproducibility of STD measurement of EVLW

Whilst there are a plethora of studies measuring $EVLW_{STD}$ in human subjects, few authors make any assessment of the reliability of the measurements obtained. Table 5.2 summarises studies examining the reliability of single thermodilution measurements in humans; it is noteworthy that it contains so few studies. Quoted values for the CV range from 4.8 to 8% with a least significant change value for $EVLW_{STD}$ of 7.8-12%, suggesting the reproducibility of $EVLW_{STD}$ to be ‘good’.

Table 5.2. Reproducibility of single indicator trans-pulmonary thermodilution measurements.

<i>Study</i>	<i>Pop</i>	<i>N (n)</i>	<i>Dup.</i>	<i>Statistic</i>	<i>CO</i>	<i>GEDV</i>	<i>ITBV</i>	<i>EVLW</i>	<i>PVPI</i>
Gondos et al. ⁴⁰⁴ (2009)	General ICU	30 (30)	2 3 4 5	CE CE CE CE	4.7% 4.9% 4.9% 4.8%	4.9% 4.8% 4.8% 4.8%	- - - -	- - - -	- - - -
Craig et al. ⁴⁰⁵ (2010)	Intensive care – ALI/ARDS	44	3	CV	-	-	-	5.2%	-
Tagami et al. ⁴⁰² (2010)	Ventilate ICU	30	3	CV	-	-	-	7.4%	-
Monnet et al. ³⁷⁶ (2011)	Haem. unstable ICU	91 (91)	3 3	Precision LSC	8% 12%	8% 12%	-	8% 12%	-
Tagami et al. ³⁷⁴ (2012)	Hypothermic post cardiac arrest	88 (462)	3	CV LSC	4.8% 7.8%	5.2% 8.5%	-	4.8% 7.8%	7.4% 12.1%
Wolf et al. ⁴⁰⁶ (2013)	Elective neurosurgery	101 (635)	‘At least’ 3	CV				10.1%	

N, number of patients; n, number of measurement sets; CE, coefficient of error; CV, coefficient of variation; LSC, least significant change.

How many duplicates?

The manufacturers of both TPTD monitors available for clinical use recommend that for each measurement, a triplicate set of thermodilution measurements are performed and the average values taken^{407, 408}. There is little to suggest where the recommendation for triplicate injections originated⁴⁰⁹, but it is likely that it stems from observations of the precision of TCTD cardiac output measurement; Stetz et al demonstrated that in order to reliably detect a clinical change of 12 to 15%, three measurements per determination would be required⁴¹⁰.

The validity of direct extrapolation of such recommendations to TPTD measurements is however questionable. On one hand, due to the longer transit

time of indicator during TPTD, by making an assessment over a greater number of heartbeats, the effects of arrhythmias and variations in CO to respiration might be minimised, and so TPTD has the potential to be more reproducible than TCTD. On the other hand the long transit time provides the potential for greater susceptibility to errors resulting from baseline temperature drift during the course of the measurement³⁷³. Furthermore, whilst a given number of replications may be adequate for the measurement of CO (the only volume *measured* directly by TCTD), measurement of derived parameters from TPTD may require an increased number of replications. In order to measure EVLW by TPTD, cardiac output and the mean transit time and downslope time of thermal indicator must be determined. Each measurement has the potential for error, whilst synchronous variation may improve the precision of the estimate, asynchronous variation could potentially decrease precision, an effect which Godje et al describe “*is a basic problem in mathematically combining multiple measurements to a new parameter*”³⁷⁵.

In recent years, several authors have tackled the question of how many thermodilution replicates are required in order to provide a clinically acceptable level of precision. Tagami et al, examined the precision of TPTD measurement in 88 patients following successful resuscitation after cardiac arrest, a population undergoing (and being re-warmed following) therapeutic hypothermia and as such high risk for variability secondary to changes in thermal baseline³⁷⁴. By performing 10 successive thermodilution estimates of CI, GEDVI and pulmonary vascular permeability index (PVPI - see section 5.6), Tagami et al explored the effect of number of replications on the coefficient of variation, precision and least significant change statistics for the estimates concluding that in order to maintain precision less than 10% for all variables, at least three injections are required³⁷⁴. Monnet et al assessed the relationship between precision and number of replications in 91 haemodynamically stable intensive care patients and similarly observed that three replications are required to maintain precision less than 10% for CI, global end-diastolic volume index (GEDVI) and extravascular lung water index ELWI³⁷⁶.

^Q Extravascular lung water index (ELWI), EVLW indexed to body weight (ml/kg). A discussion of the rationale for indexing EVLW is provided in Section 5.4.5.

From the work of Tagami³⁷⁴, Monnet³⁷⁶ and others (Table 5.2), it is evident that the precision of directly measured variables (CO and GEDV) is greater than for derived variables (EVLW and PVPI), where mathematical compounding or errors is a risk.

In contrast to the conclusions of Tagami³⁷⁴ and Monnet³⁷⁶, in a study of 30 general ICU patients, Gondos et al⁴⁰⁴ concluded that a coefficient of error of less than 5% (their predefined criteria of ‘scientific precision’) could be reliably obtained for CI and GEDVI by taking the average of just two individual injections. The authors made no assessment of the precision of EVLW estimates obtained from two injections.

5.4.3 Validity of STD measurement of EVLW

5.4.3.1 Criterion validity

Since the original description of the STD technique by Sakka et al⁴⁰³ and their validation against $EVLW_{TDD}$ there have been a large number of studies exploring the validity of $EVLW_{STD}$ against the ‘gold standards’ of gravimetry and $EVLW_{TDD}$.

Comparisons with gravimetry

There have been four animal studies which compare $EVLW_{STD}$ to gravimetric EVLW (Table 5.3). These studies examine the relationship both in control animals and animals with pulmonary oedema, demonstrating across a wide range of EVLW values that there is good agreement between $EVLW_{GRAV}$ and $EVLW_{STD}$, but a systematic over-estimation of EVLW by STD techniques (mean bias +2.4 to +5.4ml/kg, Table 5.3).

Much of the discrepancy observed between $EVLW_{STD}$ and $EVLW_{GRAV}$ in the studies of Katzenelson et al and Rossi et al is likely to be reflected by the fact that these two studies use a commercially available STD TPTD monitor validated for human use, which determines EVLW based on the ‘ $ITBV=1.25 \times GEDV$ ’ relationship described by Sakka et al⁴⁰³. As Kirov et al point out in their article of the same name, “*animals and humans are not the same*”⁴¹¹. Whilst in humans the linear regression equation for the relationship between ITBV and GEDV is $ITBV=1.25 \times GEDV - 28.4\text{ml}$ (the 28.4ml intercept is dropped for simplicity in commercially available monitors), in pigs the linear regression equation has been reported to be $ITBV=1.73 \times GEDV - 7.7\text{ml}$ ⁴¹² and in

sheep $ITBVI=1.43 \times GEDVI + 13.48 \text{ ml/kg}^{411}$. In their study comparing $EVLW_{STD}$ with $EVLW_{GRAV}$ in pigs, Rossi et al simultaneously establish the regression equation $ITBV=1.52 \times GEDV + 49.7 \text{ ml}$ (Table 5.3)⁴¹³. By substituting the $ITBV=1.25 \times GEDV$ equation for this one, the bias between $EVLW_{STD}$ and $EVLW_{GRAV}$ reduced to a statistically not significant $+2.34 \text{ ml/kg}^{413}$.

Table 5.3. Studies examining the validity of transpulmonary thermodilution derived EVLW verses gravimetry.

Author (year)	Species	N	Pathology	Correlation ($EVLW_{STD}$ vs $EVLW_{GRAV}$)	Mean Bias ($EVLW_{STD} - EVLW_{GRAV}$)	Mean $EVLW_{STD}/EVLW_{GRAV}$
Kirov et al ⁴¹⁴ (2004)	Sheep	18	Overall	$r=0.85$; $p<0.01$	$+4.9 \text{ ml/kg}$	
		4	Sham			1.44
		7	ALI - IV LPS			1.66
		7	ALI - IV OA			1.54
Katzenelson et al ⁴¹⁵ (2004)	Dogs	15	Overall	$r=0.97$; $p<0.001$	$+3.01 \text{ ml/kg}$	
		5	Control			1.33
		5	IV OA			1.13
		5	Hydrostatic oedema			1.19
Rossi et al ⁴¹³ (2006)	Pigs	15	Overall	$r=0.94$; $p<0.001$	$+5.4 \text{ ml/kg}$	
		6	Sham			$+5.11 \text{ ml/kg}$
		5	LPS			$+5.74 \text{ ml/kg}$
Kuzkov et al. ⁴¹⁶ (2010)	Sheep	11	Control / OA	$r=0.85$; p not provided.	$+2.4 \text{ ml/kg}$	

LPS, lipopolysaccharide; OA, Oleic acid.

Kirov et al (Table 5.3) substituted the human coefficient of 1.25 with an ‘ovine’ coefficient of 1.34 previously established by the authors⁴¹⁴. Even with this correction however, $EVLW_{STD}$ continued to overestimate $EVLW_{GRAV}$ (mean bias $=+4.91 \text{ ml/kg}$)⁴¹⁴. The authors hypothesise this could be explained by “*heat exchange of the thermal indicator with extra-vascular intrathoracic structures, and by recirculation of indicator*”⁴¹⁴, the same explanations offered by others for the systematic overestimation of $EVLW_{GRAV}$ by TDD^{400, 417}.

Comparisons with thermo-dye dilution measurement of EVLW

Thermo-dye dilution (TDD) techniques (in which ITBV is directly measured and therefore not dependent on relationship between ITBV and GEDV) have been used as a ‘gold standard’ in comparison to which the validity of STD has been explored.

Neumann compared $EVLW_{STD}$ and $EVLW_{TDD}$ in 13 pigs with lung injury induced by oleic acid injection⁴¹², and observed a systematic over estimation of $EVLW_{TDD}$ by

EVLW_{STD} of 0.5-1ml/kg, which could be ameliorated by correcting the ITBV:GEDV relationship to $ITBV=1.73 \times GEDV - 7.7 \text{ml}$ which he determined from the population. Clearly, the requirement to establish the ITBV:GEDV relationship in the study population (by TDD) undermines the value of a STD technique. As such, validity of STD (when compared to TDD) depends on the reliability and linearity of the $ITBV=1.25 \times GEDV$ relationship; if the relationship is true, that is ITBV derived by calculation from GEDV (ITBV_{STD}) is equivalent to ITBV measured by TDD (ITBV_{TDD}), then EVLW_{STD} will equal EVLW_{TDD}.

As previously discussed, Sakka et al derived the $ITBV=1.25 \times GEDV$ in a cohort of 57 critically ill patients and then validated the relationship in a further 209 patients⁴⁰³. Sakka et al conclude their paper with the comment that “*further validation studies are needed in the future to test our algorithm in other patient populations*”⁴⁰³. Despite this, and similar calls from others^{382, 412}, there are only two studies which have subsequently attempted to confirm in humans the relationship observed by Sakka et al⁴⁰³ (Table 5.4).

Table 5.4. Studies examining the validity of transpulmonary thermodilution derived EVLW verses thermo-dye dilution in humans.

Author (year)	N	Population	Correlation (EVLW_{STD} vs EVLW_{TDD})	Mean Bias (EVLW_{STD} – EVLW_{TDD})	ITBV=a.GEDV+b
<i>Buhre et al</i> ⁴¹⁸ (1998)	10	Neurosurgery			a = 1.16 b = +86
<i>Sakka et al</i> ⁴⁰³ (2000)	57 (n=209)	Critically ill	r=0.96; p<0.0001	-0.2ml/kg	a= 1.25 b = -28.4
<i>Reuter et al</i> ⁴¹⁹ (2002)	19	Post-op cardiac surgery			a = 1.10 b = +180
<i>Kuntscher et al</i> ⁴²⁰ (2003)	18 (n=290)	Burns	r=0.83; p<0.01	+1.5ml/kg	
<i>Hofmann et al</i> ⁴²¹ (2005)	174	Critically ill	r=0.94; p value not provided	0.0ml/kg	
<i>Michard et al</i> ²⁴⁸ (2005)	48 (n=192)	Surgical intensive care	r=0.96; p value not provided	-0.5ml/kg	a = 1.16 b = +97

N, number of subjects; n, number of comparisons.

From Table 5.4, it can be seen that bias between EVLW_{STD} and EVLW_{TDD} tends to be modest. It must be appreciated however, that this data reflects mean bias. Sakka et al observe that EVLW_{STD} tends to overestimate EVLW_{TDD} at low/normal values and underestimate EVLW_{TDD} at higher values, as can be appreciated in Figure 5.8.

Michard et al similarly observed underestimation of $EVLW_{TDD}$ by STD at higher levels of EVLW, suggesting that a systematic correction factor should be applied at $EVLW_{STD}$ levels $> 7\text{ml/kg}$ ²⁴⁸.

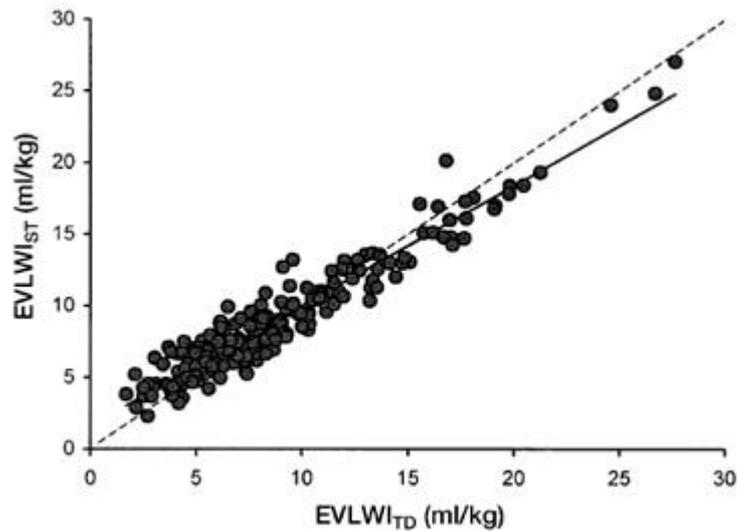


Figure 5.8. Relationship between extravascular lung water determined by single-thermo and thermo-dye dilution in 209 critically ill patients. Line of identity is dashed. From Sakka et al (2000)⁴⁰³.

Reuter et al determined the relationship between ITBV and GEDV in 19 post-operative patients following cardiac surgery (Table 5.4)⁴¹⁹. By TDD they determined the relationship to be $ITBVI = 1.16 \times GEDVI + 180\text{ml/kg}$. They then estimated $ITBV_{STD}$ using this equation, and the conventional $ITBV_{STD} = 1.25 \times GEDV$. By comparing these estimated figures to $ITBV_{TDD}$ they demonstrated greater bias using the '1.25' relationship (33ml/m^2 vs 0.5ml/m^2 ; no statistical comparison provided).

Analogous to Mihm et al's gravimetric validation of $EVLW_{TDD}$ in organ donors⁴²², Tagami et al performed a similar study comparing $EVLW_{STD}$ and $EVLW_{GRAV}$ in 30 lung specimens harvested at autopsy⁴⁰². Whilst unfortunately they did not measure gravimetric EVLW, Tagami et al observed good association between $EVLW_{STD}$ and post-mortem lung weight ($r=0.90$; $p<0.001$).

In summary it is evident that both $EVLW_{TDD}$ and $EVLW_{STD}$ systematically overestimate $EVLW_{GRAV}$, but that the relationship between $EVLW_{TDD}$ and $EVLW_{STD}$ is dependent on EVLW level, with $EVLW_{STD}$ tending to underestimate $EVLW_{TDD}$ (and so approximate $EVLW_{GRAV}$) as EVLW rises.

5.4.3.2 Concurrent validity

In a prospective observational cohort of 51 patients admitted to a mixed ITU with shock, Chew et al examined the utility of EVLW measurement as a diagnostic tool in the diagnosis of ALI/ARDS⁴²³. By utilising an EVLW cut-off of >10ml/kg, they demonstrated that EVLW added diagnostic value; an ELWI >10ml/kg increased the post-test odds ratio for the diagnosis of lung injury (ALI, ARDS or LIS>2.5) by up to three-fold whilst an ELWI <10ml/kg reduces the post-test odds by almost half. The sensitivity and specificity of ELWI for the diagnosis of both ALI and ARDS were approximately 70 percent⁴²³.

Clinically, it would be arguably of more value if EVLW could serve as an early marker of impending ALI/ARDS allowing for example, identification of at risk patients and targeting of therapies. Le Tourneau et al measured EVLW on admission to intensive care in 29 patients⁴²⁴. They demonstrated that an EVLW cut off of 10ml/kg had had a positive predictive value of 83%, and negative predictive value of 70% to predict progression to acute lung injury. Whilst these values are impressive, it must be acknowledged that the author's claim that "*extravascular lung water predicts progression to acute lung injury in patients with increased risk*" is a bold claim from the sample size presented (8 patients had 'progression to ALI' in the study) and that the positive predictive value quoted though noteworthy may be over estimated in the context of the high prevalence of 'progression to ALI' found in the study⁴²⁵. Nonetheless the potential for EVLW measurement to identify patients at risk of progressing to ALI over two days before they fulfil ALI/ARDS criteria is an exciting one.

5.4.3.3 Construct validity of EVLW measurement

Though no established criteria exist against which the construct validity of a technique seeking to measure ALI/ARDS can be compared, the 'Berlin' ARDS Definition Task Force⁵⁰ provided a clinical, physiological and pathological framework from which constructs can be created^R. Thus, if association is found between measured values of EVLW and the *clinical* findings of decreased oxygenation and typical radiological appearances; *physiological* evidence of increased shunt or decreased lung compliance and; the presence of diffuse alveolar

^RDiscussed in detail in Section 5.2.1.

damage in *pathological specimens* (in animal models or *ex-vivo* human specimens) then it could be concluded that EVLW has construct validity as a measure of pulmonary oedema / lung injury. There have been numerous such findings (either directly sought or collected as a by product during clinical studies) suggestive of the construct validity of EVLW in the measurement of pulmonary oedema in critically ill humans with or without ALI/ARDS using both TDD and STD techniques (Table 5.5).

As can be seen from Table 5.5, association has been observed between EVLW measurements and oxygenation (determined as $\text{PaO}_2/\text{FiO}_2$ ^{402, 405, 423, 426-434}, respiratory index⁴³⁵, oxygenation index⁴⁰⁵), chest X-ray scores^{423, 428, 430, 436, 437}, lung injury score^{402, 405, 423, 428-430, 432, 433}, venous admixture⁴³⁸, pulmonary compliance^{405, 430, 432}, pulmonary dead space fraction (V_D/V_T)⁴²⁹ and level of PEEP^{427, 439} in both post-operative and critically ill intensive care patients. In addition EVLW has been shown to be associated with severity of illness (determined as Sequential Organ Failure Assessment (SOFA) score in critically ill patients⁴²⁹).

Whilst there have been few studies directly comparing EVLW with biological markers of infection, inflammation or lung injury, EVLW has been demonstrated to be associated with levels of procalcitonin⁴³⁹, neutrophil elastase⁴⁴³ and endothelin-1⁴³⁰ in peripheral blood.

Whilst there are many studies suggesting construct validity of EVLW measurement several have been negative^{428, 438, 441}. The failure to find any association between EVLW and physiological variables in the studies of Patroniti et al⁴³⁸ and Groeneveld et al⁴⁴¹ could potentially be explained by the modest sample size in each study (n=14 and 16 respectively). Furthermore in the study of Groeneveld et al⁴⁴¹ TDD estimates of EVLW were made in post-operative patients following major vascular surgery where the degree of lung injury (and therefore variation in EVLW and other physiological variables) might be expected to be modest. Perhaps more surprising is the finding of no identifiable relationship between EVLW and fluid balance in several studies^{381, 428, 433}.

Table 5.5. Studies exploring the construct validity of extravascular lung water measurement in humans.

<i>Author (year)</i>	<i>Population</i>	<i>Method</i>	<i>N</i>	<i>Comparator (EVLW vs....)</i>	<i>Findings</i>
Baudendistel et al. ⁴³⁶ (1982)	Blunt trauma following RTA	TDD	12 (n=70)	CXR score	r=0.623; 'significant,' p not provided
Halperin et al. ⁴⁴⁰ (1985)	Intensive care patients with respiratory failure	TDD	12 (n=73)	CXR score	r=0.51; p<0.05 No correlation between Δ EVLW and Δ CXR score
Davey-Quinn et al. ⁴³¹ (1999)	Intensive care – ALI & ARDS	TDD	11	PaO ₂ /FiO ₂	ELWI independent predictor of PaO ₂ /FiO ₂ (B=-2.8±0.45; p<0.0001)
Szakmany et al. ⁴²⁷ (2004)	Intensive care – septic shock and ALI/ARDS	STD	23	PaO ₂ /FiO ₂ PEEP	r=-0.36; p<0.001 r=0.56; p<0.001
Martin et al. ⁴²⁸ (2005)	Medical intensive care – severe sepsis	STD	29	PaO ₂ /FiO ₂ LIS CXR score Fluid bal.	r=-0.52; p<0.0001 r=0.42; p<0.0001 r=0.53; p<0.0001 No association
Patroniti et al. ⁴³⁸ (2005)	Intensive care – ARDS	TDD	14	Compliance PaO ₂ /FiO ₂ Venous admixture	-0.43; NS 0.47; NS -0.57; p<0.05
Wan et al. ⁴²⁶ (2005)	Intensive care – septic shock	STD	23	PaO ₂ /FiO ₂	Δ ELWI vs Δ PaO ₂ /FiO ₂ over consecutive measurement days: r = -0.33; p <0.01
Groeneveld et al. ⁴⁴¹ (2006)	Post-operative - major vascular surgery	TDD	16	Pulmonary leak index PaO ₂ /FiO ₂ Compliance CXR score LIS	No significant difference in any parameter in patients with EVLW ≤ 7 compared with EVLW > 7.
Kuzkov et al. ⁴³⁰ (2006)	Intensive care – septic shock and acute lung injury	STD	38	Compliance PaO ₂ /FiO ₂ LIS CXR score Endothelin 1	Day 1 r =0.48 Day 3 r=0.51 r=-0.5 r=-0.49 r=0.46 r=0.53 r=0.39 (p<0.01 for all) Significantly higher in patients with EVLW ≥ 7
Sato et al. ⁴³⁵ (2007)	Post-operative - oesophagectomy	STD	23	Respiratory index	r=0.64; p<0.00001
Berkowitz et al. ⁴³³ (2008)	Medical and surgical intensive care	STD	30 (225)	LIS PaO ₂ /FiO ₂ Fluid bal.	EVLW _{PBW} : r=0.62; EVLW _{PBW} : r=-0.50; p<0.01 for all No association
Oshima et al. ⁴³² (2008)	Post-operative – oesophagectomy	STD	25	PaO ₂ /FiO ₂ Compliance LIS	r=-0.36; p=0.014 r=-0.625; p=0.0001 r=0.614; p=0.0001

Author (year)	Population	Method	N	Comparator (EVLW vs....)	Findings
Phillips et al. 429 (2008)	Intensive care – sepsis induced ARDS	STD	19	LIS SOFA PaO ₂ /FiO ₂ VD/VT	r=0.53; p=0.02 r=0.61; p=0.006 r=-0.53; p=0.02 r=0.62; p=0.005
Bognar et al. 439 (2010)	Intensive care - >20% burns	STD	28	PEEP PCT	r=0.50; p=0.017 r=0.60; p=0.008
Chung et al. 442 (2010)	Intensive care – severe sepsis	STD	67	SOFA	Day 1 r=0.70; p<0.001 Day 3 r=0.77; p<0.001
Craig et al. 405 (2010)	Intensive care – ALI/ARDS	STD	44	LIS Oxygenation index PaO ₂ /FiO ₂ Compliance	r=0.50; p=0.0005 r=0.56; p=0.0001 r=-0.57; P<0.0001 r=-0.06; p=0.71
Tagami et al. 402 (2010)	Ventilated ITU	STD	30	LIS PaO ₂ /FiO ₂	r=0.61; p<0.001 r=-0.41; p<0.02
Tagami et al. 443 (2011)	HDU/ITU – community acquired pneumonia	STD	14 (6 in ITU)	Plasma neutrophil elastase	Day 1 r=0.88, p<0.02 Day 2 r=0.83, p<0.04
Aman et al. 381 (2012)	Intensive care – septic and nonseptic	TDD	63	PaO ₂ /FiO ₂ Fluid balance	ΔPaO ₂ /FiO ₂ associated with ΔEVLW (r=0.36; p=0.004) ΔEVLW no assoc. with fluid administration
Chew et al. 423 (2012)	Intensive care – SIRS and 'circulatory failure'	STD	51	PaO ₂ /FiO ₂ CXR score PEEP LIS	r=-0.37 to -0.49; p=0.001 r=0.26 to 0.46; p=0.002 No association Increasing ELWI with increasing strata of LIS (p<0.01)
Kushimoto et al. 434 (2012)	Intensive care – multicentre predominantly ALI/ARDS	STD	266	PaO ₂ /FiO ₂	r=-0.21; p<0.01
Mallat et al. 444 (2012)	Intensive care – septic shock	STD	55	LIS PaO ₂ /FiO ₂ Compliance	r=0.52, 0.55, 0.6 r=-0.32, -0.37, -0.37 days 1-3 respectively, p<0.05 for all. No association over all 3 days.
Wolf et al. 406 (2013)	Intensive care - elective neuro-surgery	STD	101	Fluid balance	EVLW increased by 3.4% per litre fluid gain (p=0.04)
Brown et al. 437 (2013)	Intensive care – ALI/ARDS	STD	59 n=476	CXR score	r=0.35; p<0.001

STD – 'single' thermodilution. TDD – thermo-dye dilution. LIS – 'Lung Injury Score'. Fluid bal., fluid balance. LIS, lung injury score.

Positive fluid balance has been associated with poor oxygenation, increased lung injury score, and prolonged ventilator requirement in patients with ALI/ARDS⁴⁴⁵. It might be expected therefore that patients with more positive fluid balance are likely to have greater EVLW. Aman et al explored the relationship between fluid loading and changes in EVLW in 63 mechanically ventilated patients. By defining changes in EVLW of $\geq 10\%$ as a positive response, the authors observed that increases in EVLW following fluid loading are dependent on cardiac index and pulmonary vascular filling (determined as PBVI) but independent of the volume and type of fluid administered. One hypothesis for the lack of association between EVLW and fluid balance may be the existence of a threshold effect for the influence of fluid balance on EVLW such that in studies where standard practice (in the wake of the US ARDS Network Fluid and Catheters Treatment trial⁴⁴⁵) may be to restrict fluid administration, fluid balance does not accumulate sufficiently to levels where a dependent increase in EVLW might be expected. Such a hypothesis is supported by the finding of Phillips et al who observed a linear relationship between EVLW and fluid volume administered in a porcine model of haemorrhage and resuscitation where fluid volumes administered were commonly in excess of 50ml/kg; visual inspection of the scatter plot of fluid volume vs ELWI provided by the authors suggest that with fluid volumes of <50ml/kg such linear association is unlikely to be present⁴⁴⁶.

5.4.3.4 Predictive validity of EVLW measurement for mortality

There have been a large number of studies assessing the prognostic validity of EVLW (Table 5.6). ELWI has been demonstrated to be consistently higher in non-survivors than survivors in critically ill patients with a wide variety of pathologies, both with and without ARDS. This might be anticipated as ELWI is likely to reflect overall severity of illness; however in the study by Craig et al, this effect was maintained after adjustment for covariates reflecting severity of illness⁴⁰⁵, suggesting that in critically ill patients with ALI/ARDS ELWI has prognostic value in addition to being a simple marker of disease severity. Similarly several other authors found EVLW to be an independent predictor of mortality^{442, 447, 448}.

Though the estimates of AUROCC, sensitivity and specificity of ELWI reported in (Table 5.6) are encouraging, many of the studies listed have a small sample size, (all but two are in less than 100 patients, most less than 50) resulting in broad

confidence intervals for the estimates. In attempt to provide some clarity, Zhang et al recently published a systematic review in which they conducted a meta-analysis exploring the relationship between ELWI and mortality in critically ill patients⁴⁴⁹. Pooling the results of 11 studies including 670 individual patients they demonstrated significantly higher ELWI in nonsurvivors than in survivors (mean difference of 5.06 ml/kg [95%CI -7.523 to -2.58]). Though their analysis was hampered by significant heterogeneity ($I^2=90\%$), small sample sizes in many studies and variations in body weight to which EVLW was indexed (actual or predicted body weight), Zhang et al report an overall 81% sensitivity [95% CI 72-88] and 66% specificity [95% CI 0.55-0.76] for ELWI for the prediction of mortality in critically ill patients.

Table 5.6. Summary of studies examining predictive validity of EVLW.

Author (year)	Population	Method	N	Survivors v. nonsurvivors	AUROC	Cut off	Sens	Spec
Davey-Quinn et al. ⁴³¹ (1999)	Intensive care – ALI/ARDS	TDD	11	Initial ELWI higher in nonsurvivors. (31.0 vs 20.7 ml/kg; p=0.013)	-	-	-	-
Sakka et al. ⁴⁴⁷ (2002)	Surgical intensive care	TDD	373	Maximum ELWI higher in nonsurvivors. (10.2 vs 14.3ml/kg; p<0.001)	0.649	9.2ml/kg 6.5ml/kg	69.4%	50.8%
Martin et al. ⁴²⁸ (2005)	Medical intensive care	STD	29	EVLW higher in nonsurvivors (14 ml/kg vs 8.0 ml/kg; p<0.001)	-	-	-	-
Kuzkov et al. ⁴³⁰ (2006)	Intensive care – septic shock and acute lung injury	STD	38	Day 3, EVLW higher in nonsurvivors. (6.6 vs 11.1ml/kg; p<0.05)	-	-	-	-
Yang et al. ⁴⁵⁰ (2006)	Intensive care – septic shock	STD	50	Baseline: No significant difference in EVLW between survivors and nonsurvivors. Day 3. ELWI significantly higher in nonsurvivors. (14.3 vs 8.1 ml/kg; p=0.001)	0.74	7.5ml/kg	83%	54%
Chung et al. ⁴⁵¹ (2008)	Medical intensive care – severe sepsis	STD	33	Proportion of patients surviving with ELWI > 10ml/kg: 15% vs 68%; p=0.0008	-	10ml/kg	88.2%	68.7%
Philips et al. ⁴²⁹ (2008)	Intensive care	STD	19	Day 1.ELWI higher in nonsurvivors. (20.6 vs 11.6ml/kg; p=0.002).	0.988	16ml/kg	100%	86%
Bognar et al. ⁴³⁹ (2010)	Intensive care - >20% burns	STD	28	Day 1 & 3. ELWI higher in nonsurvivors (data not provided; p<0.01, <0.001 respectively)	-	-	-	-
Chung et al. ⁴⁴² (2010)	Intensive care – severe sepsis	STD	67	Day 1. Pulmonary sepsis - ELWI higher in nonsurvivors (25.0 vs 11.8ml/kg; p=0.001). Non-pulmonary sepsis - ELWI higher in nonsurvivors (22.0 vs 12.8 ml/kg; p=0.012).	0.88	10ml/kg	94.7%	66.7%

Author (year)	Population	Method	N	Survivors v. nonsurvivors	AUROC	Cut off	Sens	Spec
Craig et al. 405 (2010)	Intensive care – ALI/ARDS	STD	44	First reading within 48hrs of diagnosis. ELWI higher in nonsurvivors. (17.5 vs 10.6ml/kg; p=0.003)	0.80	16ml/kg	75%	78%
Chew et al. 423 (2012)	Intensive care – SIRS and 'circulatory failure'	STD	51	First measurement (within 6 hours of admission). ELWI higher in non survivors. (10.6 vs 9.1ml/kg; p=0.05)	-	10ml/kg	-	-
Mallat et al. 444 (2012)	Intensive care – septic shock	STD	55	ELWI higher on day 3 in non survivors (p<0.001), (no difference day 1 & 2). OR of death ELWI = 1.7 per SD (95% CI 1.1-3.7; p=0.02).	0.85	14ml/kg	75%	76%
Brown et al 437 (2013)	Intensive care – ALI/ARDS	STD	59	Baseline ELWI higher in non survivors. (17 vs 12 ml/kg; p=0.05)	0.68	-	-	-
Jozwiak et al. 448 (2013)	Medical intensive care - ARDS	STD	200	Day 1. ELWI not significantly different between non survivors and survivors. Maximum ELWI higher in non survivors (24 vs 19 ml/kg p<0.001)	-	21ml/kg (max)	54%	73%

STD, 'single' thermodilution; TDD, thermo-dye dilution; AUROC, area under the receiver operating characteristic curve; Sens., sensitivity; Spec., specificity.

5.4.4 Sensitivity and specificity of extravascular lung water measurement.

Rather than examining the sensitivity and specificity of EVLW according to the numerical definitions of the terms, this section is concerned with the following questions: Firstly, are changes in EVLW *specific* to changes in interstitial and alveolar oedema? Secondly, how sensitive are TPTD techniques to small changes in EVLW?

5.4.4.1 Specificity of EVLW measurement

Clinical measurement of EVLW is made on the assumption that EVLW is “the morphologic correlate of pulmonary oedema”²⁹, in the belief that the extravascular thermal volume is the fluid volume of the lung interstitium and alveolar spaces with which thermal indicator may exchange. It is plausible that the presence of pleural or pericardial effusions could provide a further extravascular fluid volume into which cold indicator could distribute, leading to an artefactual over-estimate of the EVLW volume.

Pulmonary oedema

Bongard et al created a porcine hydrostatic pulmonary oedema model by variable inflation of a left atrial balloon²⁹. By titrating cuff inflation to a predetermined level of EVLW (measured by TDD) and examining histological autopsy specimens at progressively increasing levels of EVLW, they describe a familiar progression of pulmonary oedema evolving from inter-alveolar septal thickening, to perivascular cuffing before fulminant alveolar flooding. Bongard et al provide compelling evidence for the ability of TPTD to characterise progressive accumulation of pulmonary oedema, by demonstrating that perivascular cuff width:vessel diameter correlated linearly with EVLW ($r=0.87$; $p<0.0001$) and inter-alveolar septal width was linearly related to EVLW in animals with $EVLW > 11.2\text{ml/kg}$ ($r=0.89$; $p<0.001$). Alveolar flooding did not occur until EVLW exceeded 11.4ml/kg , but then increased linearly with EVLW ($r=0.87$; $p<0.001$)²⁹.

Pleural effusion

Blomquist et al systematically evaluated the effects of incremental increases in pleural fluid volume (warmed normal saline introduced bilaterally via intercostal

catheters) on $EVLW_{TDD}$ in otherwise healthy dog lungs⁴⁵². They reported a slight, but not statistically significant rise in EVLW and though “*a minor, and for practical purposes negligible loss of thermal indicator [cold] to the pleural fluid could not be excluded*”, they ultimately concluded that installation of up to 20ml/kg of fluid into the pleural cavity has no effect on $EVLW_{TDD}$. In 30 human autopsies in patients who had undergone $EVLW_{STD}$ measurement in the 48 hours preceding autopsy, Tagami et al examined the relationship between $EVLW_{STD}$ and lung weight. They report no significant difference in the relationship between EVLW and lung weight (as a surrogate estimate of ‘true’ EVLW) in patients with pleural effusion volumes of less than or more than 500 mL, suggesting no effect of pleural effusion on EVLW measurement. Several authors have similarly reported that pleural fluid volume does not contribute to measured EVLW in clinical studies in medical intensive care patients undergoing thoracocentesis^{453, 454}. Saguel et al in fact observed a statistically significant increase in EVLW following ‘large volume thoracocentesis’, hypothesising that expansion of lung tissue following removal of pleural fluid may both lead to further fluid extravasation (re-expansion pulmonary oedema), and lead to increased perfusion of previously atelectatic regions of lung thus increasing the volume of lung ‘visible’ to cold indicator.

5.4.4.2 Sensitivity of EVLW measurement

Fernandez-Mondejar et al examined the ability of STD EVLW measurement to detect ‘small changes’ in EVLW (defined by the authors as 10-20%) in pigs both with and without pulmonary oedema⁴⁵⁵. By measuring $EVLW_{STD}$ immediately before and after intratracheal administration of 50mls of saline solution (so increasing EVLW (alveolar fluid) by 50ml), they were able to demonstrate that STD technology was able to detect the increase in EVLW. In normal lungs a mean of 84% and in oedematous lungs 77% of the administered bolus was detected. Putting these results in context with the observations of Bongard et al²⁹ (above) which suggest that increases in EVLW in excess of 100% are required before hypoxaemia or chest radiography changes are observed makes the exciting suggestion that EVLW measurement may be able to sensitively detect sub-clinical increases in EVLW. As in 2005 when Fernandez-Mondejar et al made their observations, “*the clinical significance of these changes... has yet to be elucidated*”⁴⁵⁵.

Interpretation of 'sensitivity' in terms of how small a change in EVLW can be detected by TPTD techniques must incorporate examination of the 'least significant change' (LSC) values determined from studies examining the reproducibility of the technique (Table 5.2). From Table 5.2, it can be seen that quoted LSC values for $EVLW_{STD}$ range from 7.8-12%; that is to say that observed changes in EVLW of less than ~10% cannot reliably be interpreted as clinical changes and may represent measurement artefact.

5.4.5 Indexing of EVLW values

Raw data obtained from both STD and TDD estimates of EVLW return an absolute value for EVLW; that is volume of EVLW measured in millilitres. It is unsurprising to observe however that larger people have larger lungs, and more lung water. It has become conventional therefore to provide EVLW data indexed for (actual) body weight. Such indexing of a physiological trait is performed to remove its dependence on height, weight or gender and thus facilitate a comparison between patients⁴⁰⁶. Yet in the context of EVLW, indexing to actual body weight might be ineffectual. Recommendations from the American Thoracic Society (ATS)/European Respiratory Society Task Force on pulmonary function standards, clearly states that "*lung volumes are related to body size, with standing height being the most important factor*"³³⁸. Crapo et al performed a large observational study aiming to determine 'reference spirometric values' by performing pulmonary function testing in 251 health men and woman⁴⁵⁶. They report that the addition of weight to regression equations predicting lung volumes on the basis of height and age did not improve predictability of the equations; suggesting minimal influence of weight on pulmonary volumes. It is interesting to observe the prediction equations for pulmonary volumes on the basis of age and height are different for males and females⁴⁵⁶; there have been no suggestions that EVLW be indexed any differently according to sex.

In 1974 in response to the problem that weight based estimation of creatinine clearance might result in gentamicin toxicity in obese patients, Devine published equations for calculation of predicted body weight (PBW), providing a height based estimate of lean body weight⁴⁵⁷. In recognition that lean body weight represents "*the weight at which 99% of the body's metabolic processes occur*", this and other

similar calculations of lean or ideal body weight have been incorporated both into pharmacokinetic and wider clinical practice⁴⁵⁸; the US ARDS Networks trial of low tidal volume ventilation for example recommends a tidal volume of 6ml/kg based on *predicted* body weight⁹⁹.

In obese subjects it seems implausible that lung size increases in proportion to increases in actual body weight (indeed in obese subjects lung volumes are reported to be reduced due to reductions in absolute and chest wall compliance⁴⁵⁹). As such, EVLW indexed to actual body weight might be falsely low in obese subjects whilst EVLW indexed to predicted body weight might reflect a patient's condition more accurately^{406, 429}. This is supported by a number of studies which have demonstrated that the predictive validity of EVLW measurement (for mortality) is improved when EVLW is indexed for ideal body weight^{405, 429}. Similarly, Berkowitz et al observed that indexing EVLW to PBW resulted in a stronger correlation with Lung Injury Score and PaO₂:FiO₂ ratio⁴³³. In contrast Mallet et al were unable to demonstrate any improvement in the predictive value of ELWI_{PBW} over ELWI_{ACT}⁴⁴⁴ whilst Chew et al demonstrated ELWI_{PBW} to have a weaker statistical relationship to mortality than ELWI_{ACT}⁴²³.

Nonetheless, on the strength of the evidence suggesting improved predictive validity, indexing to PBW has become accepted practice; so much so that both commercially available clinical monitors provide EVLW indexed to PBW by default^{407, 408}. It wasn't until 2013 that the relationship between EVLW and biometric variables was explored in more detail, challenging the validity of indexing to PBW. Wolf et al performed a multivariate analysis of raw and indexed EVLW data examining the relationship with age, gender, height, body surface area and actual and predicted body weight in a cohort of 101 elective neurosurgical patients. They observed that indexing EVLW to height was the only method of indexing where a value could be obtained independent (as is desirable) of any statistically significant relationship to age, height, weight or gender⁴⁶⁰. Huber et al performed a similar analysis in 234 consecutive intensive care patients finding markedly different ELWI values between different weight based methods of indexing; the difference being most pronounced in female patients with BMI \geq 30kg/m². Huber et al conclude that height is the only biometric parameter independently associated with EVLW and that "EVLW should be indexed to height"⁴⁶¹. Supporting such a conclusion, the authors performed a post-hoc analysis

demonstrating ELW_{HEIGHT} to have greater discriminatory value than ELW_{PBW} in determining a population of patients with $PaO_2/FiO_2 < 200\text{mmHg}$ (AUROCC 0.77 vs 0.71).

5.4.6 What is normal range of EVLW?

5.4.6.1 Determining normality

Establishing a normal range for EVLW has been challenging with several authors reporting a lack of consensus as to what constitutes ‘normal’,^{402, 405}. In laboratory medicine, standard methods for determining the normal range are to make nonparametric estimates of the 95% reference interval in at least 120 healthy individuals⁴⁶³. Measurement of EVLW requires invasive haemodynamic monitoring and as such, large cohorts of healthy patients with the appropriate monitoring simply do not exist, or arguably would be unethical to pursue. Studies attempting to establish a reference range for EVLW have necessitated more innovative approaches.

5.4.6.2 Quoted normal ranges

‘Normal’ EVLW is variously quoted in the literature as $<5\text{ ml/kg}$ ⁴³¹, $3\text{-}8\text{ml/kg}$ ⁴⁰⁶, $5\text{-}7\text{ml/kg}$ ³⁸², $<7\text{ml/kg}$ ^{74, 382, 405, 464-466}, $<7\text{-}10\text{ ml/kg}$ ^{438, 467} or $<10\text{ ml/kg}$ ^{428, 433, 434, 468}. Many authors provide no indication to determine on what basis the quoted value has been derived, many others simply reference the un-explained values provided by others. It seems likely that the origin of many of the values lies in animal data; Lewis et al (for example) report that “*numerous studies show that EVLW [is] 6-7 ml/kg in normal animals measured by the thermal technique*”⁴⁶⁹.

5.4.6.3 Studies attempting to identify ‘normal’ EVLW in humans

In 1983 using the TDD technique, Sibbald et al sought to identify the normal range for EVLW. From a group of 79 critically ill patients requiring invasive haemodynamic monitoring, the authors identified a sub-group of 16 critically ill patients with no radiological evidence of pulmonary oedema, no evidence of systemic infection and normal pulmonary capillary wedge pressure. The mean \pm SD

EVLW in this subgroup was 5.6 ± 1.8 ml/kg suggesting that the upper limit of the normal range (+2SD) would be around 9 ml/kg⁴⁷⁰.

Normal range derived from autopsy specimens

In their autopsy study, Tagami et al attempted to define a normal range for $EVLW_{STD}$ ⁴⁰². In 30 autopsy specimens who had undergone $EVLW_{STD}$ measurement in the 48 hours preceding autopsy, Tagami et al derived a regression equation reflecting the relationship between $EVLW_{STD}$ and lung weight. Then by reference to 'normal' lung weights derived in a previous study, using their regression equation they estimate that normal $ELWI_{STD}$ values (indexed to predicted body weight) were 7.5 ± 3.3 ml/kg (\pm SD) in males and 7.3 ± 3.3 ml/kg in females. Whilst this approach is undoubtedly novel it has significant limitations. Firstly, given that by definition all of the subjects were deceased it seems likely that EVLW could have increased as disease progressed in the interval between $EVLW_{STD}$ estimation and death (though the authors report that $EVLW_{STD}$ was determined "*just before death*", no timings are provided). Secondly, as highlighted by the authors, cardiopulmonary resuscitation was performed in 16 cases (53%); the effects of which upon EVLW are unknown. Thirdly, as discussed by Zhang et al⁴⁷¹, the authors calculate 'normal' EVLW from a regression equation determined by observing the relationship between EVLW and lung weight in critically ill patients. The proportion EVLW makes of lung weight is however likely to vary as EVLW increases, making extrapolation of this relationship questionable.

Studies measuring EVLW in peri-operative patients

Table 5.7 summarises the available data from studies utilising EVLW measurement in the peri-operative period. Where a pre-operative value of EVLW is reported, this may provide some insight into the 'normal' EVLW, though it must be emphasised that by definition, patients in these populations are not 'healthy', 'normal' subjects. Indeed in some circumstances for example patients undergoing lung resection, patients may have significant perfusion deficits such that EVLW might be underestimated.

Table 5.7. Studies reporting ‘normal’ pre-operative values for EVLW in patients undergoing elective surgery.

<i>Author (year)</i>	<i>N</i>	<i>Method</i>	<i>Population</i>	<i>Pre-operative ‘normal value’ (ml/kg)</i>
Byrick et al (1977) ⁴⁷²	17	TDD	Cardiac surgery	5.77 ± 0.24
Sivak et al (1982) ⁴⁷³	9	TDD	Cardiac surgery	5.47 ± 1.67
Honore et al (2001) ⁴⁷⁴	13	TDD	Cardiac surgery	6.9 ± 3.3
von Spiegel et al (2002) ⁴⁷⁵	8	TDD	Cardiac surgery	6.7 ± 1.7
	10			5.8 ± 1.0
Michelet et al (2006) ¹⁴⁹	10	STD	Oesophagectomy	5.4 ± 1.1
	52			~5.5
Licker et al (2008) ⁴⁷⁶	20	STD	Pneumonectomy	9.1 ± 4.4

STD, ‘single’ thermodilution; TDD, thermo-dye dilution

In summary it might pragmatically be concluded from the assembled expert opinion and clinical data and that ELWI > 10 ml/kg is likely to be pathological, ELWI < 7 ml/kg is likely to be normal, but that any absolute cut-off defining the upper limit of ‘normal’ EVLW is likely to lie in a grey area between 7-10 ml/kg.

5.4.7 Sackett’s test as applied to ‘single indicator’ trans-pulmonary thermodilution measurement of extravascular lung water

Has there been an independent, “blind” comparison with a “gold standard” of diagnosis?

Yes. EVLW_{STD} has been evaluated against gravimetric EVLW in animals and as far as possible in humans, and against EVLW_{TDD}. Sakka et al performed a classical derivation / replication study in 57 / 209 critically ill patients demonstrating the ITBV=1.25xGEDV was well maintained⁴⁰³.

Has the diagnosis test been evaluated in a patient sample that included an appropriate spectrum of mild and severe, treated and untreated, disease...?

Yes. Sakka et al’s validation study included critically ill patients with a wide variety of different pathologies⁴⁰³. Studies have however shown the criterion validity of EVLW_{STD} to be compromised in conditions of altered regional distribution of pulmonary blood flow and in some aetiologies of acute lung injury. No assessment has been made of whether such changes influence construct or predictive validity and so are of significance in clinical practice.

Was the setting for this evaluation, as well as the filter through which study patients passed, adequately described?

Yes. On the whole the majority of the available literature refers to well conducted scientific evaluations.

Have the reproducibility of the test result (precision) and its interpretation (observer variation) been determined?

Yes. Reproducibility values have been well established in several studies. In addition, Tagami et al have provided validation to the widespread practice of performing three duplicate thermodilution injections³⁷⁴. Clinicians and researchers must be aware of the limitations of the technique (and consequently observe the 'least significant change' values observed). Inter-observer variation has been neglected by the literature to date.

Has the term normal been defined sensibly as it applies to this test?

Whilst a definitive upper limit of the normal range has not been defined, normality has been pursued as far as is practicable. The concept of normal below 7ml/kg, definitely pathological above 10ml/kg, with an area of uncertainty in between, though unwritten appears well established.

If the test is advocated as part of a cluster or sequence of tests, has its individual contribution to the overall validity of the cluster or sequence been determined?

Not fully. Chew et al reported that ELWI measurement increased the post-test probability of ALI/ARDS, where pre-test probability was that of 'being in intensive care with shock'⁴²³. In general however authors have examined the role of ELWI as a diagnostic tool, early predictor of subsequent lung injury development, or predictor of mortality in isolation. Further studies are required examining the diagnostic or predictive information ELWI adds to clinical variables (such as oxygenation, lung injury score, lung injury prediction score and acute physiology and chronic health evaluation score).

Have the tactics for carrying out the test been described in sufficient detail to permit their exact replication?

Yes. The methodology of TPTD is well described, with little variation between studies.

Has the utility of the test been determined?

No. Whilst several authors have promoted the incorporation of ELWI into a novel definition of ALI/ARDS, this has not yet been established. Greater consensus regarding normal or cut off values representing ALI/ARDS would be needed first. The (Berlin) ‘ARDS Definition Task Force’ examined the possibility of incorporating ELWI into a novel definition of ALI/ARDS, but concluded *“at the present time, technology to measure EVLW is relatively costly, invasive, not widely available and has significant methodological limitations”*⁶⁶.

The effects of pathology (including lung injury resulting from a range of differing aetiologies), and other ‘methodical limitations’ are discussed in section 5.5.

5.5 Literature review: The influence of pulmonary ventilation-perfusion relationships on thermodilution EVLW measurement

In this section factors, both physiological and pathological, which can influence trans-pulmonary thermodilution measurement of extravascular lung water are discussed. Particular attention is paid to EVLW measurement in patients with ALI/ARDS, and other pathophysiological states which may be encountered in the early post-operative period following lung resection.

By the nature of the indicator dilution technique involved, TPTD methods for measuring EVLW (both STD and TDD) can only measure lung water in perfused areas of lung and so rely upon a homogeneous distribution of pulmonary perfusion in order to accurately determine EVLW; a large perfusion deficit will lead to underestimation of EVLW.

In many circumstances, this assumption of a homogeneous distribution of pulmonary perfusion may be unjustified. In the normal lung, pulmonary perfusion is physiologically heterogeneous with an almost linear decrease in blood flow from bottom to top of an upright lung²³. In the diseased lung, many authors have demonstrated further heterogeneity to the distribution of pulmonary perfusion⁴⁷⁷⁻⁴⁸⁰. Regional pulmonary perfusion is influenced by many factors pertinent to the lung resection population or critically ill population in which EVLW measurement might be desirable. The effects of hypoxic pulmonary vasoconstriction, acute lung injury, vascular obstruction and positive end-expiratory pressure will each be considered in turn.

5.5.1 Hypoxic pulmonary vasoconstriction

By a direct effect of low partial pressure of oxygen in alveolar gas on vascular smooth muscle, hypoxic pulmonary vasoconstriction (HPV) serves to direct blood away from hypoxic regions of the lung thus maintaining ventilation-perfusion relationships²³. Any factor influencing HPV is likely therefore to alter the distribution of pulmonary blood flow and so influence EVLW measurement.

The effect of such changes in pulmonary blood flow on $EVLW_{STD}$ measurement were elegantly demonstrated by Easley et al⁴⁷⁹. In a canine model of ALI Easley et al reported a linear relationship between $EVLW$ and a reference value ('CT water') obtained from computed tomography. This relationship was lost however following pharmacological manipulation of the distribution of pulmonary perfusion following the administration of lipopolysaccharide endotoxin (LPS), which according to the authors will disrupt hypoxic pulmonary vasoconstriction, but not acutely alter the distribution of lung water (Figure 5.9). This resulted in an abrupt increase in the measured $EVLW$ value due, the authors hypothesise, to the recruitment of "previously thermally silent" areas of lung.

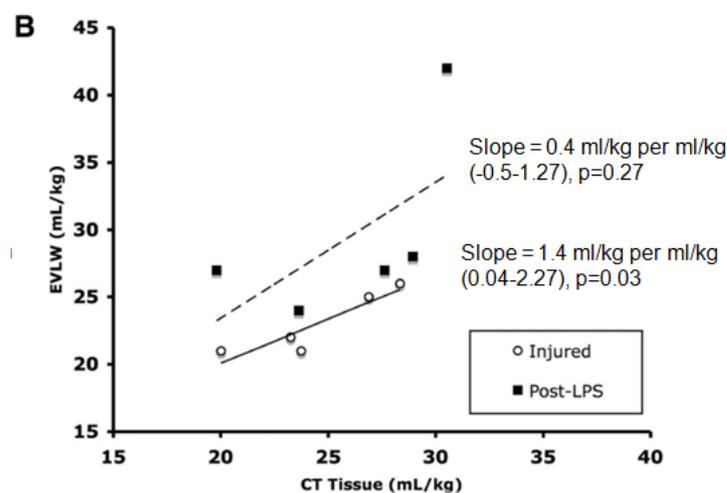


Figure 5.9. Relationship between single thermodilution derived $EVLW$ and computed tomography derived 'tissue water'.

Following disruption of hypoxic-pulmonary vasoconstriction by administration of lipopolysaccharide, the distribution of pulmonary perfusion is altered leading to loss of the linear relationship between $EVLW$ and CT 'tissue water'. From Easley et al⁴⁷⁹.

It should be appreciated that in addition to pathophysiological processes (such as in ALI as discussed below), the effects of HPV may also be altered by administration of anaesthetic drugs; volatile anaesthetic agents are described as inhibitors of HPV⁴⁸¹ whilst the intravenous anaesthetic agent propofol is described as augmenting HPV⁴⁸². The clinical significance of such findings are uncertain, though in patients undergoing thoracic surgery (the subject of this thesis), oxygenation during the period of one lung ventilation has been reported to be better maintained in patients with undergoing propofol anaesthesia compared to volatile anaesthesia; an effect attributed to maintenance of HPV⁴⁸³.

5.5.2 Acute lung injury

The reported effects of lung injury on pulmonary perfusion are inconsistent. Whilst it is generally accepted that hypoxic pulmonary vasoconstriction serves to homeostatically divert blood away from areas of injury and so maintain ventilation-perfusion relationships, demonstrating such an effect has been challenging. Work in animal models has demonstrated changes in pulmonary perfusion with the onset of lung injury^{484, 485} (interpreted to reflect HPV, direct vascular injury or mechanical compression of vasculature due to oedema). Schuster et al. were however unable to replicate such findings in humans with non-ALI or ALI pulmonary oedema, hypothesising that in humans (in this study at least), the effects of HPV are blunted in ALI⁴⁷⁸. Whilst this could reflect physiological differences between humans and animal models, it is also likely that pulmonary perfusion (and indeed the integrity of HPV mechanisms) will differ according to the severity and mechanism of lung injury.

5.5.2.1 Type of lung injury

The implications of differences in pulmonary blood flow occurring in ALI on EVLW measurement are illustrated in a study by Roch et al who compare $EVLW_{TDD}$ with 'gold-standard' gravimetric measures in two different porcine models of lung injury⁴⁸⁶. In one group ALI was induced by intra-tracheal hydrochloric acid, purportedly generating a heterogeneous lung injury; in the other group ALI was induced by intravenous oleic acid leading to a diffuse homogenous lung injury. Both models induced a significant lung injury as evidenced by the onset of hypoxaemia and a significant increase in gravimetric EVLW, however whilst in the (homogenous) IV oleic acid group good correlation was observed between $EVLW_{TDD}$ and $EVLW_G$ ($r=0.88$, $p<0.0001$), in the (heterogeneous) hydrochloric acid group no correlation between $EVLW_{TDD}$ and $EVLW_G$ could be observed. Carlile and Gray made a similar observation in canine models of focal (hydrochloric acid inhalation) and diffuse lung injury (alloxan or α -naphthylthiourea intra-pulmonary arterial)⁴⁸⁷. They demonstrated a marked reduction in the ratio of $EVLW_{TDD}$ to extravascular lung mass in animals with focal lung injury despite a similar severity of lung injury in both groups. Strikingly, $EVLW_{TDD}$ was reduced when compared to baseline in all 11 of the 15 animals exposed to focal (HCl acid induced) injury. The authors hypothesise that such a discrepancy reflects redistribution of pulmonary blood flow

away from injured areas in conditions of focal injury; a supposition which is supported by the observation of correlation between the EVLW / ELM and shunt ($r=0.70$)⁴⁸⁷.

Carlile et al offer an alternative to the 'thermally silent' lung tissue theory to explain the underestimation of EVLW in patients with acute lung injury⁴⁸⁵. They suggest that it is not an absence of perfusion to injured lung units that leads to inaccuracies but that indicator is detained in injured lung units. Carlile et al estimate the regional mean transit time (in injured lung units) to be in the region of 40 seconds. As such, this detained indicator will reach the sensor after the point at which the mono-exponential decay curve is truncated (to avoid recirculation artefact - Section 5.3.1.2), and so will be 'lost' to the sensor.

5.5.3 Vascular obstruction

'Thermally silent' lung tissue (i.e. lung tissue which is not perfused, as would occur in vascular obstruction) cannot be accessed by thermal indicator, and so makes no contribution to TPTD derived EVLW. By its very existence however, the lung tissue concerned would contribute to $EVLW_{GRAV}$; as such it would be expected that the presence of thermally silent lung tissue (due to vascular obstruction or any other mechanism) would lead to underestimation of EVLW. This is illustrated by the study of Schreiber et al, who measured $EVLW_{TDD}$ before, during and after branch pulmonary artery occlusion in pigs⁴⁶². They demonstrated a significant reduction in measured EVLW during occlusion followed by a return to baseline when occlusion was released. Whilst such overt arterial occlusion is unlikely to go unnoticed in the clinical environment, a similar reduction might be expected in more diffuse microvascular obstruction. Exploring the effects of diffuse, small vessel occlusion, Oppenheimer et al embolised glass beads 500 μ m in diameter into mongrel dogs⁴⁸⁸. They observed good agreement between gravimetric and TDD derived EVLW in normal animals, embolised animals without lung injury and animals with lung injury but not embolised, but significant underestimation in EVLW in embolised animals with lung injury⁴⁸⁸. Beckett et al conducted a similar study subjecting dogs to a 'low' or 'high' dose embolic shower⁴⁸⁹. In 'high' dose embolisation the authors demonstrated a reduction in $EVLW_{TDD}$ (as seen by Oppenheimer et al⁴⁸⁸) alongside a reduction in TDD measured pulmonary blood volume (PBV). In contrast, following

'low' dose embolisation they observed a paradoxical increase in $EVLW_{TDD}$ alongside a significant reduction in measured PBV. This they attribute to the TDD technique, suggesting that small areas of injured lung are accessed thermally by diffusion from adjacent lung units, but are 'silent' to dye perfusion; as a result PBV (measured from the dye-dilution curve) is underestimated and EVLW (measured from the thermo-dilution curve) is consequently assumed to compose a greater proportion of the ITTV (Equation 5.17 $EVLW_{TDD} = ITTV - ITBV$, where $ITBV = PBV + GEDV$). There are no studies of STD EVLW measurement in similar models of vascular obstruction to allow comparison with TDD techniques. One might hypothesise that the potential for spurious overestimation of EVLW as observed by Beckett et al⁴⁸⁹ in 'low' dose embolisation (in reality probably more of a theoretical entity than a clinical one) might not exist but that similar potential for underestimation of EVLW in cases of vascular obstruction still exists.

5.5.4 Positive End-Expiratory Pressure

The potential effects of positive end expiratory pressure (PEEP) on both the development and thermodilution measurement of EVLW are multiple and have been the subject of much debate with studies reporting increases, decreases and no effect of PEEP on EVLW. Whilst the discrepancies between the various reports may partly be accounted for by variations in the methodology of EVLW measurement, variations in the model of ALI studied and timing of PEEP application^{483, 490}, some common themes emerge. It is apparent that PEEP may affect the value of EVLW obtained following thermodilution measurement by either directly influencing the amount of EVLW present (a 'true' effect) or by artefactually influencing the measured value of ELVW due to alterations in pulmonary blood flow.

5.5.4.1 Direct effects

Positive end-expiratory pressure is widely used in critically ill patients with acute lung injury with the aim of maintaining functional lung volume and preventing damaging atelectotrauma^{90, 305}. It is intuitive therefore that if application of PEEP is able in some way to directly improve lung injury, or arrest the development of injury, then PEEP may lead to decreased EVLW. Such an effect has been demonstrated in animal models^{490, 491}. Colmenero-Ruiz et al⁴⁹⁰ studied the effects

of PEEP on the accumulation of $EVLW_{TDD}$ in a porcine (oleic acid) model of lung injury. PEEP was applied at the onset of lung injury. They observe that though the effects of PEEP on oxygenation were evident (compared to controls) early in the experimental protocol, any effect on EVLW took longer to occur with significant differences in EVLW between PEEP and zero-PEEP controls taking 180 minutes to emerge. The same group subsequently demonstrated (in the same porcine / oleic acid model of lung injury) that the protective effect of PEEP on EVLW accumulation is dependent on application of PEEP early in the disease process⁴⁹¹. This observation potentially explains why other authors have failed to observe a direct effect of PEEP on EVLW when PEEP has been applied late in the experimental protocol. In both of these studies, the reduced EVLW in the PEEP groups was confirmed gravimetrically, confirming the presence of a direct effect of PEEP on EVLW accumulation^{490, 491}.

5.5.4.2 Indirect effects

Application of PEEP however has the potential to indirectly increase or decrease the measured value of EVLW. Firstly it is possible that high levels of PEEP may directly compress pulmonary blood vessels leading to vascular obstruction (and an increase in the amount of thermally silent lung tissue - an increased West Zone 1), and consequently an artefactual reduction in measured EVLW. In a canine model, Hedenstierna et al⁴⁹² demonstrated marked reductions in pulmonary blood flow following the application of ~20cmH₂O PEEP. Blood flow was greatly reduced in the uppermost portions of the lung with Zone I conditions being demonstrated half to two thirds of the way down the upper and middle lobes. Interestingly such changes could be negated by maintaining cardiac output at the same level as before the onset of PEEP⁴⁹². Secondly, by recruitment of alveoli, and subsequent redistribution of pulmonary perfusion to recruited lung areas (decreasing the amount of thermally silent lung tissue), PEEP may increase measured EVLW. Such an effect was demonstrated by Carlile et al in a canine model of lung injury⁴⁸⁵. Administration of 15cmH₂O of PEEP led to an acute and reversible increase in perfusion of the injured lung area which was paralleled by an increase in measured EVLW⁴⁸⁵.

5.6 Literature review: Trans-pulmonary thermodilution estimates of pulmonary vascular permeability

Ratios of EVLW to TPTD derived blood volumes have been utilised in an attempt to provide an estimate of pulmonary vascular permeability. These ratios are intended to reflect EVLW in the context of, or indexed to preload, and were first described in 2001 by Honore et al⁴⁷⁴. The concept is intuitive; a high EVLW in a hypovolaemic patient (and therefore an elevated ratio) would suggest capillary permeability is the primary pathology whilst low EVLW in a patient with elevated preload (and therefore a low ratio) would suggest capillary permeability to be intact. Similarly the diagnosis of hydrostatic pulmonary oedema is suggested by high EVLW in a patient with high preload and therefore a normal ratio of EVLW to preload (Figure 5.10).

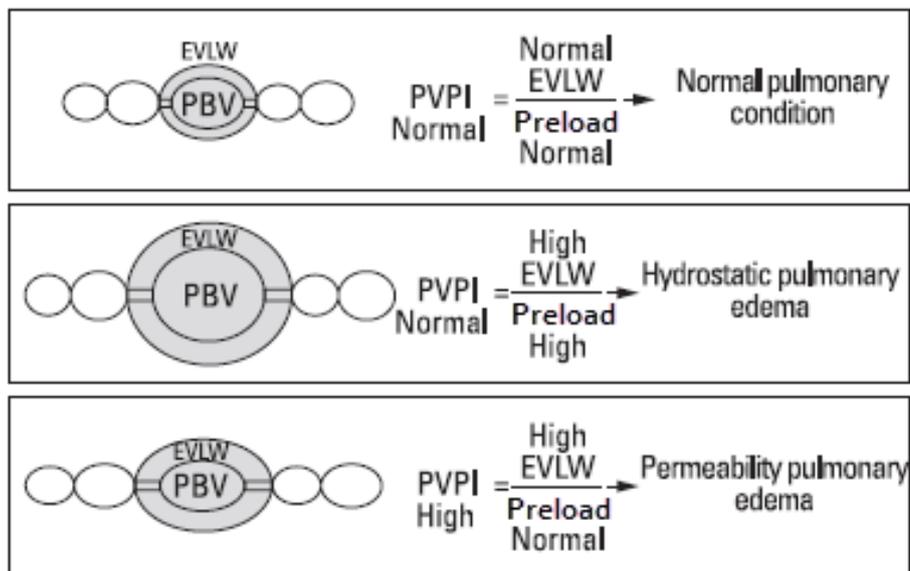


Figure 5.10. Schematic diagram explaining rationale behind measuring ratios of EVLW to preload indices as indicative of pulmonary permeability.

PVPI, pulmonary vascular permeability index. Modified from Sakamoto et al⁴⁹³.

Before considering the utility of these ratios however, I will first discuss the concept of TPTD derived measurement of preload.

5.6.1 Trans-pulmonary thermodilution derived indices of preload

In cardiovascular physiology, attempts to measure preload involve direct or indirect assessment of right or left ventricular end diastolic volume⁴⁹⁴. In general, rather

than as a measure of preload per se, the motivation for such measurements is an attempt to ascertain preload responsiveness. That is, an attempt to identify where a patient lies on the Frank-Starling curve and so identify the potential for a meaningful increase in stroke volume following fluid administration. Intrathoracic blood volume (ITBV)^{430, 467, 474, 495}, global end-diastolic (GEDV)^{79, 495} and pulmonary blood volume (PBV)^{79, 423, 424, 434, 443, 467, 495, 496} are indices of cardiac preload derived from TPTD to which EVLW has been indexed in the estimation of pulmonary vascular permeability. When considering these variables as measures of cardiac preload a number of important observations require to be made.

Firstly, it must be emphasised that none of these ‘volumes’ are measured during STD TPTD. The only volumes measured directly by STD are cardiac output (from the area under the thermodilution-time curve), intra-thoracic thermal volume (ITTV - as the volume of distribution of the thermal indicator) and pulmonary thermal volume (PTV - from the linear down-slope of the logarithmic thermodilution curve). Global end-diastolic volume is then derived by subtraction of PTV from ITTV (Equation 5.20, Page 319). ITBV and PBV are derived from these measurements based on the $ITBV = 1.25 \times GEDV$ relationship (Equation 5.20) described by Sakka et al³⁹⁶, where PBV is the difference between ITBV and GEDV:

$$PBV = ITBV - GEDV$$

Equation 5.23

Substituting Equation 5.20 into Equation 5.23 yields:

$$PBV = 0.25 \times GEDV$$

Equation 5.24

Secondly, it must be appreciated that the volumes derived have little if any anatomic equivalent, and as such can only be considered “virtual volumes”. The normal range of GEDV provided by the manufacturer of the most widely studied TPTD monitor in clinical use (PiCCO, Pulsion Medical Systems) is 680-800mls; a range several times larger than the combined end-diastolic volumes of the right and left heart in reality⁴⁹⁷.

There is nonetheless a strong and consistent evidence base suggesting that STD derived ITBV and GEDV are robust markers of cardiac preload. Whilst detailed examination of this data is beyond the scope of this thesis, a review article Della Rocca et al, summarises 18 articles in which GEDVI and ITBVI were compared to CI or SVI in a range of patient populations⁴⁹⁸. In all of these studies moderate to good association was observed between GEDVI and ITBVI and SVI or CI, or between changes in GEDVI and ITBVI and corresponding changes in SVI or CI. In many of these studies TPTD derived volumes consistently out-performed other 'static' estimates of preload such as central venous pressure and pulmonary artery occlusion pressure⁴⁹⁸. Hofer et al compared GEDVI derived from TPTD to left ventricular end-diastolic area index measured by transoesophageal echocardiography. Good agreement was observed between changes in GEDVI and corresponding change in LVEDAI ($r=0.81$; $p<0.001$) with a mean bias between percentage changes in the two parameters of just -3.2% ⁴⁹⁹. This suggests that whilst the absolute value of GEDV may be considered to be a 'virtual volume', its physiological behaviour closely tracks 'anatomic volumes'.

Pulmonary blood volume has not been subjected to the same degree of investigation as a marker of preload as ITBV and GEDV; indeed there is little rationale to use an estimate of pulmonary blood volume as a surrogate estimate of cardiac chamber volumes. There might however be an appropriate rationale for incorporating PBV into an index of pulmonary vascular permeability. Analogous to the common historical practice of using central venous *pressure* as a surrogate for right ventricular end-diastolic *volume*, EVLW is indexed to preload indices (*volumes*) as surrogates for pulmonary capillary hydrostatic *pressure* (P_c - Section 1.2.2). Intuitively, *pulmonary* blood volume may be a better surrogate for *pulmonary* capillary pressure, than *cardiac* volumes.

5.6.2 Reproducibility of pulmonary vascular permeability indices

The reproducibility of PVPI measurement has to an extent been covered above, alongside the reproducibility of EVLW measurement. Where the available literature examining reproducibility of EVLW was sparse, the available evidence base of studies directly examining the reproducibility of PVPI measurement is limited to the study of Tagami et al. Table 5.2 (which summarises the available literature

from studies examining the reproducibility of EVLW, GEDV, ITBV, PBV and PVPI measurement), clearly demonstrates that whilst reproducibility of directly measured variables (CO and GEDV), is good, reproducibility is poorer for derived variables. As such, measurements of PVPIs, which rely on precise measurement of CO and PTV directly from the STD curve and subsequent derivation of EVLW and ITBV or PBV are at risk of compounded errors. This is reflected by the increased 'least significant change' (LSC) values for these indices. As a consequence clinicians and researchers must be aware of the errors involved and the substantially larger 'least significant change values' that need to be demonstrated by the monitor before this can be interpreted as a clinical change.

5.6.3 Validity of pulmonary vascular permeability indices

Attempts to establish the validity of PVPIs are challenged by the technical complexities involved in determining a 'gold standard' measure of pulmonary vascular permeability. In addition, having been first described in 2004⁴¹⁵, the available evidence base for PVPIs is small when compared to EVLW (TDD measurement of EVLW began as early as 1966)⁵⁰⁰.

Criterion validity

In clinical practice the diagnosis of increased pulmonary vascular permeability pulmonary oedema is generally made on clinical grounds; the presence of cardiac failure is excluded and as such, in the face of pulmonary oedema, pulmonary vascular permeability is assumed to be increased. No gold standard measure of pulmonary vascular permeability exists⁵⁰¹. Historically, in animal models, increased protein flux in cannulated lymph vessels has been considered pathognomic of capillary leak³⁰, whilst in humans the presence of protein in pulmonary oedema fluid and increases in the oedema fluid protein to plasma protein ratio have been studied¹¹⁶. More recently, several studies have used radioisotopes to facilitate non-invasive, quantitative evaluation of permeability; the extent and rate of accumulation of radiolabelled protein in the lung reflecting permeability^{125, 467, 495}. Notably, Schuster's group have conducted numerous studies using Positron Emission Tomography (PET scanning) to evaluate pulmonary capillary permeability in patients with ARDS, but have not reported any comparison with TPTD derived indices⁵⁰².

Groeneveld et al performed two studies examining criterion validity of TPTD derived pulmonary vascular permeability indices using pulmonary leak index (PLI, as determined by assessing the rate of transport of ^{67}Ga labelled transferrin) as a criterion. These studies demonstrated modest associations between ^{67}Ga -transferrin determined PLI and EVLW/ITBV, EVLW/PBV and EVLW/GEDV both in septic⁴⁶⁷ and non septic patients⁴⁹⁵ ($r=0.43-0.50$ for all; $p\leq 0.05$). The association appeared particularly strong when PLI was low (as occurred in extra-pulmonary sepsis - PLI vs EVLW/PBV, $r=0.71$, $p=0.02$)⁴⁶⁷, but was lost in patients with pneumonia and high PLI (r -values not provided). The authors conclude that TPTD derived permeability indices are “*imperfect measures of increased protein permeability*”⁴⁶⁷. Unfortunately these two studies were not performed in parallel so no simultaneous estimate of the indices ability to distinguish between the different aetiologies of increased EVLW could be made.

Concurrent validity

Much of the clinical potential of PVPIs measurement concerns the possibility of their use in aiding clinicians in distinguishing between patients with pulmonary oedema (and raised EVLW) of hydrostatic or increased permeability aetiology. It is on this question that most investigators studying PVPIs have concentrated. As with EVLW, some proponents have gone as far as to suggest that PVPIs could be incorporated into a novel definition for ALI/ARDS^{73, 74, 79}.

Animal studies

In the first study to examine the utility of TPTD derived PVPIs, Katzenelson et al studied the EVLW/ITBV in three groups of dogs; control animals, animals subjected to ALI induced by intravenous injection of oleic acid and dogs who developed hydrostatic pulmonary oedema following the inflation of a left atrial balloon and administration of excess intravenous fluids ($n=5$ per group)⁴¹⁵. EVLW/ITBV was markedly increased in animals with ALI and only modestly increased compared to controls in those with hydrostatic pulmonary oedema (Figure 5.11).

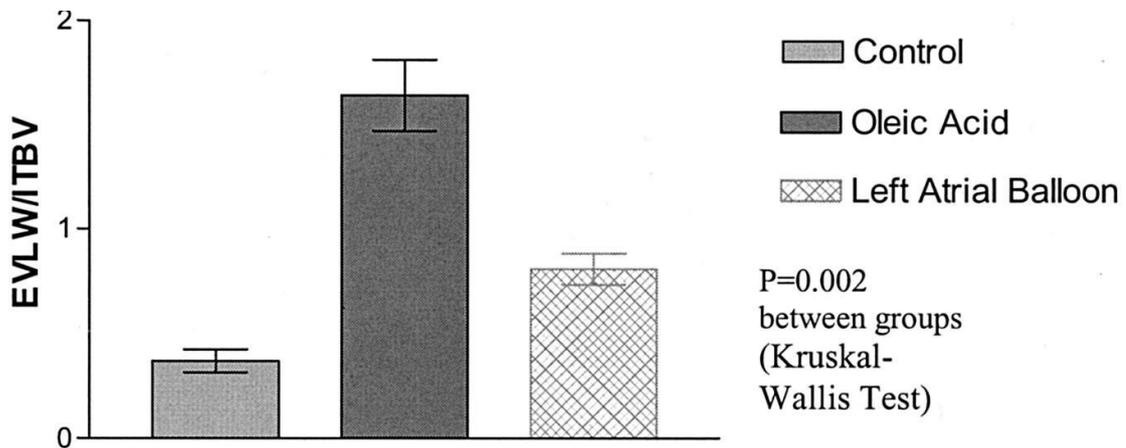


Figure 5.11. Trans-pulmonary thermodilution derived pulmonary vascular permeability in an animal model of pulmonary oedema.

From Katzenelson et al (2004)⁴¹⁵.

Though animals in the hydrostatic oedema group exhibited markedly elevated levels of EVLW, these animals had an elevated preload, so maintaining a relatively low level of EVLW/ITBV.

Human studies

Three human studies have examined the role PVPs might play in the diagnosis of ALI/ARDS. In a study analogous to the animal study performed by Katzenelson et al (discussed immediately above⁴¹⁵), Monnet et al retrospectively identified two cohorts of medical intensive care patients; one with hydrostatic pulmonary oedema and one with ALI/ARDS⁷⁹. Studying both the ratio of EVLW/PBV and EVLW/GEDV, Monnet et al demonstrated significantly higher values of both ratios in patients with ALI/ARDS. Perhaps of greater significance however are the observations that $EVLW/PBV \geq 3$ and $EVLW/GEDV > 1.8 \times 10^{-2}$ both had 85% sensitivity and 100% specificity for diagnosis of ALI/ARDS (AUROCC = 0.92 ± 0.04 for both), strongly suggesting a potential diagnostic role for PVPs. An accompanying editorial urges caution however; firstly observing that Monnet et al restricted their study to a population with relatively high EVLW values ($ELWI \geq 12$) and secondly highlighting the existence of population of 5 patients (~15% of the study's sample) who had $ELWI \geq 12$ ml/kg, $EVLW/PBV < 3$ yet were clinically classified as having ALI/ARDS⁷³.

In a mixed ITU population, Chew et al examined the utility of EVLW measurement as a diagnostic tool in the diagnosis of ALI/ARDS⁴²³. EVLW/PBV was higher in patients with ALI and ARDS compared to those without ALI/ARDS (2.1 vs 1.6; $p=0.02$ and 2.3 vs 1.7; $p<0.05$ for ALI/ARDS respectively). Though not specifying the cut off value of EVLW/PBV used, the authors demonstrated that EVLW/PBV added

diagnostic value; EVLW/PBV increased the post-test odds ratio for the diagnosis of ALI or ARDS by 1.8 and 1.6 times respectively. A 'negative' EVLW/PBV reduced the post-test probability markedly. The sensitivity and of EVLW/PBV for the diagnosis of both ALI and ARDS was 87% and 89% respectively, whilst specificity was 47% and 52% respectively.

In the largest study to report measurement of PVPIs to date, Kushimoto et al performed a prospective, multi-centre observational study seeking to establish "quantitative differential criteria of ALI/ARDS on the basis of PVPI" (PVPI defined as EVLW/PBV)⁴³⁴. Two hundred and sixty-six adult intensive care patients requiring mechanical ventilation with PaO₂/FiO₂ <300mmHg and bilateral infiltrations on chest radiography were enrolled. An expert panel (blinded to the PVPI values) retrospectively classified patients into categories of ALI/ARDS (n=207), cardiogenic oedema (n=26) and pleural effusion without atelectasis (n=33). The authors demonstrated that PVPI was higher in ALI/ARDS patients than in patients with cardiogenic oedema or pleural effusion with atelectasis (3.2 vs 2.0 vs 1.6 respectively). In the group of ALI/ARDS patients, ELWI increased with PVPI (r=0.73; p<0.01) and admission PVPI had an area under the receiver operating characteristic curve of 0.89 (CI 0.84-0.94) for prediction of ALI/ARDS. The authors, arguing that TPTD is a relatively invasive measurement, chose to select a cut off value in favour of maximising specificity and report that PVPI values of 2.6 to 2.85 provided a 'definitive diagnosis' of ALI/ARDS with a specificity 0.90 to 0.95 respectively and that a PVPI value < 1.7 to 2.0 ruled out an ALI/ARDS with a specificity of 0.95 and 0.90; sensitivity of the proposed cut offs was 0.54, 0.64 and 0.50, 0.70 respectively. Again this study is not without its limitations as an accompany editorial points out; exclusion of several patients was questionable, blinding was poor and the size of the non ALI/ARDS group was limited⁵⁰³.

A further limitation of several of the studies examining the diagnostic utility of PVPIs is that the authors chose to exclude patients with EVLW in the borderline area of 10-12ml/Kg^{79, 434}. Arguably it is in the patients with less overt pulmonary oedema that such a diagnostic tool may be of greatest value to the clinician.

Predictive validity

Le Tourneau et al measured EVLW on admission to intensive care in 29 patients⁴²⁴. They demonstrated that PVPI (defined as EVLW/PBV) on the day of admission was

higher in patients who went on to develop acute lung injury, compared to patients who did not (3.32 vs 1.58; $p=0.03$). Unfortunately the authors did not present any assessment of the sensitivity or specificity of a defined level of PVPI in the diagnosis of ALI/ARDS.

Construct validity

As with EVLW (Table 5.5), construct validity of PVPIs is suggested by observed association with pulmonary compliance⁴³⁰, PaO₂/FiO₂ ratio^{79, 423, 430}, chest X-ray score⁴²³ and Lung Injury Score^{423, 430, 441} (Table 5.11).

The study of Tagami et al which demonstrates strong correlation between PVPI (defined as EVLW/PBV) and plasma neutrophil elastase is particularly worthy of comment (Table 5.8)⁴⁴³. By demonstrating such strong association between thermodilution measurements and an objective assessment of the severity of the disease process, these authors offer further suggestion (so strengthening 'construct validity') that permeability indices reflect the pathophysiological process taking place at an alveolar level.

Table 5.8. Studies examining the construct validity of trans-pulmonary thermodilution derived indices of pulmonary permeability in humans.

Author (year)	Population	Method	N	Index	Comparator	Association / measure of validity
Groeneveld et al. (2006) ⁴⁴¹	Post-operative - major vascular surgery	TDD	16	EVLW/ITBV EVLW/PBV	LIS	EVLW/ITBV and EVLW/PBV higher in patients with LIS >1 (p<0.05 and not significant respectively).
Kuzkov et al. (2006) ⁴³⁰	Intensive care – septic shock and ALI	STD	38	EVLW/PBV EVLW/ITBV	Compliance PaO ₂ /FiO ₂ LIS	On day 1 of ITU stay: r=-0.43, r= -0.47 respectively; p<0.01 r=-0.58, r=-0.58 respectively; p<0.01 r=0.52, r=0.53 respectively; p<0.01 (Variables reported also to correlate on day 3, but data not provided).
Monnet et al. (2007) ⁷⁹	Intensive care	STD	48 (36 with ALI/ARDS)	EVLW/PBV EVLW/GEDV	PaO ₂ /FiO ₂	In patients with ALI/ARDS, EVLW/PBV associated with (r=0.42; p not provided). No comparison made for EVLW/GEDV.
Tagami et al. (2011) ⁴⁴³	HDU/ITU – community acquired pneumonia	STD	14 (6 in ITU)	EVLW/PBV	Plasma neutrophil elastase	Correlation observed between PVPI and plasma neutrophil elastase on day 1 (r=1.0, p<0.001) and day 2 (r=0.98, p<0.001) but not day 4 (r=0.17, p=0.74).
Chew et al. (2012) ⁴²³	Intensive care – SIRS and ‘circulatory failure’	STD	51	EVLW/PBV	PaO ₂ /FiO ₂ CXR score PEEP LIS	r=-0.37 to -0.49; p=0.001 r=0.26 to 0.46; p=0.002 No association Increasing ELWI/PBV with increasing strata of LIS (p<0.01)

N, number of patients. STD, single thermodilution; TDD, thermo-dye dilution; LIS, lung injury score.

5.6.4 Factors influencing trans-pulmonary thermodilution derived indices of pulmonary vascular permeability

Factors influencing the measurement of EVLW by STD have been described in detail in Section 5.5. Whilst all of these factors will be applicable to the measurement of PVPs given that these indices are ratios of EVLW to other volumes, many of the same limitations will also apply to the measurement of PBV, ITBV and GEDV. Perhaps the greatest limitation of $EVLW_{STD}$ is its dependence on a uniform distribution of pulmonary perfusion such that if regional perfusion varies, measured EVLW can be artefactually reduced as the thermal indicator is unable to gain access to hypo-perfused areas of the lung ('thermally silent' lung tissue).

Schreiber et al determined ITBV and GEDV using a thermo-dye dilution technique in a porcine model where pulmonary perfusion was varied by clamping of the right lower and middle lobe branch of the pulmonary artery⁴⁶². They observed that that pulmonary artery clamping led to an approximately 10% reduction in measured ITBV and GEDV, which was reversible on removing the clamp. Whilst the authors offer a number of hypotheses as to why measured ITBV and GEDV might decrease, it would seem that these preload indices exhibit perfusion dependence in a similar way to EVLW. Whilst such an observation may be disadvantageous to those seeking to use these measures as markers of preload in order to guide volume therapy, it may be an asset when combining them with EVLW to estimate pulmonary vascular permeability. If EVLW and ITBV / GEDV are similarly affected by pulmonary perfusion defects, then whilst the absolute values measured may be artefactually reduced their ratio may remain a true reflection. Unfortunately, the ratio determined will reflect the ratio of EVLW to ITBV / GEDV in the perfused lung and not in the hypo-perfused and likely pathological areas. There are no reports on the effect of pulmonary perfusion distribution on TPTD measured PBV, however as PBV_{STD} is obtained mathematically as one fifth of GEDV it is inherent that its measurement will also be perfusion dependent.

5.6.5 Comparisons between described pulmonary vascular permeability indices

As discussed above, three different ratios of EVLW to preload have been described as PVPIs. In what is such a limited evidence base there is little guidance on which may be the more robust marker. It is evident from Table 5.8, that the ratio of EVLW to PBV has been most extensively studied; this is unsurprising considering both of the STD TPTD monitors currently commercially available provide this index as standard^{408, 504}, describing it simply as “pulmonary vascular permeability index” (PVPI).

Groeneveld’s group studied the ratio of EVLW to PBV and ITBV in septic patients⁴⁴¹ and EVLW to PBV, ITBV, and GEDV in non septic patients⁴⁹⁵. In their comparisons between these indices and ⁶⁷Gallium labelled transferrin derived pulmonary leak index (PLI - the closest to a gold standard criterion reported in the PVPIs literature), there is little difference between the performance of the indices ($r=0.43-0.50$ for all; $p\leq 0.05$), though the authors report in their study of septic patients that they “*observed a tendency to a closer relationship of PLI to EVLW/PBV than to EVLW/ITBV*”, though they provide no statistics to support their conclusion. Kuzkov et al examined the ratio of EVLW to PBV and ITBV and observed little if any difference in performance between them⁴⁹⁶ (Table 5.8). Given that ITBV and PBV are each determined by mathematical manipulation of GEDV it is perhaps unsurprising that the performance of the three indices is similar.

5.6.6 What is normal range of pulmonary vascular permeability indices?

Determining a ‘normal’ range for TPTD derived PVPIs faces the same problems as determining a normal range for EVLW; PVPIs have not been (and are unlikely to be) measured in large cohorts of healthy patients from which a normal range can be derived. In the case of PVPIs this is further complicated by the comparatively smaller evidence base spread across the three different indices. As with EVLW, rather than a normal range per se, it is the threshold at the upper limit of the normal range at which normal can be said to become pathological that is of primary interest to clinicians.

5.6.6.1 Quoted normal values

Quoted normal values for the three PVPIs are provided in Table 5.9. The majority of authors provide no evidence to suggest from where the normal values have been derived.

As PVPI is an index which is derived as the ratio of two values, it would seem appropriate to determine the normal range by examination of the normal ranges of the two components. Whilst the upper limit of 'normal' ELWI is poorly defined, the normal ranges of PBV, ITBV and GEDV are less well described. This is further compounded by varied descriptions of the indices; whilst most authors describe the indices as a ratio of volumes (in ml), some authors study a ratio of indexed volumes (where EVLW is indexed to weight and ITBV and GEDV indexed to body surface area)⁷⁹.

For purposes of illustration, 'normal' values of PBV, ITBV and GEDV (quoted by the manufacturers of the two clinically available monitors^{505, 506}) are also provided in Table 5.12. From these values, based on the dimensions of the average UK man (of height 175.3cm, weight 83.6kg and body surface area 1.995m²⁵⁰⁷) estimations of 'normal' values of the respective indices have been derived; 'stringently' as lowest quoted 'normal' EVLW against highest 'normal' denominator, and 'permissively' as highest EVLW over lowest denominator. This exercise serves to highlight the wide range of potentially 'normal' values of PVPIs.

Table 5.9. Quoted normal ranges of pulmonary vascular permeability indices, preload indices and derived values.

Description	Value
EVLW/PBV	'about 1' ⁴⁴¹ , <1.5 ⁴²³ , 3.0 ⁵⁰⁵ , 3.0 ⁵⁰⁶
EVLW/ITBV	0.2-0.32 ⁴⁷⁴ , 0.2-0.3 ⁴⁴¹ , 0.2-0.3 ⁵⁰⁸
EVLW/GEDV	No normal values quoted
ELWI (ml/kg)	Conservative upper limit 7.0 ^A Permissive upper limit 10.0 ^A
PBV (ml)	No normal values quoted
ITBVI (ml/m ²)	850-1000 ⁵⁰⁵ , 850-1000 ⁵⁰⁶
GEDI (ml/m ²)	680-800 ⁵⁰⁵ , 650-800 ⁵⁰⁶
Derived 'Stringent' upper limit of normal ^B	EVLW/ITBV: 0.29 EVLW/GEDV: 0.36
Derived 'Permissive' upper limit of normal ^B	EVLW/ITBV: 0.49 EVLW/GEDV: 0.64

^A As described in the text – 'What is the normal value of EVLW?', Section 5.4.2.6; ^B Derivation as described above, 'stringent' – lowest 'normal' EVLW to highest 'normal' preload index, 'permissive' – highest 'normal' EVLW to lowest 'normal' preload index.

5.6.6.2 Studies measuring PVPIs in peri-operative patients

Honore et al reported a normal range for EVLW/ITBV of 0.2-0.32 in elective cardiac surgical patients⁴⁷⁴. The authors described the patient cohort as having "ELWI values within the normal range (<7ml/kg) and ITBVI values at the top of the normal range", concluding (but with little evidence provided to support their conclusion) that "in our study these values were compatible with an intact pulmonary permeability"⁴⁷⁴. Groeneveld et al measured PVPIs in patients undergoing major vascular surgery⁴⁴¹. Though no pre-operative values are provided, observed median values of EVLW/ITBV and EVLW/PBV in patients with ELWI≤7 and Lung Injury Score ≤ 1 (suggesting no clinical evidence of increased permeability) were 0.21-0.22 and 1.1 respectively. Whilst sub-clinical levels of capillary permeability cannot be excluded these values are in keeping with quoted 'normal' values (Table 5.9).

In summary, it appears 'normal' values of PVPIs are poorly defined. Furthermore whilst there is no consensus regarding which index to report, nor whether to perform a ratio of volumes, or indexed volumes, it seems unlikely that established normal values will be defined.

5.6.7 Sackett's test as applied to trans-pulmonary thermodilution measurement of pulmonary vascular permeability indices

1. *Has there been an independent, "blind" comparison with a "gold standard" of diagnosis?*

Only partially. Some of the problem lies with lack of 'gold standard' comparators. Groeneveld et al's two studies comparing PVPIs to ⁶⁷Gallium labelled transferrin derived pulmonary leak index are the closest to 'gold standard' comparisons available^{467, 495}; in these studies PVPIs performed as "imperfect measures of increased protein permeability"⁴⁶⁷.

2. *Has the diagnostic test been evaluated in a patient sample that included an appropriate spectrum of mild and severe, treated and untreated, disease...?*

Yes. PVPIs have been evaluated in a wide range of lung injury aetiologies, though unfortunately, for clarity, many studies have avoided the 'grey area' of mildly elevated EVLW, the setting in which TPTD measurement might arguably be of greatest value.

3. *Was the setting for this evaluation, as well as the filter through which study patients passed, adequately described?*

Yes. On the whole the majority of the available literature refers to well conducted scientific evaluations.

4. *Have the reproducibility of the test result (precision) and its interpretation (observer variation) been determined?*

Reproducibility has been evaluated in several studies. Though reproducibility is at the edge of what can be considered clinically acceptable, provided clinicians and researchers are aware of the limitations of the technique (and consequently observe the 'least significant change' values reported), then reproducibility should be no impediment to future adoption of the technique. As with EVLW, inter-observer variation has been relatively neglected by the literature to date.

5. *Has the term normal been defined sensibly as it applies to this test?*

No. Quoted and theoretical normal values differ over a wide range. Similarly there is no consensus regarding which index to report, nor whether to perform a ratio of volumes, or indexed volumes.

6. *If the test is advocated as part of a cluster or sequence of tests, has its individual contribution to the overall validity of the cluster or sequence been determined?*

No. The studies of Monnet et al⁷⁹, Chew et al⁴²³ and Kushimoto et al⁴³⁴, all reported that PVPs may be utilised to make the diagnosis of ALI/ARDS with acceptable sensitivity and specificity (depending on the cut-off chosen and the desire to pursue sensitivity over specificity or vice versa). All three studies however sought to examine the utility of PVPs alone rather than in combination with clinical findings; in reality, PVPs values will be interpreted in the context of clinical findings. A study of the contribution of PVPs to aid the clinical diagnosis of ALI/ARDS would be a valuable addition.

7. *Have the tactics for carrying out the test been described in sufficient detail to permit their exact replication?*

Yes. The methodology of TPTD is well described, with little variation between studies.

8. *Has the utility of the test been determined?*

No. Whilst several authors have promoted the incorporation of PVPs into a novel definition of ALI/ARDS, this has not yet been established. Greater consensus regarding normal values and which of the three potential PVPs is most valid is likely to be required first. Whilst the (Berlin) 'ARDS Definition Task Force' advocate the development of "*reproducible and valid methods for the direct measurement of pulmonary vascular permeability*" (which would be) "*important advances over current methods of assessing the presence and origin of lung oedema, and could be incorporated into the future definition of ARDS⁶⁶*", the same "*significant methodological limitations⁶⁶*" as applied to EVLW apply to PVPs.

5.7 Literature review: Trans-pulmonary thermodilution measurement of extravascular lung water and pulmonary vascular permeability indices in patients undergoing lung resection

This investigation concerns the application of trans-pulmonary thermodilution (TPTD) measurement of extravascular lung water (EVLW) and pulmonary vascular permeability indices (PVPIs) in patients undergoing lung resection. A number of authors have suggested that quantification of EVLW may be of value in this population^{48, 115, 363, 509-511}, and there have been several clinical studies, conducted in the lung resection population where TPTD derived EVLW has been a study endpoint^{235, 245, 476}.

There are however a number of theoretical considerations, concerning the methodology of single thermodilution (STD) TPTD that at very least require consideration, and at worst may compromise its validity in the lung resection population. So much so in fact that several experts recommend against TPTD measurements in patients undergoing lung resection. In their original validation of the STD technique, Sakka et al, when discussing patients undergoing pneumonectomy concluded that *“we therefore advise against using the single thermodilution technique under these circumstances”*³⁹⁶. Schreiber et al have also counselled that *“therapeutic consequence based on transpulmonary double indicator measurement in these [lung resection] patients... may be misleading”*⁴⁶².

As will be discussed, the validity of TPTD monitoring following lung resection has seen little study. It is of concern therefore that $EVLW_{STD}$ is being employed as an endpoint to clinical studies; in some circumstances to confirm the safety of clinical practices. Haas et al, and Assaad et al, for example use the absence of a post-operative rise in EVLW as confirmation of the safety of liberalized fluid administration protocols^{235, 245}.

5.7.1 Theoretical considerations in application of TPTD techniques following lung resection

Theoretical considerations potentially compromising the validity of TPTD measurements in patients undergoing lung resection include:

- Changes in the ITBV:GEDV ratio
- Shortened pulmonary transit time
- Physiological changes occurring in spontaneously rather than mechanically ventilated patients
- Post-operative hyperinflation

Each will be explored in turn.

5.7.1.1 Changes in the ITBV:GEDV ratio

The fundamental assumption intrinsic to STD techniques is that of there being a linear relationship between intrathoracic blood volume (ITBV) and global end-diastolic volume (GEDV) (such that the ratio of ITBV:GEDV remains constant at 1.25) as reported by Sakka et al^{392, 403}. Such an assumption does not allow for the possibility of independent changes in pulmonary blood volume PBV and/or global end-diastolic volume, yet both ITBV and GEDV have the potential to change independently following lung resection.

Changes in intrathoracic blood volume following lung resection

Resection of lung tissue is likely to result in reduced pulmonary blood volume and so lead to a reduction in ITBV independent of GEDV⁵¹². Findings from studies exploring the changes in the relationship of ITBV:GEDV following lung resection are summarised in Table 5.10. In the three animal models, there is a relatively consistent fall in ITBV (13-21%) and PBV (22-30%) following pneumonectomy which is reflected in a significant reduction in the ITBV:GEDV ratio. Failure to account for a theoretical reduction in PBV following lung resection would lead to an overestimation of ITBV and a consequent underestimation of EVLW. It should also be appreciated that in the two studies examining the regression equation for the relationship between ITBV and GEDV, both demonstrated increases in the intercept value. As this value is conventionally ignored in the

Table 5.10. Studies investigating changes in blood volumes and ITBV:GEDV following lung resection.

Author (year)	Species	Methodology	Sample size, study protocol	Observed changes in blood volumes following lung resection
Kirov et al ⁴¹¹ . (2006)	Sheep	TDD	N=93 Mechanical ventilation (n=51) Pneumonectomy (n=42)	ITBV _{TDD} - ↓21% following pneumonectomy (p<0.05) PBV _{TDD} - ↓30% following pneumonectomy (p<0.05) GEDV _{TDD} - ↓16% following pneumonectomy (p<0.05) ITBV:GEDV – ↓ from 1.46 to 1.39 following pneumonectomy (p<0.05) Regression equation – Pre pneumonectomy ITBVI=1.43(GEDVI)+13.48, post pneumonectomy ITBV=1.21(GEDVI)+73.72.
Kuzkov et al ^{A 512} . (2007)	Sheep	STD TDD	N=18 Sham operation n=4 Left pneumonectomy, n=7 Right pneumonectomy, n=7	ITBV _{TDD} - ↓18% following left pneumonectomy, 19% fall following right (both p<0.05) PBV _{TDD} - ↓22% following left pneumonectomy (p<0.05), 23% fall following right (NS) ITBV:GEDV - ↓ from 1.47 to 1.43 following left pneumonectomy, from 1.48 to 1.45 following right (both not significant).
Kuzkov et al ^{A 512} . (2007)	Sheep	STD TDD	N=12 Pneumonectomy followed by 4 hrs: Injurious vent n=6 (12ml/kg, ZEEP) Protective vent n=6 (6ml/kg, 2cmH ₂ O PEEP)	Injurious ventilation resulted in a significant ↓ in ITBV:GEDV 1.46 to 1.30 (p<0.05) Moderate correlation in EVLW _{STD} bias and ITBVI _{TDD} /GEDVI _{TDD} . (r=0.49, P<0.01)
Roch et al ⁵⁰⁹ . (2005)	Pigs	STD TDD	Pneumonectomy N=27	ITBVI _{TDD} – ↓13% post pneumonectomy (p<0.01) ITBV:GEDV - Change from 1.42 to 1.29 following pneumonectomy (no p-value provided) Regression equation – Pre pneumonectomy ITBVI=1.29(GEDVI)+ 49ml, post-pneumonectomy ITBVI=1.42(GEDVI)+53ml.
Naidu et al ⁵¹³ . (2009)	Humans	STD TDD	N=3 Pneumonectomy, n=1 Lobectomy, n=2	Marked variability observed in ITBV:GEDV for approximately 2 hours post-operatively before remaining relatively stable for the remaining 12 hours. Post resection ITBV:GEDV – LUL = 1.36, LLL = 1.23, LPn = 1.15.

^AKuzkov et al. Parallel investigations reported in a single paper. STD – ‘single’ thermodilution. TDD – thermo-dye dilution. ITBV(I) – Intrathoracic blood volume (index), GEDV(I) – Global end-diastolic volume (index), PBV – Pulmonary blood volume. LLL – left lower Lobectomy, LPn – left pneumonectomy, LUL – left upper Lobectomy.

two commercially available thermodilution monitors, an increase in the intercept could further compromise the ability of the monitor to make an accurate estimate of EVLW following lung resection; ignorance of an increased intercept value would lead to underestimation of ITBV and consequent overestimation of EVLW.

Interestingly, the study of Kuzkov et al⁵¹² demonstrated a more pronounced change in ITBV:GEDV in sheep exposed to an injurious ventilation protocol. The injurious ventilation protocol led to the development of lung injury as evidenced by a significant fall in PaO₂:FiO₂ ratio and a significant increase in gravimetrically determined EVLW. There have been no studies specifically examining changes in the ITBV:GEDV in patients with lung injury (and two lungs), though the potential for lung injury and consequent redistribution of pulmonary blood flow to compromise the validity of STD is well recognised and has been discussed in detail in Section 5.5. The changes observed by Kuzkov et al represent a relative decrease in pulmonary blood volume, or increase in GEDV. It is plausible that both could occur in the context of lung resection and lung injury. Vascular constriction (in response to hypoxic pulmonary vasoconstriction or extrinsic compression by oedematous lung tissue) might lead to a reduction in the volume of the already compromised pulmonary vascular bed. The potential for GEDV to increase in the face of right ventricular dysfunction is discussed below. Pneumonectomy in combination with lung injury could lead to markedly increased right ventricular afterload, leading to RV dilatation and consequently increased GEDV. In support of this hypothesis, both pulmonary artery pressure and pulmonary vascular resistance index increased significantly in animals subjected to pneumonectomy and an injurious ventilation protocol.

The single human study by Naidu et al⁵¹³ demonstrates that whilst ITBV:GEDV is relatively uniform pre-operatively, there are large and inconsistent changes in ITBV:GEDV following lung resection (Figure 5.12). This study is limited by its small sample size; though published under the title of 'work in progress report', this study has been abandoned due to technical difficulties with the indocyanine green reader (personal communication B. Naidu, April 2012).

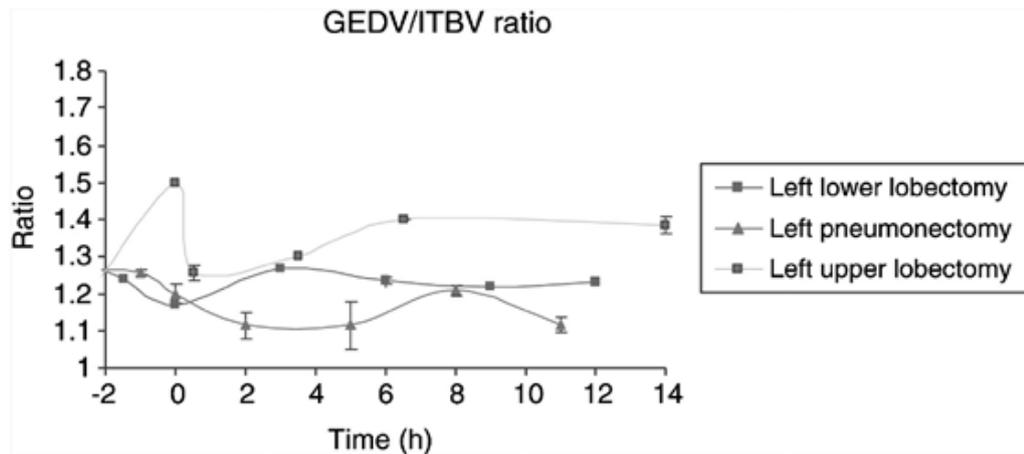


Figure 5.12. Changes in GEDV/ITBV ratio in three patients undergoing lung resection.
From Naidu et al (2009)⁵¹³.

Changes in global end-diastolic volume following lung resection

Several studies have described a reduced right ventricular (RV) function following both lobectomy and pneumonectomy⁵¹⁴⁻⁵¹⁸. RVEF appears to be at its lowest on post-operative day two with incomplete recovery evident by three weeks^{514, 515}. Such dysfunction is associated with increased RV end-diastolic (EDV) and end-systolic volumes (increases in RVEDV in excess of 40% have been reported⁵¹⁷). Such increases in RVEDV will lead to increased GEDV, potentially altering the ratio of ITBV:GEDV. In theory, increases in GEDV independent of PBV would lead to overestimation of ITBV and therefore underestimation of EVLW.

5.7.1.2 Shortened pulmonary transit time

Kuzkov et al and Schreiber et al have hypothesised that lung resection will lead to a shortening of the time that the thermal indicator takes to transit the pulmonary vascular bed^{147, 462}; according to the Venturi effect⁵¹⁹, if an unchanged cardiac output is forced to pass through a restricted pulmonary circulation post-operatively, velocity and therefore transit time of indicator would be expected to decrease. Reduced mean transit time of indicator would result in an under-estimation of intrathoracic thermal volume (ITTV is derived from the product of cardiac output and mean transit time - Section 5.3.2.1, Equation 5.9) and a consequent under-estimation of EVLW. It must be emphasised however that these concerns are theoretical; there have been no

^s The potential and importance of RV dysfunction in the post-operative period is discussed more extensively in Chapter 7, 'future work'.

studies examining the effect of lung resection on pulmonary transit time or the velocity of pulmonary blood flow. It is possible that following lung resection, recruitment of previously hypoperfused pulmonary vasculature might mitigate any restriction. Against this hypothesis are the consistent observations of increased pulmonary vascular resistance following lung resection suggesting there is some 'restrictive' effect within the pulmonary vascular bed^{125, 174}.

5.7.1.3 Spontaneous vs. mechanical ventilation

The constant relationship between ITBV and GEDV observed and subsequently validated by Sakka et al^{392, 396} was made in critically ill patients undergoing positive pressure ventilation. Similarly, subsequent validation studies of TPTD measurement of EVLW were made in ventilated cohorts. If TPTD were to be a useful monitoring methodology in the post-operative period following lung resection however, validity would be required in spontaneously breathing patients.

During mechanical ventilation, increases in intra-thoracic pressure result in reduced inferior vena caval blood flow and a reduction in pre-load to the right ventricle⁵²⁰. Reduced preload (and consequently reduced GEDV) in the context of an unchanged pulmonary blood volume would result in an increase in the ITBV:GEDV ratio. Kirov et al⁴¹¹ determined ITBV/GEDV ratio by TDD and demonstrated an increased ITBV:GEDV (1.46 compared with 1.31 ($p < 0.05$)) in mechanically ventilated sheep when compared to spontaneously breathing. ITBV was unchanged, whilst GEDVI was 16% lower in mechanically ventilated animals ($p < 0.05$).

In many studies of EVLW measurement in humans following lung resection, post-operative estimates made whilst spontaneous breathing are compared with a mechanically ventilated pre-operative estimates (Table 5.11, right most column); potentially leading to a relative underestimation of EVLW post-operatively.

5.7.1.4 Post-operative hyperinflation

Hyperinflation of the residual lung tissue is well described after pneumonectomy as excessive negative pressure in the operative hemithorax leads to acute mediastinal shift^{114, 158}. Similarly, following lobectomy, residual lung tissue on the operative side expands to a degree to occupy the post-lobectomy space.

As pulmonary volume increases, pulmonary vascular resistance rises as a result of both increased transmural pressure and a stretching effect, leading to thinning of alveolar walls and mechanical compression²³. Pulmonary inflation to supra-normal volumes post-operatively is likely therefore to be associated with increased pulmonary vascular resistance, altering the distribution of pulmonary perfusion and potentially further reducing the 'visible' portion of pulmonary vasculature. Conversely post-operative recruitment of previously hypo-perfused pulmonary vasculature could increase the volume of lung 'visible' to the thermal indicator.

5.7.2 Reproducibility and validity of single thermodilution EVLW and PVPI measurement after lung resection

Though the reproducibility and validity of STD measurement of EVLW has to some degree been established in ventilated patients without lung resection, despite theoretical concerns, there has been little attention to the reproducibility of the technique in either spontaneously breathing patients or patients undergoing lung resection.

Similarly, the validity of EVLW measurement following lung resection has received little attention. The limited data available to assess criterion and construct validity are presented below; no assessment of concurrent or predictive validity has been made.

5.7.2.1 Criterion validity

Both Kuzkov et al⁵¹¹ and Roch et al⁵⁰⁹ have compared $EVLW_{STD}$ with the 'gold standards' of $EVLW_{TDD}$ and $EVLW_{GRAV}$ in animal models of pneumonectomy (sheep and pigs respectively).

Roch et al assessed bias between $EVLW_{GRAV}$ and both $EVLW_{TDD}$ and $EVLW_{STD}$ in two lung and one lung (post-pneumonectomy) animals⁵⁰⁹. In two lung animals Roch et al observed that $EVLW_{TDD}$ underestimated and $EVLW_{STD}$ marginally overestimated $EVLW_{GRAV}$ (mean bias -1ml/Kg and $+1.5\text{ml/kg}$ respectively). Following pneumonectomy however, whilst $EVLW_{TDD}$ maintained good agreement with $EVLW_{GRAV}$ (mean bias $+2\text{ml/kg}$), $EVLW_{STD}$ markedly overestimated $EVLW_{GRAV}$ (mean bias $+5\text{ml/kg}$)⁴⁸⁷. This observed increase in the overestimation of $EVLW_{GRAV}$ by $EVLW_{STD}$ following pneumonectomy is at odds with much of the theory presented; a fall in PBV relative to GEDV, reduced mean transit time of thermal indicator and post-operative hyperinflation (altering the distribution of pulmonary perfusion) are each expected to lead to an underestimation in EVLW. Roch et al were no more able to explain the apparent paradox in their findings than the author (B. Shelley)⁴⁸⁷. It must be emphasised however, that though at odds with the theory presented, the findings of Roch et al represent those of a single study and are inconsistent with the findings of others.

Kuzkov et al determined EVLW by TDD, STD and gravimetrically following pneumonectomy in a sheep model⁵¹¹. Though these authors didn't assess mean bias between $EVLW_{STD}$ and $EVLW_{GRAV}$ in two-lung animals pre-operatively, post-operative mean bias was $+3\text{ml/kg}$. This post-operative value includes a control group of animals undergoing 'sham' surgery (lateral thoracotomy only, no lung resection) in whom the bias between $EVLW_{STD}$ and $EVLW_{GRAV}$ appears similar (from inspection of the scatter / Bland-Altman plots provided, reproduced in Figure 5.13) to that in animals undergoing pneumonectomy.

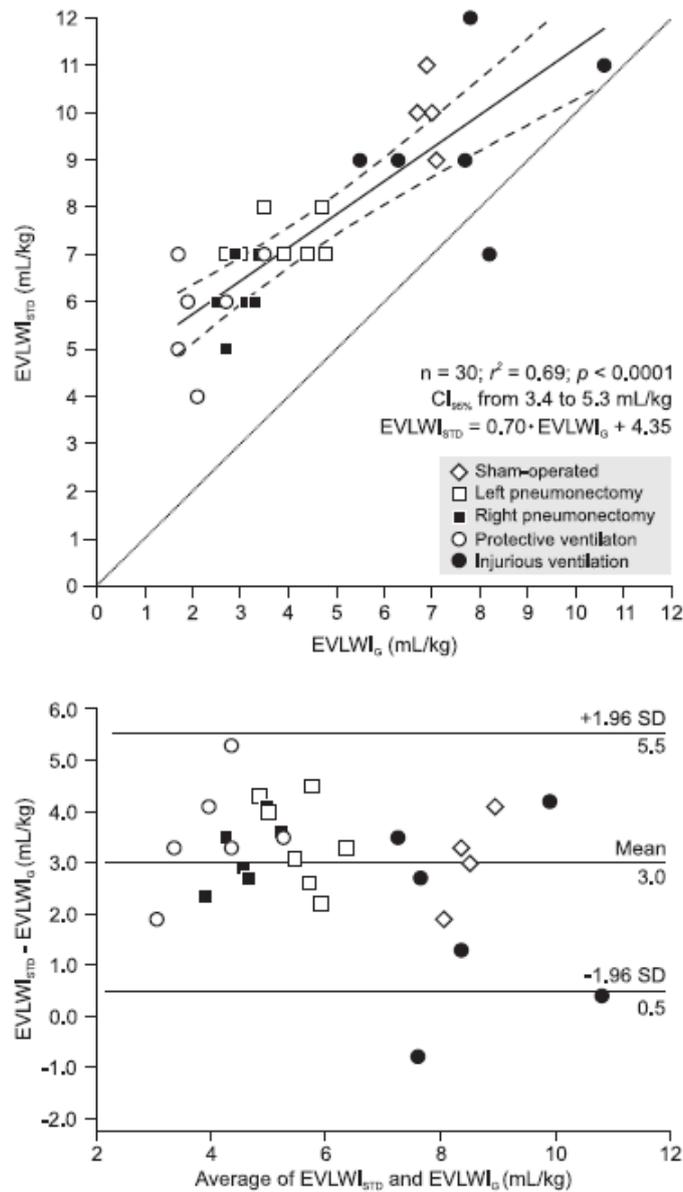


Figure 5.13. Linear regression analysis and Bland–Altman plot for comparison between single thermodilution and gravimetric estimates of EVLW following pneumonectomy in sheep.

From Kuzkov et al (2007)⁵¹¹.

There are no reports from which to assess criterion validity of PVPs following lung resection.

5.7.2.2 Construct validity

Animal studies

In Kuzkov et al's sheep model of pneumonectomy followed by injurious ventilation, EVLW_{STD} can be observed to rise in parallel with the development of lung injury, represented by the constructs of falling PaO₂/FiO₂ and increased shunt (no statistical comparison made)¹⁴⁷. Similarly, in two further reports from

the same research group, utilizing the same model of pneumonectomy and injurious ventilation in sheep, Subarov et al present data in which EVLW can be observed to rise in parallel with increasing venous admixture and lung injury score and falling oxygenation and both total lung and chest compliance^{228, 521}.

In all three studies, PVPI (as EVLW indexed to pulmonary blood volume) is observed to rise in parallel to EVLW^{147, 228, 521}.

Human studies

In 2008, Licker et al studied the effects of inhaled bronchodilators on the resolution of pulmonary oedema in 24 patients undergoing lung resection, using EVLW to quantify pulmonary oedema. Post-operative administration of bronchodilator led to decreases in mean EVLW and increases in mean PaO₂/FiO₂. Change in PaO₂/FiO₂ following bronchodilator administration was associated with change in EVLW (R²=0.55, p<0.001). Licker et al also report a parallel reduction in both mean chest X-ray score and mean EVLW on post-operative day one compared to immediately post-operatively (no statistical comparison were made)⁴⁷⁶.

Licker et al observe a significant fall in PVPI (as ELWI / GEDVI) following administration of bronchodilator on the day of surgery, but not on post-operative day one⁴⁷⁶.

5.7.3 Observed changes in EVLW following lung resection

Human studies evaluating the observed changes in EVLW following lung resection are summarised in Table 5.11; it can be seen that there is some variety in the findings, with EVLW being observed to fall, remain the same or increase post-operatively.

Table 5.11. Summary of studies reporting changes in EVLW determined by TPTD after lung resection in humans.

Author (year)	Study design	Study population*	Change Pre- vs Post-op	Subsequent post-op change	B/L
Kuzkov et al ⁵¹² (abs) 2005	Prospective observational No intervention	N=7 All P	↓35% (p=0.02)	↑55% to a peak at 36hrs	N/S
Kuzkov et al ⁵¹¹ (abs) 2007	Prospective observational No intervention	N=27 P=16 L=11	P: ↓30% (p<0.05) L: no sig change	P: ↑24% to peak at 36hrs “significantly” L: no sig change	MV
Licker et al ⁴⁷⁶ (2008)	Randomised cross-over trial of inhaled bronchodilator	N=21 B=5 L=16	↑31% (p=0.009)	No sig. diff on POD 1 vs pre-op	SB
Leo et al ⁵²² (2008)	Prospective observational - No intervention	N=15 All P	↓32% “significantly decreased”	No sig. change over 1 st 24hrs	MV
Haas et al ²³⁵ (2012)	Prospective observational - SVV guided, goal directed fluid therapy	N=27 (11 having lung resection) B= 2 L= 9	N/S	No sig. change pre- vs 24hrs post-op	MV
Assaad et al ^{234, 245} (abs) (2012)	Prospective observational - Liberalized fluid protocol	N=11 P=1 L=8 W=2	N/S	No sig change to POD 3	MV

P, pneumonectomy; BL, bi-lobectomy; L, lobectomy; SL, sub-lobar resection. B/L, whether baseline measurement made whilst mechanically ventilated (MV) or spontaneously breathing (SB). N/S, not specified.

5.7.3.1 Immediate post-operative changes

What might be expected to occur following lung resection?

If one considers a lung with a given EVLW, it is assumed that that the EVLW is uniformly distributed throughout the lung (i.e. that EVLW per unit lung tissue is uniform throughout the lung). Following lung resection therefore it would be anticipated that the lung resection would result in a reduction in total EVLW in proportion to the volume of resected lung tissue (with the unlikely assumption, that the resection takes place in such fashion that EVLW is unaltered). That is to say that the volume of EVLW per unit lung is unchanged, but there is less lung therefore less total EVLW post-operatively.

As such one might expect to observe an approximate fall in EVLW of 47 / 53% for a left / right pneumonectomy respectively and between 10% and 26% for an anatomic lobectomy (based on a 19 segment model of pulmonary anatomy).

Studies reporting immediate peri-operative changes

Several human studies using STD following lung resection report either a less substantial reduction than might be anticipated^{511, 512, 522} in EVLW post-operatively or recognise no change^{234, 235} (Table 5.11).

There are several possible explanations for the fall in EVLW to be less than hypothesised: Firstly, resection of lung tissue may result in a proportional reduction in EVLW as hypothesised, but the EVLW per unit of residual lung tissue is increased as a result of pathological processes occurring during the peri-operative period. Secondly, resection of lung tissue may result in a proportional reduction in EVLW as hypothesised, but following lung resection, STD measurement of EVLW results in a systematic overestimation of lung water (as reported by Roch et al)⁵⁰⁹.

It is notable from Table 5.11, that Licker et al⁴⁷⁶ are the only group to report an increase in EVLW (immediately) post-operatively whilst all others either demonstrate no significant change or a fall in EVLW post-operatively. This is potentially explained by the following observations: Firstly Licker et al made their baseline assessments of EVLW prior to the induction of anaesthesia / onset of mechanical ventilation; as such, they compare an estimate of EVLW made whilst spontaneously breathing pre-operatively with one made whilst spontaneously breathing post-operatively. The majority of the others (as far as it can be deduced from the text) appear to have made their baseline estimate of EVLW after induction of anaesthesia and so compare a mechanically ventilated baseline value with a spontaneously breathing post-operative value, an observation that will lead to a relative underestimation of EVLW post-operatively. Secondly, Licker et al report data from a predominantly lobectomy group. As such, the lesser anatomical reduction in EVLW seen following lobectomy may be insufficient to mask a pathological increase observed following resection.

It seems likely that whilst total EVLW might reduce following lung resection due to the anatomical loss of lung tissue (and its associated EVLW), due to the parallel intra-operative insults to both the operative and contra-lateral lung (discussed in detail in Chapter 1) resulting in development of sub-clinical lung injury, EVLW per unit of lung tissue is likely to be increased post-operatively.

Such disparity of study findings may also be symptomatic of the relatively small number of patients included in each study or differences in baseline cardio-respiratory function or intra-operative conditions (ventilator settings, duration of OLV, fluid administration, etc). Unfortunately as several of the investigations were only reported in abstract form it is not possible to obtain this data for all studies.

5.7.3.2 Changes during the post-operative period

In both their studies^T, Kuzkov's group observed a significant increase in EVLW over the first 36 hours post-operatively^{511, 512}. Though this might represent the development of lung injury in a high risk group of patients (pneumonectomy), the authors observed that this change *“was not accompanied by changes in pulmonary artery pressure, pulmonary artery occlusion pressure and PaO₂/FiO₂”*. As these papers were only published in abstract form, it is difficult to apply much further analysis. Whilst it is possible these changes represent sub-clinical oedema formation, it is plausible that these changes represent fluctuation in the GEDV:ITBV ratio occurring post-operatively. Naidu et al observed marked changes in GEDV:ITBV occurring immediately post-operatively, before relative stability from 4-6 hours onwards.

All other studies demonstrate no increase in mean EVLW post-operatively.

Only the study of Licker et al reported PVPI post-operatively (ELWI/GEDVI)⁴⁷⁶. This demonstrated increased PVPI compared to baseline on post-operative day one (1.3 vs 2.0, p<0.05) before return to baseline on post-operative day two.

^T Which may in fact represent a preliminary sub-group of the same group of patients.

5.7.4 Summary

Whilst the reproducibility and validity of STD measurement of EVLW and PVPI appear to be well established in the general critical care population, it is clear that as regards EVLW and PVPI measurement after lung resection, the literature is in its infancy. There are significant theoretical considerations which appear to challenge the validity of STD measurement following lung resection though these have yet to be explored fully in humans. Regrettably there are also methodological concerns concerning the use of TPTD in patients with lung injury, and so it seems that the very population in which the author is seeking to apply TPTD monitoring (i.e. for the detection of lung injury following lung resection), is the very population in which its application may be most challenging.

It has been suggested that adjustment of the GEDV/ITBV relationship might improve the validity of TPTD monitoring following lung resection²⁴⁸. This concept is discussed in detail in Appendix three, and is a secondary aim of this Investigation IV. The primary aim is to establish the reproducibility and construct validity of trans-pulmonary thermodilution derived extravascular lung water and pulmonary vascular permeability index in patients undergoing lung resection.

5.8 Methods

5.8.1 Ethical approval

The need for ethical approval was waived following correspondence with the Scientific Officer and Service Manager of the West of Scotland Research Ethics Committee (WOSREC). This was because the project was considered by the committee to be “*an evaluation of a new CE marked device with a view to introducing the device into routine clinical practice*” (personal communication Dr Judith Godden, WOSREC Scientific Officer/Manager, February 2012).

5.8.2 Patient population

This patient population is a sample of convenience of nine patients undergoing elective lung resection by open thoracotomy at Golden Jubilee National Hospital^U. Inclusion criteria were, age greater than 16 years and planned elective open lung resection (by lobectomy or pneumonectomy) for presumed primary lung cancer. Patients were excluded if they were pregnant, were undergoing lung resection for non malignant disease or secondary malignancy, were planned to undergo a wedge / segmental lung resection, or a resection via a thoracoscopic / minimal access technique. In addition, patients were excluded if they had a contraindication to femoral arterial catheterisation e.g. peripheral vascular disease, or localised skin infection. Anaesthetic technique was standardised, to total intravenous anaesthesia with Propofol and Remifentanil by target controlled infusion; post-operative analgesia was provided by thoracic epidural analgesia.

5.8.3 Trans-pulmonary thermodilution

TPTD measurements were performed using the Edwards Lifesciences EV1000 clinical platform in combination with the VolumeView set according to the manufactures instructions (Edwards Lifesciences, Irvine, California, USA). The VolumeView set comprises VolumeView sensor, thermodilution manifold and the

^U Support for loan of equipment and provision of consumables was provided for this ‘pilot study’ by Edwards Lifesciences (Irvine, California, USA). The sample size of nine patients was dictated by the limits of the support available.

femoral arterial catheter. The components and set up are described in Figure 5.14.

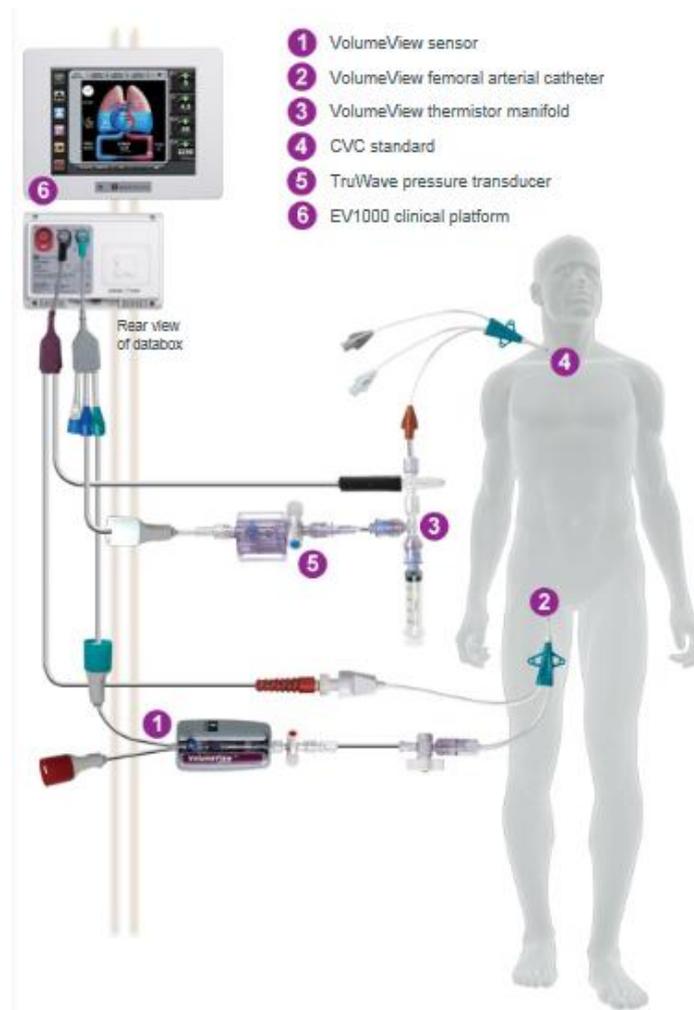


Figure 5.14. Schematic representation of the patient and equipment set-up for transpulmonary thermodilution monitoring using the Edwards E1000 clinical platform.
From: <http://www.edwards.com/eu/products/mininvasive/Pages/volumeviewsetup.aspx>.

The VolumeView femoral arterial transducer is a 4 or 5F, 16cm cannula, equipped with a monitoring port (providing conventional arterial access for blood pressure monitoring and blood aspiration) and incorporating a thermister. This was inserted by the author (B Shelley), under ultrasound guidance in the patients' femoral artery (on the contra-lateral side to the surgery) prior to the induction of general anaesthesia using an aseptic seldinger technique. Arterial pressure monitoring was instituted by connecting the monitoring port of the femoral arterial cannula to the VolumeView sensor. In addition to routine arterial pressure monitoring, following calibration by TPTD, the VolumeView allows continuous cardiac output monitoring by pulse contour analysis (not used for this investigation). Central venous cannulation was performed under

ultrasound guidance immediately following induction of anaesthesia using a triple lumen, 16cm, 7F, cannula (Edwards Lifesciences). The VolumeView thermodilution manifold was then connected to the distal lumen of the central venous cannula. This manifold allows monitoring of injectate temperature by the EV1000 system. The VolumeView sensor, thermodilution manifold, femoral arterial thermistor were then connected to the EV1000 monitor as shown in Figure 5.14.

TPTD monitoring was then performed by rapid injection of known volume (15 or 20ml) of cold normal saline through the thermodilution manifold; the temperature change in the femoral artery is determined by the thermistor in the femoral arterial cannula and leads to generation of a thermodilution curve (Figure 5.15). From this curve, cardiac output, global end-diastolic volume index (GEDV) and extravascular lung water index (ELWI) are determined by the monitor.

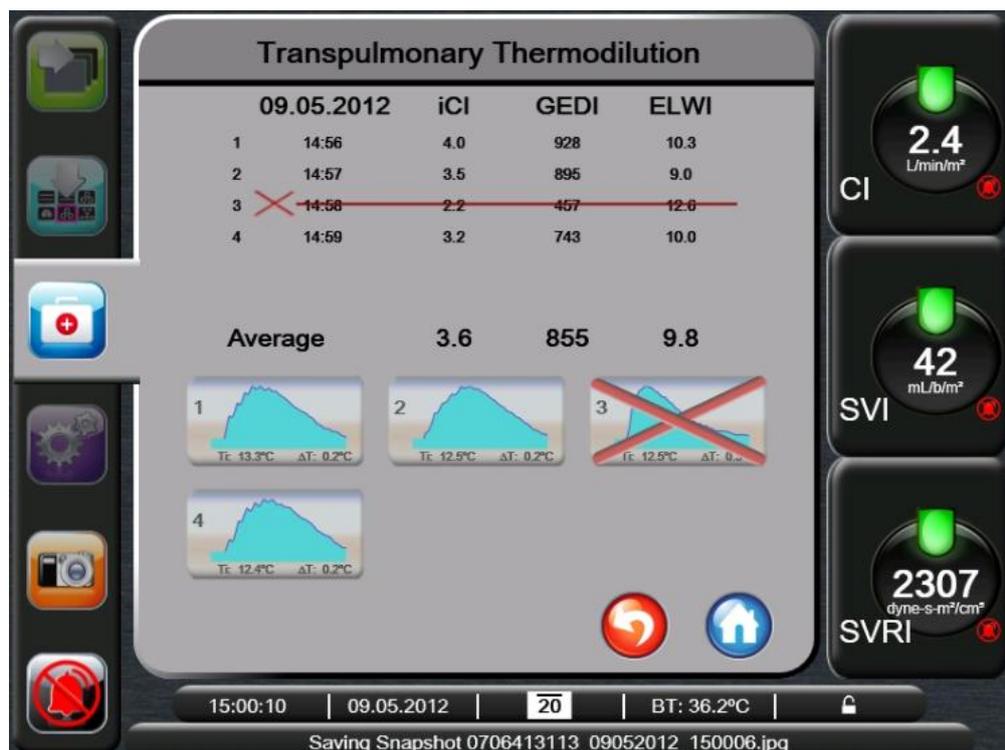


Figure 5.15. Screenshot from the Edwards EV1000 monitor demonstrating acquisition of thermodilution curves during TPTD.

The results from injection 3 lie in excess of 10% from the mean value and so have been discarded and a further injection performed. It can be appreciated that the shape of the thermodilution curve during injection 3 is different from that observed during injections 1, 2 and 4.

The manufacturers recommend performing triplicate thermodilution on each occasion and the monitor then determines the mean values from the triplicate sets (Figure 5.15).

5.8.3.1 TPTD monitoring for assessment of construct validity

For routine clinical monitoring, the manufacturers recommend performing triplicate TPTD measurements, but rejecting any measurement whose cardiac index value lies greater than 10% from the mean value⁴⁰⁸. In the example shown in Figure 5.15 therefore, the results from measurement three have been discarded and a fourth measurement has been performed. The mean value, determined from injections one, two and four is taken as the result and was used in subsequent analyses.

5.8.3.2 TPTD monitoring for assessment of reproducibility

For the purposes of assessing reproducibility, sequentially discarding curves until the returned cardiac output results lay within 10% of the mean value would lead to an artefactual overestimation of the monitor's reproducibility. Reproducibility statistics were therefore determined based on the results of the first three thermodilution injections. In the example in Figure 5.15, means and standard deviations (from which subsequently reproducibility statistics would be derived) would be determined from the results of injections one, two and three.

5.8.4 Adjustment of TPTD value for the volume of lung resected

To test the hypothesis that adjustment of TPTD derived ELWI and PVPI for the volume of lung resected would lead to an improvement in construct validity, two further estimates of ELWI / PVPI were derived by mathematical manipulation of the raw ELWI / PVPI results returned by the monitor (referred to from here onwards as 'unadjusted' results ($ELWI_{UNadj}$ / $PVPI_{UNadj}$)).

From the unadjusted data, two modified values were derived. Derivation of the modified values is described in Appendix three. Briefly, in the derivation of the first modified value, the coefficients utilised in equations used to derive ELWI and PVPI are adjusted in order to account for the hypothesised reduction in

pulmonary blood value following lung resection - resulting in the proposed 'anatomical' adjustment (yielding $ELWI_{ANadj}$ and $PVPI_{ANadj}$). In the second, TPTD derived indices were calculated using the original equations, but the ELWI and PVPI results yielded were 'corrected' to reflect that they have been determined from less than a whole lung. Correcting the result based on the number of pulmonary segments remaining following resection is the basis of the proposed 'segment corrected' result ($ELWI_{SEGcorr}$ and $PVPI_{SEGcorr}$).

5.8.5 Post-operative data collection

5.8.5.1 Clinical endpoints

Oxygenation and Chest X-ray scoring

Collection of data pertaining to oxygenation, and chest X-ray scoring were performed as described previously in Section 4.5.1.6.

Fluid balance

Post-operative fluid balance recording is routinely performed by the high dependency unit nursing staff, in the hospital critical care electronic records system (Centricity CIS, GE Healthcare, Wilmington, Massachusetts). The 'zero point' was defined as the point of admission to the high dependency unit, from which cumulative fluid balance was calculated hourly.

5.8.6 Statistical handling

5.8.6.1 Reproducibility

Reproducibility statistics were determined for each triplicate set of TPTD measurements according to the definitions provided in Section 5.2.2.1.

Median CV, CE, precision and LSC values were then derived by pooling all measurement sets. Confidence interval for medians were determined based on the Binomial distribution using Confidence Interval Analysis software, version 2.2.0, (University of Southampton, Southampton, UK)⁵²³.

Reproducibility was assessed according to the criteria described by Holm et al to represent “*usual practice*” (Table 5.12)³⁷³. The use of the cut-offs described are supported by others³⁷⁴⁻³⁷⁷.

Table 5.12. Quantitative interpretation of coefficient of variation (CV) of a clinical monitor.

CV	Interpretation
<10%	Small
10-15%	Acceptable
>15%	Poor

From Holm et al (2005)³⁷³

5.8.6.2 Construct validity

Cross-sectional construct validity

Contemporaneous values of EVLW / PVPI and post-operative PaO₂/FiO₂, CXR score and fluid balance were pooled across all time points and association determined using Pearson’s rho or Spearman rank correlation as appropriate. Strength of association was quantified according to the precedent described by Cohen⁵²⁴ (Table 5.13).

Table 5.13. Interpretation of the strength of association by correlation coefficient.

r	Interpretation
0.10	Small
0.30	Moderate
0.50	Large

From Cohen, 1988⁵²⁴.

Whilst pooled analyses such as these are a widely accepted and reported method of handling clinical data^{419, 427-429, 433, 525}, such a pooled analysis fails to take into account repeated measures (where often, as in this study, many observations are made per subject). As Bland and Altman describe, in a comparison such as this, where one is interested in whether a subject’s PaO₂/FiO₂ is related to that subject’s ELWI, the association of interest is that within-subjects, and it is therefore desirable to remove any differences observed between-subjects⁵²⁶. To assess within-subject correlation, alongside the pooled analysis as described, analysis of covariance (ANCOVA) was performed (covariate = EVLW or PVPI and factor=patient) allowing within-subject variability to be partitioned out⁵²⁶.

Longitudinal construct validity

Changes in paired EVLW / PVPI results and PaO₂/FiO₂, CXR score and fluid balance between sequential time-points were similarly subjected to pooled analysis for determination of association and ANCOVA. In addition, trending ability of EVLW / PVPI was determined by the construction of four quadrant plots and direction of change analysis. As advocated by Monge Garcia et al, to exclude random measurement error a central exclusion zone equivalent to the least significant change value for EVLW or PVPI was defined⁵²⁷. Concordance was defined as the number of data points falling into one of the quadrants of agreement, expressed as a percentage of the total number of data points used in the plot⁵²⁸.

Following Fisher's r-to-z transformation, comparisons between correlation coefficients were made according to Steiger's method⁵²⁹ using computer software available made available online by Lee and Preacher⁵³⁰. Though this method was originally described for comparison of Pearson's correlation coefficients, Myers and Siriois have reported "*treating Spearman coefficients as though they were Pearson coefficients and using the standard Fisher's z-transformation and subsequent comparison*" (in order to compare coefficients) to be more robust than alternative methods⁵³¹.

5.9 Results

5.9.1 Patient demographics

Trans-pulmonary thermodilution monitoring was instituted in 9 patients. Unfortunately in one patient, a disconnection occurred on transfer from the operating table to the patient trolley, comprising the sterility of the monitoring system and no further TPTD could be performed. As this patient had not had any post-operative TPTD measurements made, this patient's data was excluded from the study. The demographic and surgical details of the remaining eight patients are summarised in Table 5.14. Whilst planned lobar lung resection or pneumonectomy was an *a priori* inclusion criteria of the study, in one case the operative plan was modified intra-operatively and the patient underwent sub-lobar lung resection; this patient's data was included in all analyses.

Table 5.14. Demographic and surgical data from 8 patients included in reproducibility and validity of ELWI and PVPI study.

Patient	1	2	3	4	5	6	7	8
Age	64	68	61	74	70	66	63	52
Sex	M	M	F	F	M	F	F	M
Side	Right	Right	Right	Right	Right	Right	Left	Left
Resection	2x wedge	Lung	Lower lobe	Upper lobe	Middle & lower lobe	Upper & middle lobe	Upper lobe	Upper lobe
OLV time	37	106	23	107	99	176	70	57
Op. time	154	147	104	218	184	261	163	128

OLV, one lung ventilation; Op., operation. 'wedge', refers to sub-lobar resection.

5.9.2 Changes in ELWI and PVPI following lung resection

There were no peri-operative changes in ELWI for both ‘un- adjusted’ values ($ELWI_{UNadj}$) and those following ‘anatomical adjustment’ ($ELWI_{ANadj}$) Figure 5.16. Following correction of the ELWI value for the number of pulmonary segments resected ($ELWI_{SEGcorr}$), $ELWI_{SEGcorr}$ was significantly higher immediately post-operatively compared to pre-operative values ($p=0.02$, Wilcoxon signed rank test). All comparisons between pre- and post-operative values should be interpreted with caution however, as pre-operative measurements were made under conditions of positive pressure ventilation and are being compared to post-operative measurements made whilst spontaneously breathing.

Unadjusted pulmonary vascular permeability index ($PVPI_{UNadj}$) was significantly lower immediately post-operatively than pre-operatively and continued to fall up to 6 hours post-operatively (Figure 5.17). Both $PVPI_{ANadj}$ and $PVPI_{SEGcorr}$ increased significantly immediately post-operatively with little subsequent change through the monitored period. The same caution applies in the comparison of pre- vs post-operative PVPI values.

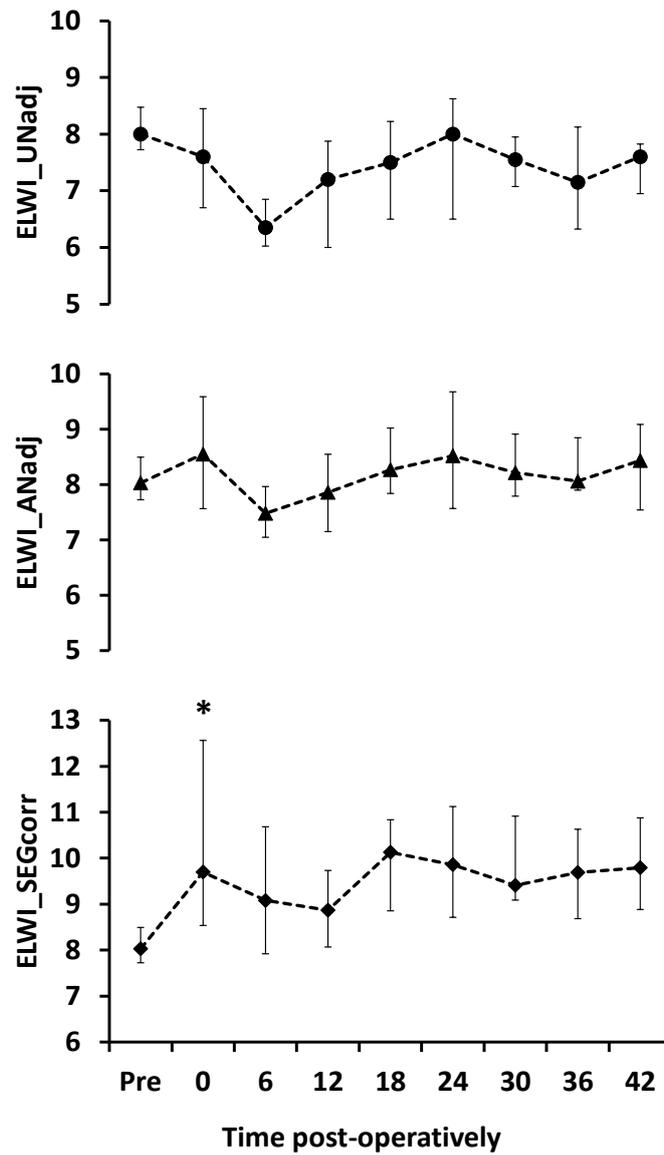


Figure 5.16. Changes in ELWI following lung resection.

UNadj, unadjusted result; ANadj, ITBV/GEDV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no. of pulmonary segments remaining. Data presented as median, IQR. * $p < 0.05$ vs pre-operative values, Wilcoxon signed rank test for both. $N=8$.

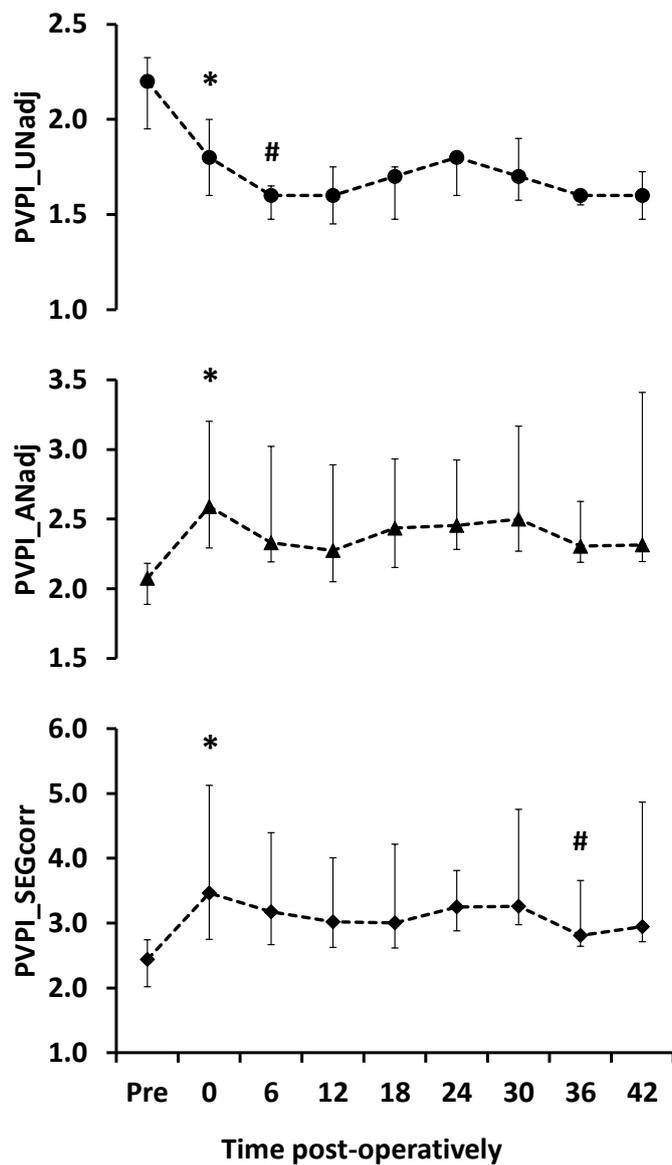


Figure 5.17. Changes in PVPI following lung resection.

UNadj, unadjusted result; ANadj, ITBV/GEDV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no. of pulmonary segments remaining. Data presented as median, IQR. * $p < 0.05$ vs pre-operative values, # $p < 0.05$ vs 0 hours (immediately post-operatively), Wilcoxon signed rank test for both. $N=8$.

Whilst there was little change in median ELWI and PVPI through the post-operative period (Figures 5.16 and 5.17), it is evident that in some individual patients, there was marked variation in both ELWI and PVPI. $ELWI_{UNadj}$ and $PVPI_{UNadj}$ values for individual patients are provided for illustration in Figure 5.18. Arguably the purpose of the remainder of this investigation is to examine the clinical significance of such variation.

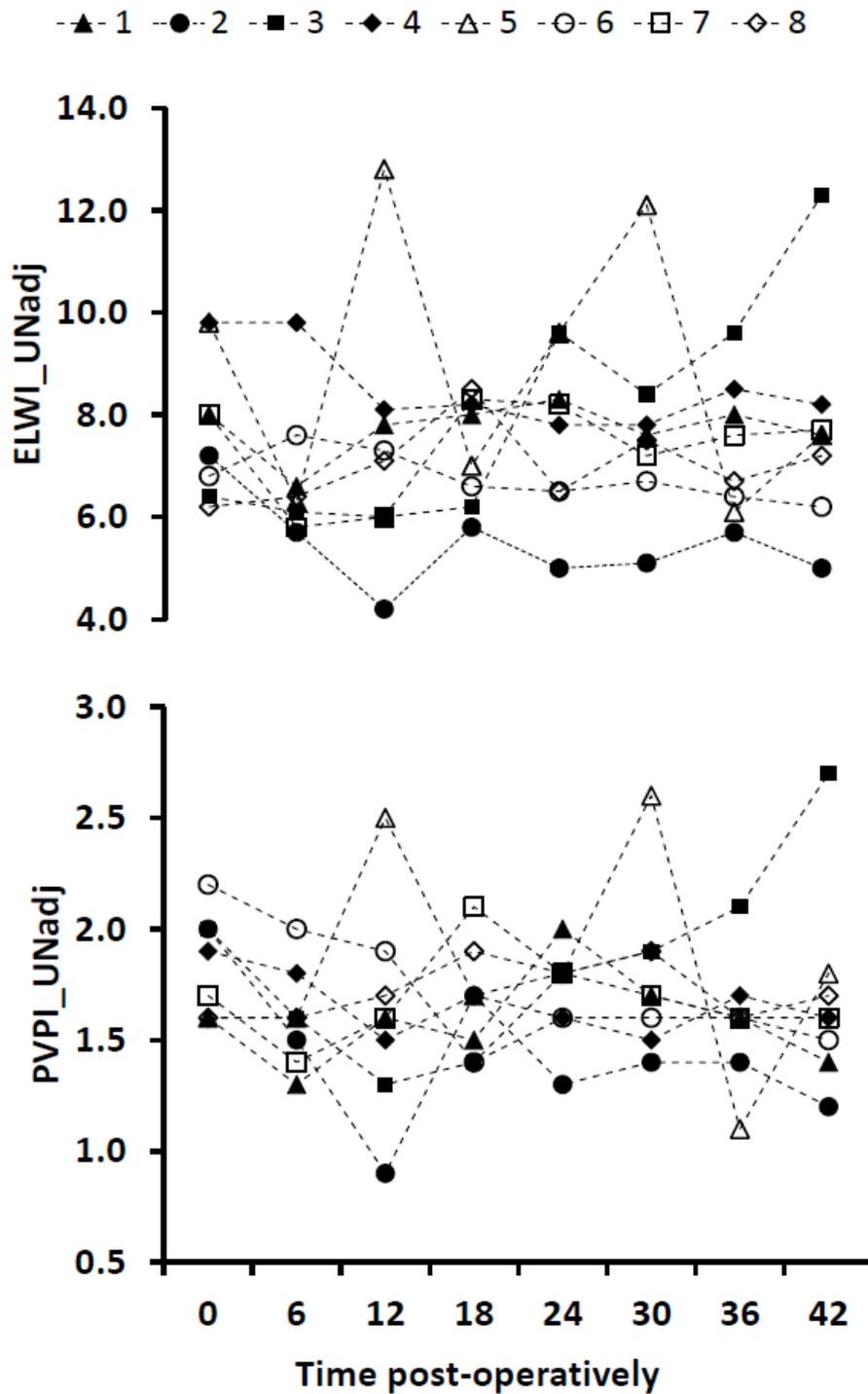


Figure 5.18 Changes in un-adjusted ELWI and PVPI over time for individual patients.

Legend indicates patient number. Patient 5 demonstrates marked peaks in ELWI and PVPI on two occasions post-operatively whilst in patient 3 both ELWI and PVPI appear to be rising from 18 to 42 hours post-operatively. No statistical comparisons made.

5.9.3 Reproducibility of TPTD derived values following lung resection

Triplicate sets of TPTD derived measurements were made immediately post-operatively and at six hourly intervals to 42 hours post-operatively in all patients, yielding 64 triplicate data sets. In addition a further 26 measurements were performed when required for calibration, following disconnection of the power supply (for example to facilitate transfer from recovery to the high dependency unit) or when the system was disconnected from the patient to allow mobilisation. All 90 measurement sets were subject to analysis in order to derive coefficient of variation (CV), precision and least significant change statistics (LSC) for the following TPTD derived variables: cardiac output (CO), global end-diastolic volume (GEDV), extravascular lung water (EVLW) and pulmonary vascular permeability index (PVPI determined as EVLW indexed to pulmonary blood volume).

Table 5.15 Reproducibility statistics for TPTD derived parameters following lung resection.

	<i>Median value</i>	<i>CV (%)</i>	<i>Precision (%)</i>	<i>LSC (%)</i>
CO	5.6 L/min (4.8-6.2 [2.6-8.1])	5.3 (4.9-6.1)	6.1 (5.7-7.0)	8.5 (7.9-9.8)
GEDV	1078 ml (960-1301 [697-1509])	6.5 (5.1-8.0)	7.5 (5.9-9.2)	9.0 (7.3-11.6)
EVLW	471 ml (412-543 [291-857])	8.3 (6.7-9.3)	9.6 (7.6-10.7)	11.5 (9.4-14.3)
PVPI	1.73 (1.57-2.20 [0.90-3.40])	13.0 (9.8-15.1)	15.0 (11.3-17.4)	17.7 (13.9-23.2)

Values are presented as median (IQR [range]). CV, precision and LSC statistics for all variables were not normally distributed and are presented as median and 95% confidence interval for the median.

The CV of all variables could be described as ‘good’, with the possible exception of PVPI where the 95% confidence interval for the mean just exceeds 15% (Table 5.15). The sequential loss in reproducibility (increasing CV, precision and LSC) from CO to GEDV to EVLW to PVPI is evident from Figure 5.19 where the distribution of the individual measurements can be observed; the tight grouping of triplicate measurements seen in cardiac output separates progressively whilst moving through the sequence described. It is also evident from Figure 5.19 that

there is some loss of reproducibility in GEDV and EVLW measurement towards the higher extremes of the measured values.

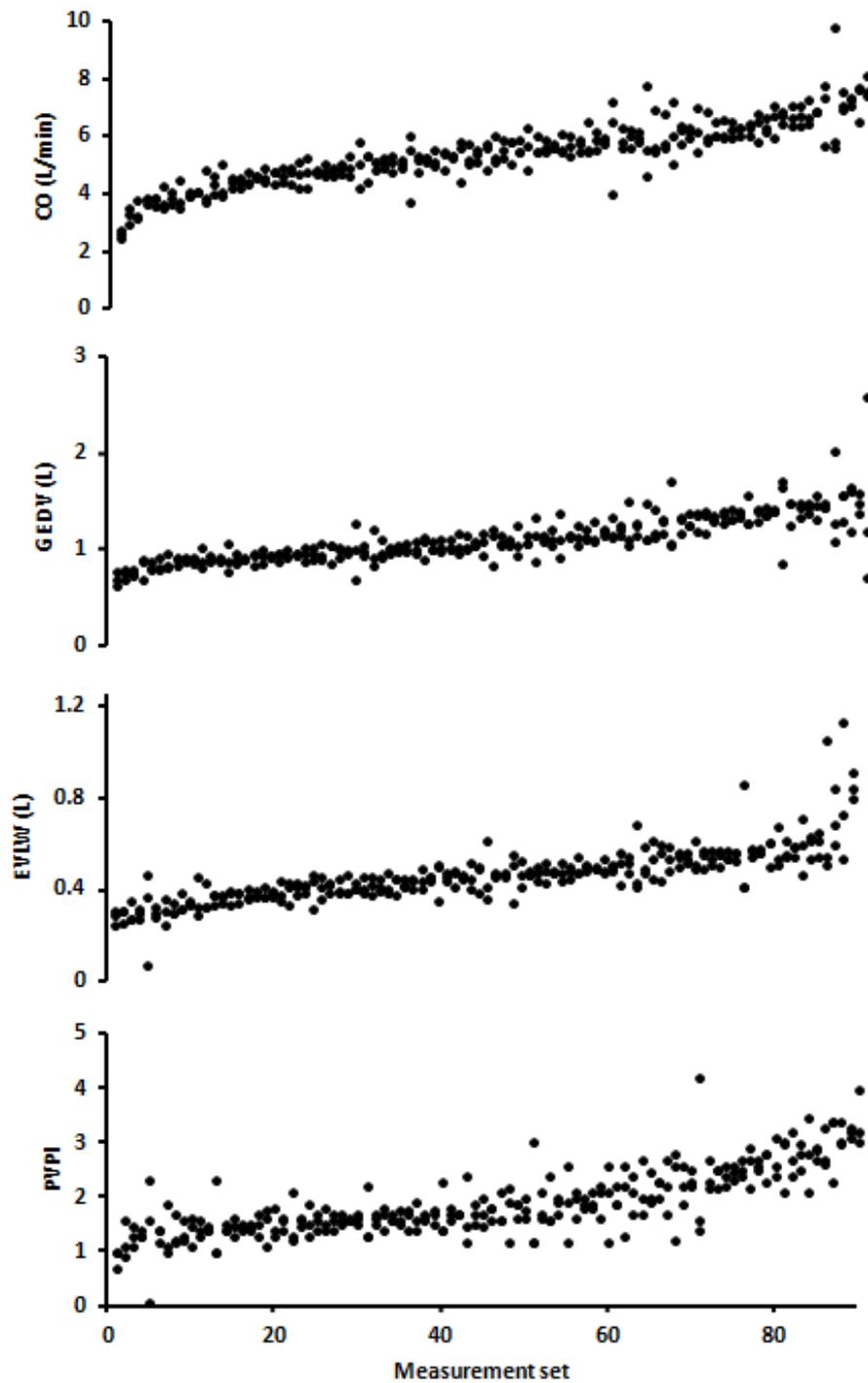


Figure 5.19. Distribution of triplicate cardiac output (CO), global end-diastolic volume (GEDV), extravascular lung water (EVLW) and pulmonary vascular permeability index (PVPI).

For each parameter, measurement sets have been ordered according to increasing mean value.

5.9.3.1 Post hoc analysis: Reproducibility of TPTD derived variables by volume of lung resected

When working with the TPTD data, it became apparent that whilst the overall median coefficients of variation for the four TPTD derived variables were acceptably low (as reported above), there was a marked variability between patients. To explore whether the variability between patients was related to the volume of lung tissue resected, comparison was made between median CV for each individual patient, and the number of pulmonary segments resected. There was a consistent positive association between CV and number of pulmonary segments resected for all four parameters (Figure 5.20, Table 5.16).

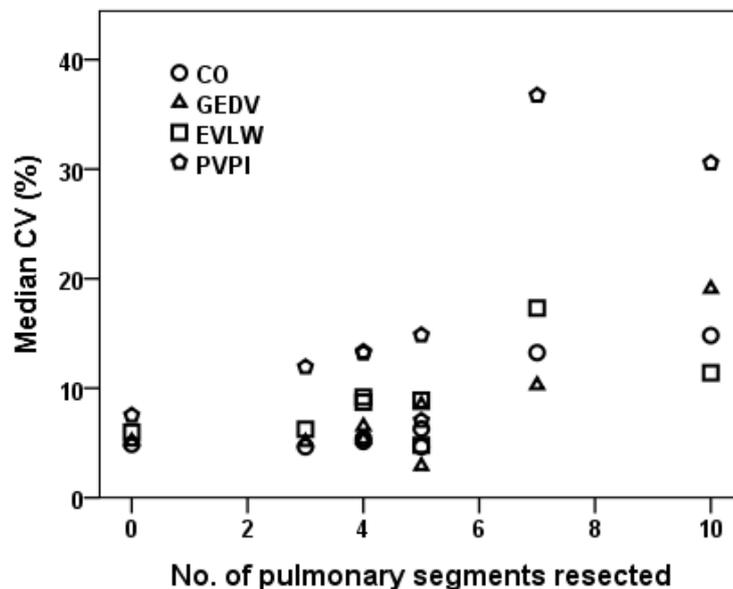


Figure 5.20. Median coefficient of variation (CV) verses number of pulmonary segments resected.

CO, cardiac output; GEDV, global end-diastolic volume; EVLW, extravascular lung water; PVPI, pulmonary vascular permeability index.

Table 5.16. Association between coefficient of variation for TPTD derived parameters and volume of lung tissue resected.

	<i>r</i>	<i>p</i>	<i>N</i>
CO	0.72	0.04	8
GEDV	0.68	0.07	8
EVLW	0.56	<0.01	8
PVPI	0.68	0.07	8

Spearman's rho.

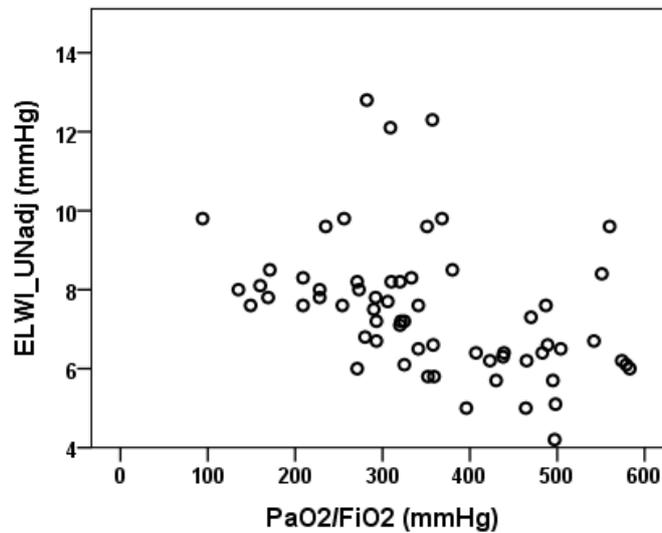
5.9.4 Construct validity of ELWI and PVPI following lung resection

5.9.4.1 Cross-sectional construct validity of ELWI

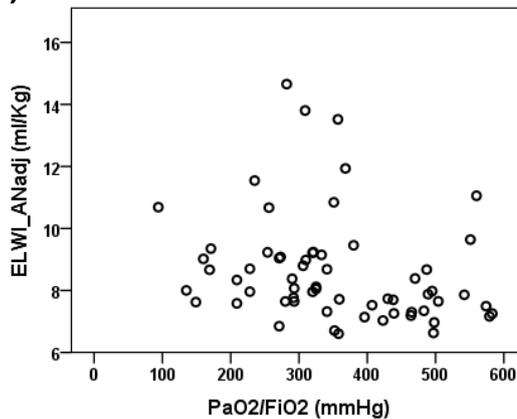
Association between ELWI and PaO₂/FiO₂

There were 62 paired ELWI and PaO₂/FiO₂ results available for analysis (Figure 5.21). There was a negative association between both ELWI_{UNadj} and ELWI_{ANadj} and PaO₂/FiO₂, though this was significantly stronger for ELWI_{UNadj} than ELWI_{ANadj} ($r=-0.52$ vs -0.36 , $p<0.01$, Fig 5.21a & b, Table 5.17). On pooled analysis there was no relationship between ELWI_{SEGcorr} and PaO₂/FiO₂ (Figure 5.21c). Following within-subject (ANCOVA) analysis, there was a moderate association between all ELWI values and PaO₂/FiO₂ with no significant differences between values (Table 5.17).

a)



b)



c)

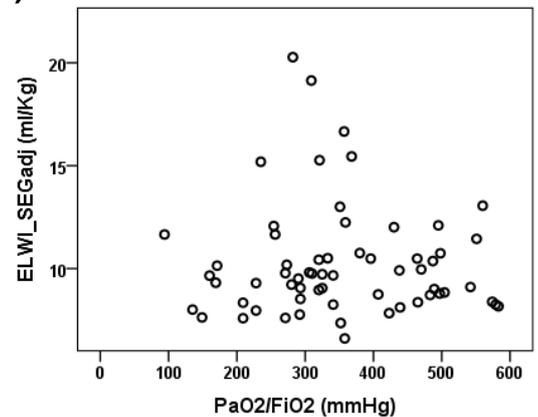


Figure 5.21 Extravascular lung water index (ELWI) versus $\text{PaO}_2/\text{FiO}_2$.

a) UNadj, unadjusted result, $r=-0.52$, $p<0.01$. b) ANadj, GEDV/ITBV adjusted by 'anatomical approach', $r=-0.36$, $p<0.01$. c) SEGcorr, result corrected to reflect no. of pulmonary segments remaining, $r=0.04$, $p=0.75$. Spearman correlation, $N=8$, $n=62$ for all.

Association between ELWI and Chest X-ray score

There were 19 paired ELWI and CXR scores available for analysis. These 19 CXRs were dual reported and the mean CXR score used for subsequent analysis. Inter-rater reliability was explored by determining Type 3 Intraclass Correlation Coefficient (two-way mixed model for agreement, average measures). This revealed 'substantial' agreement between raters ($\text{ICC}=0.72$).

There was a positive association between all ELWI values and post-operative CXR score, though this was weaker and lacked statistical significance for $\text{ELWI}_{\text{SEGcorr}}$ than for $\text{ELWI}_{\text{UNadj}}$ and $\text{ELWI}_{\text{ANadj}}$ (Figures 5.22a-c). There was however no statistically significant differences in the associations observed (Table 5.17).

On within-subject ANCOVA analysis there was a similar strong association between ELWI and CXR score for all ELWI values, with no differences between values (Table 5.17).

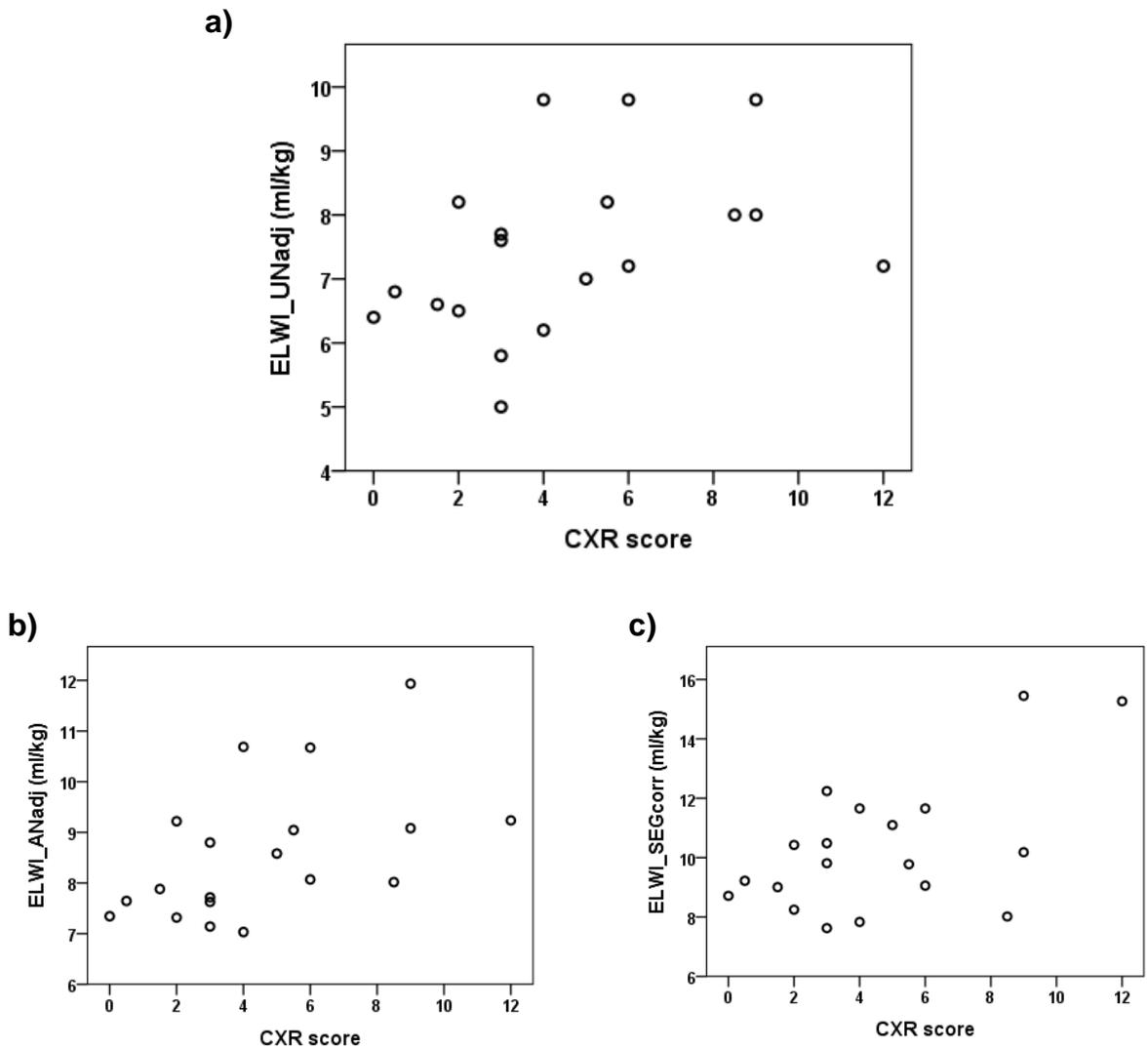


Figure 5.22. Extravascular lung water index (ELWI) versus CXR score.

a) UNadj, unadjusted result, $r=0.51$, $p=0.03$. b) ANadj, ITBV/GEDV adjusted by anatomical 'segment counting approach', $r=0.59$, $p<0.01$. c) SEGcorr, result corrected to reflect no. of pulmonary segments remaining, $r=0.40$, $p=0.09$. Spearman correlation, $N=8$, $n=19$ for all.

Association between ELWI and fluid balance

There were 56 paired ELWI and fluid balance values available for analysis. There was no association between fluid balance and ELWI values in either pooled or within-subject analyses (Figs 5.23a-c and Table 5.17).

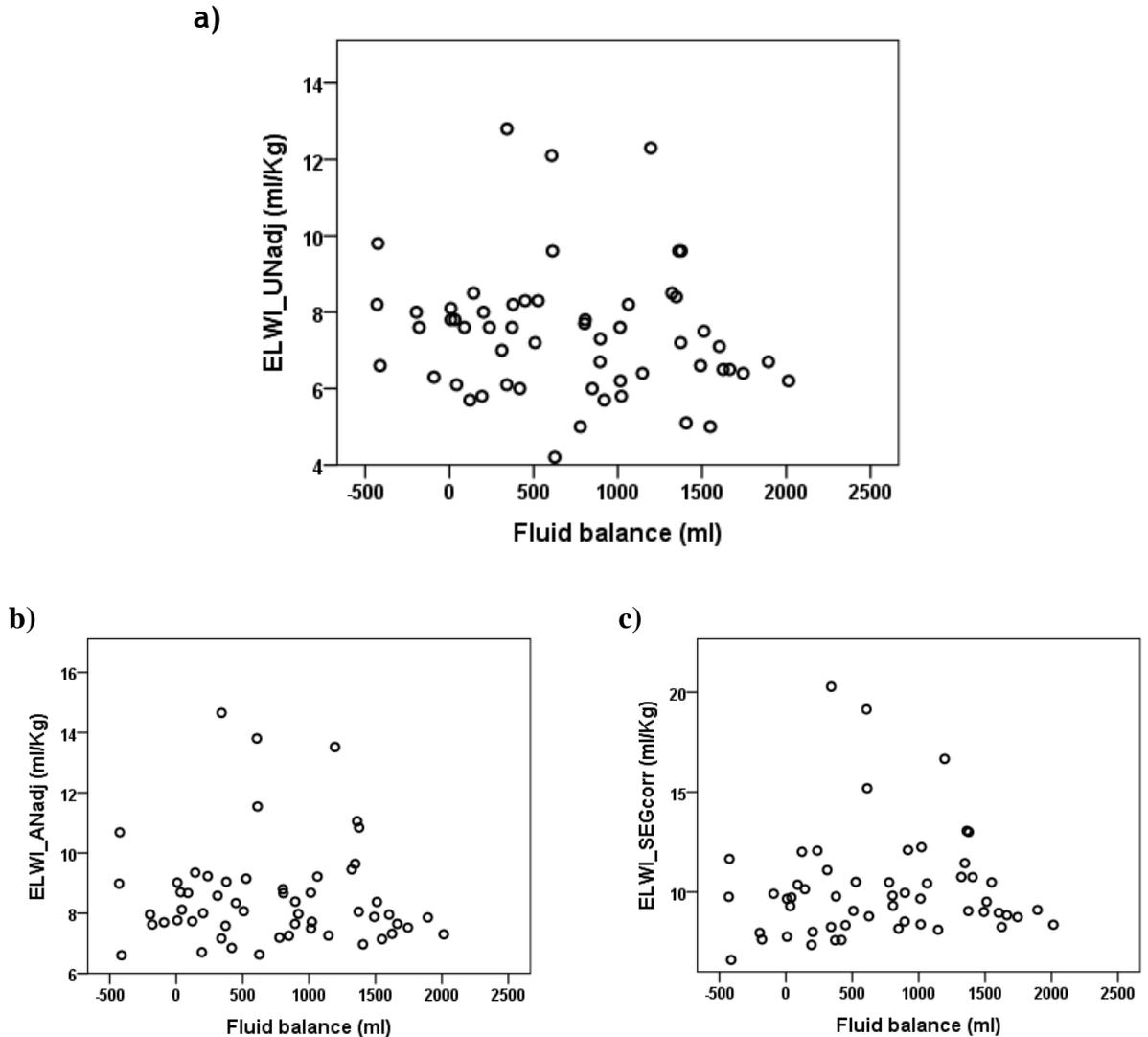


Figure 5.23 Extravascular lung water index (ELWI) versus fluid balance.

a) UNadj, unadjusted result, $r=-0.20$, $p=0.15$. b) ANadj, ITBV/GEDV adjusted by anatomical 'segment counting approach', $r=-0.11$, $p=0.44$. c) SEGcorr, result corrected to reflect no. of pulmonary segments remaining, $r=0.11$, $p=0.41$. Spearman correlation, $N=8$, $n=56$ for all.

Table 5.17. Cross-sectional construct validity of ELWI following lung resections: Association between post-operative ELWI values and oxygenation, CXR score and fluid balance.

		$ELWI_{UNadj}$	$ELWI_{ANadj}$	$ELWI_{SEGcorr}$	n	r_{max} v r_{min}^A	Pairwise comparisons ^A		
							r_{UNadj} v r_{ANadj}	r_{ANadj} v $r_{SEGcorr}$	$r_{SEGcorr}$ v r_{UNadj}
Pooled analysis (Spearman)									
PaO_2/FiO_2	r	-0.52	-0.36	0.04	62	z -4.35	-2.71	-4.41	-4.35
	p	<0.01	<0.01	0.75		p <0.01	<0.01	<0.01	<0.01
CXR score	r	0.51	0.59	0.40	19	z 1.13	-	-	-
	p	0.03	<0.01	0.09		p 0.26	-	-	-
Fluid balance	r	-0.20	-0.11	0.11	56	z -	-	-	-
	p	0.15	0.44	0.41		p -	-	-	-
Within-subject analysis (ANCOVA)									
PaO_2/FiO_2	r	-0.42	-0.40	-0.41	62	z -1.64	-	-	-
	p	<0.01	<0.01	<0.01		p 0.10	-	-	-
CXR score	r	0.57	0.57	0.64	19	z -1.24	-	-	-
	p	0.06	0.06	0.03		p 0.21	-	-	-
Fluid balance	r	-0.19	-0.20	0.18	56	z -	-	-	-
	p	0.19	0.17	0.21		p -	-	-	-

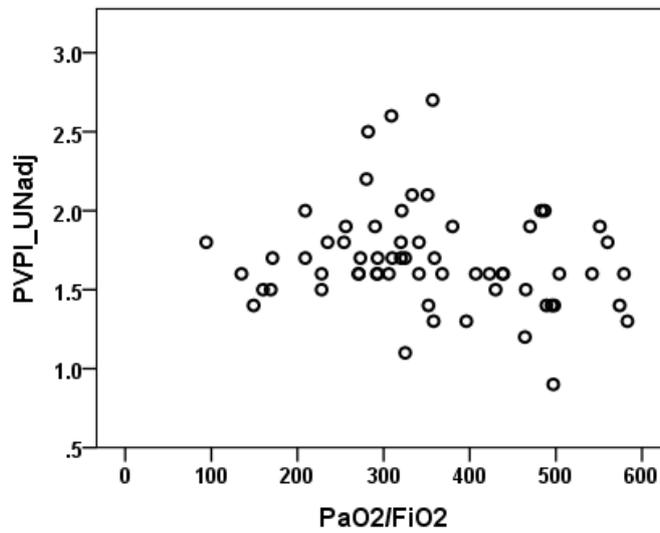
UNadj, unadjusted result; ANadj, ITBV/GEDV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no. of pulmonary segments remaining. n , number of comparisons from which result derived (N=8 patients). ^Ap-value by Steiger's method following Fisher's r-to-z transformation⁵²⁹.

5.9.4.2 Cross-sectional construct validity of PVPI

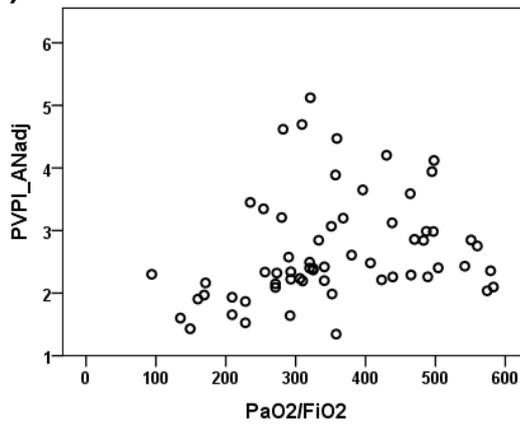
Association between PVPI and PaO₂/FiO₂

There were 62 paired ELWI and PaO₂/FiO₂ results available for analysis. For both PVPI_{ANadj} and PVPI_{SEGcorr}, there was a moderate positive association with PaO₂/FiO₂ which was supported by within-subject analysis (Figures 5.24b & c, Table 5.18). Conversely, there was a moderate (though not statistically significant, p=0.08) negative association between PVPI_{UNadj} and PaO₂/FiO₂ which was strengthened (and became statistically significant) on within-subject analysis (Figure 5.24a and Table 5.18). The observed differences in correlation coefficient between ELWI_{ANadj} and ELWI_{SEGcorr} vs ELWI_{UNadj} were significant in both pooled and within-subject analysis (Table 5.18).

a)



b)



c)

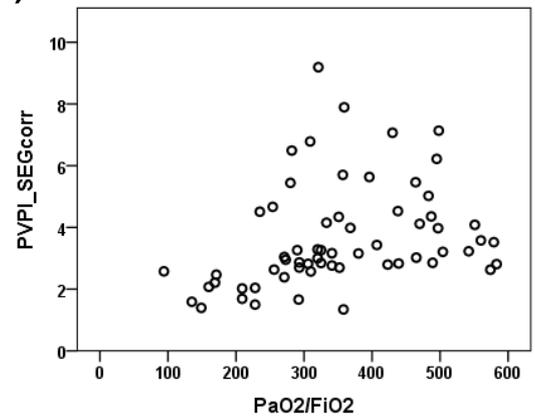


Figure 5.24. Pulmonary vascular permeability index (PVPI) versus $\text{PaO}_2/\text{FiO}_2$.

a) UNadj, unadjusted result, $r=-0.23$, $p<0.08$. b) ANadj, ITBV/GEDV adjusted by anatomical 'segment counting approach', $r=0.39$, $p<0.01$. c) SEGcorr, result corrected to reflect no. of pulmonary segments remaining, $r=0.47$, $p<0.01$. Spearman correlation, $N=8$, $n=62$ for all.

Association between PVPI and CXR score

There were 19 paired ELWI results and CXR scores available for analysis. There was no association between PVPI values and CXR scores in either pooled or within-subject analyses (Figures 5.25a-c and Table 5.18).

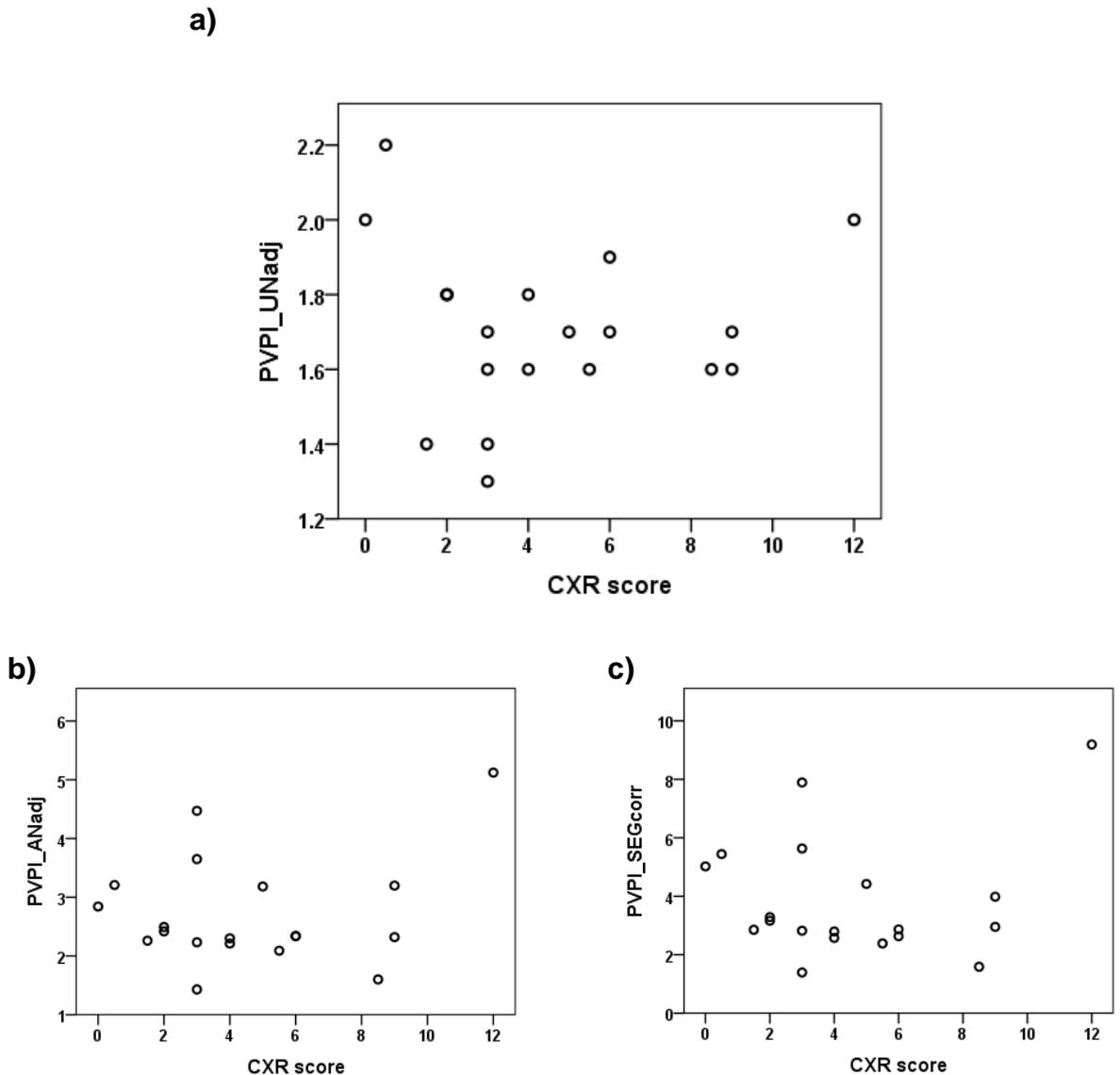


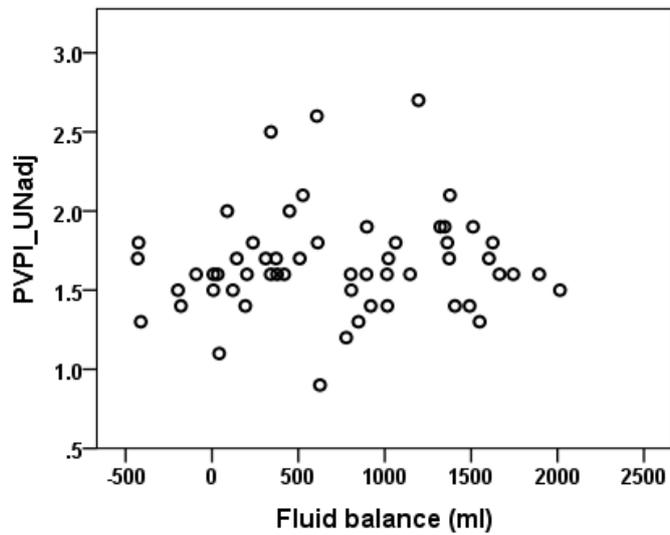
Figure 5.25. Pulmonary vascular permeability index (PVPI) versus CXR score.

a) UNadj, unadjusted result, $r=-0.08$, $p=0.74$. b) ANadj, ITBV/GEDV adjusted by 'anatomical' approach, $r=-0.06$, $p=0.82$. c) SEGcorr, result corrected to reflect no. of pulmonary segments remaining, $r=-0.16$, $p=0.50$. Spearman correlation, $N=8$, $n=19$ for all.

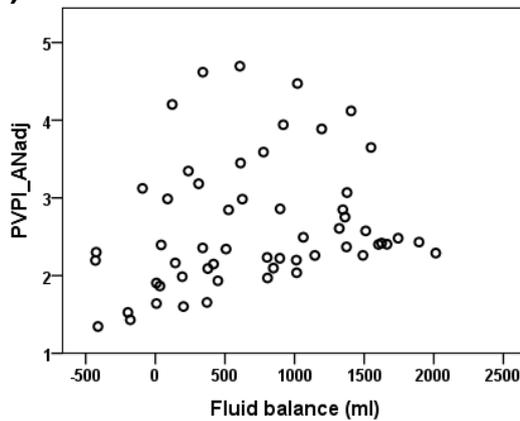
Association between PVPI and post-operative fluid balance

There were 56 paired ELWI results and post-operative fluid balance values available for analysis. Whilst there was no association between $PVPI_{UNadj}$ and post-operative fluid balance, on pooled analysis there was a moderate positive association between both $PVPI_{ANadj}$ and $PVPI_{SEGcorr}$ and post-operative fluid balance. There was no association between any PVPI parameter and fluid balance on within-subject analysis.

a)



b)



c)

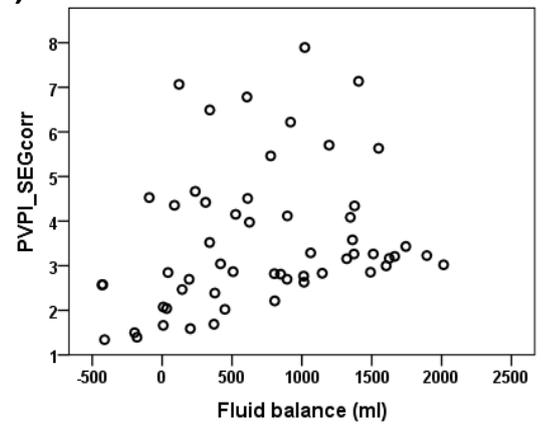


Figure 5.26. Pulmonary vascular permeability index (PVPI) versus fluid balance.

a) UNadj, unadjusted result, $r=0.11$, $p=0.44$. b) ANadj, ITBV/GEDV adjusted by 'anatomical approach', $r=0.36$, $p<0.01$. c) SEGcorr, result 'corrected' to reflect no. of pulmonary segments remaining, $r=0.38$, $p<0.01$. Spearman correlation, $N=8$, $n=56$ for all.

Table 5.18. Cross-sectional construct validity of PVPI following lung resections: Association between post-operative ELWI values and oxygenation, CXR score and fluid balance.

		<i>PVPI</i> _{UNadj}	<i>PVPI</i> _{ANadj}	<i>PVPI</i> _{SEgcorr}	<i>n</i>	<i>r</i> _{max} v <i>r</i> _{min} ^A		<i>Pairwise comparisons</i> ^A		
								<i>r</i> _{UNadj} v <i>r</i> _{ANadj}	<i>r</i> _{ANadj} v <i>r</i> _{SEgcorr}	<i>r</i> _{SEgcorr} v <i>r</i> _{UNadj}
Pooled analysis (Spearman)										
<i>PaO₂/FiO₂</i>	<i>r</i>	-0.23	0.39	0.47	62	<i>Z</i>	-5.02	-4.52	-2.82	-5.02
	<i>p</i>	0.08	<0.01	<0.01		<i>p</i>	<0.01	<0.01	<0.01	<0.01
<i>CXR score</i>	<i>r</i>	-0.08	-0.06	-0.16	19	<i>Z</i>	-	-	-	-
	<i>p</i>	0.74	0.82	0.50		<i>p</i>	-	-	-	-
<i>Fluid balance</i>	<i>r</i>	0.11	0.36	0.38	56	<i>Z</i>	-1.80	-	-	-
	<i>p</i>	0.44	<0.01	<0.01		<i>p</i>	0.07	-	-	-
Within-subject analysis (ANCOVA)										
<i>PaO₂/FiO₂</i>	<i>r</i>	-0.49	0.46	0.46	62	<i>Z</i>	-12.98	-12.98	-0.18	-12.06
	<i>p</i>	<0.01	<0.01	<0.01		<i>p</i>	<0.01	<0.01	0.86	<0.01
<i>CXR score</i>	<i>r</i>	0.30	0.43	0.35	19	<i>Z</i>	-	-	-	-
	<i>p</i>	0.34	0.17	0.28		<i>p</i>	-	-	-	-
<i>Fluid balance</i>	<i>r</i>	0.13	0.12	0.06	56	<i>Z</i>	-	-	-	-
	<i>p</i>	0.38	0.43	0.70		<i>p</i>	-	-	-	-

UNadj, unadjusted result; ANadj, GEDV/ITBV adjusted by 'anatomical approach'; SEgcorr, result corrected to reflect no. of pulmonary segments remaining. *n*, number of comparisons from which result derived (N=8 patients). ^A*p*-value by Steiger's method following Fisher's r-to-z transformation⁵²⁹.

5.9.4.3 Longitudinal construct validity of ELWI

Concordance between contemporaneous changes in ELWI and PaO₂/FiO₂

There were 54 paired sequential changes in ELWI and PaO₂/FiO₂ available for analysis. Four quadrant analysis of $\Delta\text{EVLW}_{\text{UNadj}}$ and $\Delta\text{PaO}_2/\text{FiO}_2$ revealed a weak negative association between the parameters ($r=-0.28$, $p=0.04$) (Figure 5.27a). An exclusion zone of $\Delta\text{EVLW}_{\text{UNadj}} \pm 11.5\%$ (equivalent to the LSC value for EVLW), was applied to the data such that only data points demonstrating $\Delta\text{EVLW}_{\text{UNadj}}$ greater than 11.5% in either direction were included in the concordance analysis. The concordance rate for a change in $\Delta\text{EVLW}_{\text{UNadj}}$ of greater than 11.5% to be accompanied by an opposing change in $\Delta\text{PaO}_2/\text{FiO}_2$ (of any magnitude) was 64% (Figure 5.27a). The same methodology was applied to comparisons of $\Delta\text{ELWI}_{\text{EDadj}}$, $\Delta\text{ELWI}_{\text{ANadj}}$ and $\Delta\text{ELWI}_{\text{SEGcorr}}$ versus $\Delta\text{PaO}_2/\text{FiO}_2$. There was a similar degree of association and concordance between $\Delta\text{ELWI}_{\text{ANadj}}$ and $\Delta\text{ELWI}_{\text{SEGcorr}}$ and $\Delta\text{PaO}_2/\text{FiO}_2$ as with $\Delta\text{EVLW}_{\text{UNadj}}$ and $\Delta\text{PaO}_2/\text{FiO}_2$, with no statistically significant difference observed between the performance of any of the parameters (Figures 5.27a-c, Table 5.19). The observed negative association between ΔELWI and $\Delta\text{PaO}_2/\text{FiO}_2$ was supported by with-in subject analysis (Table 5.19).

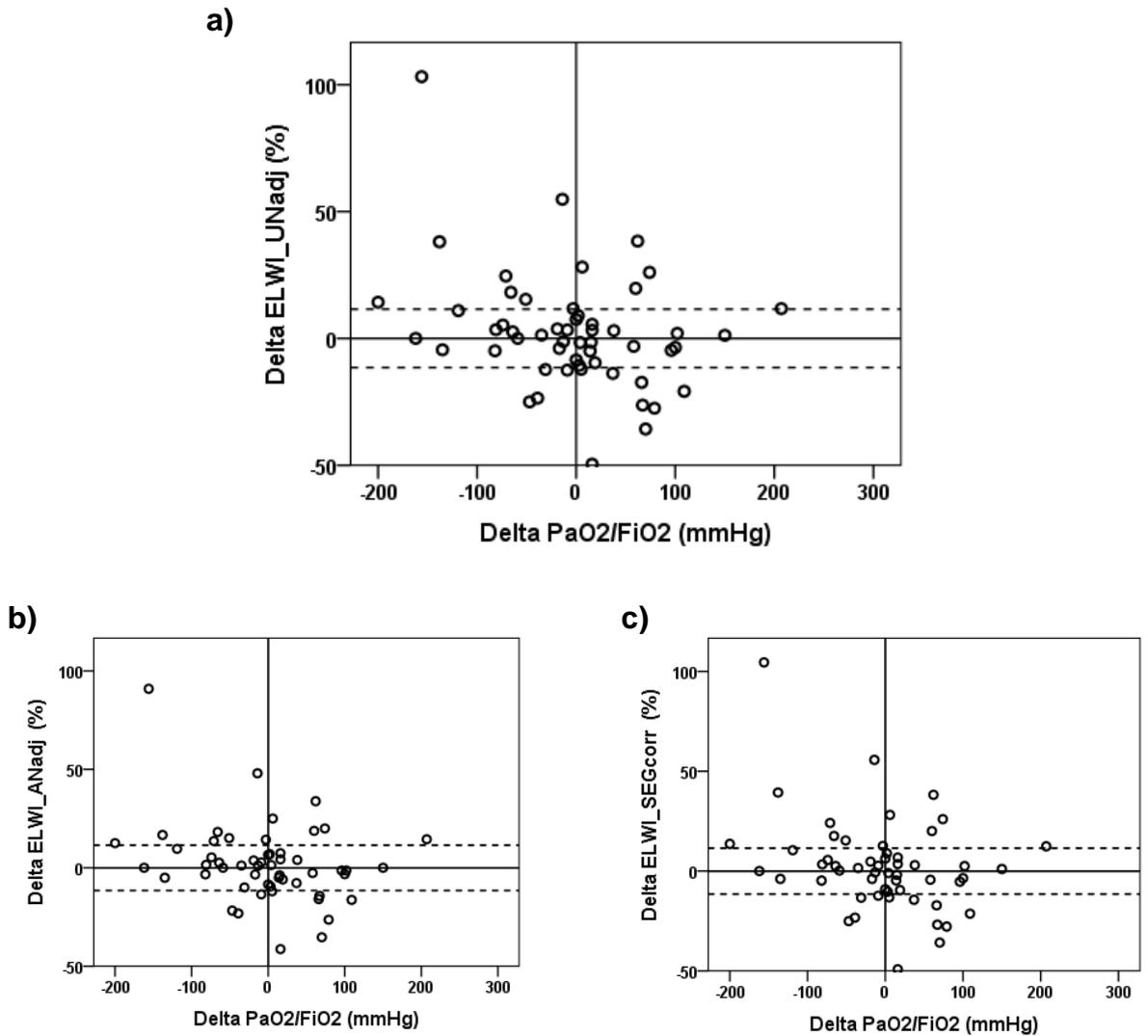


Figure 5.27. Four quadrant plot of changes in ELWI against corresponding changes in $\text{PaO}_2/\text{FiO}_2$.

a) UNadj, unadjusted result, $r=-0.28$, $p=0.04$, concordance=64%. b) ANadj, ITBV/GEDV adjusted by 'anatomical' segment counting approach, $r=-0.25$, $p=0.07$, concordance=65%. c) SEGcorr, result corrected by no. of pulmonary segments remaining, $r=-0.29$, $p=0.03$, concordance=64%. Spearman correlation. Quadrants of agreement defined as top left and bottom right. Data falling in dashed area (corresponding to ΔELWI less than $\pm 11.5\%$) not included in the concordance analysis. $N=8$, $n=54$.

Concordance between contemporaneous changes in ELWI and CXR score

There were 11 paired sequential changes in ELWI score and post-operative CXR score available for analysis. There was no significant association observed between ΔELWI and ΔCXR score for any ELWI parameter on pooled analysis (Figures 5.28a-c, Table 5.19); due to small numbers, with-in patient analysis could not be performed. Most of the returned data fell within the $\pm 11.5\%$ exclusion zone meaning concordance analyses were based on just 3-4 data points and so should be interpreted with caution (Figs 5.28a-c, Table 5.19).

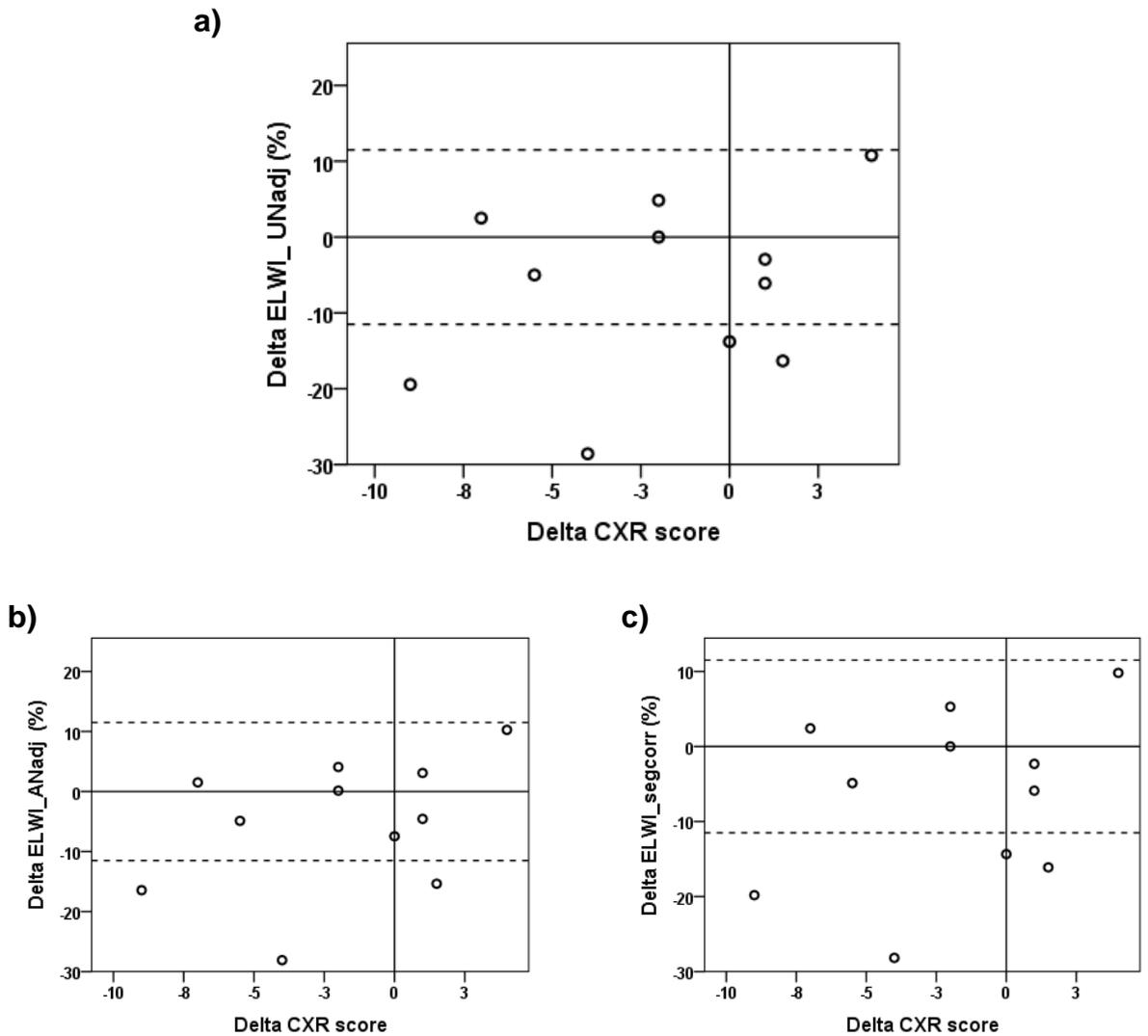


Figure 5.28. Four quadrant plot of changes in ELWI against corresponding changes in CXR score.

a) UNadj, unadjusted result, $r=0.24$, $p=0.48$, concordance=50%. b) ANadj, GEDV/ITBV adjusted by 'anatomical' approach, $r=0.37$, $p=0.26$, concordance=67%. c) SEGcorr, result corrected by no. of pulmonary segments remaining, $r=0.26$, $p=0.48$, concordance=50%. Spearman correlation. Quadrants of agreement defined as top right and bottom left. Data falling in dashed area (corresponding to ΔEVLW less than $\pm 11.5\%$) not included in the concordance analysis. $N=8$, $n=11$.

Concordance between contemporaneous changes in ELWI and fluid balance

There were 49 paired sequential changes in ELWI and post-operative fluid balance available for analysis. There was no significant association observed between Δ ELWI and Δ Fluid balance score for any ELWI parameter, either on pooled or within subject analysis (Figures 5.29a-c, Table 5.19). Concordance between Δ ELWI and Δ Fluid balance was low at 52-57%.

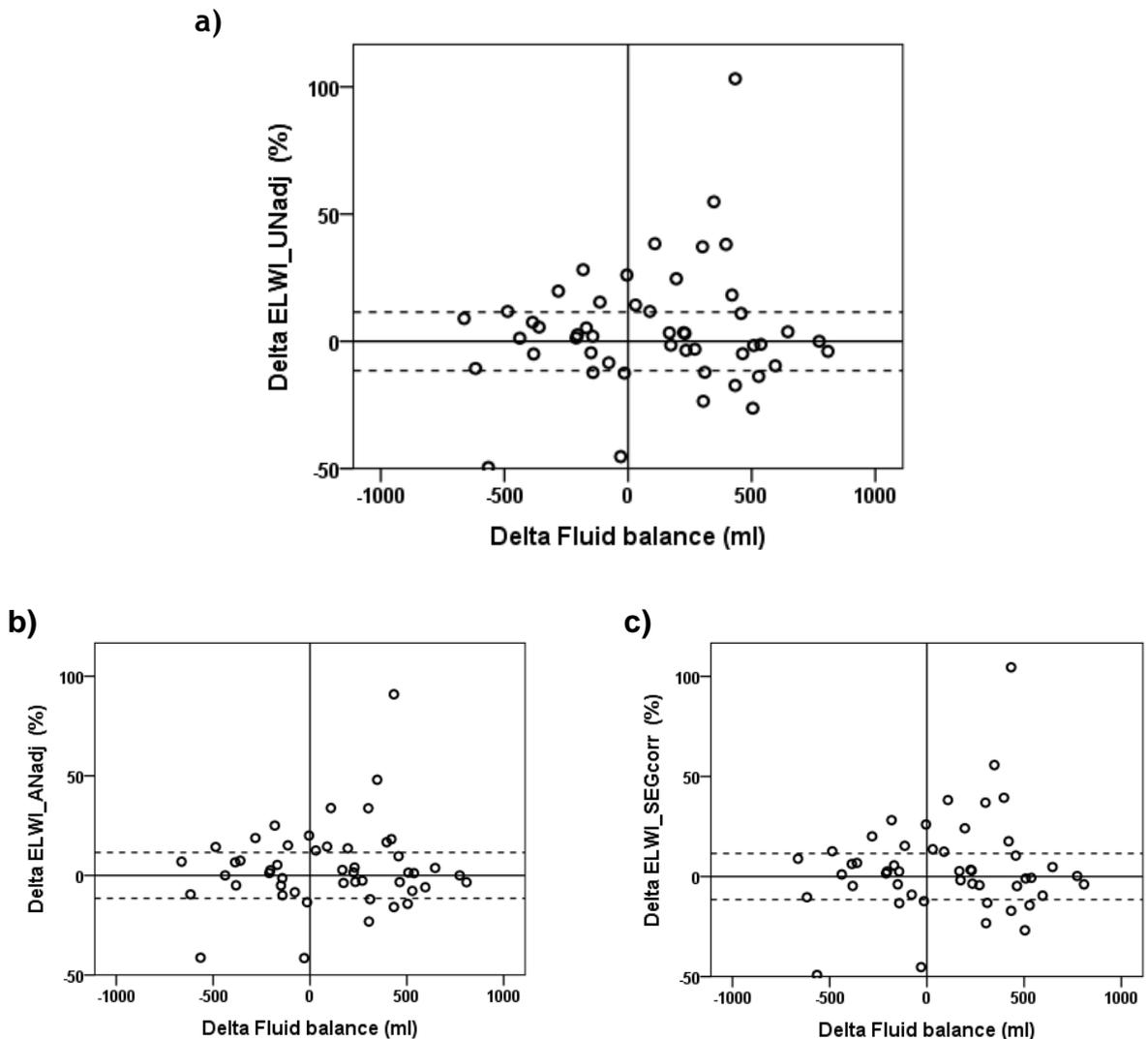


Figure 5.29. Four quadrant plot of changes in ELWI against corresponding changes in fluid balance.

a) UNadj, unadjusted result, $r=-0.08$, $p=0.58$, concordance=57%. b) ANadj, ITBV/GEDV adjusted by 'anatomical' approach, $r=-0.05$, $p=0.72$, concordance=52%. c) SEGcorr, result corrected by no. of pulmonary segments remaining, $r=-0.08$, $p=0.57$, concordance=56%. Spearman correlation. Quadrants of agreement defined as top right and bottom left. Data falling in dashed area (corresponding to Δ EVLW less than +/-11.5%) not included in the concordance analysis. N=8, n=49.

Table 5.19 Longitudinal construct validity of ELWI following lung resection: Association and concordance between changes in post-operative ELWI values and corresponding changes in oxygenation, CXR score and fluid balance.

		$\Delta ELWI_{UNadj}(\%)$	$\Delta ELWI_{ANadj}(\%)$	$\Delta ELWI_{SEGcorr}(\%)$	n	r_{max} v r_{min}^A	Pairwise comparisons (p for difference) ^A			
							r_{UNadj} v r_{ANadj}	r_{ANadj} v $r_{SEGcorr}$	$r_{SEGcorr}$ v r_{UNadj}	
Pooled analysis (Spearman)										
$\Delta PaO_2/FiO_2$	r	-0.29	-0.25	-0.29	54	z	1.848	-	-	-
	p	0.03	0.07	0.03		p	0.06			
	C	64%	65%	64%						
ΔCXR score	r	0.24	0.37	0.24	11	z	-	-	-	-
	p	0.48	0.26	0.48		p				
	C	50%	67%	50%						
$\Delta Fluid$ balance	r	-0.08	-0.05	-0.08	49	z	-	-	-	-
	p	0.58	0.72	0.57		p				
	C	57%	52%	56%						
Within-subject analysis (ANCOVA)										
$\Delta PaO_2/FiO_2$	r	-0.29	-0.25	-0.30	54	z	-1.842	-	-	-
	p	0.04	0.08	0.04		p	0.07			
ΔCXR score	r	-	-	-	11 ^B	z	-	-	-	-
	p					p				
$\Delta Fluid$ balance	r	0.14	0.15	0.14	49	z	-	-	-	-
	p	0.36	0.35	0.36		p				

UNadj, unadjusted result; ANadj, GEDV/ITBV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no. of pulmonary segments remaining. C, concordance (%). ^Ap-value by Steiger's method following Fisher's r-to-z transformation⁵²⁹. ^BWithin-patient analysis not possible due to small numbers.

5.9.4.4 Longitudinal construct validity of PVPI

Concordance between contemporaneous changes in PVPI and PaO₂/FiO₂

There were 54 paired sequential changes in PVPI and PaO₂/FiO₂ available for analysis. There was a similar degree of association between $\Delta PVPI_{UNadj}$, $\Delta PVPI_{ANadj}$ and $\Delta PVPI_{SEGcorr}$ and $\Delta PaO_2/FiO_2$, with no statistically significant difference observed between the performance of any of the parameters (Figures 5.30a-c, Table 5.20). Applying an exclusion zone of +/-17.7% (equivalent to the LSC value of PVPI), and excluding $\Delta PVPI$ values falling within this zone from analysis, the concordance between $\Delta PVPI$ values and $\Delta PaO_2/FiO_2$ was substantial at 65-81%. The observed negative association between $\Delta PVPI$ values and $\Delta PaO_2/FiO_2$ was supported by with-in subject analysis (Table 5.20).

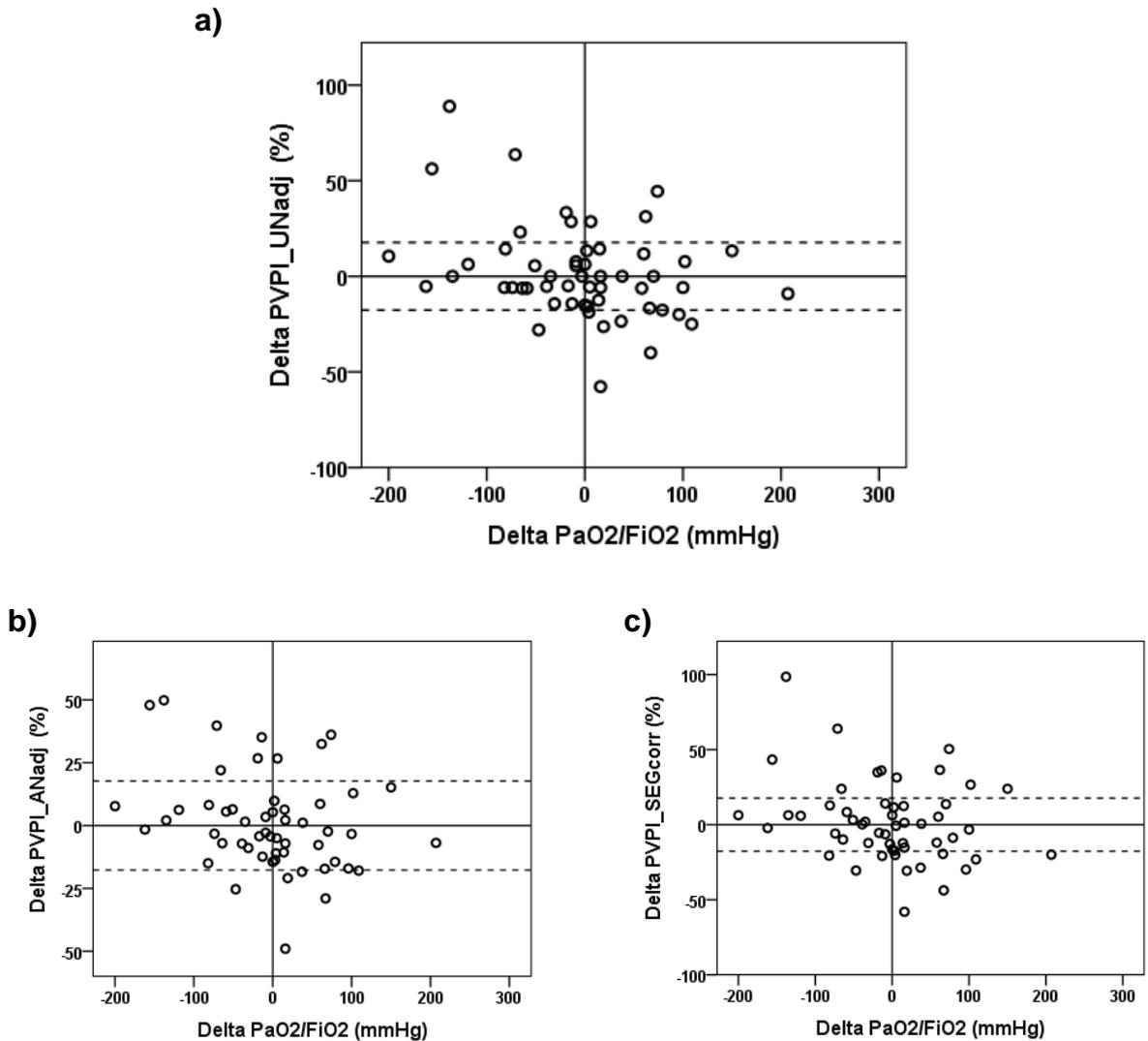


Figure 5.30. Four quadrant plot of changes in PVPI against corresponding changes in PaO₂/FiO₂.

a) UNadj, unadjusted result, $r=-0.30$, $p=0.03$, concordance=76%. b) ANadj, ITBV/GEDV adjusted by 'anatomical' segment counting approach, $r=-0.28$, $p=0.04$, concordance=81%. c) SEGcorr, result corrected by no. of pulmonary segments remaining, $r=-0.27$, $p=0.05$, concordance=65%. Spearman's rho. Quadrants of agreement defined as top left and bottom right. Data falling in dashed area (corresponding to Δ PVPI less than $\pm 17.7\%$) not included in the concordance analysis. N=8, n=54.

Concordance between contemporaneous changes in PVPI and chest X-ray score

There were 11 paired sequential changes in PVPI score and post-operative CXR score available for analysis. There was no significant association observed between Δ PVPI and Δ CXR score for any PVPI parameter on pooled (Figures 5.31a-c, Table 5.20); due to small numbers, with-in patient analysis could not be performed. All but two data points fell within the $\pm 17.7\%$ exclusion zone meaning concordance analysis could not be performed (Figures 5.31a-c, Table 5.24).

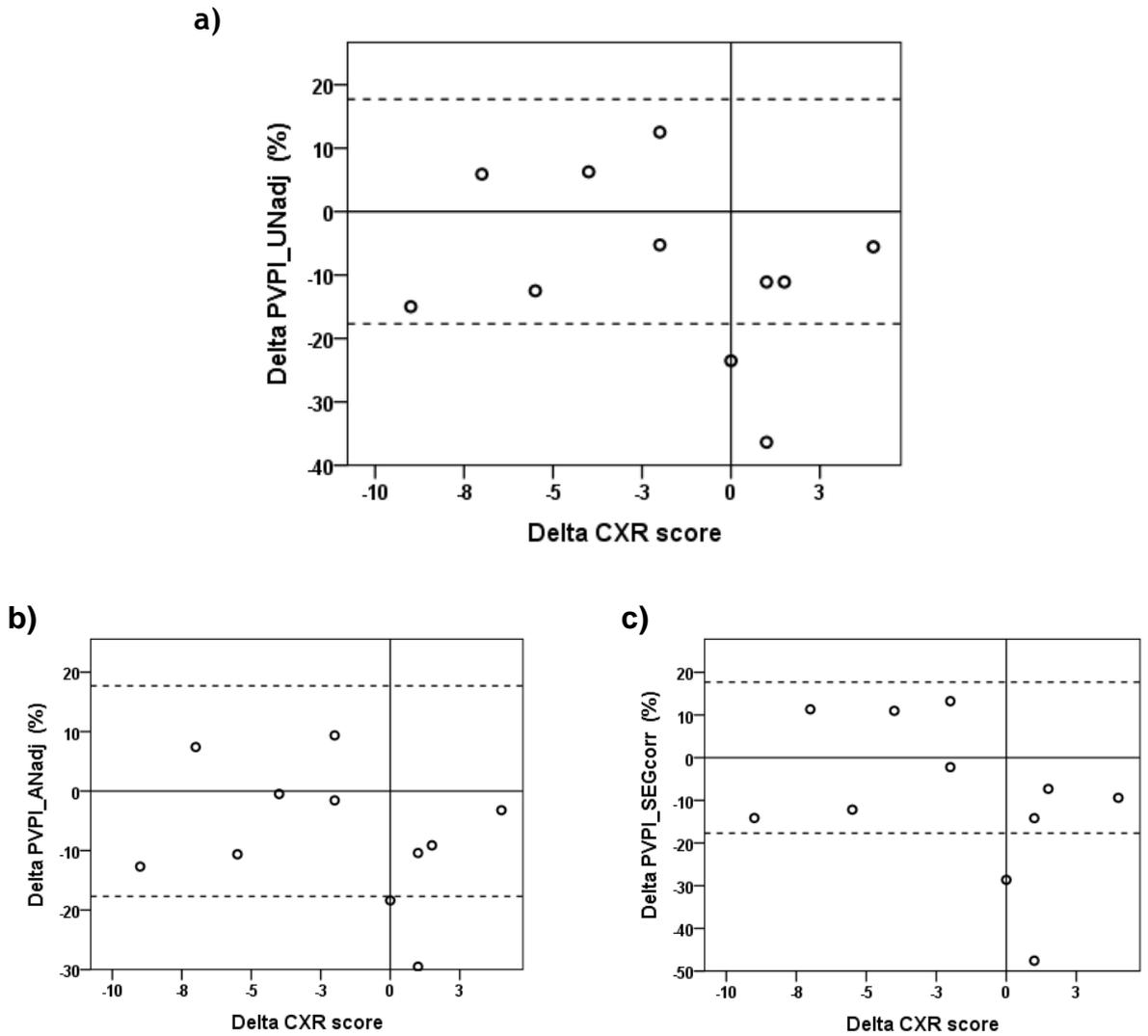


Figure 5.31. Four quadrant plot of changes in PVPI against corresponding changes in CXR score.

a) UNadj, unadjusted result, $r=-0.16$, $p=0.64$. b) ANadj, GEDV/ITBV adjusted by 'anatomical' segment counting approach, $r=-0.17$, $p=0.62$. c) SEGcorr, result corrected by no. of pulmonary segments remaining, $r=-0.30$, $p=0.38$. Spearman's rho. Data area corresponds to ΔEVLW less than $\pm 17.7\%$ (LSC value for PVPI). No direction of change analysis performed. $N=8$, $n=11$.

Contemporaneous changes in PVPI and fluid balance

There were 49 paired sequential changes in PVPI and post-operative fluid balance available for analysis. There was no significant association observed between Δ PVPI and Δ Fluid balance score for any PVPI parameter, either on pooled or within-subject analysis (Figures 5.32a-c, Table 5.20). No concordance analysis between Δ PVPI and Δ Fluid balance was performed in light of the hypothesis that PVPI would not be associated with fluid balance.

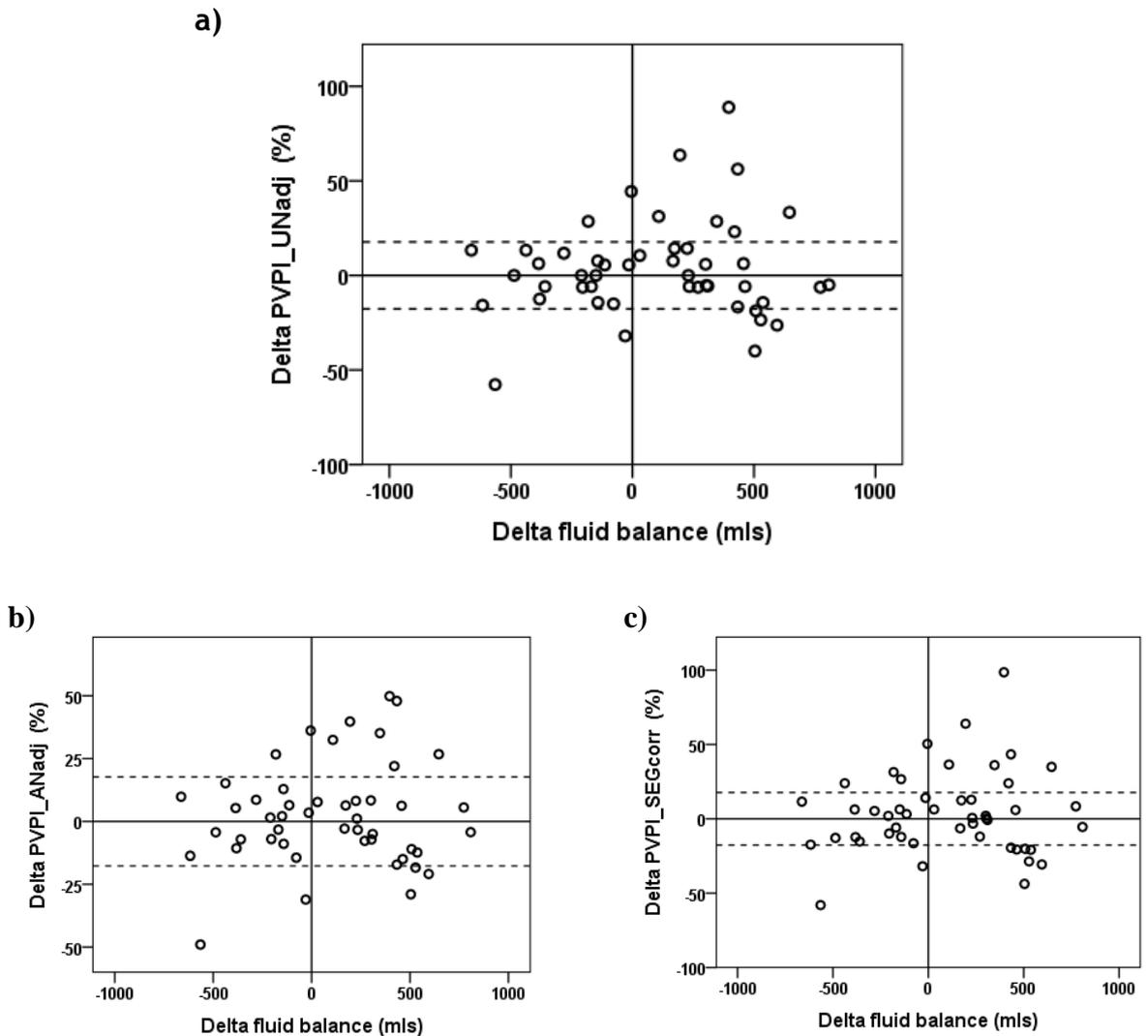


Figure 5.32. Four quadrant plot of changes in PVPI against corresponding changes in fluid balance.

a) UNadj, unadjusted result, $r=-0.06$, $p=0.70$. b) ANadj, ITBV/GEDV adjusted by 'anatomical' segment counting approach, $r=-0.05$, $p=0.73$. c) SEGcorr, result corrected by no. of pulmonary segments remaining, $r=-0.03$, $p=0.82$. Spearman's rho. Data falling in dashed area corresponds to Δ PVPI less than $\pm 17.7\%$ (LSC value for PVPI). $N=8$, $n=48$.

Table 5.20 Longitudinal construct validity of PVPI following lung resection: Association and concordance between change in post-operative PVPI values and corresponding changes in oxygenation, CXR score and fluid balance.

		$\Delta PVPI_{UNadj}(\%)$	$\Delta PVPI_{ANadj}(\%)$	$\Delta PVPI_{SEGcorr}(\%)$	n	r_{max} v r_{min}^A	<i>Pairwise comparisons (p for difference)^A</i>			
							r_{UNadj} v r_{ANadj}	r_{ANadj} v $r_{SEGcorr}$	$r_{SEGcorr}$ v r_{UNadj}	
Pooled analysis (Spearman)										
$\Delta PaO_2/FiO_2$	r	-0.30	-0.28	-0.25	54	z	0.956	-	-	-
	p	0.03	0.04	0.07		p	0.34			
	C	76%	81%	65%						
ΔCXR score	r	-0.16	-0.17	-0.30	11	z	-	-	-	-
	p	0.64	0.62	0.38		p				
	C	-	-	-						
Δ Fluid balance	r	-0.06	-0.05	-0.03	48	z	-	-	-	-
	p	0.70	0.73	0.82		p				
	C	-	-	-						
Within-subject analysis (ANCOVA)										
$\Delta PaO_2/FiO_2$	r	-0.30	-0.25	-0.25	54	z	1.54	-	-	-
	p	0.04	0.09	0.08		p	0.12			
ΔCXR score	r	-	-	-	11 ^B	z	-	-	-	-
	p					p				
Δ Fluid balance	r	0.13	0.14	0.12	48	z	-	-	-	-
	p	0.40	0.37	0.46		p				

UNadj, unadjusted result; ANadj, GEDV/ITBV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no. of pulmonary segments remaining. C, concordance (%). ^Ap-value by Steiger's method following Fisher's r-to-z transformation⁵²⁹. ^BWithin-patient analysis not possible due to small numbers.

5.9.5 Summary of results: Construct validity of ELWI and PVPI following lung resection

In an attempt to provide a summary of the multiple comparisons between ELWI and PVPI and the constructs PaO₂/FiO₂, CXR score and fluid balance made both contemporaneously (cross-sectional analysis) and longitudinally, Table 5.21 was constructed. In this table which seeks to present a point of reference from which the presence or absence of construct validity can be determined, the results of the comparisons are graded according to what extent the results support the concept of construct validity:

- ++** Consistent relationship (or lack of) demonstrated, consistent with hypotheses, within-patient analysis supports pooled.
- +** Relationship (or lack of) demonstrated, consistent with hypotheses, but is either inconsistent, or results of within-patient and pooled analyses are not consistent.
- No relationship demonstrated (or relationship demonstrated where not hypothesised), against hypothesis.
- Consistent relationship demonstrated, with-in patient analysis supports pooled, NOT consistent with hypotheses.

Table 5.21. Summary of results: Construct validity of ELWI and PVPI after lung resection.

	ELWI _{UNadj}		ELWI _{ANadj}		ELWI _{SEGcorr}	
	<i>Cross.</i>	<i>Long.</i>	<i>Cross.</i>	<i>Long.</i>	<i>Cross.</i>	<i>Long.</i>
<i>PaO₂/FiO₂</i>	++	++	++	++	+	++
<i>CXR score</i>	++	-	++	-	++	-
<i>Fluid balance</i>	-	-	-	-	-	-
	PVPI _{UNadj}		PVPI _{ANadj}		PVPI _{SEGcorr}	
	<i>Cross.</i>	<i>Long.</i>	<i>Cross.</i>	<i>Long.</i>	<i>Cross.</i>	<i>Long.</i>
<i>PaO₂/FiO₂</i>	++	++	--	++	--	++
<i>CXR score</i>	-	-	-	-	-	-
<i>Fluid balance</i>	++	++	++	++	++	++

UNadj, unadjusted result; ANadj, GEDV/ITBV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no of pulmonary segments remaining. For explanation of symbols see text. Cross, cross-sectional construct validity; Long, longitudinal construct validity.

5.10 Discussion

5.10.1 *Changes in ELWI and PVPI following lung resection*

There were no significant immediate post-operative changes in $ELWI_{UNadj}$ or $ELWI_{ANadj}$. In contrast, $ELWI_{SEGcorr}$ was significantly higher immediately post-operatively than pre-operatively, a finding which ‘feels right’ intuitively in the context of the discussion of ‘*what might be expected to occur following lung resection?*’ provided in Section 5.7.3.1. The observed $ELWI_{UNadj}$ values are in keeping with those reported by others, for a group primarily composed of patients undergoing lobectomy (Table 5.11)^{235, 245, 511}.

$PVPI_{UNadj}$ fell immediately post-operatively whilst $PVPI_{ANadj}$ and $PVPI_{SEGcorr}$ were increased. Again, the changes in adjusted values ‘feel right’; it is hard to conceive that undergoing lung resection with one lung ventilation would lead to reduced pulmonary vascular permeability. Such a finding would be in contrast with the observations of Waller et al, who used lung scintigraphy (as close to a ‘gold standard’ permeability measure as exists), to demonstrate increased pulmonary vascular permeability in 9 of 10 patients undergoing pneumonectomy and 6 of 11 patients undergoing lobectomy¹²⁵. It must be emphasised that the comparisons made pre- versus post- operatively were made in ventilated versus spontaneously breathing patients, compromising their reliability. Relative to observations made during mechanical ventilation, observations made during spontaneous breathing would be anticipated to underestimate ELWI (Section 5.7.1.3), as such all values perhaps under represent the (hypothesised) post-operative rise in ELWI per unit of lung tissue.

All post-operative observations were made in spontaneously breathing patients. There were no significant changes in median ELWI (adjusted or unadjusted) compared to immediate post-operative baseline values throughout the monitored period. Similarly, besides a fall in $PVPI_{UNadj}$ 6 hours post-operatively there were no other significant changes in median PVPI (adjusted or unadjusted). Such observations are not however at odds with the suggestion that TPTD might be a useful monitor in this post-operative population. Arguably the purpose of the monitor should be to identify the individuals, in whom (clinically)

significant changes do occur. Inspection of Figure 5.18 suggests there were marked increases in ELWI and PVPI during the monitored period in two individuals patients within the 8 patient cohort.

5.10.2 Feasibility

In general TPTD monitoring was well tolerated by patients and appears feasible in the early post-operative period following lung resection. The femoral arterial catheters appeared not to cause any discomfort and did not impair mobilisation. Interruption of the power supply or disconnection of the EV1000 monitor unit from the arterial cannula unfortunately necessitates re-calibration by triplicate thermodilution injection as the monitor has no battery nor ‘hot-start’ function. One patient suffered a femoral haematoma following unsuccessful placement of the arterial cannula which was subsequently placed without event on the contralateral side. The disconnection experienced in patient 9 was unfortunately the result of user error and a failure to check the security of the Luer-Lok connection. The author (B. Shelley) has since been informed by the manufactures that the ‘VolumeView thermistor manifold’ (which became disconnected from the central venous cannula) is available as a additional ‘spare’ (person communication, Jane Wylie, Account Manager - Scotland and NE, Critical Care, Edwards Lifesciences); had this not been the last monitoring set in the hospital then the disconnection would not have caused any problems.

5.10.3 Methodology

Before considering the results of the current study, it is important first to pay some attention to the methodology. In the opinion of the author (B. Shelley), ‘construct validity’, is seldom discussed but often implied within the medical literature. All of the studies in Table 5.5, for example, make comparison between EVLW and some ‘construct’ perceived to be supportive of the supposition that EVLW is a measure of lung injury, but none explicitly describes construct validity in its methodology. It is arguable that to Sackett et al’s “conscious clinician” (Section 5.2.), who is mandated to make some assessment of the usefulness of the diagnostic criteria³⁶⁴, the observation of association with

such ‘constructs’ is a powerful driver in the process of establishing ‘face validity’.

To offer some endorsement of the use of construct methodology to validate tests where no ‘gold standard’ exists, the example quoted in the text of Ely et al seeking to validate the Richmond Agitation-Sedation Scale (RASS)³⁷¹, was published in the *Journal of the American Medical Association (JAMA)*’ in 2003 and has since received 377 citations (Web of Science® ‘times cited’ at the time of writing (18th October 2014)). Seeking to compare diagnostic tests by comparing the strength of association between constructs is a further extension of the ‘construct validity’ methodology, but is not without precedent. The widespread acceptance that EVLW should be indexed to predicted rather than actual body weight stems from the observations of Craig et al and Philips et al, who observed that the predictive validity of EVLW measurement (for mortality) is improved when EVLW is indexed for predicted body weight (PBW)^{405, 429}. Similarly, Berkowitz et al observed that indexing EVLW to PBW resulted in a stronger correlation with Lung Injury Score and PaO₂:FiO₂ ratio⁴³³.

The testing of statistical significance between the corresponding correlation coefficients (as performed in this study) is not generally pursued. To this end the author (B. Shelley) is grateful for the advice of Dr Malachy Coulomb, Consultant in Anaesthesia and Intensive Care Medicine at the University Hospital of South Manchester, and statistical advisor to the *British Journal of Anaesthesia*. Dr Coulomb was in agreement with the author’s conclusion ; that the use of Fisher’s r-to-z transformation and comparison of the two correlation coefficients with greatest separation, followed by pairwise comparisons in the event of a significant result represented the most appropriate methodology in the absence of a suitable global test (analogous for example to one way analysis of variance across multiple groups before pairwise comparisons). It may have been equally valid however to make no statistical comparison “*and allow the correlation coefficients to stand for themselves*” (personal communication, Dr Malachy Coulomb, January 2013). It must be emphasised firstly, that no power analysis has been performed to assess the discriminatory ability of the sample size presented in distinguishing between the different adjustments when compared in this way, and secondly, multiple pairwise comparisons conducted in this way carry high risk of type I error.

It is undoubtedly a strength of this study that within-patient analysis of covariance was performed to ‘confirm’ the results of the pooled analysis. In almost all occasions this analysis was supportive of the pooled analyses, though on occasion the results were different, more often than not with the within-patient result appeared to correct a biologically ‘implausible’ pooled result. In such circumstances the question of ‘which result to believe?’ is pertinent. Bland et al suggest that the within subject analysis yields the ‘true’ result, citing an example in their manuscript where “*the correct analysis within-subjects reveals a relation which the incorrect [pooled] analysis misses*”⁵²⁶. The grading devised to summarise overall construct validity in Table 5.21, attempts to address this issue by awarding greater significance to relationships where the results of the pooled and within-patient analyses are consistent.

Finally (before discussion of the results), I shall discuss the methodology of the longitudinal analyses. Concordance analysis by the use of four-quadrant plots is classically described to compare cardiac output measurement between a novel monitor and a reference technique. In such a situation, it can be appreciated that the novel monitor, if valid, should provide identical results to the reference monitor and as such “*a concordance rate of >90% to 95% indicates reliable trending ability*”⁵²⁸. In the current investigation however, whilst it is desirable that changes in EVLW are mirrored (for example) by changes in oxygenation, concordance rates in excess of 90% are an unlikely finding, and should not be anticipated; there are of course many other causes of poor oxygenation following lung resection than pulmonary oedema. As such, concordance in excess of 50% (representing a 50:50 chance that the monitor mirrors the clinical change) are desirable, but it would require a more detailed study of the perceived clinical explanation for all changes in clinical parameters in order to identify a ‘target value’ representing ‘acceptable’ concordance.

5.10.4 Reproducibility

Holm et al describe that “*according to usual practice*” a coefficient of variation (CV) of less than 10% may be considered ‘good’; between 10 and 15% considered ‘acceptable’ and greater than 15% considered ‘poor’³⁷³. By this criteria, in the pooled analysis, the CV of all variables in the current study could be described

as 'good', with the possible exception of PVPI where the 95% confidence interval for the mean just exceeds 15% (Table 5.15). Though clinically acceptable, the CV values appear larger than those previously reported by Tagami et al for TPTD measurement in critically ill post-cardiac arrest patients³⁷⁴. There are a number of reasons why this might be the case. Firstly, Tagami et al used the PiCCO® system (Pulsion Medical Systems, Munich, Germany) whilst the EV1000 platform was used in this study. Bendjelid et al however have demonstrated equivalent reproducibility between the two devices in animals⁵²⁵. Secondly, all of the datasets subject to reproducibility analysis were obtained during conditions of spontaneous breathing; where the majority (if not all) of the measurements obtained in Tagami et al's study group will have been made during positive pressure ventilation. Using an early model 'lung water computer', Laggner et al reported that the CV for $EVLW_{TDD}$ measurement was higher in patients spontaneously breathing compared to those mechanically ventilated (16.1 vs 10.8%, $p < 0.05$)⁵³². These authors attribute the improved reproducibility seen in mechanically ventilated patients to greater "*stability of the thoracic cage*"⁵³². It is the authors' belief, that the increased CV seen in spontaneous breathing reflects the naturally varying respiratory pattern observable when spontaneously breathing, leading to variation in stroke volume between (and during) measurement sets. Furthermore, it is hypothesised that the effects of ventilation (spontaneous or mechanical) on stroke volume will be amplified following lung resection leading to further increases in variability between measurements. The results of the post-hoc analysis presented in Figure 5.20, appear to demonstrate that CV increases in proportion to the volume of lung resected. This is a novel and important finding. Whilst further research is required to provide a precise estimate of the CV in patients undergoing larger resections (bi-lobectomy and pneumonectomy), it must be appreciated that such increases in CV will lead to marked increases in the least significant change (LSC) value in these patients.

The LSC values obtained are perhaps the most useful outcome of the reproducibility study, allowing interpretation of changes in TPTD derived values in the context of whether they are likely to represent 'actual' change rather than measurement artefact. In the current cohort, of the 54, 6-hourly interval changes in ELWI and PVPI available for analysis, just 46% of the ELWI (Figure

5.27) and 31% of the PVPI changes (Figure 5.30) were greater than the corresponding LSC value, and so can be interpreted as 'true' changes.

5.10.5 Validity

5.10.5.1 Extravascular lung water index

Construct validity of unadjusted TPTD measurement of ELWI is suggested by the following observations:

- Negative association between ELWI and $\text{PaO}_2/\text{FiO}_2$
- Negative association between ΔELWI and $\Delta\text{PaO}_2/\text{FiO}_2$
- Positive association between ELWI and CXR score values

There was no association between ELWI and fluid balance, or between ELWI and longitudinal analysis of CXR scores. The lack of association with fluid balance is not surprising. Several authors have found no association between ELWI and fluid balance in critically ill patients many of whom had ALI/ARDS^{381, 428, 433}. In comparison to critically ill patients with ALI/ARDS, it is plausible to suggest that the patients in the current cohort might be expected to have relatively preserved alveolar-capillary membrane function. Furthermore, as it is routine practice in our institution to restrict fluids following lung resection, it would seem unlikely that fluid balance would have strayed into the range that would be required to influence ELWI. The lack of association between ΔELWI and ΔCXR score (and associated low concordance) seems more surprising, given the significant association between (ELWI and CXR score) though it must be appreciated that there were just 11 sequential ΔCXR results on which the analysis could be performed, and just four of these represented a change greater than the LSC value for ELWI (Figure 5.28).

Validity of 'anatomical adjustment' and 'segment correction' of ELWI

Adjustment of TPTD derived values by either manipulation of GEDV/ITBV ('anatomical' adjustment, ANadj) or indexing of the results to the number of pulmonary segments remaining (segment correction, SEGcorr) did not appear to improve the construct validity of ELWI. There were no significant differences seen in the level of association observed between unadjusted and adjusted ELWI and $\text{PaO}_2/\text{FiO}_2$ and CXR score.

There are a number of possible explanations for why adjustment of values did not improve construct validity:

1. *Type II error.* Potential for type II error in the comparisons between correlation coefficients is discussed above. Though the differences between r_{\max} and r_{\min} (of within-subject comparisons) occasionally demonstrated trends towards significance, consistency in the associations observed was striking, suggesting any clinical significance (in the face of statistical significance) may be negligible. For example, $ELWI_{UNadj}$ and $ELWI_{ANadj}$ were both significantly negatively associated with PaO_2/FiO_2 , $r=-0.42$ and -0.40 respectively, $p<0.01$ for both (Table 5.17). Following Fisher's r-to-z transformation, the p value when testing the null hypothesis that there is no difference between the two r-values observed, was 0.10. Had this p-value been 0.04, it would have been difficult to conclude that $ELWI_{UNadj}$ had greater construct validity than $ELWI_{ANadj}$ on the basis of a 0.02 change in r-value.
2. *The volume of lung resected was inadequate to invalidate unadjusted ELWI and PVPI values.* All of the animal work suggesting a reduction in PBV post-operatively and a consequent alteration in the GEDV/ITBV ratio, was performed in pneumonectomy models (Table 5.10)^{147, 411, 509}. As the current study cohort only included one patient undergoing pneumonectomy (and otherwise could be described as a lobectomy population), it is conceivable that post-operative changes in the GEDV/ITBV were insufficient to compromise the validity of unadjusted ELWI and PVPI measurement. Extrapolation of the results of this work to populations undergoing pneumonectomy should therefore be undertaken with caution.
3. *The GEDV/ITBV ratio does not change to the degree anticipated.* The hypothesis that pulmonary blood volume falls in direct proportion to the volume of lung resected may be an overestimation. Firstly, recruitment of previously hypo-perfused pulmonary vasculature may increase the 'capacity' of the pulmonary vasculature and so result in relative maintenance of PBV. This hypothesis is supported by the results of animal studies observing that PBV (determined by TDD) falls by 22-30% following pneumonectomy rather than the ~50% anticipated in the calculation of the 'anatomical

adjustment'^{411, 511}. In this context it must be appreciated that PBV determined by TDD is a 'theoretical volume' rather than an actual one, and may *not* therefore be solely determined (as might be expected) by the volume of blood in the pulmonary vasculature⁴⁹⁷.

Secondly, whilst 'true' GEDV ($GEDV_{ACTUAL}$) might be expected to increase following lung resection (Page 377), 'measured' GEDV decreases following lung resection, maintaining GEDV/ITBV ratio in the face of reduced pulmonary blood volume. In the current study, there was a small but non-significant reduction in median $GEDV_{STD}$ following lung resection (1075 vs 1041ml, $p=0.12$, Wilcoxon Signed Rank Test, data not shown), but this comparison is compromised by making comparison of invasively ventilated pre-operative values with spontaneously breathing post-operative estimates and the administration of intravenous fluids intra-operatively (both of which would be anticipated to increase $GEDV_{ACTUAL}$). In more controlled conditions, Schreiber et al observed a significant reduction in $GEDV_{STD}$ on occlusion of branch pulmonary arteries in ventilated swine⁴⁶². $GEDV_{STD}$ was reduced despite the observation of increased right ventricular end-diastolic volume; both changes reverted to baseline on removing the occlusion. Schreiber et al hypothesised this artefactual underestimation of $GEDV_{ACTUAL}$ resulted from errors in ITTV measurement due to increases in transit velocity (and hence reduction in mean transit time) through the reduced pulmonary vascular bed⁴⁶².

4. *Changes in the GEDV/ITBV occurring secondary to a reduction in PBV may be to an extent 'cancelled out' by the opposing change in the intercept value in the $ITBV=aGEDV+b$ regression equation.* As reported in Table 5.10, both Kirov et al⁴¹¹ and Roch et al⁵⁰⁹ used TDD to determine what changes occur to the relationship between ITBV and GEDV following pneumonectomy, observing the 'a' coefficient to fall, and the 'b' intercept to rise. Calculating ITBV post operatively based on an unchanged 'a' coefficient and ignoring any intercept value (as will be the case when determining $ELWI_{UNadj}$) will lead to inaccuracies that will in effect 'cancel out'.

5.10.5.2 Pulmonary Vascular Permeability Index

Construct validity of unadjusted TPTD measurement of PVPI is suggested by the following observations:

- Negative association between PVPI and $\text{PaO}_2/\text{FiO}_2$
- Negative association between ΔPVPI and $\Delta\text{PaO}_2/\text{FiO}_2$
- The absence of any association between PVPI and fluid balance

There was however no association observed between PVPI and CXR score (both contemporaneously and longitudinally). Though the number of comparisons involved in the CXR analyses was low (19 CXRs, 11 ΔCXR s), and this result could be the product of type II error, visual inspection of the scatter plots (Figures 5.25 and 5.31) does not reveal any 'signal'. Chew et al have reported a positive association between PVPI and chest X-ray score in intensive care patients with systemic inflammatory response syndrome and 'circulatory failure',⁴²³ whilst others have observed positive association between PVPIs and Lung Injury Score (of which chest x-ray scoring is a component)^{441, 496}. As has been highlighted previously however, in comparison to these critically ill patients with ALI/ARDS, the current cohort might be expected to have relatively preserved alveolar-capillary membrane function.

It is striking that in the analyses of ΔPVPI verses ΔCXR score, only two patients demonstrated changes in PVPI greater than the $\pm 17.7\%$ least significant change value; all other changes in PVPI could simply reflect measurement artefact (Figure 5.31). It seems likely therefore that the degree of pulmonary vascular permeability observed in the current cohort was insufficient to influence chest X-ray appearances. Such a supposition is supported by the concept of an 'oedema threshold'. It is well established that the relationship between pulmonary capillary hydrostatic pressure and pulmonary oedema formation is non-linear, such that below a certain threshold value, no pulmonary oedema formation is observed^{533, 534}. Furthermore, the position of such a threshold value is determined by pulmonary capillary membrane permeability, such that with increased permeability, pulmonary oedema will occur at a lower 'threshold pressure'⁵³⁵. Though pulmonary permeability may be 'sub clinically' elevated in the current cohort, in the face of (what is hypothesised to be) normal pulmonary

capillary hydrostatic pressures, it can be appreciated that modest changes in permeability would not influence oedema formation (and therefore X-ray appearances).

Validity of ‘anatomical adjustment’ and ‘segment correction’ of PVPI

As per the study hypothesis, $PVPI_{UNadj}$ was *negatively* associated with PaO_2/FiO_2 , an association which became stronger and gained clinical significance on within-subject analysis (Table 5.18). Both $PVPI_{ANadj}$ and $PVPI_{SEGcorr}$ however were both significantly *positively* associated with PaO_2/FiO_2 , with the difference in associations between $PVPI_{ANadj}$ and $PVPI_{SEGcorr}$ versus $PVPI_{UNadj}$ being highly statistically significant. Such changes are difficult to explain; it seems biologically implausible that oxygenation should improve as alveolar-capillary permeability deteriorates. This is all the more difficult to rationalise given the observed associations between both $\Delta PVPI_{ANadj}$ and $\Delta PVPI_{SEGcorr}$ versus $\Delta PaO_2/FiO_2$ are *negative*, in keeping with the hypothesis, supported by within-subject analysis and though statistically less significant, no different in strength from the negative association observed between $\Delta PVPI_{UNadj}$ and $\Delta PaO_2/FiO_2$. Whilst the observed changes are difficult to explain, it is not difficult to argue that they challenge the construct validity of adjusting PVPI values.

5.10.6 Conclusion

This study is not presented as the definitive work on the reproducibility and validity of TPTD measurement of ELWI and PVPI following lung resection. Nonetheless, the study’s findings are supportive of the reproducibility and construct validity of unadjusted ELWI and (to a lesser extent) PVPI measurements after lung resection; within the realms of the least significant change values observed, and in acknowledgement of the small sample size in which the observations were made. The study was not supportive however of the construct validity of either ‘anatomical adjustment’ or per ‘segment correction’, of ELWI and PVPI values. Furthermore care should be taken in extrapolating the findings in this lobectomy cohort to patients undergoing greater volume resection. This is especially so given the reduction in reproducibility observed as the volume of lung resected is increased.

The 'conscious clinician' is challenged by the fact that the very population in which ELWI and PVPI measurement might be desirable, those following large lung resections and with lung injury, are the very population in which theoretical concerns regarding the methodology of the measurements are greatest, and the reproducibility and validity least well established.

6 Major findings and conclusions

Framed by comprehensive, contemporary reviews of the current literature, this thesis presents the findings of a collection of investigations concerned with the prevention, incidence, mortality and detection and monitoring of post-lung resection lung injury.

6.1 Investigation I

The results of this survey of UK thoracic anaesthetic practice suggest that aspects of lung protective ventilation and fluid restriction are being widely incorporated into UK thoracic anaesthetic practice. From the current work there is no way of assessing what are the barriers to implementation of these techniques; it would be useful to explore these in order to develop strategies to improve engagement.

6.2 Investigation II

Despite what is perceived to be widespread adoption of lung protective strategies, there was no evidence from the meta-regression analyses presented to suggest that the incidence of ALI and/or ARDS following lung resection is falling. It must be emphasised that though conducted on the entirety of the available literature, meta-regression analysis is an imperfect tool and so the analysis of trends in incidence over time may still have been underpowered. It is plausible however that the finding of no association between ALI and/or ARDS incidence and time may reflect increasing baseline risk of lung injury due to increased patient co-morbidity and trends in favour of performing lung resection on patients with increasingly advanced disease. Unfortunately, in the current literature, baseline covariates were reported too infrequently to allow a multivariate meta-regression analysis to be conducted to address this hypothesis.

It is encouraging to observe that meta-regression analysis of ALI and/or ARDS mortality against time does suggest some evidence for reducing mortality. Such

a finding may reflect a reduction in the severity of lung injury developed, or better intensive care management of patients suffering lung injury.

The results of the meta-regression analyses, and the reports of individual institutions in which fluid restriction is aggressive and lung protective ventilation is uniformly practiced suggest that despite adoption of preventative strategies, post lung resection lung injury continues to occur. It is the author's opinion (B. Shelley), that ventilator induced lung injury and over-hydration represent but two parts of the complex pathophysiology of PLR-ALI. It is likely that the role of pulmonary endothelial dysfunction in the pathogenesis of PLR-ALI is under-appreciated. Recent revelations regarding the important role of the endothelial glycocalyx in the regulation of alveolar-capillary permeability may offer avenues for future investigation in addition to potential therapeutic targets.

Where Investigations I and II serve to reinforce in the author's mind the need for increased understanding of PLR-ALI, and further research into its pathogenesis and prevention; biomarker measurement and transpulmonary thermodilution (Investigations III and IV) are offered as potential aids in this quest. Both biomarker measurement and TPTD have potential to serve as bedside clinical monitors of lung injury development in order to guide clinical decision making, monitor patient progress and serve as a surrogate end point in future clinical studies.

6.3 Investigation III

Pentraxin 3 (PTX3) compared favourably with the properties of the ideal lung injury biomarker and appeared to identify a population of patients with elevated post-operative Lung Injury Score with high sensitivity. PTX3 may have a role in both prognostic and diagnostic prediction of lung injury development. Association was observed between PTX3 and indices of injury severity ($\text{PaO}_2/\text{FiO}_2$ and chest X-ray score) in addition to clinically important outcomes (duration of hospital and HDU stay). The near universal demonstration of PTX3's superiority over C-reactive protein in this investigation is encouraging, and suggests that PTX3 may have more potential than being just 'another' inflammatory marker.

Though encouraging, there remains however much further work to be done before PTX3 measurement could be routinely advocated. Firstly, the current study needs to be replicated in a larger cohort in order to confirm the predictive values observed. Secondly, the predictive value of PTX3 needs to be confirmed against the 'hard' end-points of ARDS diagnosis (as defined by the 'Berlin' definition), need for post-operative mechanical ventilation and mortality, rather than the surrogate endpoints of modified Lung Injury Score, oxygenation and chest X-ray score as studied in the current investigation. Thirdly, the post-operative kinetics of PTX3 require further exploration in order to more accurately characterise the optimal timing of blood sampling.

The negative findings of the multiple biomarker panel study serve to highlight the complexities of biomarker studies in this population. Firstly, the desire for pulmonary 'specificity' in separating the pulmonary inflammatory response from the systemic is complicated by a fall in pulmonary epithelial biomarker levels occurring secondarily to lung resection. Whilst correction of biomarker levels for the proportion of lung resected has been proposed (and appears appropriate in this investigation), this methodology requires further validation. Secondly, the transient nature of biomarker expression post-operatively makes choosing appropriate sample timing of paramount importance. Attempts to combine the results of multiple biomarkers are challenged by peak expression occurring at different time points post-operatively. As such, the post-operative kinetics of any candidate biomarker require to be explored in detail at an early stage of the investigative process. In addition, it is not known what degree of heterogeneity exists in the timing of post-operative biomarker expression.

6.4 Investigation IV

Transpulmonary thermodilution (TPTD) offers the potential to provide a relatively non-invasive method of monitoring the accumulation of extravascular lung water (EVLW) and pulmonary vascular permeability (PVPI) in the early post-operative period following lung resection. TPTD appeared feasible post-operatively in this population, and was well tolerated by patients. In general this study's findings were largely supportive of the reproducibility and construct validity of both EVLW and PVPI measurement following lung resection. It must be

emphasized however, that both indices require to be interpreted in the context of the least significant change values observed and in acknowledgement of the small sample size in which the observations were made. In addition, care should be taken in extrapolating the findings in this lobectomy cohort to patients undergoing greater volume resection.

Furthermore, it should be noted that the current study suggests that reproducibility was reduced in proportion to the volume of lung tissue resected. It appears that the very population in which TPTD monitoring may be most useful, that is in patients with or at high risk of lung injury undergoing greater volume resection, are the very population in which the reproducibility and validity are least well established, and in which theoretical concerns are greatest.

Perhaps the major barrier to optimal use of TPTD in patients undergoing lung resection is our ignorance to the post-operative changes in pulmonary blood volume and regional perfusion occurring following lung resection. Without such knowledge, any attempt to modify the single thermodilution algorithm to account for loss of lung tissue appears speculative. This study was not supportive of either the 'anatomical' or 'per segment' based corrections described.

6.5 Conclusion

Though mortality from ALI/ARDS following lung resection appears to be falling, the incidence is stable, and this condition remains the major cause of early mortality in this patient population. The UK thoracic anaesthetic community appears to be adopting lung protective practices, yet even when implemented consistently the effects of such practices appear modest. Biomarker measurement and TPTD are monitoring modalities which may serve to inform clinical decision making in this challenging patient population and act as surrogate endpoints in future research aiming to better understand the complex pathogenesis of this condition.

As advances in surgical techniques and adjuvant therapies confer survival benefits; more, older and sicker patients with more advanced disease are presenting for lung resection. It is incumbent therefore on all involved in the

care of such patients, to embrace this increasing demand, and strive to better understand and combat the causes of mortality and morbidity in this patient group.

Appendix One

7 Appendices

7.1 Appendix One – Original survey transcript

Anaesthesia for Thoracic Surgery

1. Introduction

Many thanks for agreeing to take part in the survey.

We have attempted to identify many topical / controversial aspects of thoracic anaesthetic practice and look forward to your comments. (There is space at the end for any further comments you wish to make.)

While we acknowledge that no single anaesthetic is the same as any other, please answer the questions when considering you own current routine 'first choice' practice when anaesthetising for lobectomy / pneumonectomy with one-lung ventilation - that is in the absence of any contraindications or special considerations.

Thanks again.

Ben Shelley
Clinical Research Fellow Anaesthesia,
University of Glasgow / Golden Jubilee Hospital (Clydebank)

Anaesthesia for Thoracic Surgery

2. One-Lung Ventilation

During the period of one-lung ventilation do you:

1. Ventilate with:

Pressure Control?

Volume Control?

2. Routinely use Positive End Expiratory Pressure (PEEP)?

Yes

No

If Yes, How much?

3. Ventilate with a target tidal volume?

Yes

No

If yes, What target?

4. Aim to ventilate to normocapnia?

Yes

No

5. Adopt a 'permissive' approach to hypercapnia?

Yes

No

If Yes, is there a cut off / maximal PCO_2 that you will tolerate?

Anaesthesia for Thoracic Surgery**6. Routinely ventilate with an FiO₂ = 1?**

- Yes
 No

If no, What FiO₂ do you routinely use during OLV assuming hypoxia is not an issue?

Anaesthesia for Thoracic Surgery**3. Anaesthetic technique**

Concerning your typical 'first choice' anaesthetic technique for thoracotomy and one-lung ventilation do you:

1. Routinely use a 'depth of anaesthesia' monitor?

- Yes
 No

2. Use target controlled (TCI) Propofol / TIVA?

- Yes
 No

3. Use inhalational anaesthetic agents?

- Yes
 No

4. If Yes, Which inhalational anaesthetic agents do you use?

- Sevoflurane
 Isoflurane
 Desflurane
 Other (please specify)

Anaesthesia for Thoracic Surgery**4. Analgesic Technique**

Concerning your 'first choice' POST-OPERATIVE analgesic technique following thoracotomy:

1. What (if any) method of regional anaesthesia do you employ?

(Sorry - you can only choose ONE 'first choice' answer)

- Epidural blockade
- Paravertebral blockade
- Intrathecal opioid
- Intercostal nerve blocks
- Other regional technique
- No regional technique

Anaesthesia for Thoracic Surgery**5. Epidural blockade**

Concerning epidural blockade for thoracotomy and one-lung ventilation do you:

1. Site a thoracic or lumbar epidural?

- Lumbar
- Thoracic

2. Routinely attempt to establish this and use it for intraoperative analgesia?

- Yes
- No

3. Routinely establish AND confirm your Epidural block prior to induction of anaesthesia?

- Yes
- No

4. What Local Anaesthetic / Opioid / Other additive do you use postoperatively (please specify concentrations)?**5. How is this delivered?**

- Intermittent bolus
- Continuous infusion
- Patient controlled bolus (PCEA)
- Background infusion AND Patient controlled bolus (PCEA)

Anaesthesia for Thoracic Surgery

6. Paravertebral blockade

Concerning paravertebral blockade for thoracotomy and one-lung ventilation:

1. Is this normally sited:

- Preoperatively by Anaesthetist?
 Intraoperatively by Surgeon?

2. If sited preoperatively, Do you use this for intraoperative analgesia?

- Yes
 No

**3. What Local Anaesthetic / Opioid / Other additive do you use postoperatively?
(Please specify concentrations)**

4. How is this delivered?

- Intermittent bolus
 Continuous infusion
 Patient controlled bolus
 Background infusion AND Patient controlled bolus

Anaesthesia for Thoracic Surgery

7. Intrathecal opioids

Concerning intrathecal opioids for thoracotomy and one-lung ventilation:

1. Do you routinely use:

- Local anaesthetic and intrathecal opioid?
 Intrathecal opioid alone?
 Other mixture / additive?

**2. What Local Anaesthetic / Opioid / Other additive do you use?
(Please specify concentrations)**

Anaesthesia for Thoracic Surgery

8. Intercostal nerve blockade

Concerning intercostal nerve blockade for thoracotomy and one-lung ventilation:

1. Is this normally sited:

Preoperatively by Anaesthetist?

Intraoperatively by Surgeon?

Postoperatively by Anaesthetist?

2. What Local Anaesthetic / Opioid / Other additive do you use?
(Please specify concentrations)

3. How is this delivered?

One-off series of injections

Intermittent bolus via catheter(s)

Continuous Infusion via catheter(s)

Anaesthesia for Thoracic Surgery

9. Alternative regional technique

1. If you routinely use a regional technique other than epidural / paravertebral blockade / intercostal nerve blockade please describe it here:

Anaesthesia for Thoracic Surgery**9. Alternative regional technique**

1. If you routinely use a regional technique other than epidural / paravertebral blockade / intercostal nerve blockade please describe it here:

Anaesthesia for Thoracic Surgery**10. Adjunctive analgesia**

Concerning your 'first choice' POST-OPERATIVE analgesic technique following thoracotomy:
(Assuming your first choice regional technique (if any) was successful)

1. Do you routinely prescribe systemic opioid analgesia postoperatively?

- Morphine via Patient Controlled Analgesic (PCA) device
- PRN Intravenous opiate
- PRN Subcutaneous opiate
- PRN Oral opiate
- No
- Other (please specify)

2. Do you routinely prescribe postoperative Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)?

- Yes - in all patients without allergy / contraindication
- Not routinely, but I will add them postoperatively if pain is a problem
- Never in this patient group
- Not routinely but I will give them for other indications:

Anaesthesia for Thoracic Surgery**11. Double lumen tubes**

Concerning your 'first choice' technique for lung separation during thoracotomy do you:

1. Use a double lumen endotracheal tube?

- Yes
 No

**2. For what indications would you place a LEFT-sided double lumen tube?
(Please choose all that apply)**

- Always
 Never
 All right sided surgery
 All left sided surgery except proximal left proximal main stem bronchus
 All left sided surgery except left pneumonectomy
 Other (please specify)

**3. For what indications would you place a RIGHT-sided double lumen tube?
(Please choose all that apply)**

- Always
 Never
 All left sided surgery
 Left proximal main stem bronchus lesions
 Left pneumonectomy
 Teaching
 Other (please specify)

Anaesthesia for Thoracic Surgery**13. Intra-operative fluid management**

Concerning INTRA-OPERATIVE fluid management for patients undergoing thoracotomy and one-lung ventilation for pneumonectomy:

**1. Which fluids do you routinely administer?
(Please choose all that apply)**

- Normal (0.9%) Saline
 Hartmanns / Ringers Lactate
 Gelofusine
 Other (please specify)

2. Do you administer fluids according to a mls/kg formula?

- Yes
 No

If Yes, What (mls/kg)?

**3. What is the average volume of fluids you administer intra-operatively?
(Assuming blood loss is not exceptional)**

Anaesthesia for Thoracic Surgery**14. Post-operative fluid management**

Concerning POST-OPERATIVE fluid management for patients undergoing thoracotomy and one lung ventilation for pneumonectomy:

1. What is your standard fluid prescription?

(For the immediate post operative period / night of surgery - assuming intraoperative losses were not exceptional - in an 'average' 70kg patient)

2. Alternatively, I don't have a 'standard prescription' but:

- I believe it is important to adopt a 'restrictive' approach to postoperative fluids in this patient group
- I do NOT restrict postoperative fluids in this patient group

Anaesthesia for Thoracic Surgery**15. Management of intraoperative hypotension**

Concerning the management of INTRA-OPERATIVE hypotension, do you:

1. Routinely / regularly use BOLUSES of vasopressor?

- Yes
- No

2. If Yes, Which?

(Please choose all that apply)

- Ephedrine
- Metaraminol
- Phenylephrine
- Other (please specify)

3. Routinely / regularly use vasopressors by INFUSION?

- Yes
- No

4. If Yes, Which?

(Please choose all that apply)

- Metaraminol
- Phenylephrine
- Noradrenaline
- Dopamine
- Other (please specify)

Anaesthesia for Thoracic Surgery**16. Management of postoperative hypotension**

Concerning the management of POST-OPERATIVE hypotension, do you:

1. Routinely / regularly use vasopressors by INFUSION?

- Yes
 No

2. If Yes, Which?

(Please choose all that apply)

- Metaraminol
 Phenylephrine
 Noradrenaline
 Dopamine
 Other (please specify)

Anaesthesia for Thoracic Surgery**17. Postoperative care**

Concerning (once again) 'first choice' management of our 'average' patient undergoing routine pneumonectomy:

1. Where would this patient be nursed postoperatively in your institution?

- Intensive Care Unit (ICU)
 High Dependency Unit (HDU)
 Surgical ward

Anaesthesia for Thoracic Surgery**18. Finally...**

While all answers will remain anonymous and no attempt will be made to match answers / techniques to centers, it will be helpful if you could let us know where you work in order to help gauge how representative our sample is.

1. In which centre(s) do you perform thoracic anaesthesia?

- | | |
|---|--|
| <input type="checkbox"/> Aberdeen Royal Infirmary | <input type="checkbox"/> London Harefield Hospital |
| <input type="checkbox"/> Belfast Royal Victoria Hospital | <input type="checkbox"/> London King's College Hospital |
| <input type="checkbox"/> Birmingham Heartlands Hospital | <input type="checkbox"/> London Royal Brompton Hospital |
| <input type="checkbox"/> Blackpool Victoria Hospital | <input type="checkbox"/> London St George's Hospital |
| <input type="checkbox"/> Bristol Royal Infirmary | <input type="checkbox"/> London St Mary's Hospital |
| <input type="checkbox"/> Cardiff University Hospital of Wales | <input type="checkbox"/> London The Heart Hospital |
| <input type="checkbox"/> Clydebank Golden Jubilee Hospital | <input type="checkbox"/> Manchester Royal Infirmary |
| <input type="checkbox"/> Cork University Hospital | <input type="checkbox"/> Manchester South Manchester University |
| <input type="checkbox"/> Coventry University Hospital | <input type="checkbox"/> Middlesbrough James Cook Hospital |
| <input type="checkbox"/> Dublin St James's Hospital | <input type="checkbox"/> Newcastle Freeman Hospital |
| <input type="checkbox"/> Edinburgh Royal Infirmary | <input type="checkbox"/> Norwich University Hospital |
| <input type="checkbox"/> Exeter Royal Devon and Exeter | <input type="checkbox"/> Nottingham City Hospital |
| <input type="checkbox"/> Hull Castle Hill Hospital | <input type="checkbox"/> Oxford John Radcliffe Hospital |
| <input type="checkbox"/> Leeds St James's Hospital | <input type="checkbox"/> Papworth Papworth Hospital |
| <input type="checkbox"/> Leicester Glenfield Hospital | <input type="checkbox"/> Plymouth Royal Infirmary |
| <input type="checkbox"/> Liverpool The Cardiothoracic Centre | <input type="checkbox"/> Sheffield Northern General Hospital |
| <input type="checkbox"/> London Barts and the London | <input type="checkbox"/> Southampton General Hospital |
| <input type="checkbox"/> London Guys and St Thomas's | <input type="checkbox"/> Stoke-on-Trent North Staffordshire University |
| <input type="checkbox"/> London Hammersmith Hospital | |
| <input type="checkbox"/> Other (please specify) | |

7.2 Appendix Two – Published survey manuscript

Anesthesia for Thoracic Surgery: A Survey of UK Practice

Ben Shelley, FRCA,*† Alistair Macfie, FRCA,* and John Kinsella, FRCA, MD†

Objective: The authors sought to provide a snapshot of contemporary thoracic anesthetic practice in the United Kingdom and Ireland.

Design: An online survey.

Setting: United Kingdom.

Participants: An invitation to participate was e-mailed to all members of the Association of Cardiothoracic Anaesthetists.

Intervention: None.

Measurements and Main Results: A total of 132 responses were received; 2 were excluded because they did not originate from the United Kingdom. Values are number (percent).

Anesthetic Technique: The majority of respondents (109, 85%) maintain anesthesia with a volatile anesthetic agent, with a lesser proportion (20, 15%) reporting use of a total intravenous anesthetic technique. The majority of respondents (78, 61%) favor pressure control ventilation over volume control (50, 39%); just under half (57, 45%) report the routine use of positive end-expiratory pressure (median = 5 cmH₂O [interquartile range (IQR), 4-5]). Fifty-two (40%) re-

spondents report ventilating to a target tidal volume (median = 6 mL/kg [IQR, 5-7]). Most (114, 89%) respondents routinely ventilate with an F_iO₂ less than 1.0. Thoracic epidural blockade (TEB) is favored by nearly two thirds of respondents (80, 62%) compared with paravertebral block (39, 30%) and other analgesic techniques (10, 8%). Anesthesiologists favoring TEB are significantly less likely to prescribe systemic opioids (17, 21% v 39, 100% [*p* < 0.001]). Proponents of TEB are significantly more likely to “routinely” use vasopressor infusions both intra- and postoperatively (16, 20% v 0, 0% [*p* = 0.003] and 28, 35% v 4, 11% [*p* = 0.013], respectively). Most respondents (127, 98%) report a double-lumen tube as their first choice. Many (82, 64%) report “rarely” using bronchial blockers.

Conclusions: The authors hope this survey both provides interest and serves as a useful resource reflecting the current practice of thoracic anesthesia.

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KEY WORDS: thoracic surgery, thoracic anesthesia, analgesia, ventilation

THE AUTHORS CONDUCTED a survey of UK thoracic anesthesiologists, exploring issues surrounding anesthetic and analgesic technique and perioperative management. The clinical practice of thoracic anesthesia has evolved with time as experience has been gained and greater understanding fostered through research (both in thoracic surgery and anesthesia, and the wider anesthetic and intensive care environment). Traditional texts often lag behind such changes, reflecting historic practice.¹ The present authors sought to provide a snapshot of contemporary thoracic anesthetic practice in the United Kingdom and Ireland, concentrating on areas of controversy or recent innovations.

METHODS

An invitation to participate in the survey was e-mailed to all members of the Association of Cardiothoracic Anaesthetists (ACTA). Respondents completed an online survey with data collected via the commercially available “SurveyMonkey” software (www.surveymonkey.com) during the months of July to September 2009.

Participants were requested to complete questions in the context of their “current routine ‘first choice’ practice when anaesthetising for lobectomy/pneumonectomy with one-lung ventilation. . . in the absence of any contraindications or special (patient) considerations.” Questions concerned anesthetic technique, mode of ventilation during the period of one-lung ventilation, regional analgesic technique (if any), adjunctive analgesia, perioperative fluid management, management of peri-

operative hypotension, and choice of lung separation technique. Data were assumed to be nonparametric in distribution and analyzed using Mann-Whitney *U*, chi-square, or Fisher exact tests as appropriate using SPSS v15.0 (SPSS UK Ltd, Surry, UK).

RESULTS

A total of 132 responses were received; 2 were excluded because they originated from outside the UK. This represents at least 1 reply from 39 of 42 (93%) identified centers performing thoracic surgery in the United Kingdom and Ireland, with a median response rate of 3 (range, 0-8) per center.

Anesthetic Technique

The majority of respondents (109, 85%) maintain anesthesia with a volatile anesthetic agent, with a lesser proportion (20, 15%) reporting the sole use of a total intravenous anesthetic technique. Of those favoring volatile anesthesia, sevoflurane and isoflurane are almost equally popular (42, 38% and 45, 41% of respondents respectively), with a smaller proportion (20, 19%) favoring desflurane. Only a small number of respondents (20, 16%) report routinely using a “depth of anesthesia” monitor.

Approaches to One-Lung Ventilation

Approaches to one-lung ventilation vary. The majority of respondents (78, 61%) favor pressure-control ventilation over volume control (50, 39%), and just under half (57, 45%) report the routine use of positive end-expiratory pressure (median = 5 cmH₂O [interquartile range, 4-5]). Fifty-two (40%) respondents report ventilating to a target tidal volume (median = 6 mL/kg [interquartile range, 5-7]). Forty-eight (37%) of respondents aim to ventilate to normocapnia although the majority (104, 83%) report adopting a permissive approach to hypercapnia. Figure 1 shows the range of F_iO₂ routinely used (assuming hypoxia is not an issue); the majority (114, 89%) of respondents routinely ventilate with an F_iO₂ less than 1.0.

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ANESTHESIA FOR THORACIC SURGERY

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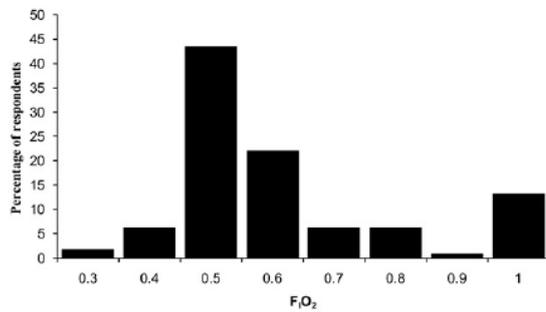


Fig 1. The range of F_IO₂ routinely used by respondents during the period of one-lung ventilation (assuming hypoxia is not an issue).

Thoracic epidural blockade (TEB) is favored by nearly two thirds of respondents (80, 62%) compared with paravertebral block (PVB) (39, 30%) and other analgesic techniques (10, 8%). "Other" analgesic techniques included intercostal nerve blocks, intrathecal opioids, other regional techniques, and no regional analgesic technique. Because of the small numbers and heterogenous nature of the group, these responses were excluded from further analysis.

Seventy-six of the 80 anesthesiologists using TEB (95%) attempt to establish the block and use it for intraoperative analgesia, with 19 (24%) reporting routinely confirming the onset of blockade before the induction of anesthesia. Postoperatively, 52 (65%) respondents maintain epidural analgesia by continuous infusion, and 24 (30%) use a continuous infusion supplemented by patient-controlled epidural analgesic top-ups.

The majority (75, 94%) of respondents report the use of extradural opioids.

Of the 39 respondents using PVB for postoperative analgesia, in a third of cases (13, 34%) the block is performed by the anesthesiologists, and in two thirds (25, 66%) the surgeon places the catheter intraoperatively. Two respondents reported (in free text) the combination of a single-shot anesthetic PVB preoperatively and a surgically placed catheter postoperatively; unfortunately, the survey was not equipped to determine whether this practice is more widespread. Most respondents (36, 94%) use a "plain" local anesthetic solution, and this generally is delivered by continuous infusion (27, 77%); 5 respondents (14%) use a patient-controlled bolus. Table 1 details respondents' attitudes toward adjunctive analgesia, perioperative fluid management, management of intra- and post-operative hypotension, and level of postoperative care cross-referenced by analgesic technique.

All of the anesthesiologists favoring PVB (39, 100%) routinely prescribe systemic opioid analgesia; in the majority (31, 80%) of cases, this constitutes morphine via a patient-controlled analgesic device. Anesthesiologists favoring TEB are significantly less likely to prescribe systemic opioids (17, 21% v 39, 100% [$p < 0.001$]).

There are no significant differences in attitudes to perioperative fluid management between proponents of TEB and PVB, with no significant differences between intra- and postoperative fluid prescribing. Proponents of TEB are significantly more likely to routinely use vasopressor infusions both intra- and postoperatively ($p = 0.003$ and $p = 0.013$, respectively [Table 1]). The vasopressor most commonly administered by infusion is norepinephrine, which is used "routinely/regularly" intraop-

Table 1. Attitudes Toward Adjunctive Analgesia, Perioperative Fluid Management, and Management of Intra- and Postoperative Hypotension Cross-Referenced by First-Choice Regional Analgesic Technique

	TEB 80 (62%)	PVB 39 (30%)	Other* 10 (8%)	p Value
<u>Adjunctive analgesia</u>				
Routine systemic opioids?				
Yes	17 (21)	39 (100)	—	<0.001†
No	63 (79)	0 (0)		
Routine NSAID use?				
Yes	26 (33)	13 (33)	—	0.92†
No	54 (68)	26 (67)		
<u>Perioperative fluids</u>				
Typical intraoperative fluids (mL)?	1,000 (750-1,500)	1,000 (750-1,500)	—	0.8‡
Typical postoperative fluids (mL/h)?	70 (70-83)	70 (65-83)	—	0.88‡
<u>Management of hypotension</u>				
Routine intraoperative vasopressor infusion?				
Yes	16 (20)	0 (0)	—	0.003§
No	64 (80)	37 (100)		
Routine postoperative vasopressor infusion?				
Yes	28 (35)	4 (11)	—	0.013†
No	52 (65)	32 (89)		

NOTE. Values are median (IQR) or number (proportion).

NOTE. Values are number median (IQR) or number (proportion).

*Excluded from further analysis because of small numbers and heterogenous nature of group.

†Chi-square test.

‡Mann-Whitney *U* test.

§Fisher exact test.

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Table 2. Most Frequently Cited Indications for the Choice of Lung Separation Technique

Technique	Indication	(%)
Left DLETT	All right-sided surgery	102 (79)
	All left-sided surgery except proximal left main bronchus lesions	43 (33)
	All left-sided surgery except left pneumonectomy	37 (28.7)
	Other	25 (19.4)
	Always	6 (4.7)
Right DLETT	Left pneumonectomy	63 (49)
	Proximal left main bronchus lesions	63 (49)
	All left-sided surgery	47 (36)
	Teaching	24 (19)
	Other*	23 (18)
Bronchial Blocker	Failure to pass a DLETT	99 (87)
	Teaching	49 (43)
Other	Anticipated difficult intubation	36 (32)
	Other	28 (25)
	(includes When SLETT/tracheotomy in situ)	8 (7)
	Routinely for VATS	8 (7)

NOTE. Values are number (proportion).

Abbreviations: VATS, video-assisted thoroscopic surgery; SLETT, single-lumen endotracheal tube; DLETT, double-lumen endotracheal tube.

*Provided opportunity for free form text, generally a heterogeneous group of responses.

eratively by 11 (8%) and postoperatively by 33 (25%) of respondents.

Most respondents (127, 98%) report a double-lumen tube as the first-choice lung separation technique for "routine" thoracotomy. Twenty-three (17.8%) respondents report "regularly" using bronchial blockers in their thoracic anesthetic practice, whereas 82 (64%) report their use "rarely." Twenty-four (19%) "never" use bronchial blockers. The most frequently cited indications for the choice of lung separation technique are described in Table 2. Forty-seven (36%) respondents report the use of a right-sided double-lumen tube for "all left-sided surgery."

DISCUSSION

The present results reflect the practice of a large number (130) of thoracic anesthesiologists in the United Kingdom and Ireland. Unfortunately, establishing a denominator for this response rate is difficult because it is unknown what fraction of the 458 ACTA members (at the time of survey release, C. Bunnell, personal communication, ACTA administrator, November 2010) actively practices thoracic anesthesia. In addition to circulating the survey within the membership of ACTA, the authors excluded all thoracic anesthesiologists who were not members of ACTA. A further limitation of the survey is that the authors did not attempt to establish the respondent's level of experience or the frequency of his/her thoracic anesthetic practice and as such were unable to assess the effect experience makes on practice. This survey is the only survey published to

date tackling wider issues of thoracic anesthesia beyond the choice of analgesic technique and exploring the effects such choices have on other aspects of practice.

Traditional teaching of thoracic anesthesia describes one-lung ventilation with a target tidal volume of 10 mL/kg, an F_{iO_2} of 1, and an intention to maintain normocapnia.² In recent years, this practice has been questioned in light of evidence that high intraoperative ventilatory pressure is an independent risk factor for acute lung injury after pulmonary resection,³ concerns over the potentially harmful effects of hyperoxia,⁴ and increasing research evidence from the wider intensive care population of the dangers of volu- and barotrauma.⁵ Yang et al⁶ recently showed a reduction in postoperative pulmonary dysfunction using a "protective" ventilatory strategy (tidal volume = 6 mL/kg) compared with a "conventional" (10 mL/kg) strategy. The results suggest that so-called "lung-protective" ventilatory techniques are being incorporated into UK thoracic anesthetic practice. Although not universal, pressure-controlled ventilation with reduced tidal volumes, an $F_{iO_2} < 1.0$, and a permissive approach to hypercapnia is commonplace.

Debate surrounding the ideal analgesic technique for thoracotomy has intensified in recent years, with the suggestion that PVB has a better side effect profile and has been associated with a reduction in complications when compared with TEB.^{7,8} Nonetheless, TEB remains the most popular analgesic technique for thoracic surgery in the United Kingdom; the approximate 2:1 split in favor of TEB over PVB observed in the survey remains consistent with previous reports,^{7,9} although 1 recent survey suggested a swing in practice toward PVB (50% TEB:40% PVB).¹⁰ It has been postulated that excessive fluid administration in response to hypotension seen with TEB may contribute to the development of pulmonary complications.¹¹ The results of the present survey challenge this suggestion, revealing that attitudes toward perioperative fluid management are similar between proponents of TEB and PVB, with anesthesiologists favoring TEB significantly more likely to treat hypotension by the administration of vasopressors by infusion during the intra- and postoperative period.

The National Confidential Enquiry into Perioperative Deaths 1997 to 1998 highlighted the importance of correct double-lumen endotracheal tube (DLETT) placement in ensuring the safe practice of one-lung ventilation.¹² The proximal takeoff of the right upper lobe bronchus makes correct positioning of a right-sided DLETT more technically challenging than when positioning a left-sided DLETT.¹³ Consequently, it has been suggested by some that right-sided DLETTs should not be used routinely in thoracic anesthetic practice.¹⁴ The present survey suggests that although favoring a left-sided tube in many circumstances, right-sided DLETTs are used widely by thoracic anesthesiologists in the United Kingdom. Sixty-four percent of anesthesiologists report rarely using bronchial blockers in their thoracic anesthetic practice, whereas only 17.8% report routine use. Nonetheless, it would seem that bronchial blockers are relied on by many in the event of failed or difficult double-lumen intubation. "Teaching" was the 2nd most frequently reported indication for bronchial blockade; although not widely used in routine clinical practice, it is reassuring that this essential skill is being passed on to trainees. It is interesting to reflect anecdotally that a significant number of respondents voiced

their desire to increase their use of bronchial blockade. Any future study may find it interesting to explore the reasoning behind and barriers preventing such a change in practice.

The Society for Cardiothoracic Surgery, UK, and Ireland First National Thoracic Surgery Activity & Outcomes Report suggests that approximately 4,500 open-lung resections are performed in the United Kingdom each year¹⁵; such relatively small case numbers mean that many of the controversies highlighted by this survey are unlikely to be resolved by random-

ized trial data. The authors hope this survey both provides interest and serves as a useful resource to trainees and experienced practitioners alike regarding the current practice of thoracic anesthesia in the United Kingdom and Ireland.

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7.3 Appendix Three – Power calculation for meta-regression analyses

Hedges and Pigott provide methodology for power analysis of meta-regression analyses²⁸⁵. By working through this method, the power of the meta-regression analyses performed in Investigation II could be retrospectively derived.

7.3.1 Formulae and methodology

In a meta-regression analysis, the relationship between moderator variables and effect size can be modelled by the relationship:

$$\theta_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \dots + \beta_p x_{ip} + \varepsilon_i$$

Equation 7.1

Where it is supposed that the effect size parameter (θ_i) is linearly related to p moderator variables x_1, x_2, \dots, x_p , where $\beta_0 \dots \beta_p$ are unknown regression coefficients. As under random effects, the moderator variables do not explain all of the variation in effect size, the relationship is governed by a study specific random effect, ε_i .

For any individual regression coefficient, the test of the (null) hypothesis that $\beta_j=0$, where β_j is the regression coefficient for the relationship between the effect size and the j th moderator variable, uses the test statistic:

$$Z_j^* = \frac{\hat{\beta}_j^*}{\sqrt{\sigma_{ij}^*}}$$

Equation 7.2

Where, σ_{ij} is the variance of $\hat{\beta}_j$,

and σ_{ij}^* is given by the j^{th} diagonal element of the matrix:

$$\Sigma^* = [X'(V^*)^{-1}X]^{-1}$$

Equation 7.3

Where, X is a k by $(p+1)$ design matrix, whose first column is a vector of ones and whose other elements are x_{ij} , and
 V^* is the conditional covariance matrix of the effect size estimates under random effects:

$$V^* = \text{Diag}(v_1 + \hat{\tau}^2, v_2 + \hat{\tau}^2, \dots, v_k + \hat{\tau}^2)$$

Equation 7.4

Where, v_i = the variance of study i , and
 $\hat{\tau}^2$ = tau squared, the variance of the true effect size.

Power for a two sided test of this relationship can be computed by:

$$\text{Power} = 1 - \Phi\left(c_{\alpha/2} - \frac{\beta_j}{\sqrt{\sigma_{ij}^*}}\right) + \Phi\left(-c_{\alpha/2} - \frac{\beta_j}{\sqrt{\sigma_{ij}^*}}\right)$$

Equation 7.5

Where, $\Phi(x)$ is the standard normal cumulative distribution function, and
 c_α is the $100(1-\alpha)$ percent critical value of the standard normal distribution.

7.3.2 Application to the present study

7.3.2.1 Power to detect effect sizes reported by Licker et al

Licker et al reported a 2.9% decrease in the incidence of ALI, from a baseline of 3.8% over a study period of 5.3 years (OR 0.85)¹⁵⁵. The regression coefficient for the relationship between logit event rate and year can be determined as the natural logarithm of the odds ratio, hence for the study of Licker et al:

$$\beta = \log_e(0.85) = -0.1628$$

Thus, we wish to determine the power of the current analysis to test the hypothesis that $\beta = -0.1628$.

From the 15 studies included in the meta-regression analysis of ALI incidence verses year, the study variance (v_i) and a pooled estimate of Tau squared can be estimated $\hat{\tau}^2$ (and are in fact provided in the data output of the Comprehensive Meta-analysis software).

From v_i and $\hat{\tau}^2$, the covariance matrix V^* can be constructed (Equation 7.4). Using this solution for V^* , solution of Equation 7.3 is possible where the values of x in the design matrix X are the median years of study conduct of the 12 studies in the analysis. Solution of Equation 7.3, yields the second diagonal element of Σ^* , the variance of $\hat{\beta}_j$ (σ_{ij}). Solving Equation 7.3 in this way for the current analysis of ALI incidence yields:

$$\sigma_{ij} = 0.003967$$

With these known values of β and σ_{ij} , Equation 7.5 can be solved to yield the power of the current investigation to detect the effect size observed by licker et al:

$$Power = 1 - \Phi\left(c_{\alpha/2} - \frac{\beta_j}{\sqrt{\sigma_{ij}^*}}\right) + \Phi\left(-c_{\alpha/2} - \frac{\beta_j}{\sqrt{\sigma_{ij}^*}}\right)$$

Hence:

$$Power = 1 - \Phi\left(1.96 - \frac{-0.1628}{\sqrt{0.003967}}\right) + \Phi\left(-1.96 - \frac{-0.1628}{\sqrt{0.003967}}\right)$$

($c_{\alpha} = 100(1-\alpha)$, which for $\alpha=0.05$ equals 1.96)

Hence:

$$Power = 0.73$$

7.3.2.2 Power to detect effect sizes reported by Tang et al

Tang et al reported a 1.6% reduction in the incidence of ARDS form a baseline of 3.2% over a study period of 5.4 years (OR 0.94)¹¹⁰.

Using the same methodology as above,

$$\beta = \log_e(0.94) = -0.066$$

and $\sigma_{ij} = 0.000861$ as calculated from the variance and median year of study conduct values in the 15 studies included in the analysis of ARDS incidence.

Hence:

$$Power = 1 - \Phi\left(1.96 - \frac{-0.066}{\sqrt{0.000861}}\right) + \Phi\left(-1.96 - \frac{-0.066}{\sqrt{0.000861}}\right)$$

Hence:

$$Power = 0.61$$

It can be appreciated from these analyses that the current study, based on the totality of available literature, lacks sufficient power to confidently test for the effect sizes reported by Licker and Tang and colleagues within the pooled incidence estimates.

7.3.2.3 What was the power of the current analysis?

Given the negative findings of the analyses examining the incidence of ALI and/or ARDS against time, rather than ‘what was the power of the current analysis?’ a more pertinent question is perhaps ‘what effect size (β or OR) was the current study powered to detect?’

Once the variance of $\hat{\beta}_j$ (σ_{ij}), is known (as calculated above), for the current analyses, it is a relatively straightforward step to deduce the power from the relationship Equation 8.5. This step is simplified by utilising power analysis software (e.g. Minitab Ltd., Coventry, UK) where power is similarly deduced based on the cumulative distribution function of the standard normal distribution.

7.4 Appendix Four – Summary of published literature describing biomarkers of ALI/ARDS in patients undergoing lung resection

Table 7.1. Summary of studies measuring lung injury biomarkers in exhaled breath condensate in patients undergoing lung resection

Biomarker	Effect of lung resection?	Association with outcome?	Comment
pH	↓ vs T0 ¹⁶⁴ ↓ during OLV ³¹⁵	Not tested	No association with duration of OLV ¹⁶⁴
Leukotriene B4	↑ vs T0 ¹⁶⁴	Not tested	No association with duration of OLV ¹⁶⁴
Hydrogen peroxide	↑ vs T0 ^{163, 164}	Not tested	No association with duration of OLV ¹⁶⁴ , ↑ only after L not P ¹⁶³
8-Isoprostane	↔ vs T0 ¹⁶⁴	Not tested	
Myeloperoxidase	Not detectable pre-op ¹⁶⁴	Not tested	
Interleukin-1β	↑ vs T0 on POD 3 and 7 ⁵³⁶	Not tested	

L, lobectomy; P, pneumonectomy; T0, baseline (pre-operative) value; OLV, one-lung ventilation. Arrows refer to biomarker level being increased (↑), decreased (↓) or unchanged (↔).

Table 7.2. Summary of studies measuring lung injury biomarkers in urine in patients undergoing lung resection

Biomarker	Classification	Effect of lung resection?	Association with outcome?	Comment
Malondialdehyde	Oxidative stress	↑ iPO ¹⁶³	Not tested	Greater ↑ L vs P ¹⁶³

L, lobectomy; P, pneumonectomy; iPO, immediately post-operatively. Arrows refer to biomarker level being increased (↑), decreased (↓) or unchanged (↔).

Table 7.3. Summary of studies measuring lung injury biomarkers in plasma in patients undergoing lung resection

Biomarker	Classification	Effect of lung resection?	Association with outcome?	Comment
Krebs von den Lungen (KL)-6	Epithelial	↓iPO and POD1 ³¹⁵ ↓ vs baseline on POD 2, 7, 14 & 28 ³¹⁶	Not tested	Fall in proportion to volume of resected lung ^{315, 316} No association with duration of OLV, P _{plat} , V _T ³¹⁵
Surfactant protein (SP)-D	Epithelial	Unchanged iPO vs T0 ³¹⁵ , significant fall POD1 ³¹⁵	Not tested	Fall in proportion to volume of resected lung ³¹⁵ No association with duration of OLV, P _{plat} , V _T ³¹⁵
Receptor for advanced glycation end products (RAGE)	Epithelial	↑iPO vs T0 ³¹⁵	Not tested	No association with duration of OLV, P _{plat} , V _T ³¹⁵
von Willebrand factor (vWF)	Endothelial	↑iPO and POD1 ³¹⁵	Not tested	No association with duration of OLV, P _{plat} , V _T ³¹⁵
Vascular endothelial growth factor (VEGR)	Endothelial	No change ⁵³⁷	Not tested	No change 2h PO and POD3 ⁵³⁷
Angiopietin (Ang)-1	Endothelial	↓POD1 and POD3 ⁵³⁷	Not tested	
Ang-2	Endothelial	↑POD1 and POD3 ⁵³⁷	Not tested	Greater ↑ after VATS vs open resection
soluble-VEGF receptor (sVEGFR)-1	Endothelial	↑POD1 and POD3 ⁵³⁷	Not tested	
sVEGFR-2	Endothelial	↓POD1 and POD3 ⁵³⁷	Not tested	
Lactate dehydrogenase	Pro-inflammatory	↑ vs T0 on POD 2,7,14,28 ³¹⁶	Not tested	Greater ↑ in L vs SL ³¹⁶
Tumour necrosis factor (TNF)-α	Pro-inflammatory	Not detected ³¹⁵ No change ²⁴¹ ↑intra-op at 3h ⁵³⁸	Not tested	Not detected at iPO and POD1 ³¹⁵ No change iPO ²⁴¹ No difference low TV vs high TV ⁵³⁸ ↓POD 1,2,3 w. β2-agonist vs. control ⁵³⁹

Biomarker	Classification	Effect of lung resection?	Association with outcome?	Comment
TNF-soluble receptor(sr)-1	Plasma	No change ⁵⁴⁰	Not tested	
TNF-soluble receptor(sr)-2	Plasma	↑POD1 ⁵⁴⁰	Not tested	
Myeloperoxidase	Plasma	↑iPO, 24hPO (but NS) ¹⁶²	Not tested	
PMN elastase	Pro-inflammatory	↑iPO and 24hPO ⁵⁴¹	Not tested	↑ iPO in P only not L ⁵⁴¹ ↑ 24hPO in L only not P ⁵⁴¹
C-reactive protein	Pro-inflammatory	↑POD1 ³¹⁰ ↑ vs baseline on POD 1,2,7,14,28 ³¹⁶ ↑ vs T0 4hPO and POD 1,2,3,5 ¹⁸⁰ ↑ peak on POD2 ^{180, 311}	↑ at baseline in patients with complications ^{v 310} Peak higher in pts with complications ^{w,542} ↑ in pts w. infection. AUROCC for predicting infection 0.66 (CI 0.58-0.73) ³¹¹	AuROCC for baseline CRP vs pulmonary complications = 0.86 ³¹⁰ Greater ↑ in L vs SL ³¹⁶ Greater ↑ in P or L vs VATS SL ⁵³⁶ Greater ↑ in open vs VATS ¹⁸⁰
Interleukin-1	Pro-inflammatory	↑intra-op at 3h ⁵³⁸	Not tested	No difference low TV vs high TV ⁵³⁸
Interleukin(IL)-1β	Pro-inflammatory	No change ^{241, 361, 540}	Not tested	No change iPO ²⁴¹ ↓POD 1,2,3 w. inhβ2-agonist vs. control ⁵³⁹
IL-6	Pro-inflammatory	↑ iPO and POD1 ^{241, 310, 312, 315} , POD3 & 7 ³¹² ↑ during OLV and during TLV ¹⁶⁶ ↑intra-op at 3h ⁵³⁸ ↑ peak on POD1 ^{180, 311, 536}	↑ at baseline and greater ↑ POD1 in patients with complications (p=0.1) ^{K 310} ↑IL-6 on POD 7 in pts w. complications ^L ; OR (univariate) = 1.06 ³¹² Levels slower to fall in patients w. complications ⁵⁴²	Modest ↑ “barely clinically significant” ^{m166} No assoc w. duration OLV, P _{plat} , V _T ³¹⁵ AuROCC for baseline IL-6 vs pulmonary complications = 0.79 ³¹⁰ No difference low V _T vs high V _T ⁵³⁸ No difference low TV vs high TV ⁵³⁸ ↓POD 1,2,3 w. inhβ2-agonist vs. control ⁵³⁹ Greater ↑ in open vs VATS ^{180, 361}
IL-12	Pro-inflammatory	No change ⁵³⁸	Not tested	No change intra-op ⁵³⁸

^v Major complications occurred in 9/153 (5.9%); pneumonia (n = 4), ARDS (n=3), myocardial infarction (n=1), pulmonary embolism (n=1), and acute renal failure (n=1).

^w Described “such as infectious or cardiac complications”

Biomarker	Classification	Effect of lung resection?	Association with outcome?	Comment
IL-8	Pro-inflammatory	Not detected ³¹⁵ ↑during OLV and TLV ³⁶² No change ²⁴¹ ↑intra-op at 3h ⁵³⁸ Peak at 4hPO ³⁶¹	Not tested	Not detected at iPO and POD1 ³¹⁵ No change iPO ²⁴¹ No difference low V _T vs high V _T ⁵³⁸
Albumin	Pro-inflammatory	↓during OLV and TLV ³⁶²	Not tested	Modest↓, ? significance AuROCC for baseline albumin vs complications = 0.86 ³¹⁰
Procalcitonin	Pro-inflammatory	↑ peak on POD2 ⁵⁴² ↑ peak on POD1 ³¹¹	Peak higher in pts w. complications ^{x542} ↑ in pts w. infection. AUROCC for predicting infection 0.92 (CI 0.87-0.96) ³¹¹	Appears to be ↑ on POD1, no stats provided ⁵⁴² ↑ in P vs L / VATS ³¹¹ No difference in pts with non-infective complications (data not provided) ³¹¹
IL-10	Anti-inflammatory	No change ^{241, 538} ↑iPO, 4,8h PO (no stats) ³⁶¹	Not tested	No change iPO ²⁴¹ ↓ POD 1,2,3 w. inhβ2-agonist vs. placebo ⁵³⁹ Greater ↑open vs VATS ³⁶¹
Protein thiol	Oxidative stress	↓iPO ¹⁶²	Not tested	Greater ↓P and BiL vs L ⁵⁴³
Protein carbonyl	Oxidative stress	↑iPO ¹⁶²	Not tested	Greater ↑ P vs L ¹⁶²
Malondialdehyde	Oxidative stress	↑iPO, 6hPO ^{157, 165} ↑ during O/TLV ^{166 157, 165}	↑assoc w. major complications ^{y165}	↑ proportional to duration OLV ^{157, 165}
Thrombomodulin (TM)	Coagulation / endothelial	↓POD1 ³¹⁴	? ↑ levels linked to poor oxygenation PO (see text) ³¹⁴	↓ in proportion to volume of resected lung tissue ³¹⁴

SL, sub-lobar resection; L, lobectomy; P, pneumonectomy; T0, baseline (pre-operative) value; OLV, one-lung ventilation; POD, post-operative day; iPO, immediately post-operatively; P_{peak}, peak airway pressure; V_T, tidal volume; AuROCC, area under the receiver operating characteristic curve; NS, not significant. Arrows refer to biomarker level being increased (↑), decreased (↓) or unchanged (↔).

^x Described “such as infectious or cardiac complications”.

^y “respiratory failure, cardiac arrhythmias and pulmonary hypertension”

Table 7.4. Summary of studies measuring lung injury biomarkers in bronch-alveolar lavage / epithelial lining fluid in patients undergoing lung resection

Biomarker	Classification	Effect of lung resection?	Association with outcome?	Comment
Albumin	Permeability	↑during OLV and TLV ³⁶² ↑after OLV and 2hPO ¹⁴⁸	Not tested	
Protein	Permeability	↑after OLV and 2hPO ¹⁴⁸	Not tested	
Soluble intracellular adhesion molecule-1	Endothelial	↓ after OLV and 2hPO ¹⁴⁸	Not tested	↓ only in low tidal volume group ¹⁴⁸
PMN elastase	Pro-inflammatory	↑after OLV and 2hPO ¹⁴⁸	Not tested	
Interleukin(IL)-1β	Pro-inflammatory	↑iPO ²⁴¹	Not tested	↑PO only with propofol vs volatile anaesthesia ²⁴¹
IL-6	Pro-inflammatory	↑iPO ²⁴¹	Not tested	↓after 30mins OLV w. inhβ2-agonist vs. placebo ⁵³⁹
IL-8	Pro-inflammatory	↑after OLV ¹⁴⁸ , iPO ²⁴¹ and 2hPO ¹⁴⁸ ↑during OLV and TLV ³⁶²	Not tested	Greater ↑ PO with propofol vs volatile anaesthesia ²⁴¹ ↑ proportional to duration OLV ³⁶² ↓after 30mins OLV w. inhβ2-agonist vs. placebo ⁵³⁹
IL-10	Anti-inflammatory	↓ after OLV ¹⁴⁸ ↑iPO(NS) ²⁴¹	Not tested	↓in low tidal volume ventilation group only ¹⁴⁸ ↑iPO only with propofol vs volatile anaesthesia ²⁴¹ ↓after 30mins OLV w. inhβ2-agonist vs. placebo ⁵³⁹
IL-12 p70	Pro-inflammatory	No change ²⁴¹	Not tested	No change iPO ²⁴¹
Tumour necrosis factor-α	Pro-inflammatory	↑after OLV ¹⁴⁸ , PO ²⁴¹ and 2hPO ¹⁴⁸	Not tested	No ↑ after OLV in low tidal volume vent ¹⁴⁸ ↑iPO only with propofol vs volatile anaesthesia ²⁴¹ ↓after 30mins OLV w. inhβ2-agonist vs. placebo ⁵³⁹

SL, sub-lobar resection; L, lobectomy; P, pneumonectomy; T0, baseline (pre-operative) value; OLV, one-lung ventilation; POD, post-operative day; iPO, immediately post-operatively; P_{peak}, peak airway pressure; V_T, tidal volume; NS, not significant. Arrows refer to biomarker level being increased (↑), decreased (↓) or unchanged (↔).

7.5 Appendix Five - Derivation of adjusted values of ELWI and PVPI

The Edwards EV1000 transpulmonary thermodilution (TPTD) monitor used in this study incorporates the facility to download the results of all individual thermodilution procedures. Results are provided in Microsoft Excel format and include the TPTD derived parameters cardiac output, stroke volume, global end-diastolic volume (GEDV), intra-thoracic blood volume (ITBV), extravascular lung water (EVLW) (and their corresponding ‘index’ values indexed to predicted body weight) and pulmonary vascular permeability index (PVPI).

In Bendjelid et al’s validation paper of the “new transpulmonary thermodilution system” (EV1000), the authors provide the following formulae describing the derivation of TPTD derived indices⁵²⁵:

$$EVLW = (CO \times DSt) - (0.25 \times GEDV)$$

Equation 7.6

Where *DSt* is the exponential downslope time of the thermal indicator, derived from the thermodilution curve. Though *DSt* is not provided in the results downloaded from the EV1000 monitor, rearrangement of Equation 7.6 allows *DSt* to be derived from the results provided:

$$DSt = \frac{EVLW + (0.25 \times GEDV)}{CO}$$

Equation 7.7

By deconvolution of the result in this way, the raw variables required to compute values of ELWI, pulmonary blood volume (PBV) and PVPI are then available.

7.5.1 Rationale for derivation of adjusted values

The methodology for single indicator TPTD relies on the fundamental assumption of the linear and continuous relationship between ITBV and GEDV (observed by Sakka et al³⁹⁶) such that:

$$ITBV = 1.25 \times GEDV$$

Equation 7.8

As ITBV is the sum of the PBV and GEDV, this relationship can be simplified as:

$$PBV = 0.25 \times GEDV$$

Equation 7.9

[It can now be seen from Equations 6.1 and 6.4, that the EV1000 monitor derives EVLW by subtracting the calculated PBV from the pulmonary thermal volume (derived from the thermodilution curve as the product of CO and DSt)].

It seems unlikely that this relationship between PBV and GEDV would remain constant following lung resection, where a proportion of the pulmonary vasculature has been excised. The hypothesis being tested in this investigation therefore is that modification of the derived values of EVLW, PBV and therefore PVPI to account for the lung resected will make a better assessment of the post-resection EVLW and PVPI and so improve validity of the indices. Such an adjustment could be made in two principal ways. Firstly the '0.25' coefficient in Equation 7.6 could be adjusted to reflect a hypothesised new relationship, and EVLW calculated with this revised equation - resulting in the proposed 'anatomical' adjustment (yielding $ELWI_{ANadj}$ and $PVPI_{ANadj}$). Secondly, TPTD derived indices could be calculated using the original equations, but the ELWI and PVPI results corrected to reflect that they have been determined from less than a whole lung. Correcting the result based on the number of pulmonary segments remaining following resection is the basis of the proposed 'segment corrected' result (yielding $ELWI_{SEGcorr}$ and $PVPI_{SEGcorr}$).

7.5.2 Derivation of the 'anatomical adjustment'

If the consequent reduction in PBV following resection is assumed to be proportional to the volume of lung tissue resected, then this relationship can be 'adjusted' to account for the assumed reduction in PBV. Based on a 19 segment model of pulmonary anatomy, any pneumonectomy or lobar resection can be expressed in terms of the number of segments resected. If Equation 7.9 is then considered to represent the relationship between PBV and GEDV for 19 segments of lung, then the relationship for n/19 segments can be derived by:

$$PBV_{ANadj} = \frac{n}{19} \times 0.25 \times GEDV$$

Equation 7.10

Where n is the number of pulmonary segments remaining after resection.

Substitution of Equation 7.10 into Equation 7.6 allows EVLW to be derived based on the anatomically adjusted coefficient:

$$EVLW_{ANadj} = (CO \times DSt) - \left(\frac{n}{19} \times 0.25 \times GEDV \right)$$

Equation 7.11

PVPI_{ANadj} can then be derived as the quotient of ELVW_{ANadj} and PBV_{ANadj}:

$$PVPI_{ANadj} = \frac{EVLW_{ANadj}}{PBV_{ANadj}}$$

Equation 7.12

7.5.3 Derivation of the 'segment correction'

If it is considered that the EVLW value yielded from Equation 7.6 describes the EVLW per unit of lung tissue, where ordinarily the unit of lung tissue is defined as both lungs (i.e. 19 segments), then following resection where the volume of residual lung tissue is less than 19 segments, this equation might be expected to overestimate EVLW by a factor proportional to the volume of lung tissue resected.

Thus if ordinarily the measured value provides EVLW per 19 segments of lung, then division of the value by 19 yields the EVLW per segment. Similarly, if it assumed that the total EVLW measured by the monitor post-operatively represents the 'true' total value, but for n/19 remaining segments, then the EVLW per segment can be derived:

$$EVLW_{per\ segment} = \frac{EVLW}{n}$$

Equation 7.13

To allow rationale comparison of this value per segment between patients undergoing lung resections of differing sizes, this per segment value is multiplied

by 19 yielding an ELVW ‘corrected’ according to the no. of pulmonary segments from which it is derived:

$$EVLW_{SEGcorr} = \frac{EVLW}{n} \times 19$$

Equation 7.14

Similarly, but in fact reciprocally, the derived value of PBV is corrected to reflect that the measured value (calculated from GEDV on the assumption that the pulmonary circulation is complete) is likely to be an overestimate of PBV by a factor proportional to the volume of lung resected, thus:

$$PBV_{SEGcorr} = \frac{PBV}{19} \times n$$

Equation 7.15

7.5.4 Simulation study reflecting hypothesised effect of un-adjusted EVLW and PVPI values

In order to explore the hypothesised effect of adjusting EVLW and PVPI values as proposed above, a simulation study was performed. Using the baseline data (i.e. pre lung-resection) from the eight patients included in the reproducibility and construct validity study (Chapter 5), ‘anatomically adjusted’ and ‘segment corrected’ EVLW and PVPI values were derived as described above. Values were derived from the baseline data, making adjustment for resection of 3, 4, 5, 9 and 10 pulmonary segments (representing the volume of lung tissue resected at right upper lobectomy, left lower lobectomy, right lower lobectomy, left pneumonectomy and right pneumonectomy respectively). The resulting bias, representing the degree to which EVLW and PVPI would be overestimated by unadjusted values should the anatomically / segment corrected values represent the true situation, was then determined as the difference between unadjusted and adjusted values and is displayed in Figure 7.1.

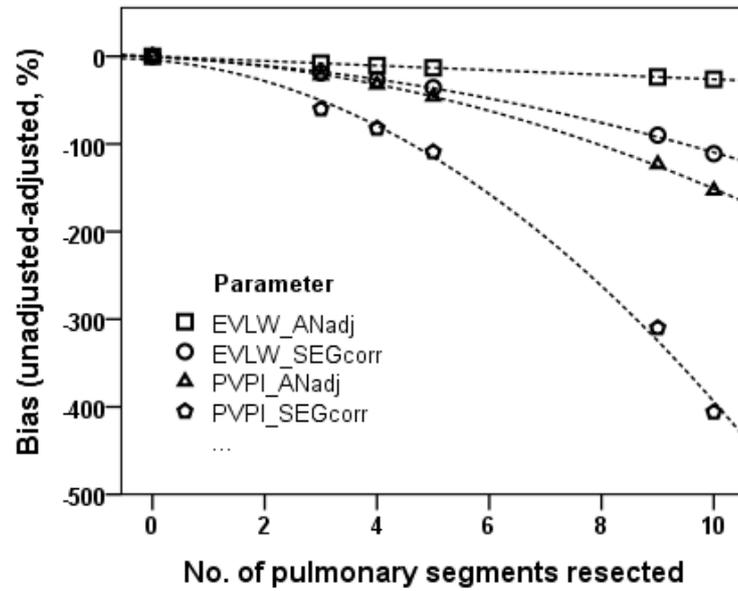


Figure 7.1. Simulated bias resulting from non-adjustment of EVLW and PVPI, in comparison to anatomically adjusted and segment corrected values.

ANadj, ITBV/GEDV adjusted by 'anatomical approach'; SEGcorr, result corrected to reflect no of pulmonary segments remaining.

It can be seen that anatomical adjustment of the parameters leads to a more conservative estimate of the degree of overestimation. Anticipated bias ranges from -7.8% for $EVLW_{ANadj}$ to -406.1% for $PVPI_{SEGcorr}$.

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List of Presentations and Publications

Oral presentations:

- Is the incidence of ALI / ARDS following lung resection falling over time? A meta-regression analysis 2013
Association of Cardiothoracic Anaesthetists (Cambridge)
- Precision and construct validity of extravascular lung water measurement following lung resection 2013
European Association of Cardiothoracic Anaesthetists (Barcelona)
- Adjustment of extravascular lung water calculation for volume of resected lung does not improve construct validity in thoracic surgical patients 2013
Glasgow and West of Scotland Society of Anaesthetists / Glasgow Anaesthetic Research Club (Glasgow)
- The precision of trans-pulmonary thermodilution measurements after lung resection 2012
Association of Cardiothoracic Anaesthetists (Newcastle)
- Adjustment of extravascular lung water calculation for volume of resected lung does not improve construct validity in thoracic surgical patients 2012
Anaesthetic Research Society (London)
- The novel biomarker pentraxin 3 may aid risk stratification in the early post-operative period following lung resection 2012
European Association of Cardiothoracic Anaesthetists (Amsterdam)
- Early Experience with a Panel of Acute Lung Injury Biomarkers after Lung Resection 2011
Glasgow and West of Scotland Society of Anaesthetists (Glasgow)
- Early Experience with a Panel of Acute Lung Injury Biomarkers after Lung Resection 2011
Association of Cardiothoracic Anaesthetists (Glasgow)

Poster presentations:

- Early Experience with a Panel of Acute Lung Injury Biomarkers after Lung Resection 2012
Scottish Intensive Care Society (St Andrews)
- Lung protective ventilation during one-lung anaesthesia: A survey of UK practice 2010
European Association of Cardiothoracic Anaesthetists (Edinburgh)
- Analgesic technique and attitudes towards management of hypotension and peri-operative fluid balance in thoracic anaesthesia: A survey of UK practice. 2010
European Association of Cardiothoracic Anaesthetists (Edinburgh)
- Lung protective ventilation during one-lung anaesthesia: A survey of UK practice 2009
Association of Cardiothoracic Anaesthetists (Blackpool)
- Analgesic technique and attitudes towards management of hypotension and peri-operative fluid balance in thoracic anaesthesia: A survey of UK practice. 2009
Association of Cardiothoracic Anaesthetists (Blackpool)
- Lung isolation techniques for thoracic surgery: A survey of UK practice. 2009
Association of Cardiothoracic Anaesthetists (Blackpool)

List of publications

Abstracts:

1. Shelley B, Macfie A, Kinsella J. Trends in the incidence and mortality of post-lung resection lung injury over time; a meta-regression analysis. *Critical Care Medicine* 2014, in press.
2. Shelley B, Macfie A, Tanner O, Galley H, Kinsella J. Pentraxin 3 as a novel biomarker of lung injury; evidence from a human one-lung ventilation model. *Critical Care Medicine* 2014, in press.
3. Shelley B, Tanner O, Macfie A, Kinsella J. Precision and construct validity of extravascular lung water measurement following lung resection (abstract). *Journal of Applied Cardiopulmonary Pathophysiology* 2013; 17(Supp 1):175-6.

4. Shelley B, Macfie A, Kinsella J. The precision of trans-pulmonary thermodilution measurements after lung resection (abstract). *Anaesthesia* 2013; 68(7): 787.
5. Shelley B, Tanner O, Macfie A, Kinsella J. Adjustment of extravascular lung water calculation for volume of resected lung does not improve construct validity in thoracic surgical patients (abstract). *British Journal of Anaesthesia* 2013; 110(5): 860P-885P.
6. Shelley B, Macfie A, Kinsella J, Galley HF. The novel biomarker pentraxin 3 may aid risk stratification in the early post-operative period following lung resection (Abstract). *Journal of Applied Cardiopulmonary Pathophysiology* 2012; 16(Supp 1):222-3.
7. Shelley B, McSharry C, Macfie A, Kinsella J. Biomarkers of Acute Lung Injury after Lung Resection (Abstract). *Anaesthesia* 2012; 67:554.
8. Shelley B, Macfie A, Schraag S. Analgesic technique and attitudes towards management of hypotension and perioperative fluid balance in thoracic anaesthesia: a survey of UK practice (Abstract). *Journal of Cardiothoracic and Vascular Anaesthesia* 2010; 24(3):S48-49.
9. Shelley B, Macfie A, Schraag S. Lung isolation techniques for thoracic surgery: a survey of UK practice (Abstract). *Journal of Cardiothoracic and Vascular Anaesthesia* 2010; 24(3):S49.

Full publications

10. Eldawlatly A, Turkistani A, Shelley B, El-Tahan M, Macfie A, Kinsella J. Anesthesia for thoracic surgery: A survey of middle eastern practice. *Saudi Journal of Anaesthesia* 2012; 6: 192-6.
11. Shelley B, Macfie A, Kinsella J. Anaesthesia for Thoracic Surgery - A Survey of UK Practice. *Journal of Cardiothoracic and Vascular Anaesthesia*. 2011; 25(6):1014-7. PMID:21868249.

Book chapters:

12. Shelley B. Post-thoracotomy acute lung Injury (chapter contributor). In Smith R, Higgins M, Macfie A. *Oxford Handbook of Cardiothoracic Intensive Care*. Oxford. Oxford University Press, 2014. Pages 177-8.