Evolution of Mesothelioma from Benign Asbestos Pleural Inflammation

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Summary

Pleural Mesothelioma (PM) is an incurable disease with a poor life expectancy. It is most commonly associated with asbestos exposure and the latency period from exposure to PM presentation is usually 20-50years. PM can present after a period of benign pleural inflammation, but the true rate of PM evolution in such cases, and factors promoting evolution, remain unclear. There has been an increase in mesothelioma research in recent years, leading to improved understanding of mesothelioma biology and subsequent treatment advances, but the prognosis remains poor. There have been improvements in PM diagnostics with the addition of ancillary testing including BRCA1-associated protein 1 (BAP1), Methylthioadenosine Phosphorylase (MTAP) and Cyclin-Dependent Kinase Inhibitor 2A (CDKN2A), but their use in prognostication of PM is not fully understood.

The aim of this thesis is to further our knowledge of benign asbestos pleural inflammation and early mesothelioma. The introduction of this thesis outlines the current knowledge of the pathophysiology, clinical characteristics and diagnostics of benign pleural inflammation and PM.

Chapter 2 describes the Meso-ORIGINS Feasibility Study which includes a prospective study to explore the feasibility of a surveillance protocol for patients with asbestos associated pleural inflammation (AAPI), including repeat pleural sampling. This chapter also describes a retrospective study carried out to determine more precisely the rate of evolution of AAPI to PM, and to identify baseline predictors of PM evolution. 296 AAPI patients (39 prospective, 257 retrospective) were recruited/selected. 21/39 prospective recruits were histologically diagnosed (target n=27). Repeat LAT was technically feasible and acceptable in 13/28(46%) and 24/36(67%) cases with complete follow-up data. Mesothelioma evolution was confirmed histologically in 36/257 retrospective cases (14%(95%CI 10.3-18.8) and associated with malignant CT features (OR 4.78(95% CI 2.36-9.86) and age (OR 1.06(95% CI 1.02-1.12).

Chapter 3 describes a systematic review and meta-analysis which aims to define the true rate of PM evolution from benign pleural inflammation, and identify any

characteristics that may give rise to a higher risk of PM evolution. 17/265 identified studies were included, describing 2607 NSP cases and 146 PM evolutions. The summary point estimate of PM evolution was 5.44%(95% CI 3.37-7.51), with significant heterogeneity ($p<0.001$, I^2 82.7%). Higher PM evolution rate was associated with ≥50% asbestos exposure by cohort and high PM incidence settings. Lower evolution rate was associated with surgical NSP biopsies.

Chapter 4 describes a retrospective cohort study performed to evaluate the prognostic utility of CDKN2A and BAP1 testing in PM and benign AAPI. 155 PM cases were included and CDKN2A and BAP1 loss were observed in in 63.2% and 57.4%, respectively. Stage I prevalence was high (60.7%). PM median OS was 12.4 months (95% CI: 11.2-15.9). Adverse OS was associated with non-epithelioid histology, higher performance status and later stage. CKDN2A/BAP1 status was not associated with OS, including by histological subtype or when both genes were lost. Among 42 eligible AAPI cases, CDKN2A and BAP1 loss were observed in 1/42 (2%) and 2/39 (5%), respectively. 28/42 cases died during follow-up; 7/28 (25%) had post-mortem examinations. No PM evolutions were observed.

The findings in these chapters have been essential in the development and delivery of the Meso-ORIGINS study, which forms a major part of the PREDICT-Meso International Accelerator. We have emphasised the importance of longitudinal tissue sampling spanning the period preceding PM development and the use of multi-omic testing to define the biological processes driving PM evolution and survival once PM is established.

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I dedicate this thesis to my daughter Maddie, who despite the sleepless nights, makes everything worthwhile.

Declaration

The work presented in this thesis was undertaken during my tenure as a Clinical Research Fellow at the Pleural Disease Unit, Queen Elizabeth University Hospital, Glasgow, and at the Institute of Cancer Sciences, College of Medical and Veterinary Life Sciences, at the University of Glasgow. I was supervised by Professor Kevin Blyth and Dr Selina Tsim.

All of the work reported in this thesis was undertaken by me, with the assistance of a number of colleagues who have been acknowledged in the previous section, and in the specific chapters of this thesis.

The three results chapters of this thesis have been published or written in preparation for submission, and therefore this thesis is submitted in alternative format.

The writing of this thesis constitutes my own work, prepared solely by me.

Signed……..

Katie Ferguson, September 2024

List of Abbreviations

AAPI	Asbestos Associated Pleural Inflammation
BAP-1	BRCA1-associated protein 1
BAPE	Benign Asbestos Pleural Effusion
bFGF	Basic Fibroblast Growth Factor
BTOG	British Thoracic Oncology Group
BTS	British Thoracic Society
CABG	Coronary Artery Bypass Graft
CDKN2A	Cyclin-Dependent Kinase Inhibitor
CI	Confidence Intervals
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRP	C-Reactive Protein
CRUK	Cancer Research United Kingdom
CT	Computed Tomography
CT-GNB	Computed Tomography-Guided Needle Biopsy
CTPA	CT Pulmonary Angiogram
CTU	Clinical Trials Unit
CXR	Chest x-ray
DPT	Diffuse Pleural Thickening
ERK	Extracellular Signal-related Kinases
FISH	Fluorescence in situ hybridisation
GEMM	Genetically Engineered Mouse Model
H&E	Haematoxylin and Eosin
HD	Homozygous Deletion
IGF	Insulin Like Growth Factor
IQR	Interquartile Range
IHC	Immunohistochemistry
IIDB	Industrial Injuries Disablement Benefit
IL	Interleukin
IMIG	International Mesothelioma Interest Group
IPC	Indwelling Pleural Catheter
ITH	Intra-Tumour Heterogeneity
KM	Kaplan-Meier

LAT	Local Anaesthetic Thoracoscopy
LDH	Lactate Dehydrogenase
MDT	Multi-disciplinary Team
MFT	Manchester University NHS Foundation Trust
MIS	Mesothelioma-In-Situ
MPM	Malignant Pleural Mesothelioma
MRI	Magnetic Resonance Imaging
MTAP	Methylthioadenosine Phosphorylase
NEL	Non Expansile Lung
NWE	North-West England
NGS	Next Generation Sequencing
NHSGGC	National Health Service Greater Glasgow & Clyde
NSP	Non-Specific Pleuritis
NVP	Negative Predictive Value
OS	Overall Survival
PAI	Plasminogen Activator Inhibitor
PDGF	Platelet-derived Growth Factor
PIS	Patient Information Sheet
PM	Pleural Mesothelioma
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QPI	Quality Performance Indicators
QUIPS	Quality in Prognosis Studies
RNS	Reactive Nitrogen Species
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
SMN	Scottish Mesothelioma Network
SV40	Simian virus 40
TB	Tuberculosis
TFPI	Tissue Factor Pathway Inhibitor
TGF-b	Transforming Growth Factor Beta
TNF-α	Tumour Necrosis Factor Alpha
TS	Tumour Suppressor
TUS	Thoracic Ultrasound
TUS-GNB	Thoracic Ultrasound-Guided Needle Biopsy

US	Ultrasound
VATS	Video-Assisted Thoracic Surgery
VEGF	Vascular Endothelial Growth Factor
VOC	Volatile Organic Compounds

Publications relating to this thesis

1. **Ferguson K**, Blyth KG, Benign Pleural Thickening, Fibrosis and Plaques, Editor(s): Sam M Janes, Encyclopedia of Respiratory Medicine (Second Edition), Academic Press, 2022, Pages 499-509, ISBN 9780081027240.
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2. **Ferguson K**, Neilson M, Mercer R, et al. Results of the Meso-ORIGINS feasibility study regarding collection of matched benign-mesothelioma tissue pairs by longitudinal surveillance. BMJ Open 2023;13:e067780.
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Presentations to Learned Societies

1. An update regarding the Meso-ORIGINS feasibility study and the PREDICT-Meso Accelerator Network
Ferguson K, Tsim S, Ferguson J *et al*
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2. Preliminary Results of the Meso-ORIGINS Feasibility Study
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4. Preliminary results of the Meso-ORIGINS feasibility study: retrospective element regarding BAPE-mesothelioma evolution rate
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5. The frequency of BAP1 and MTAP loss in Benign Asbestos Pleural Effusion
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6. Evolution of mesothelioma following initial biopsies showing benign pleural inflammation: a meta-analysis
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7. Mesothelioma Evolution following a diagnosis of Benign Pleural Inflammation: A Systematic Review and Meta-Analysis

Ferguson K, Neilly M, Roche J *et al*

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CHAPTER 1: INTRODUCTION

1 Chapter 1: Introduction

1.1 General Introduction

Pleural mesothelioma (PM) is an incurable disease with a poor life expectancy. Until recently, chemotherapy was the only licenced first line treatment, but is palliative and only extends life expectancy by an average of 3 months (1). Advances in drug therapies have been lacking, other than the recent discovery of combination immune checkpoint inhibition in mesothelioma, which later saw immunotherapies nivolumab and ipilumab approved as standard first line treatment for PM in 2022 (2).

We know that Mesothelioma can present following a period of benign pleural inflammation, which can last years. These cases with apparently benign pleural inflammation are often referred to as 'Non-Specific Pleuritis' (NSP) (3-5). This is a common diagnosis, with retrospective studies showing it accounts for up to 1/3 of thoracoscopic diagnoses (4). When benign pleuritis is coupled with asbestos exposure, the term Asbestos associated pleural inflammation (AAPI) can be used. A significant proportion of patients with NSP or AAPI will evolve into Mesothelioma, and it is currently unclear whether this reflects false negative biopsies or this is a genuine precursor to PM. Current literature suggests that around 12% of patients with benign pleuritis will develop PM within two years (3), and this would likely be even higher in a population with known asbestos exposure. Current clinical practise involves monitoring these patients in outpatient pleural clinics, and then performing a repeat pleural biopsy if the patient develops any concerning features to suggest PM. It is not entirely clear whether these cases represent an initial missed diagnosis of mesothelioma, if there is a true progression from benign to malignancy, or if some of these patients should be classed as 'mesothelioma in situ' (MIS). If we were able to identify such patients at high risk of developing Mesothelioma at an earlier stage, we may be able to intervene earlier. Prospectively studying patients who are asbestos exposed with known benign pleural inflammation gives us a unique opportunity to study early PM biology. The general aim of this thesis is to define the optimal protocol to recruit this group of patients to a clinical trial where repeat biopsies are taken, and whether we can identify the patients who will

progress to PM at an earlier stage by identifying factors that make this more likely.

1.2 Pleural Anatomy

The pleural space is bounded by the pleurae, which are two distinct linings made up of one mesothelial cell layer, with associated basement membrane and connective tissue. The visceral pleural layer surrounds the lung, and the parietal pleura lines the inner thoracic wall, the surface overlying the diaphragm and the lateral mediastinum. In health, the pleural space is filled with a tiny amount of fluid, which is secreted by the pleural mesothelial cells. This fluid acts as lubrication, which encourages the lungs to move freely and smoothly with respiration, and acts to couple the chest wall and the lung together by capillary action (6).

1.3 Pleural Response to Injury

1.3.1 Pathophysiology of Pleural Fibrosis

The mesothelial cells and basement membrane which make up the pleura play an important role in the inflammatory response to injury. This pleural reaction can either promote healing or cause an abnormal reaction which may ultimately lead to the development of pleural fibrosis. This pathological response involves dysregulation of intra-pleural fibrin pathways, which causes increased fibrin production and deposition, as well as reducing fibrin clearance. Although the true pathological process behind the promotion of the fibrin matrix remains unclear, there have been several pro-inflammatory pathways identified which may play a role. These are outlined in Figure 1.1.

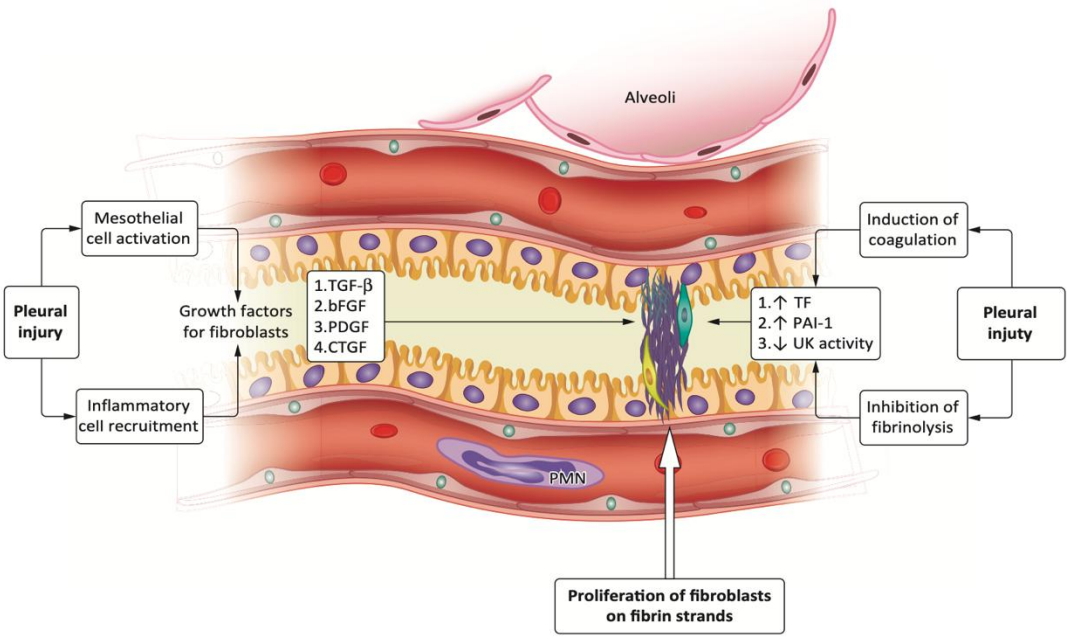

Figure 1.1 Pathogenesis of Pleural Fibrosis

(7)

Over-production of Transforming Growth Factor Beta (TGF-b) has been implicated in several diseases causing fibrosis. TGFB plays a key role in regulating cell proliferation, migration, differentiation and extracellular matrix formation. It is produced by the mesothelial cells which line the pleura, and also the cells which may infiltrate the pleura (malignant or inflammatory) (8). Furthermore, exudative pleural effusions, which are generally caused by inflammatory or malignant conditions, have high levels of TGF-b in comparison to transudates (9). There are also reports of sheep and rabbit models with pleural fibrosis secondary to intra-pleural TGF-b injection (10). TGF-b promotes production of collagen and matrix formation by recruiting fibroblasts. It reduces fibrinolysis by reducing tissue plasminogen activators and increasing mesothelial cell production of plasminogen activator inhibitor (PAI)-1 and 2 (11, 12). Basic Fibroblast growth factor (bFGF) is also known to increase mesothelial cell production and can be implicated in the pathogenesis of pleural fibrosis. Talc pleurodesis is a procedure performed in patients usually with malignant pleural effusions to attempt to fuse the visceral and parietal pleura, with the goal of ceasing pleural fluid production. Previous authors have hypothesised that bFGF plays a key role in effecting pleurodesis since human studies have shown that following administration of talc pleurodesis, pleural fluid aspiration and analysis

shows higher levels of bFGF in the patients whom pleurodesis has been successful, compared to those who have unsuccessful pleurodesis, which is defined by ongoing pleural fluid production (13).

The chemokine vascular endothelial growth factor (VEGF) has also been implicated in pleural fibrosis. It increases vascular permeability in response to inflammation of the pleura, permitting a shift of pro-coagulant fluid into the pleural space. Previous studies using rabbit empyema models have shown that injection of intra-pleural anti-VEGF antibodies decreases pleural angiogenesis and fibrosis (14). Type I and IV collagen proteins and fibronectin glycoproteins, which are also released by mesothelial cells, recruit inflammatory cells and maintain vascular permeability which promotes inflammation (15). The coagulation pathway and fibrinolysis inhibition are also activated in response to injury and inflammation of the pleura. This causes increased activity of tissue factor and PAI-1 (pro-coagulant) and reduced activation of urokinase and tissue factor pathway inhibitor (TFPI) (15), with the ultimate result of fibrin formation and subsequent pleural fibrosis.

1.3.2 Clinical Implications of Pleural Fibrosis

Parietal pleural fibrosis alone does not usually cause any symptoms or influence lung function. However, if fibrosis involves the visceral pleural surface, then there is likely to be a clinical consequence. Causes of visceral pleural fibrosis include asbestos exposure, certain drug exposures, previous infections (including bacterial and tuberculous empyema), previous coronary artery bypass graft (CABG), and renal failure with previous uremic pleuritis. Visceral pleural fibrosis can lead to non-expandable lung (NEL). NEL can categorised further into two sub-groups—trapped lung and entrapped lung, although this is a relatively old distinction, with limited impact in clinical practice today (16).

Trapped lung occurs when there is no ongoing acute pleural inflammation or malignant involvement of the pleura, and a mature, fibrous membrane (or visceral peel) develops, potentially preventing full lung expansion. The diagnosis can only be made if there is a documented chronicity and stability of the lung changes, with no acute inflammation. The pleural effusion is caused by excess of negative pressures in the pleural space, and therefore there is usually no

mediastinal shift even if the effusion is large, and the fluid is typically transudative (17).

Patients are usually asymptomatic and interventions to drain pleural fluid are not necessary. Rarely, if a patient is symptomatic of a restrictive ventilatory defect from trapped lung, surgical decortication may be attempted to improve lung expansion. When visceral pleural fibrosis leads to fusion of the visceral and parietal pleural surfaces, a fibrothorax can ensue. This is the most severe form of visceral pleural fibrosis as it results in contracture of the hemithorax and a progressive restrictive ventilatory defect. The most common causes of fibrothorax include haemothorax which is undrained/incompletely drained and chronic bacterial or tuberculous empyema. On the other hand, entrapped lung is defined by the presence of active pleural inflammation, malignancy or haemothorax. This can improve or resolve completely with appropriate treatment of the underlying cause.

1.4 Pleural Effusion

In health, the volume of pleural fluid is very small and is maintained by a balance of hydrostatic and oncotic pressure within parietal and visceral pleural vessels, and by the pleural lymphatics which drain the fluid (6). Essentially, a pleural effusion is an abnormal collection of pleural fluid in the pleural space. Pleural effusions can be classified into transudates and exudates using Light's criteria. The differential diagnosis of a pleural effusion and classification into transudates and exudates is summarised in Table 1.1.

Table 1.1 Pleural effusion aetiology and classification

Transudates	**Exudates**
Peritoneal dialysis	Infection
Nephrotic syndrome	Tuberculosis
Cardiac failure	Malignancy
Liver cirrhosis	Pulmonary Infarction
Hypoalbuminaemia	Pulmonary Embolism
	Rheumatoid Arthritis

Pathology causing inflammation results in increased vascular permeability, which can result in leakage of protein-rich fluid into the pleural space, resulting in an exudative pleural effusion. Neoplastic processes also alter vascular anatomy and can obstruct lymphatic drainage resulting in a pleural exudate. However, case series have shown that malignant pleural effusions can be transudative in 5% of cases (18). Some conditions which typically cause transudates can also turn into exudates, such as chronic cardiac failure and patients treated with diuretics. Conversely, transudative effusions tend to be caused by conditions associated with altered hydrostatic pressure, permeability and oncotic pressure, including heart failure, and hypoalbuminaemia (seen in cirrhosis and renal failure).
To classify pleural effusions into transudates or exudates a sample of pleural fluid is often analysed for pH, glucose, protein, and lactate dehydrogenase (LDH). A pleural effusion can be classed as exudative according to Light's criteria if one or more of the following criteria are met: pleural fluid protein to serum protein ratio is greater than 0.5, pleural fluid and serum LDH ratio greater than 0.6, or pleural fluid LDH greater than 2/3 times the upper limit of normal serum LDH (19).

1.5 Asbestos

The term 'asbestos' describes a group of naturally occurring fibrous material. These can be classified by their shape into serpentines and amphiboles based on morphology (20). The serpentine asbestos fibres have a curly appearance microscopically and include chrysotile. 95% of commercial asbestos use is with chrysotile, commonly referred to as 'white asbestos' due to its colour (21). Amphiboles are microscopically straight or rod-like and include crocidolite ('blue asbestos'), actinolite, amosite ('brown asbestos'), anthophyllite and tremolite (20). Amphiboles are far more hazardous than the serpentine fibres. Asbestos products have high tensile strength, are flexible and can be woven, and are resistant to chemical, thermal and electrical degradation (22). The risk from asbestos is thought to be highest when small fibres can be inhaled or ingested, such as when larger pieces are disturbed.

Although there is evidence of historical use of asbestos, it was not in popular use until the late 1800s at the start of the Industrial Revolution. Asbestos was mined

in every continent of the world. A stark increase in the use of asbestos was seen in 1920s and 1930s with its use in thousands of products including car brake pads and clutches, roofing and flooring, insultation for electric wiring, cement, and thermal insultation for homes (20).

Shipyards are now well known for being a site of widespread asbestos exposure, even in workers who deny directly coming into contact with asbestos. Other common occupations that were typically high risk for asbestos exposure include construction workers, factory workers, engineers, plumbers, boilermakers, pipefitters, mechanics, railroad workers, and hairdressers. People working in buildings built with asbestos such as schools and hospitals were also at higher risk of exposure.

Unfortunately, children and family members of asbestos exposed workers were also at risk of exposure. Workers could bring asbestos fibres home with them, including on their uniforms which were often washed by their wives. Furthermore, even living near to an area where asbestos is in use such as a mine or shipyard can pose a risk to the local residents.

The health risks of asbestos eventually started to be recognised and in 1982 Sweden was the first country to introduce a ban asbestos use (23). In the UK, the more harmful Amphibole asbestos has been banned since 1985. However, it wasn’t until 1999 that the importation, supply and use of all asbestos was banned in the UK (24). Despite more than 50 countries banning the use of asbestos since, it continues to be mined and exported in developing nations where there is a high need for affordable building material. Furthermore, despite the banning of its use in all 28 countries of the European Union, asbestos is still legal in the United States. Other countries still mining or using asbestos include China, India and Russia, where there is expected to be an epidemic of asbestos related diseases.

1.6 Benign Asbestos Related Pleural Disease

Asbestos exposure is associated with a range of clinical conditions, each with different clinical implications. These include pleural effusions, plaques, diffuse pleural thickening (DPT), lung cancer and mesothelioma.
The pleural fibrosis related to asbestos can either be individual areas of pleural plaque, or more diffuse. When pleural fibrosis is diffuse, this is often referred to as DPT. Table 1.2 summarises the clinical features of both pleural plaques and DPT.

Table 1.2 Characteristics of pleural plaques and diffuse pleural thickening

	Pleural Plaques	**Diffuse Pleural Thickening**
Location	Parietal pleura	Visceral pleura
Laterality	Usually bilateral	Usually unilateral
Latency period	20-40 years	10-15 years
Aetiology	Asbestos only	Multiple (e.g. asbestos, malignancy, empyema)
Symptoms	Asymptomatic	Breathlessness, chest pain

1.6.1 Pleural Plaques

Pleural plaques are the most common clinical entity resulting from asbestos exposure (25). Plaques are individual areas of hyaline fibrosis, made from collagen fibres and can become calcified. Plaques can sometimes be clearly seen on plain x-rays, and usually easily visible on computed tomography (CT) if larger and/or calcified. There is usually a 20–40-year interval from when a person is exposed to asbestos and the occurrence of pleural plaques. The usual position of plaques is the parietal pleura of the postero-lateral chest wall between the 7th and 10th ribs, the lateral chest wall between the 6th and 9th ribs, the dome of the diaphragm, and the mediastinal pleura (26). Plaques are usually found on both sides of the thorax, and the costophrenic angles and apical areas of the lung are usually spared. As plaques are positioned on the parietal pleura, they do not cause any restriction on respiration or even cause any respiratory symptoms (27).

While presence of pleural plaques is not associated with lung cancer or mesothelioma, patients in Scotland can claim compensation if plaques are identified on imaging. However, there are some reports in the literature of an association between pleural plaques and mesothelioma, including Pairon et al. They reported an elevated risk of mesothelioma in patients who had plaques, after controlling for other competing factors including age, latency, type and duration of asbestos exposure (25). They also reported an association with pleural plaques and lung cancer (HR: 2.41, 95% CI: 1.21–4.85 after adjustment for smoking and asbestos exposure) (28).

1.6.2 Diffuse Pleural Thickening

DPT is another clinical condition which can be related to asbestos exposure. DPT has many causes (including haemothorax or drug exposure) but is most commonly caused by asbestos exposure. It may occur as a result episodes of acute pleuritis, or can be caused by an extension of interstitial fibrosis to the visceral pleura (29). A patient may have co-existing DPT and pleural plaques, but these have a very different clinical course and can easily be distinguished on radiological imaging. As previously described, pleural plaques involve the parietal pleura, whereas DPT involves the visceral pleura (30). Cumulative doses of asbestos exposure increase the risk of DPT, which is not true of pleural plaques (31). DPT usually only involves one side of the thorax, including the costophrenic recess. It can progress to involve a complete lobe, or the entire lung and fissures (30). DPT can cause adhesions between the visceral and parietal pleurae and can even result in obliteration of the pleural space in advanced cases (32). DPT can be seen on chest x-ray (CXR), but CT is generally more sensitive and specific in order to secure the diagnosis.

In the United Kingdom, the definition of DPT is set by the Department for Work and Pensions for adjudication of Industrial Injuries Disability Benefit (IIDB). They previously used CXR to define DPT, requiring ‘obliteration of the costophrenic angle’ as well as associated unilateral or bilateral pleural thickening (33). However, with the increase in the availability of CT scanning over the past decade, the CXR appearance of obliteration of costophrenic angle was removed from the definition (34). Rounded atelectasis may also occur following many different causes of acute pleuritis, and can therefore be associated with DPT.

The term describes an area of atelectasis within the lung itself, beside an area of pleural thickening, where lung tissue is drawn into the pleural/sub-pleural are of fibrosis. When seen on CT, this looks like a rounded or oval sub pleural opacity. It also may be associated with the “comet tail sign”, when bronchovascular bundles converge on, and can appear to swirl around the region of the sub-pleural lesion or rounded atelectasis (35). Strands of fibrosis may be seen coming into the mass from all sides, and this is typically described as “Crow’s feet” (36).

Unlike pleural plaques, DPT is often associated with respiratory symptoms, such as breathlessness and chest pain. Lung function tests may also be abnormal with reduced Forced vital capacity, total lung capacity and diffusion capacity (37). Clinically, when assessing a patient with suspected DPT, the key differential diagnosis is PM, which is a progressive disease and more likely to be symptomatic at time of diagnosis.

There is no specific treatment for DPT, other than supportive measures. There have been no good quality trials of specific interventions for DPT thus far. Surgical decortication is sometimes considered in severe cases, but there would need to be very careful exclusion of any underlying lung disease that may be contributing to symptoms which could influence postoperative lung re-expansion. For example, pleural decortication would not have successful results in cases with clinically significant asbestosis (30). Patients are entitled to make a claim to the IIBD for compensation if diagnosed with DPT related to asbestos exposure at work.

1.6.3 Benign Asbestos Pleural Effusion

Asbestos exposure can cause an acute pleuritis which occurs within approximately 10 years of asbestos exposure (38). Asbestos exposure causes an acute pleurisy, inflammation and usually resolves, with repeated episodes likely resulting in DPT. After a longer latency period, acute pleuritis can present as a pleural effusion, otherwise known as benign asbestos pleural effusion (BAPE). The exact pathogenesis behind BAPE remains relatively unknown. Usually there is an exudative pleural effusion which may be bloody. The effusion is usually reported as lymphocyte-rich, but could also contain other cells including

erythrocytes, neutrophils, mesothelial cells, or eosinophils. Patients with BAPE are often diagnosed incidentally on CXR as it tends not to cause any symptoms. BAPE may also present with chest pain or breathlessness if the pleural effusion is large. BAPE is a diagnosis of exclusion and can only be diagnosed once a clinician has excluded other causes of pleural inflammation/acute pleuritis including malignancy. PM could present very similarly to BAPE and should be excluded as a priority. Therefore, patients with a potential diagnosis of BAPE are usually considered for surgical or medical thoracoscopy to obtain pleural biopsies.

While BAPE may remain static or completely resolve after a period of observation, some cases will progress to PM. One UK study reported that 12% (95% CI: 4.5%–26.4%) of patients diagnosed with benign pleuritis following Local Anaesthetic Thoracoscopy (LAT) progressed to mesothelioma within two years (3). Only 45% of the patients with benign pleuritis in this study had been exposed to asbestos. One would expect cases of BAPE to have a significantly higher risk of mesothelioma progression. Therefore, close surveillance is required for this patient group, with repeat biopsy if clinically indicated.

1.7 Asbestos as a Carcinogen

Both groups of asbestos fibres are harmful to the mesothelial cell, and all types of asbestos are classified are carcinogenic. However, crocidolite is thought to be more carcinogenic than serpentine fibres (39). The risk of mesothelioma is also dependent on the intensity and duration of asbestos exposure (40). It is generally thought that inhaled asbestos fibres can reach the visceral pleura and the pleural space via the alveoli following inhalation, or through the lymphatics (41). There is evidence that shows that the shape, length and width of the fibres affects how easily the fibres are inhaled and whether they travel through the lung and reach the pleural space, where they are retained, with the longer and thinner fibres doing so more easily (42, 43).

The exact carcinogenic effect of asbestos to the mesothelium is not fully understood, but asbestos is thought to injure the pleura both directly and indirectly and at least four possible explanations have been described in the literature (39). Following inhalation, asbestos fibres enter the lung and reach

pleural space and the mesothelial surface, where they cause repeated patterns of damage, inflammation, and healing. Eventually this may lead to pleural scarring and fibrosis (plaques) or malignancy (mesothelioma) (39). This inflammatory reaction results in recruitment of macrophages which attempt to phagocytose the asbestos, and various cytokines are released including Tumour necrosis factor alpha (TNF-α), reactive oxygen species (ROS) and reactive nitrogen species (RNS). There are in vitro studies describing how ROS and RNS are produced in response to asbestos fibres which in turn induce cell damage by damaging DNA and causing strand breaks (44). Asbestos fibres also induce phosphorylation of the mitogen-activated protein kinases and extracellular signal-related kinases (ERK1 and ERK2) and drive increased kinase activity of ERK2. NF-kB is a protein complex that controls DNA transcription, cytokine production and cell survival (45). Asbestos causes mesothelial cells to produce TNF-α, and express TNF-α receptors. TNF-α activates the NF-KB pathway which encourages survival of the damaged mesothelial cells and promotes tumour growth, rather than cell death (46). Another theory describing the mechanism of asbestos related mesothelial injury is that the asbestos fibres actually perforate the mitotic fibres during cell division, and therefore chromosomal damage occurs. In addition to the asbestos related cell damage described above, many other growth factors and cytokines are implicated in the asbestos related development of PM including platelet-derived growth factor (PDGF)-ß, insulin like growth factor (IGF)-1, TGF-α, interleukin (IL-8), VEGF and VEGF-C (47).

1.8 Pleural Mesothelioma

Mesothelioma is a rare tumour arising from the mesothelial cells in the pleura, peritoneal or pericardial lining. PM accounts for 97% of cases. Asbestos is the most common cause of PM and 90% of patients report a history of asbestos exposure (48). Other rare causes include the Simian virus 40 (SV40), a virus that typically infects monkeys, which humans were exposed to via contaminated polio virus vaccines in the 1960s (49). There is also a link between mesothelioma and exposure to ionising radiation and some mineral fibers other than asbestos. Erionite is a silicate found mostly in volcanic regions and has been linked to mesothelioma outbreaks in Turkey, where mesothelioma was reported to be

causing 50% of deaths in three small remote villages in the Cappadocia region (50).

There is also a genetic association with mesothelioma, with the autosomal dominantly inherited germline BRCA1-associated protein 1 (BAP-1) cancer syndrome accounting for a small proportion of mesotheliomas (51).

Due to the long latency period of Mesothelioma (30-40years), the cases of Mesothelioma we see today are a result of the widespread industrial use of asbestos during 1950-1980. The incidence of Mesothelioma in the UK is currently at its peak, with levels expected to fall as a result of the bans that were implemented decades ago. However, global death rates are rising, and an epidemic is predicted in the BRIC countries (including Brazil, Russia, India and China) where asbestos is still used today (52-54).

Mesothelioma is an incurable disease with a poor prognosis, with a median survival of 8 to 14 months (40, 55, 56). Prognosis is largely dependent on tissue subtype and performance status, with non-epithelioid mesotheliomas being associated with a worse prognosis.

1.9 Diagnostic Pathway of Pleural Disease

1.9.1 Presentation

Pleural disease can present with breathlessness, cough, chest pain, or can be asymptomatic and identified incidentally on imaging. PM is considered more likely in patients presenting with chest wall pain, and constitutional symptoms including weight loss, fatigue, and anorexia. A detailed clinical history should be taken to narrow down diagnosis of pleural disease, including enquiring about infective symptoms, smoking history, occupational exposures including asbestos, risk factors for Tuberculosis (TB) (ethnicity, travel, close contacts), symptoms of connective tissue disease (rashes, joint pains), and a drug history.

1.9.2 Imaging

1.9.2.1 Chest Radiograph

A CXR is a simple and non-invasive first line investigation for patients presenting with symptoms that could be explained by pleural disease. Pleural plaques, pleural thickening and effusions can be identified. A pleural effusion can usually be detected on CXR when there is >250mls fluid. It can also allow the clinician to identify evidence of non-expansile lung which can be the result of diseased visceral pleura and has clinical significance for future fluid management.

1.9.2.2 Computed Tomography

Contrast-enhanced CT scan is recommended when investigating pleural disease, as a surveillance tool in benign pleural disease, and for staging of PM. Features which are more indicative of pleural malignancy on CT include nodular pleural thickening, mediastinal pleural thickening, parietal pleural thickening greater than 1cm, and circumferential pleural thickening (57). However, up to 40% of patients with mesothelioma do not have any of these features with imaging reported as benign, and the sensitivity even lower for arterial phase CT (CT pulmonary angiography) (58).

1.9.2.3 Pleural Ultrasound

Pleural ultrasound (US) is a useful tool in the diagnostic work-up for patients with suspected mesothelioma. All diagnostic pleural aspirations require bedside US guidance by an appropriately trained physician. Pleural fluid either appears anechoic (simple) or echogenic (complex/septated). Septations and loculations which may not be clear on CT can be easily identified on US and indicate that the effusion is exudative. Ultrasound may also identify pleural nodules/thickening that may be amenable to percutaneous biopsy. However, ultrasound is operator dependent and identifying some pathologies such as nodules or pleural thickening would require an experienced physician/operator.

1.9.2.4 MRI

Magnetic resonance Imaging (MRI) is an excellent tool for imaging the pleura but is less widely available than CT and less familiar to general radiologists. MRI has higher sensitivity for diagnosis of malignant pleural disease than CT and is particularly useful in the early stage of disease when there is only minimal pleural thickening (59). It is useful in cases where iodinated intravenous contrast is contraindicated, such as allergy or poor renal function. MRI provides particularly good soft tissue images, and therefore is often used in cases of mesothelioma where there is suspected chest wall or diaphragm invasion, and for ensuring patients are staged appropriately prior to surgical assessment

1.9.3 Exhaled Breath Analysis

There has been interest in recent years in using exhaled breath as a non-invasive screening tool for PM. Breath contains volatile organic compounds (VOC) which have been associated with detection of other diseases such as asthma, chronic obstructive pulmonary disease (COPD) and some tumours (60, 61). VOCs are also released by the mesothelium in response to asbestos-induced oxidative stress (62). By sampling the breath of 64 subjects and analysing through chromatography-mass spectrometry, Lamote et al. were able to discriminate PM from asbestos-exposed participants with 100% sensitivity and 91% sensitivity (63). However, this study only included 14 PM patients and therefore further larger prospective study is required before exhaled breath could be a useful PM screening tool. Furthermore, this study was a ‘convenience cohort’ analysis rather than ‘intention to diagnosis’, and therefore the true performance of their technique is unproven when comparing mesothelioma to non-mesothelioma and other pleural disease.

1.9.4 Pleural Fluid Analysis

As discussed previously, pleural fluid aspiration is a useful diagnostic tool in pleural disease. Pleural aspiration can be performed by a trained clinician at the bedside, using US guidance. Fluid colour and consistency is noted, and samples can be sent to microbiology for culture, biochemistry for protein, glucose and

LDH analysis, and cytology to assess for malignant cells. The fluid pH should also be tested when infection is included in the differential.

Benign asbestos pleural effusions and effusions secondary to malignancies, including mesothelioma, tend to be exudative. Macroscopic appearances of pleural fluid may infer a particular diagnosis. Clear/straw-coloured fluid implies a transudative effusion, whereas a heavily blood stained effusion suggests exudates including malignancy, BAPE, post cardiac surgery, infarction, infection or trauma (64).

A cellular differential count may aid the diagnostic process. Lymphocytic effusions occur in tuberculosis, renal/liver failure, post CABG, rheumatoid, and lymphoma. Neutrophilic effusions occur in inflammatory diseases such as pleural infection or parapneumonic effusion, and eosinophilic effusions in drug reactions, Churg-Strauss and malignancy.

Pleural fluid aspirate for cytology alone yields a diagnosis in 60% of cases, with differing sensitivities depending on tumour type (65). A second pleural aspirate increases sensitivity by 10%, but there is no added benefit in a third sample (66). For suspected PM the diagnostic sensitivity is only 19% (95% CI 11-30%) (18). The recommended sample volume sent for cytological analysis is at least 50-60mls, but the optimum volume is still regularly disputed (67, 68). The sensitivity also varies depending on fluid cellularity and processing technique. Pleural cytology in PM is usually bland and is often difficult to distinguish from reactive mesothelial hyperplasia. Furthermore, epithelioid subtypes tend to shed malignant cells into a pleural effusion but sarcomatoid effusions do not, meaning a diagnosis of PM on cytology from a sarcomatoid patient would be highly unlikely (69). A diagnosis of PM on cytology alone is therefore controversial, especially with the medico-legal implications associated with PM. Some of the cytological features that may increase the possibility of mesothelioma include scalloped borders of cell clumps, variations in cytoplasm staining, varying density of cytoplasm staining, and low nuclear to cytoplasmic ratios (70) (71). However, these features may also be present in effusions with benign reactive mesothelial proliferations. There has been recent interest in the use of ancillary

tests to diagnose mesothelioma in cytology which is described later in the chapter (see Pleural Histology section 1.9.6).

1.9.5 Pleural Biopsy

Pleural biopsy is considered the gold standard to diagnose malignant pleural disease. Biopsies can either be taken blindly using the blind closed 'Abrams' needle, image guided (CT or US) cutting needles, local anaesthetic thoracoscopy (LAT) or video-assisted thoracic surgery (VATS). The Abrams needle method is no longer recommended by the British Thoracic Society, other than in regions with a high incidence of TB due to its low diagnostic rate of 57%, and higher complication rates (72) (73). The benefit of a VATS procedure is that large pleural biopsies can be taken, and the pleural space accessed even if it is loculated or fixed. However, a general anaesthetic is required, and it may not be feasible in patients with poor performance status or multiple comorbidities. VATS is highly sensitive with rates of around 95% for diagnosing malignancy (74), with similar results reported for LAT (75) . However, there been no prospective randomised studies to compare the diagnostic sensitivities of LAT and VATS. LAT is a medical alternative to VATS, avoiding the need for a general anaesthetic. LAT can be performed in patients with a poorer performance status and/or co-morbidities who may be unfit for a surgical procedure and general anaesthetic. LAT is performed under local anaesthetic and conscious sedation. A thoracoscope is inserted into the pleural space allowing direct visualisation of the pleural surface. Pleural fluid can be drained and multiple biopsies can be taken. Clinicians also have the opportunity to perform talc ploudrage or place an Indwelling Pleural Catheter (IPC) during LAT, for definitive management of the pleural effusion.

LAT is a well-tolerated procedure with a high diagnostic sensitivity (PM sensitivity 92.6%, specificity 100% n=1369 cases) and is associated with a low complication rate (0% mortality in over 2000 diagnostic LAT cases across 28 studies and a 1.8% major complication rate in over 4500 LAT cases across 47 studies) (75).

LAT feasibility assessment first includes thoracic ultrasound to assess the pleural space. It allows clinicians to assess for the lung 'sliding sign' which is required to

allow safe entry into the pleural space in the presence of minimal pleural fluid, as well as the presence of septations which may preclude LAT (76). However, LAT requires specific training and expertise and is not available in all centres in the UK.

Image guided biopsy (either CT or US) can be considered in some cases if CT imaging demonstrates a focal area of pleural thickening or nodularity. Image guided biopsies are a useful alternative if LAT is unavailable or is not feasible – either due to patient co-morbidities or a 'fixed' and/or loculated pleural space on US assessment. A prospective randomised study showed that the sensitivity for diagnosis of malignancy with a CT-guided cutting-needle biopsy was 87%, which is only slightly lower than LAT (77). The diagnosis of mesothelioma often relies on thoracoscopic appearances and multiple larger biopsies from different areas can be taken at LAT. This is key in identification of non-epithelioid tumour which could change the diagnosis, such as from epithelioid to biphasic and ultimately directing treatment towards immunotherapy rather than chemotherapy given differential response in non-epithelioid patients (78).

1.9.6 Pleural Histology

1.9.6.1 Mesothelioma

PM can be divided into three subtypes based on histology -epithelioid, sarcomatoid and biphasic (combination of both epithelioid and sarcomatoid). Firstly, mesothelial origin should be established by analysing both morphology and immunohistochemistry (IHC) (79). Mesothelioma has a variety of histological patterns and can look fairly similar to other malignancies on routine Haematoxylin and Eosin (H&E) staining. Therefore, a panel of mesothelial and epithelial immunomarkers are recommended to establish the diagnosis. The approach depends on histological subtype. These antibodies have varying sensitivity and specificity for mesothelioma and therefore the International Mesothelioma Interest Group (IMIG), and the British Thoracic Society (BTS), suggest a IHC panel with two mesothelial markers (such as Calretinin, cytokeratin 5/6, Wilms Tumour-1, D-240) and two epithelial markers (such as TTF1, CEA, Ber-EP4) and further markers can be requested if the diagnosis is still not clear (57, 71). These are generally useful for identifying PM subtypes but

discriminating malignant from benign pleuritis or mesothelial proliferation is not reliable using the standard panel of IHC markers. Furthermore, IHC is less useful in identifying sarcomatoid PM, in which the sensitivity and specificity of the usual IHC markers is reduced (57). The advantage of IHC is that it is widely available, quick and less expensive than other supplementary tests such as Fluorescence in situ hybridisation (FISH).

1.9.6.2 Heterogeneity in Mesothelioma

PM is a very heterogenous malignancy, with regards to making a diagnosis, patient prognosis, and individual response to therapies. One of the most important stages in the diagnosis of mesothelioma is sub-typing into epithelioid or non-epithelioid, as this has significant implications for prognosis and treatment options. However, Bueno et al. compared the histology of 282 diagnostic pleural biopsies with their histological specimens at surgery (extrapleural pneumonectomy) and found that subtype analysis was only correct in only 80% of cases (80). This highlights the importance of taking several large pleural biopsies from different areas of the pleura under direct visualisation at either LAT or VATS. However, this study was performed between 1988 and 2000, and there have been subsequent advances in diagnostic techniques including the use of ancillary testing.

There is also evidence to show that PM cases show intra-tumour heterogeneity (ITH). Understanding the biology behind this heterogeneity is clinically important as it influences responses to anti-cancer therapies. Kiyotani et al. initially showed spatial heterogeneity within mesothelioma through whole-exome sequencing using DNA extracted from three different positions in 6 surgically resected pleural tumours, and the outcome showed distinct somatic mutations and immune microenvironment signatures (81). Comertpay et al. also showed that mesothelioma is a polyclonal malignancy through investigating the clonality patterns of 15 tumours using the Human Androgen Receptor assay. The study implied the presence of synchronous primary tumours in PM, with their own somatic mutation and immune signature (82).

1.9.6.3 Distinguishing Benign Pleural Disease from Mesothelioma

Interpretating pleural histology and differentiating benign from malignant pleural pathology can be challenging. Early-stage mesothelioma can have similar clinical, radiological and thoracoscopic appearances as a benign proliferation, and therefore expert histopathology interpretation is key. Patients undergoing investigation for suspected pleural malignancy are frequently found to have non-specific pleuritis/pleural fibrosis/reactive mesothelial hyperplasia at biopsy. Histological features including increased cellularity, cytological atypia, architectural atypia, necrosis, and mitoses are unhelpful as these can be present in both benign and malignant proliferations (71). The diagnosis of PM hinges of the demonstration of sub-pleural tissue invasion (e.g., fat, skeletal muscle) (83). Further difficulties arise when entrapment of mesothelial cells occurs in benign proliferations, mimicking invasion (84).

The term ‘mesothelioma in situ’ has been recently introduced to describe a cohort of patients with concerning pathological features but no definitive mesothelioma diagnosis. Churg et al. describe MIS as a single layer of surface mesothelial cells showing loss of BAP1, no evidence of tumour by imaging and or/by direct examination of the pleura, and no invasive mesothelioma developing for at least 1 year (85).

Recent studies have demonstrated frequent loss of tumour suppressor genes, including BAP1, methylthioadenosine phosphorylase (MTAP) and cyclin-dependent kinase inhibitor (CDKN2A) in PM (86, 87). In addition, longitudinal animal studies report genomic events linked to these (e.g., CDKN2A hypermethylation) in asbestos-driven pleural inflammation before the development of invasive PM (88). These genomic events are not specific to mesothelioma but their presence or absence in pleural biopsies may allow the differentiation between PM and benign pleural disease.

1.9.6.4 BAP1

BAP1 is a protein that acts as a tumour suppressor gene and has both germline and somatic mutations. It can be easily detected by IHC and has a proven role in distinguishing benign from malignant mesothelial proliferations. BAP1 somatic

mutations in mesothelioma were first reported in 2011 (89). There have been many studies published following this, validating its use in differentiating benign mesothelial proliferation from PM and it is now a widely reported in pathology laboratories across the world.

One of the larger studies in the literature by Cigognetti et al. included a series of biopsies which included 212 mesotheliomas, 12 benign mesothelial tumours, and 42 reactive mesothelial proliferations. BAP1 loss was seen in 66% of mesothelioma biopsies but was retained in all benign mesothelial tumours. Furthermore, all 6 cases (100%) of benign mesothelial proliferation with BAP1 loss subsequently progressed to mesothelioma, compared to only 3/36 (8%) biopsies with retained BAP1 that progressed to mesothelioma. This study suggests that BAP1 IHC is 100% specific in differentiating benign from malignant mesothelial proliferation (90). However, the discovery and increased acknowledgement of MIS, which requires BAP1 loss for diagnosis, show that this is not the case, with most cases of MIS never evolving into PM, and by definition none have PM (91).

A meta-analysis was performed to assess the diagnostic accuracy of BAP1 in mesothelioma. 12 studies were included with a total 1824 patients, and results showed that BAP1 loss had a pooled sensitivity of 0.56 (95% CI, 0.50-0.62) and specificity of 1.00 (95% CI, 0.95-1.00) in differentiating between mesothelioma and non-mesothelioma. The studies also report that BAP1 is more accurate in diagnosing epithelioid than biphasic or sarcomatoid mesotheliomas (92). This is supported in the literature with one study reporting BAP1 mutations in approximately 61% of epithelioid mesotheliomas, but only 36% of sarcomatoid mesotheliomas (93).

There does not seem to be a consensus in the literature for an agreed cut-off value for the loss of BAP1 staining. Hida et al. used receiver operating curve (ROC) analysis to set the cut off value for BAP1 IHC in their study (94). They used a cut-off value of 19.7% of cells showing BAP1 expression and found a sensitivity of 67.5% and specificity of 100% for PM. Liu et al also used ROC analysis to establish a cut-off value of less than 19.7% cells expressing BAP1 as ‘BAP1 loss’.

Using that cut-off value, the sensitivity and specificity were 67.5% and 100% respectively for PM (95).

1.9.6.5 CDKN2A

CDKN2A is a tumour suppressor gene which is present in all normal cells and encodes a number of proteins including p16. It is located on chromosome 9p21 which can be detected by FISH. In PM, both copies of CDKN2A are lost, known as homozygous deletion (HD). This can be detected in both pleural fluid cytology and pleural histology specimens (79). Wu et al. compared the frequency of p16 deletion in benign fibrous pleurisy and PM (96). The study of 60 patients showed hemizygous or homozygous deletion in 66.7% of epithelioid PM, 87.5% biphasic PM and 100% of sarcomatoid cases, but deletion was not seen in the benign cases. This is supported by more recent work from Marshall et al., which reports the sensitivity, specificity, NVP and PPV as 50%, 100%, 39%, and 100% respectively, in a large retrospective study of 206 patients. They proposed that at least 20% of cells should have homozygous deletion to class as a positive test. They also showed that homozygous deletion of p16 is associated with a worse survival (OR 4.4, 95% CI 1.84-11.14, p0.001) (97), and this is supported by previous studies (98) (96).

1.9.6.6 MTAP

The MTAP gene is also located on chromosome 9p21 and can be detected by IHC. MTAP and CDKN2A are usually codeleted in pleural mesothelioma biopsies (99). However, whether MTAP IHC can reliably act as a surrogate marker for CDKN2A has been frequently debated. IHC is more widely available, quicker and cheaper to perform in laboratories. For MTAP analysis, loss of nuclear and cytoplasmic staining is viewed as loss, whereas for BAP1, only loss of nuclear staining is classed as loss (100). Chapel at al. studied 56 malignant mesothelial biopsies and showed that loss of MTAP shows 78% sensitivity and 96% specificity for detecting p16 homozygous deletion, with good interobserver agreement and interlaboratory agreement (101). Illei et al. also showed that of 95 cases of PM, 70 cases (74%) had homozygous deletion of CDKN2A, and MTAP was also deleted in 91% of these cases, with no cases identified with MTAP deletion but no CDKN2A loss (99). Further studies are required to show the true value of these

molecular events, as well as identifying the best cut-off for MTAP staining loss, which varies between the current literature. One large study of 125 cases of pleural mesothelioma from the National Reference Centre MESOPATH from 2003-2018 recommends a cut off of ≤30% for MTAP cytoplasmic expression loss, which showed a 97% specificity and 69% sensitivity (102).

1.10 Summary

As discussed in the preceding sections, patients with AAPI are at significant risk of developing mesothelioma and it is often a challenge to differentiate between true benign disease, MIS and early-stage mesothelioma. There have been significant advances in the diagnosis of mesothelioma and its differentiation from benign pleuritis with the additional use of IHC or FISH to detect loss of tumour suppressor genes. The frequency of BAP1, MTAP and CDKN2A loss have not been reported before in a large cohort of patients with benign asbestos associated pleuritis with prolonged follow-up. This is of critical importance in the tissue processing pipeline within PREDICT-Meso. The pipeline will require histological assessment of biopsies collected at the benign stage (pre-mesothelioma). In addition, the prognostic influence of combined and singular molecular events in patients with proven invasive PM is uncertain.
In the PREDICT-Meso CRUK Accelerator Network, we aim to define, *in human subjects,* the key biological events that drive or permit evolution of PM. Within PREDICT-Meso, there is a prospective observational study called Meso-ORIGINS, which is aiming to recruit 590 patients with a diagnosis of AAPI, and perform biological surveillance over a 2-year period preceding the diagnosis of PM. This prospective study has used a final study design and surveillance protocol based on the outcome of my research included in this thesis, which includes the Meso-ORIGINS Feasibility study as a major component.

Meso-ORIGINS, once complete, will facilitate unprecedented surveillance of the key early biological events in PM tumorigenesis. These will be interrogated within other elements of the PREDICT-Meso network for mechanisms and potential druggable targets, using a suite of pre-clinical models being developed across Europe, including a genetically engineered mouse model (GEMM) based in Glasgow.

1.11 Aims of Thesis and Hypothesis

The overall aim of this thesis is to expand on the current understanding of benign asbestos pleural inflammation and its evolution to mesothelioma. This thesis aims to determine the true rate of AAPI to PM evolution, and to identify whether there are any predictive markers. In addition, the thesis aims to explore the feasibility of recruiting patients with AAPI to a prospective study where repeat biopsy samples are taken.

I have undertaken studies on patients with AAPI, to address three specific aims. This work has also proved key in the development of the future Meso-ORIGINS study, and for wider PREDICT-Meso Network planning.

Chapter 2: The Meso-ORIGINS Feasibility Study. Prospective Element -to explore the technical feasibility and patient acceptability of a surveillance protocol for patients with AAPI, including LAT and alternative strategies such as blood tests, breath tests, imaging, and pleural fluid sampling. Retrospective Element -to determine more precisely the rate of evolution of AAPI to PM, and to identify baseline predictors of transition to PM.

Chapter 3: Mesothelioma evolution following a diagnosis of benign pleural inflammation: A systematic review and meta-analysis. This study aims to review the current literature describing the evolution of non-specific pleuritis to mesothelioma, and define the true rate of mesothelioma progression. We also aim to identify any study or subject characteristics that may give rise to a higher risk of developing mesothelioma.

Chapter 4: Prognostic utility of CDKN2A and BAP1 testing in Pleural Mesothelioma and Asbestos-Associated Benign Pleural Inflammation. This study aims to test the prognostic utility of CDKN2A/BAP1 status when testing was performed in diagnostically challenging PM cases, and to compare event frequency with a cohort of patients with AAPI with no PM evolution over 2-year follow-up.

CHAPTER 2:

RESULTS OF THE MESO-ORIGINS FEASIBILITY STUDY REGARDING COLLECTION OF MATCHED BENIGN-MESOTHELIOMA TISSUE PAIRS BY LONGITUDINAL SURVEILLANCE

2 Chapter 2: Results of the Meso-ORIGINS feasibility study regarding collection of matched benign-mesothelioma tissue pairs by longitudinal surveillance

2.1 Introduction to this paper

As outlined in the introductory chapter, a significant number of patients with benign pleuritis will evolve to PM. This chapter reports the Meso-ORIGINS Feasibility study, which was designed to assess the feasibility of delivering, and inform the design of, the future prospective study called Meso-ORIGINS. Meso-ORIGINS is a prospective study which aims to recruit patients with AAPI, to create a longitudinal cohort of cases, some of which will develop PM and thus creating a valuable cohort of AABPI-PM tissue pairs for research and drug development pipelines. The Feasibility study results were essential for the study design and creating the study protocol, as well as addressing important areas of uncertainty such as sample size and recruitment feasibility.

The data collected in this first results chapter, including the reported evolution rate of AAPI to PM, was included in the Meta-analysis described in chapter 3, and the database of AAPI cases collated for the feasibility study was used for the study of molecular events in AAPI and PM in chapter 4.

The current study described in this chapter is a multi-centre feasibility study with a prospective element recruiting patients with benign asbestos pleural inflammation, and a retrospective database study. The primary objective was to determine whether it would be possible to recruit sufficient numbers of eligible patients within the time available to the future main study, based on a proposed surveillance protocol including LAT. The primary endpoint was recruitment rate. The secondary objective was to explore patient acceptability of various surveillance methods including repeat LAT, imaging, bloods, pleural fluid sampling and breath tests. The secondary endpoint was the outcome of a patient acceptability questionnaire. The study was published in BMJ open in 2022, see Section 1.3 for citation and author contributions. In the text that follows, the acronym MPM is used. This is because publication of this paper preceded the

change in terminology to PM. However, these terms are interchangeable for the purposes of this body of work.

2.2 Abstract

Objectives

To assess key elements of the design for Meso-ORIGINS, an ambitious, UK-wide, prospective study that will collect ≥63 matched benign-mesothelioma tissue pairs through longitudinal surveillance and repeat biopsy of patients with asbestos-associated pleural inflammation (AAPI).

Design

A multi-centre, mixed methods feasibility study, comprising a prospective observational element, evaluating recruitment feasibility, technical feasibility of repeat local anaesthetic thoracoscopy (LAT) and patient acceptability, and a retrospective cohort study focused on AAPI-mesothelioma evolution rate, informing sample size.

Setting

4 UK Pleural Disease Centres (February 2019-January 2020).

Participants

Patients with AAPI (history or typical imaging plus appropriate pleural histology) were eligible for both elements. In August 2019, eligibility for the prospective element was broadened, including addition of radiological AAPI for technical feasibility and patient acceptability endpoints only. Retrospective cases required ≥ 2-years follow-up.

Outcome Measures

A prospective recruitment target was set a priori at 27 histological AAPI cases (or 14 in any 6-months). Technical feasibility and patient acceptability were determined at 6-month follow-up by thoracic ultrasound (TUS) surrogates and questionnaires, respectively. Retrospective MPM evolution rate was defined by proportion (95% CI). Baseline predictors of evolution were identified using logistic regression.

Results

296 AAPI patients (39 prospective, 257 retrospective) were recruited/selected. 21/39 prospective recruits were histologically diagnosed (target n=27). Repeat LAT was technically feasible and acceptable in 13/28(46%) and 24/36(67%) cases

with complete follow-up data. Mesothelioma evolution was confirmed histologically in 36/257 retrospective cases (14% (95%CI 10.3-18.8) and associated with malignant CT features (OR 4.78(95% CI 2.36-9.86) and age (OR 1.06(95% CI 1.02-1.12).

Conclusions

Our initial eligibility criteria were too narrow. Meso-ORIGINS will recruit a broader cohort, including prevalent cases, any biopsy type and patients with malignant CT features. A range of re-biopsy techniques will be allowed, accounting for technical and patient factors. The sample size has been reduced to 500.

Trial Registration
ISRCTN12840870

KEY WORDS: Mesothelioma, Pleural Effusion, Non-specific Pleuritis, Asbestos

2.3 Citation

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2.4 Author Contribution

The study design was conceived by KGB and KF. KF, RM, JK, KM, HW, ST, NM, NR, ME and KGB contributed to patient recruitment and data collection. Statistical analyses were performed by KGB, KF and MN. KF and KGB prepared the manuscript. KF, MN, RM, JK, KM, HW, ST, NM, NR, ME and KGB reviewed and approved the final manuscript.

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2.5 Manuscript

2.5.1 Background

Malignant Pleural Mesothelioma (MPM) is an aggressive cancer caused by prior asbestos exposure. Despite recent positive clinical trials, most new drug therapies for MPM have failed, with only 6% reaching phase III in a recent survey (103). The outlook for MPM patients therefore remains poor, with a median survival of less than a year (104). The development of new drugs for MPM poses several unique challenges. The MPM tumour genome is dominated by tumour suppressor loss, with few protein-alternating mutations in obviously druggable oncogenes(86, 105). MPM is also typically associated with high tumour burden, even at the earliest detectable stage of disease (106), probably reflecting the voluminous size of the pleural cavity in which it develops. A better understanding of the processes that drive or permit evolution of MPM is required for development of more effective therapies. This is the focus of the PREDICT-Meso (PRE-malignant DrIvers Combined with Target-drug validation in Mesothelioma) International Accelerator, funded by CRUK/FAECC and IARC.

PREDICT-Meso seeks to take advantage of a unique window of opportunity presented by the disease course of MPM, which typically develops 30-50 years after initial inhalation of asbestos fibres. To date, investigators have been unable to utilise this latent period to collect human tissue samples before and after MPM develops for the purpose of target identification, drug discovery and validation. However, several recent small studies have shown that in some patients, MPM may be preceded by an episode of pleural effusion and apparently benign inflammation, which requires clinical follow-up because of a risk of MPM evolution over the following years. An often-quoted example of this risk was reported by Davies et al, who found that 12% (95%CI 5-26%) patients with ‘non-specific pleuritis’ at local anaesthetic thoracoscopy (LAT) developed MPM within 2 years (3). It is not clear whether this observation is a genuine precursor of MPM or simply reflects false negative biopsies in patients with thoracoscopically-occult MPM. However, the former hypothesis is certainly plausible, with a preceding inflammatory trigger promoting MPM via pro-angiogenic and

immunosuppressive factors known to exist within pleural effusions (107, 108). Whatever the truth of the presentation, this sequence of events provides a unique opportunity in which to study early MPM biology by creating rare in-patient tissue pairs combining preceding pleural inflammation and subsequent invasive MPM. Collection of this material will be performed in the Meso-ORIGINS study (Mesothelioma Observational study of RIsk prediction and Generation of paired benign-meso tissue samples, Including a Nested MRI Sub-study), which is embedded in the PREDICT-Meso programme (see www.predict-meso.com). The tissue collected will be utilised by an international team of pre-clinical scientists for multiomic target identification and for development of a suite of pre-clinical models and high-throughput drug screening. Tissue and other samples (including blood, exhaled breath and imaging) will be banked and used for parallel risk prediction studies designed to identify patients who could reasonably be recruited to future early intervention trials.

The primary objective of Meso-ORIGINS is to create a prospective, longitudinal cohort of patients with asbestos-associated pleural inflammation (AAPI), of whom at least 63 patients will develop MPM over the 2-year study follow-up. A minimum of 63 matched benign-MPM tissue pairs are needed to adequately power the downstream bioinformatics and drug development pipelines. The current feasibility study was performed to address key areas of uncertainty regarding the Meso-ORIGINS study design, including the minimum sample size of AAPI patients needed to generate 63 benign-MPM evolutions within 2 years, recruitment feasibility of the initial sample size estimate (n=590, based on the 12%, 95%CI: 5-26%) rate reported by Davies et al) (3) and the technical feasibility and acceptability to patients of the proposed 2-year surveillance +/- repeat biopsy strategy. The ‘ideal’ protocol from a scientific perspective would involve initial and repeat biopsies using LAT, since this allows complete visual inspection of the entire pleural space and collection of numerous full-thickness pleural biopsies. However, given the exploratory nature of the design, it was not clear at the point of conception, whether reliance on LAT biopsies for eligibility would unduly restrict recruitment. It was also not clear whether it would be technically feasible to perform repeat LAT after fluid drainage, given the potential for auto-pleurodesis or pleural space septation. Furthermore, we were unsure whether patients would find it acceptable to consent to repeat biopsy by LAT (or any

other invasive method). It was also considered essential to improve the precision of the sample size estimate for the main study, since the wide confidence intervals surrounding the MPM evolution rate point estimate reported by Davies et al (12% (95%CI 5-26%))(3) meant the true sample size needed could be as high as 1260 (if the true MPM evolution rate was 5%), making the main study unfeasible. The Meso-ORIGINS Feasibility Study was therefore a mixed methods study incorporating a prospective observational cohort study focused on recruitment feasibility, technical feasibility and patient acceptability, plus a retrospective cohort study focused on improved precision of the sample size estimate.

2.5.2 Methods

2.5.2.1 Design and setting

The overall design involved a prospective observational study and a retrospective cohort study. Both elements were multi-centre and recruited or selected, respectively, patients with AAPI from one of four UK pleural disease centres: (1) Queen Elizabeth University Hospital, Glasgow (2) Southmead Hospital, Bristol (3) Churchill Hospital, Oxford and (4) Wythenshawe Hospital, Manchester. This study was sponsored by NHSGGC and recruited for 12 months (February 2019-January 2020). The protocol was approved by NHS Health Research Authority South Central-Hampshire B Research Ethics Committee (reference 18/SC/0617) and was registered (ISRCTN12840870).

2.5.2.2 Objectives and endpoints

Prospective Observational Study

The primary objective was to determine whether it would be possible to recruit sufficient numbers of eligible patients within the time available to the main study (41 months). This was initially based on eligibility criteria and a surveillance and re-biopsy protocol that required LAT sampling at both timepoints. However, these strict criteria were broadened after 6 months by protocol amendment (see Protocol Amendments section 1.5.6). The primary endpoint was recruitment rate. Recruitment feasibility was defined a priori as

recruitment of 27 eligible participants over 12 months (or 14 patients during any 6-month period). This threshold was based on planned delivery of the main study in 25 sites, which would translate into 590 cases over 41 months (27 patients being equivalent to 2.25 patients/month over 4 sites). The study team have recently recruited 747 patients from 23 UK sites over 3-years to a similar study (109) and considered this size of study deliverable.

The primary objective also included assessment of the technical feasibility of repeat LAT. However, it was not considered ethical to directly test this until the study was proven deliverable. This was therefore assessed indirectly, using established sonographic markers (110) (see Online Supplement, Section 1), and is reported separately to recruitment rate, given the broader eligibility criteria deployed post-amendment.

Online Supplement Section 1: Thoracic Ultrasound Assessment of LAT Feasibility

Introduction to Method

The purpose of this study specific instruction is to provide guidance to researchers involved in the Meso-ORIGINS feasibility study on thoracic ultrasound (TUS) assessment of local anaesthetic thoracoscopy (LAT) feasibility. This assessment is conducted at Visit 2 in all participants. Researchers are required to have attained at least Level 1 RCR TUS competency and to be experienced in LAT. The final judgement regarding the feasibility of LAT and US-guided needle biopsy should be made by the site Principal Investigator, or a suitably experienced delegate.

INSTRUCTIONS

TUS should be performed with patient lying on the unaffected side in the lateral decubitus position. The following information should be recorded on each TUS case report form:

- Patient position
- Is the pleural effusion present or absent?
- If pleural effusion is present, document:
    - size of effusion (in number of rib spaces)
    - the maximum depth of fluid (in centimetres)
    - echogenicity of the effusion (echogenic or non-echogenic)
    - approximate number of septations at site of potential LAT (none, 0-5, 5-10)
- If pleural effusion is absent, document:
    - is lung sliding visible?
    - number of positions lung sliding is demonstrated
    - is there a suitable site for US guided needle biopsy?
- Whether repeat LAT is feasible based on the above information in the opinion of the site principal investigator who would be performing the LAT.

The secondary objective was to explore patient acceptability, including reasons for patients declining repeat LAT, and the acceptability of alternative resampling methods, including pleural fluid aspiration, pleural needle biopsies, imaging, blood and breath tests. The secondary endpoint was the outcome of a simple unvalidated patient acceptability questionnaire (see Online Supplement Section 2).

Online Supplement Section 2: Patient Acceptability Questionnaire

NHS Greater Glasgow and Clyde	**Meso-ORIGINS Patient Acceptability Questionnaire**
Meso-Origins Feasibility Study	
INVESTIGATOR:	**REGISTRATION DATE:** DD / MON / YYYY
SITE:	**PATIENT TRIAL IDENTIFIER:**

The following questions relate to different types of follow up tests and scans that may help us diagnose Mesothelioma at an early stage. We are interested in whether you would have these performed at follow-up clinic visits over the next 2 years. The clinic visits and tests would be every 6 months so you would have them 4 times during this period:

1. WOULD YOU CONSENT TO A BLOOD TEST WHEN YOU COME TO CLINIC ?

Yes ☐ **No** ☐

Please give reasons you would not want to have this:

If the tests were less frequent (e.g. once per year, instead of once every 6 months) would you be willing to have this test?

Yes ☐ No ☐

2. WOULD YOU CONSENT TO A BREATH TEST WHEN YOU COME TO CLINIC ?

Yes ☐ **No** ☐

Please give reasons you would not want to have this:

If the tests were less frequent (e.g. once per year, instead of once every 6 months) would you be willing to have this test?

Yes ☐ No ☐

3. WOULD YOU CONSENT TO ANOTHER CT SCAN SHORTLY BEFORE YOU COME TO CLINIC ?

Yes ☐ **No** ☐

Please give reasons you would not want to have this:

If the tests were less frequent (e.g. once per year, instead of once every 6 months) would you be willing to have this test?

Yes ☐ No ☐

4. WOULD YOU CONSENT TO AN MRI SCAN SHORTLY BEFORE YOU COME TO CLINIC ?

Yes ☐ **No** ☐

Please give reasons you would not want to have this:

If the tests were less frequent (e.g. once per year, instead of once every 6 months) would you be willing to have this test?

Yes ☐ No ☐

The next two questions relate to your views on having repeat fluid or biopsy samples taken during the next 2 years.

These procedures would be performed by the same doctors who did your recent tests. The test would be performed based on careful assessments made during your clinic visits and only if it was thought to be safe and appropriate.

5. WOULD YOU CONSENT TO REPEAT PLEURAL FLUID SAMPLING?

Yes ☐ **No** ☐

Please give reasons you would not want to have this:

6. WOULD YOU CONSENT TO REPEAT THORACOSCOPY AND BIOPSY?

Yes ☐ **No** ☐

Please give reasons you would not want to have this:

Retrospective Cohort Study

The primary objective was to determine the rate of MPM evolution more precisely than previous smaller studies, thereby improving the precision of the sample size estimate for Meso-ORIGINS. The primary endpoint was the MPM evolution rate, and its associated 95%CI. This was defined as the number of eligible patients diagnosed with MPM within 2 years of the index diagnosis of AAPI, divided by the total number of AAPI patients. The secondary objective was to identify baseline predictors of MPM evolution; the intention being to use any features identified to refine the eligibility criteria for the main study and maximise the MPM evolution rate therein. The secondary endpoint was the output of a logistic regression model based on baseline data.

2.5.2.3 Eligibility

Patients with AABPI were sought for both studies, using similar eligibility criteria, with appropriate adjustments to address the different objectives.

Prospective Observational Study

Inclusion Criteria

Participants were subject to all of the following: (1) history of asbestos exposure or compatible radiology, e.g., pleural plaques (2) histological findings compatible with AAPI on any previous pleural biopsy (e.g., benign fibrinous pleurisy, non-specific pleuritis, atypical mesothelial proliferation) or a confident radiological diagnosis based on CT imaging (must include pleural effusion +/- pleural thickening or plaques) and exclusion of other causes (e.g. following pleural fluid aspiration) (3) informed written consent (4) prognosis ≥ 6 months. Note, criterion (2) was broadened from an initial definition that allowed only histological diagnoses made by LAT after feedback from sites (see Protocol Amendments section 1.5.6).

Exclusion Criteria

Participants were excluded if any of the following criteria were met: (1) histological or cytological diagnosis of MPM or any secondary pleural malignancy (2) diagnosis of pleural infection, empyema or granulomatous pleuritis.

Retrospective Cohort Study

Inclusion Criteria
Potential cases were subject to all the following: (1) history of asbestos exposure or compatible radiology, e.g., pleural plaques (2) compatible histological findings on any previous pleural biopsy (e.g., benign fibrinous pleurisy, non-specific pleuritis, atypical mesothelial proliferation).

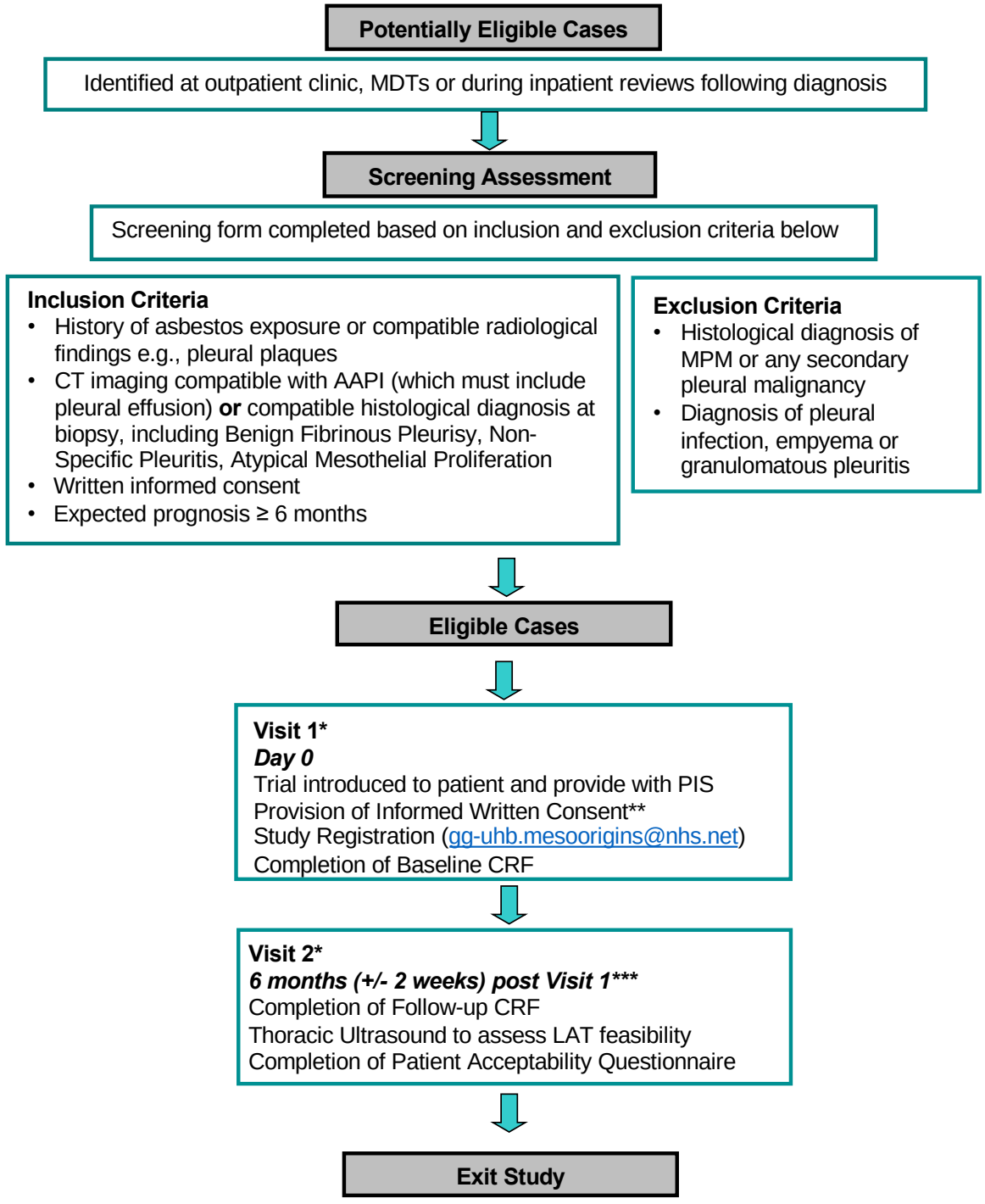
Exclusion Criteria
Cases were excluded if any of the following were met (1) <2 years follow-up at eligibility assessment (2) histological or cytological diagnosis of MPM or any secondary pleural malignancy (3) diagnosis of pleural infection, empyema or granulomatous pleuritis.

2.5.3 Screening, consent, and study interventions

Prospective observational study
Study activities are summarised in the flow chart in the online supplement (Online Supplement Section 3). Eligibility was assessed during outpatient clinic attendances or inpatient encounters. Following provision of a patient information sheet and informed written consent, baseline data was recorded at visit 1. This included asbestos exposure history, demographics, computed tomography (CT) and chest radiograph (CXR) findings. CT findings were codified into benign or malignant, based on previously reported descriptors (58, 111) and by the presence of pleural plaques. CXR was used to classify effusion size as small or large (<50/≥50% hemithorax opacification). A second study visit was completed 6 months later but could be completed at any time during the 6-month follow-up period if the patient presented with progressive ipsilateral pleural disease suggestive of possible MPM evolution, which was recorded if it occurred.

Following a single protocol amendment 6 months into recruitment, visit 2 could also be combined with visit 1 if the diagnosis of AAPI had been made ≥6 months prior to enrolment (since this amendment allowed recruitment of prevalent not

just incident cases, see section 1.5.6 Protocol Amendments). At visit 2, a TUS scan was performed to assess the technical feasibility of repeat LAT according to a standardised protocol (see Online Supplement Section 1). Established sonographic markers(110), including the presence of sufficient fluid, the presence of septations and evidence of 'lung sliding' were recorded and used to classify LAT feasibility, in addition to the feasibility of a TUS-guided needle biopsy (TUS-GNB), based on visualization of suitable and accessible target lesions. At visit 2, patients were also asked to complete a simple, unvalidated patient acceptability questionnaire (see Online Supplement Section 2) regarding repeat sampling options, including breath tests, blood tests, pleural fluid sampling and LAT.



Combined with routine clinic attendance
*** Provide another opportunity for patients to provide consent if required*
**** Can occur as early as 2 month following biopsy if symptomatic recurrence of pleural effusion or any other manifestation of progressive ipsilateral pleural disease. If patient recruited ≥6months after initial BAPE diagnosis, then visit 1 and 2 can be combined at day 0.*

Figure 2.1 Online Supplement Section 3: Prospective Study Flowchart

Retrospective cohort study

Potentially eligible cases were identified from existing databases at each site, supplemented by pathology department and electronic health records. Baseline data corresponding to the date of the index AAPI diagnosis were recorded, matching those collected in the prospective study. These were supplemented by baseline blood results, including haemoglobin, neutrophil, lymphocyte and platelet counts, C-reactive protein (CRP), albumin, lactate dehydrogenase (LDH) and total protein, and baseline pleural fluid measurements, including total protein, LDH, glucose, macroscopic appearance (e.g., blood-stained) and cytology.

2.5.4 Sample size and statistical analysis

For the prospective study, a formal sample size calculation was not possible. We planned to recruit up to 54 patients over 12 months, of whom we expected at least 50% (n=27) to be meet the primary endpoint. Each of the 4 study centres performs 30-50 LATs/year (total 120-200/year), generating a potentially recruitable cohort of 40-60 patients, based on a historical incidence of non-specific pleuritis in 30% of LAT cases (3). Simple descriptive statistics were used to report the primary and secondary endpoints. Baseline data are reported as mean (SD) or median (IQR), depending on distribution, or percentage (%).

The maximum sample size available for the retrospective cohort study was considered to be 300, based on a historical incidence of non-specific pleuritis in 30% of LAT cases[8] and an estimated total of 1000 cases in the LAT databases at the 4 study centres. Assuming a similar MPM evolution rate as previously reported by Davies et al (12%, 95% CI: 5-26%), which was based on 5 MPM evolutions in 42 AAPI patients) (3), we projected 36 evolutions in the estimated 300 AAPI cases available, with an associated 95% CI of 9-16 %. The increased precision in this estimate (95%CI of 21% (3) previously v 7% here) was deemed acceptable for the primary endpoint of the retrospective study. It was acknowledged that smaller numbers of AAPI cases in the retrospective study would proportionately reduce the precision achieved. The primary endpoint of the retrospective study is reported as a proportion with associated 95%CI computed by the modified Wald method (112). Minimum samples sizes for the

subsequent main Meso-ORIGINS study were computed using prediction intervals for binomial data, as proposed by Lu & Jin (113). The sample size of 300 provided adequate power for the secondary endpoint (a logistic regression model for MPM evolution) to test up to 7 candidate predictor variables, assuming a minimum of 5 events per predictor variable (114). Baseline features with a univariate $p<0.05$ were included in multivariate model building, assuming no collinearity was observed. Regression results are reported as odds ratios (OR (95%CI)) for MPM Evolution. Statistical analyses were performed in GraphPad Prism v9.1.0 (San Diego, USA) and R (v.4.0.0, Vienna, Austria)

2.5.5 Protocol amendments

A single amendment to the prospective study was implemented in August 2019 following review of screening data and site feedback. This broadened eligibility to include histological diagnoses made by any pleural biopsy (previously LAT only) and prevalent cases in clinic follow-up (previously incident cases only). The amendment also allowed recruitment of radiological diagnoses after exclusion of other causes (previously histological only), maximising numbers for the secondary objectives. It was acknowledging that patients without histological confirmation would not contribute to the primary endpoint regarding recruitment rate. Primary endpoint data regarding technical feasibility of repeat LAT based on TUS data are therefore reported separately. This amendment also allowed compression of Visits 1 and 2 into a single visit if recruitment occurred ≥6 months after diagnosis in prevalent cases.

2.5.6 Patient and public involvement

Input from patients to the final design of Meso-ORIGINS was a key goal of the current study and is reflected in the secondary objectives. All patient facing materials used were reviewed by lay members of the research ethics committee. The Meso-ORIGINS Study Management Group includes a named PPI representative, who is fully involved in study design and delivery. Details of wider PPI activities of the PREDICT-Meso team can be found at www.predictmeso.com/ppi-and-public-engagement.

2.5.7 Results

2.5.7.1 Prospective Study

Primary objective: recruitment and technical feasibility

39 patients were recruited over the 12-month study period (Glasgow (21), Manchester (12), Bristol (5), Oxford (1)). A study flow chart is presented in Figure 2.2.

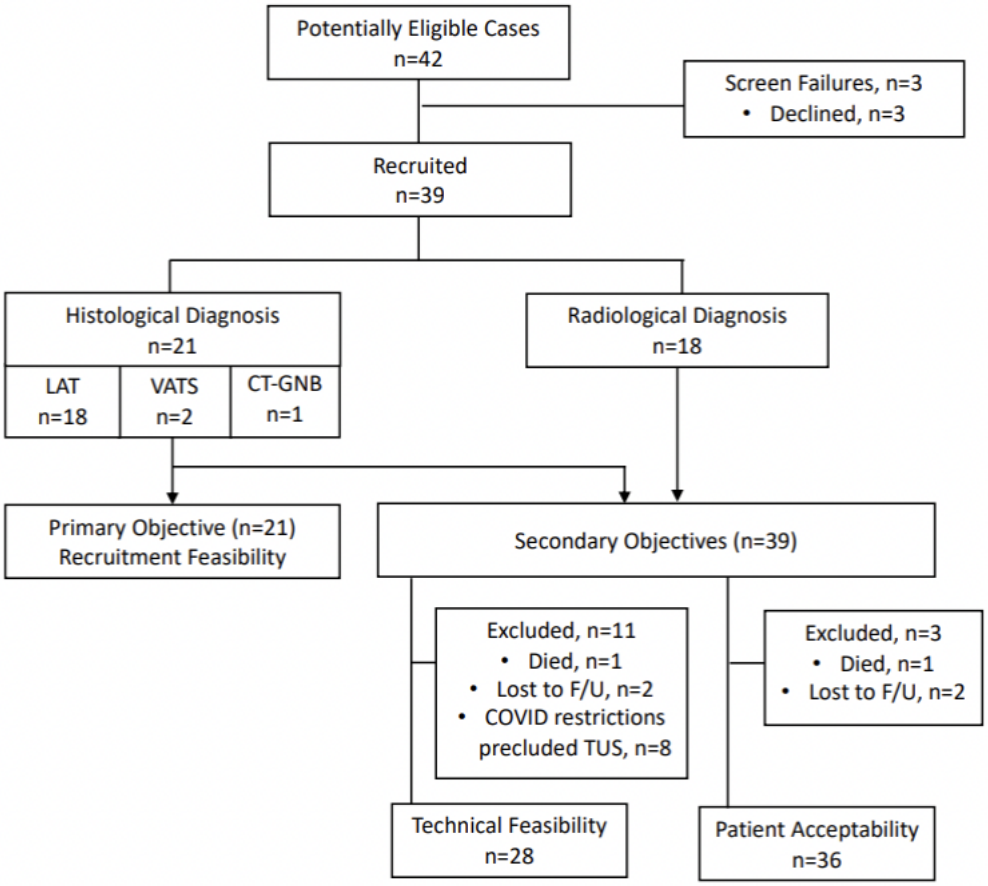


Figure 2.2 Prospective study flowchart

The prospective study flowchart summarises patient recruitment, and numbers of patients completing study activities and reasons for unavailable data. Recruits are separated into those with histological diagnosis, who were eligible to contribute to the primary endpoint and those with radiological diagnosis who were not. Follow-up data regarding the technical feasibility of local anaesthetic thoracoscopy (LAT) and patient acceptability were combined from histological

and radiological diagnoses for the secondary endpoint analyses. VATS, Video assisted thoracoscopic surgery; CT-GNB, Computed Tomography-Guided Needle Biopsy.

21/39 (54%) recruits had a histological diagnosis, meaning the target of 27 was not achieved, see Figure 2.3.

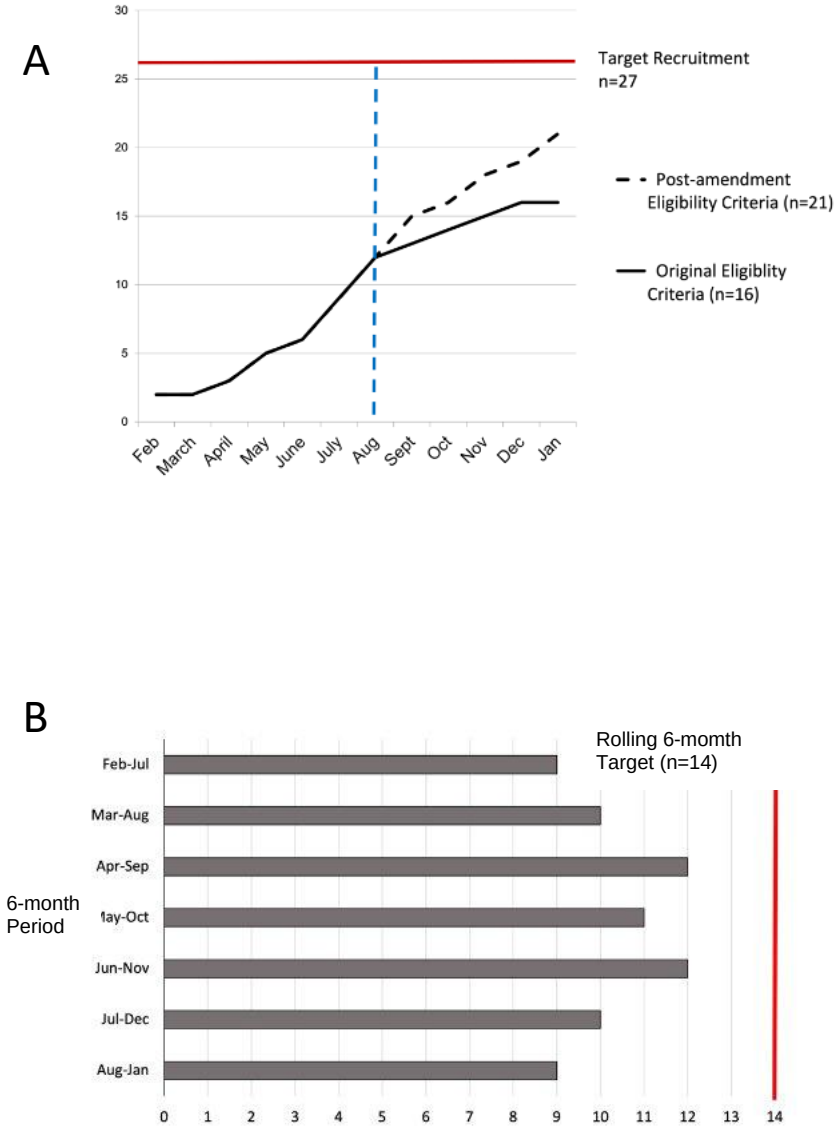


Figure 2.3 Recruitment to the prospective study

The recruitment target for the prospective study was 27 patients over 12 months or 14 patients in any 6-month period. Panel A shows cumulative recruitment of patients eligible to contribute to the primary endpoint over 12-month (histological diagnoses only). The solid line shows patients recruited using the original eligibility criteria, based around local anaesthetic thoracoscopy (LAT) biopsies showing asbestos-associated pleural inflammation (AAPI) in incident cases only. The dashed line shows the total number of patients recruited following a single protocol amendment (blue vertical line) which broadened the eligibility to include biopsies of any type and prevalent cases (n=21). The solid line continuing after the protocol amendment shows the number of recruits that would have been achieved if the original criteria had been retained (n=16).

Panel B summarises the cumulative recruitment over rolling 6-months recruitment periods. The target of 14 recruits in any 6-month period was not achieved. Higher recruitment was observed in periods encompassing the broadened eligibility criteria (August onwards). In several 6-month periods cumulative recruitment approached the alternative threshold of 14 (see April-September and June-November).

Of the histological cases recruited, only 2/21 (9.5%) were diagnosed by surgical thoracoscopy (or video assisted thoracoscopic surgery (VATS)). Baseline characteristics of the recruited population are summarised in Table 2.1

Table 2.1 Baseline characteristics of the prospective and retrospective cohorts recruited to the Meso-ORIGINS feasibility study in 4 UK pleural centres.

	Prospective Study (n=39)	Retrospective Study (n=257)
Age	76 (52-88)	72 (36-90)
Male Gender	39 (100%)	243 (95%)
Asbestos Exposed • Based on history • Based on imaging features only, e.g., plaques	39 (100%) 39 (100%) 0 (0%)	257 (100%) 236 (92%) 21 (8%)
Pleural Effusion Characteristics: • Right sided • Unilateral • <50% of hemithorax on erect chest radiograph	 19 (49%) 37 (94%) 33 (85%)	 126 (49%) 236 (92%) 201 (78%)
CT Findings • Pleural Plaques • Malignant Features	 31 (79%) 5 (13%)	 167 (65%) 68 (26%)

Values are reported as median (range) or n (%)

There were no statistically significant differences between baseline features in histological v radiological diagnoses (see Online Supplement Section 4).

Table 2.2 Online Supplement section 4: Comparison of baseline features in histological versus radiological diagnoses

Characteristic	Histological Diagnosis (n=21)	Radiological Diagnosis (n=18)	p-value
Age	74 (53-84))	76.5 (63-88)	0.2872
Male Gender	21 (100%)	18 (100%)	>0.9999
Asbestos Exposed	21 (100%)	18 (100%)	>0.9999
Pleural Effusion Characteristics: • Right-sided • Unilateral • <50% of hemithorax on erect chest radiograph	 12 (57%) 21 (100%) 16 (76%)	 7 (39%) 17 (94%) 17 (94%)	 0.3406 0.4615 0.1897
Findings on CT imaging • Pleural Plaques • Malignant Features	 15 (71%) 4 (19%)	 16 (89%) 1 (6%)	 0.2472 0.3489

CT: Computed Tomography. Values are reported as median (range) or n (%).

A complete assessment of LAT technical feasibility could not be completed in 11/39 patients, who could not attend Visit 2 due to COVID-19 restrictions, precluding the prerequisite TUS examinations. In the 28/39 cases with TUS data, a pleural effusion was detected in 20/28 (71%) and LAT was technically feasible in 13/28 (46%). A detailed summary of TUS findings can be found the Online Supplement Section 5. Of the 15/28 non-feasible cases, effusion was observed in 9/15 (60%). Effusions were generally small (median 1 (range 1-3) rib spaces and 2/9 were severely septated. Lung sliding was absent from 7/9 non-feasible cases with effusions suggesting small, fixed spaces. In the remaining 6/15 non-feasible cases without effusion, sliding was observed in 4/6, at a median of 3.25 (range 2-6) positions. This suggests these spaces might be accessible by pneumothorax induction in centres with appropriate training. TUS-GNB was technically feasible in 3/28 (11%). Therefore, re-biopsy by LAT or TUS-GNB was feasible in 16/28 (57%).

Online Supplement section 5

Local Anaesthetic Thoracoscopy (LAT) feasibility was assessed indirectly by thoracic ultrasound (TUS) at the single follow-up visit in the prospective study. 28/39 cases recruited completed this visit and had data available for analysis.

A pleural effusion was present in 20/28 (71%) patients and LAT was deemed technically feasible in 13/28 (46%). TUS features associated with LAT feasibility are summarised in the table below. TUS-guided needle biopsy (TUS-GNB) was recorded as a technically feasible alternative in 3/28 (11%) recruits. Re-biopsy by LAT or TUS-GNB was therefore feasible in 16/28 (57%) assessable patients.

Table 2.3 Results of LAT Feasibility Assessment

TUS feature	LAT Feasible n=13	LAT Not Feasible n=15	p-value
Pleural effusion present	11/13 (85%)	9/15 (60%)	0.2213
Character of effusion when present			
• Size (Median # of rib spaces occupied by fluid)	3 (1-4)	1 (1-3)	0.0097
• Any septations reported	1/11 (9%)	2/9 (22%)	0.5658
• Septations judged severe enough to preclude LAT	0/11 (0%)	2/9 (22%)	>0.9999
• Associated lung sliding	8/11 (73%)	2/9 (22%)	0.0698
Lung sliding present	10/13 (77%)	6/15 (40%)	0.0671
Character of Lung Sliding when present			
• Sliding associated with effusion	8/13 (62%)	2/15 (13%)	0.0163
• Sliding associated with no effusion	2/13 (15%)	4/15 (27%)	0.6546
• Extent of sliding (median # of positions with sliding)	4 (1-6%)	4.5 (2-8)	0.4080

Values reported as simple proportions, median (range) or n (%)

Conclusion

LAT feasibility was frequently associated with the presence of a reasonably large pleural effusion, which was rarely septated, and the presence of lung sliding.

Pleural effusion was commonly observed in cases in which LAT was deemed non-feasible, but the effusion tended to be smaller and more frequently septated. Lung sliding was observed in a significant proportion of apparently non-feasible cases (40%), including those without pleural effusion (27%). In the future Meso-ORIGINS study, these dry but not pleurodesed spaces might be accessible by pneumothorax induction in centres with appropriate training and support. Image-guided biopsy, including by TUS-GNB will be an alternative method in some patients in whom LAT is not feasible.

Secondary Objective: patient acceptability

Acceptability questionnaires were completed by 36/39 patients (see Figure 1). In 9/36, questionnaires were completed by telephone due to COVID restrictions. Repeat investigation was deemed acceptable by LAT in 24/36 (67%), by needle aspiration in 29 (81%), by breath test or CT scan in 35/36 (97%) and by blood test or MRI scan in 36/36 (100%). Image guided pleural biopsy was not explicitly assessed in this questionnaire, but responses regarding pleural fluid aspiration are taken as a surrogate for this, given their similarity in terms of patient experience and risk.

Post hoc analysis regarding Mesothelioma evolution

Mesothelioma was subsequently diagnosed in 4/39 patients recruited to the prospective study (10.3%, 95%CI 3.5-24.2%). Repeat sampling was deemed feasible in all 4 cases and confirmed histologically by CT guided biopsy in 3/4. In the 4th case, LAT was planned based on clear radiological progression but not performed due to deteriorating patient fitness.

2.5.7.2 Retrospective Study

Primary Objective: MPM evolution rate

Flow through the study is summarised in Figure 2.4.

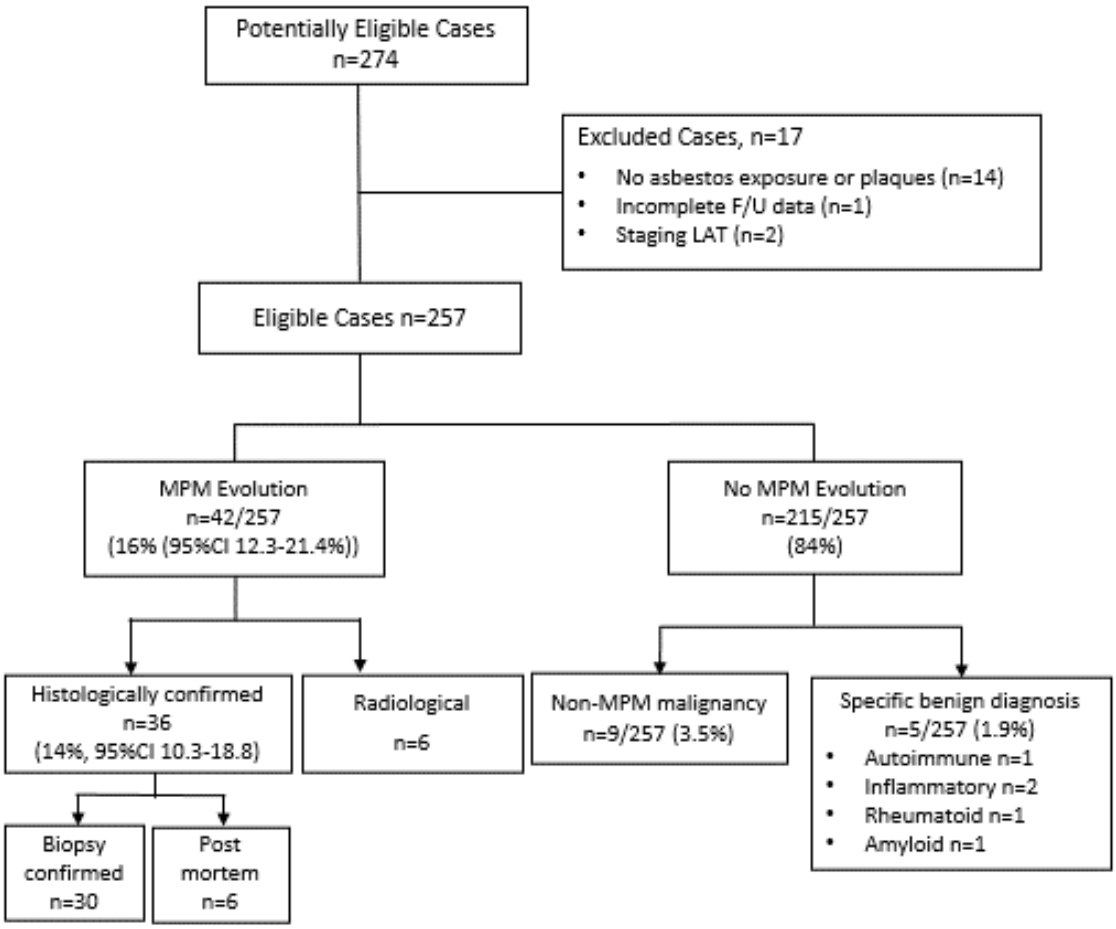


Figure 2.4 Retrospective study flowchart summarising the selection and screening of historical cases of asbestos-associated pleural inflammation (AAPI) diagnosed at the 4 study centres.

Baseline characteristics of the eligible population (n=257) cases are listed in Table 2.1. MPM evolution was recorded in a diagnosis of MPM was made within 2-years of the index diagnosis of AAPI. MPM evolution occurred in 42/257 (16%, 95%CI: 12.3-21.4%) and was confirmed histologically by repeat biopsy or at post-mortem in 36/257 (14%, 95% CI: 10.3-18.8). The median time to repeat biopsy was 3.5 months (IQR 2-9.5), excluding cases confirmed post-mortem.

Secondary Objective: baseline predictors of MPM evolution

Of 11 candidate predictor variables tested by univariate logistic regression, including blood and pleural fluid results, only malignant CT features (OR 4.41 (95% CI 2.22-8.9), p <0.0001) and age (OR 1.06 (95% CI 1.02-1.11), p=0.0055) were associated with MPM evolution (see Table 2). These retained independent associations in subsequent multivariable analyses (age OR 1.06 (95% CI 1.02-1.12, p <0.0001); malignant CT OR 4.78 (95% CI 2.36-9.86, p <0.0001).

Table 2.4 Outcome of logistic regression testing the association between baseline features and subsequent evolution of mesothelioma in patients with benign pleural inflammation recruited to the retrospective study

Baseline Predictor	Univariate OR (95% CI)	p value	Multivariate OR (95% CI)	p value
Age	1.06 (1.01-1.11)	**0.009**	1.06 (1.02-1.11)	<0.0001
Pleural plaques on CT	0.89 (0.45-1.81)	0.735	-	-
Malignant CT report	4.41 (2.22-8.90)	**<0.0001**	4.78 (2.36-9.86)	<0.0001
Asbestos exposure	0.42 (0.16-1.25)	0.096	-	-
Large effusion (>50%)	1.85 (0.84-3.88)	0.122	-	-
Haemoglobin	1.01 (0.99-1.03)	0.369	-	-
Neutrophils	0.91 (0.76-1.07)	0.259	-	-
Lymphocytes	1.29 (0.76-2.14)	0.339	-	-
Platelets	1.00 (0.99-1.01)	0.254	-	-
C-reactive protein	0.99 (0.99-1.01)	0.864	-	-
Albumin	0.99 (0.93-1.02)	0.610	-	-

2.5.8 Discussion

In this multi-centre feasibility study, we tested several critical elements of an initial design for Meso-ORIGINS, which will perform biological surveillance of a large cohort of patients with AAPI and collect matched benign-MPM tissue pairs in the minority who evolve into MPM. In Meso-ORIGINS, downstream

bioinformatics and pre-clinical pipelines require at least 63 benign-MPM tissue pairs during a recruitment window of 41 months. Matched benign-benign pairs from 145 patients without MPM evolution are also required for comparative analyses.

In the prospective feasibility study reported here, 4 UK centres recruited 39 patients in 12 months, following broadening of the original inclusion criteria 6 months into the study. This allowed recruitment of prevalent (not just incident) cases and patients diagnosed using techniques other than LAT. Radiological diagnoses did not contribute to the primary endpoint regarding recruitment rate but added additional cases for the secondary objectives regarding technical feasibility and patient acceptability. 21/39 (54%) patients recruited had a histological diagnosis of AABPI at registration, meaning the a priori threshold for recruitment feasibility based on the original design was not met. However, post-amendment recruitment was higher than it would have otherwise been (see Figure 2a) and surgically diagnosed cases were notably under-represented. The prospective study also demonstrated that LAT was not technically feasible at follow-up in 54% cases, and highlighted barriers to LAT delivery, which could be overcome in the main study, e.g., using pneumothorax induction prior to LAT in cases without effusion. Repeat LAT was acceptable to most, but not all patients (24/36 (67%)), mandating a range of resampling options in the main study. TUS-guided needle biopsy (TUS-GNB) was feasible in additional 3/28 (11%) patients and acceptability for this was reassuringly high 29/36 (81%) and near universal for breath tests, CT scans (both 97%), blood tests and MRI scans (both 100%).

In the retrospective element, we observed MPM evolution in 42/257 patients within 2-years of an AAPI diagnosis (16%, 95%CI 12.3, 21.4%), and confirmed histologically in 36/257 (14%, 95% CI: (10.3-18.8)). The median time to biopsy confirmed MPM evolution was only 3.5 months. Using multivariable logistic regression, age (OR 1.06, (95% CI 1.02-1.12)) and particularly malignant CT features (OR 4.78, (95% CI 2.36-9.86)) were independently associated with MPM evolution.

2.5.8.1 Strengths and limitations

We employed a mixed methods approach, with prospective and retrospective elements specifically testing different elements of the proposed Meso-ORIGINS design. The study centres involved are also representative of future Meso-ORIGINS sites, maximising the generalisability of our results to that study. There is potential for recall bias in the retrospective cohort study, although each participating centre maintains a prospective database which should minimize this. COVID restrictions also meant we were unable to complete face-to-face follow-up visits in 11/39 participants in the prospective study, reducing the volume data available to assess LAT feasibility and patient acceptability.

2.5.8.2 Implications for main study design

Eligibility criteria and re-biopsy strategy

The data collected revealed important flaws in the original eligibility criteria, which limited inclusion to LAT-diagnosed incident cases. Although LAT is desirable at baseline and re-biopsy given the number and size of biopsies available (57, 115), this design would make the study unfeasible. While the a priori recruitment target (n=27) might have been achieved if the broader eligibility criteria been deployed earlier, further changes will be made for the main study, including greater engagement with surgical thoracoscopy centres. The prospective study also demonstrated that a range of re-biopsy strategies will be needed in the main study, since LAT is likely to be unfeasible for technical reasons including auto-pleurodesis or extensive fluid loculation, based on TUS surrogates of these events reported in nearly half of the patients reported here. The current study also demonstrated that although all re-biopsy strategies, including LAT, were acceptable to most patients, this was not universal (67% for LAT, 81% for pleural fluid aspiration, which involves a similar experience and risk to TUS-GNB). The main study protocol will therefore include a dedicated screening visit for patients eligible for re-biopsy, which will allow the full range of resampling options to be explored based on their technical feasibility (principally based on TUS appearances for LAT and TUS-GNB) and the individual preferences of the patient and investigator. The options for re-biopsy will include LAT, which will be the preferred option given the number and size of the

samples available, TUS-GNB or CT-GNB. CT-GNB was not assessed here but is routinely used in clinical practice. It is expected that the addition of this option will maximise the numbers in which some form of repeat biopsy can be acquired, since LAT and TUS-GNB were only technically feasible in a total of 16/28 (57%) cases.

Sample size

Based on the histologically confirmed MPM evolution rate reported here (14%, 95% CI: 10.3-18.8)), the target sample size of the main study has been reduced from 590 to 500. Using prediction intervals (PI) for binomial data, as proposed by Lu & Jin (113), 500 AAPI cases will generate 63 (95% PI 41, 89) MPM evolutions, assuming 10% loss to follow-up (i.e., 450 cases completing follow-up). 500 recruits will also generate 387 (95% PI 361, 409) participants in whom MPM will not evolve within 2 years. Based on the prospective cohort study findings, repeat benign biopsies (by either LAT or TUS-GNB) will be technically feasible in an estimated 228 (95% PI 152, 300) participants in whom MPM does not evolve within 2 years, exceeding the number of Benign-No MPM evolution tissue pairs required (n=145), even when the less-than-universal acceptability of repeat biopsies is accounted for.

2.5.9 Conclusion

The current feasibility study has allowed refinement of the eligibility criteria for Meso-ORIGINS and has prompted significant changes to the re-biopsy strategy and sample size estimate. The study, which forms a major part of the PREDICT-Meso International Accelerator, opened to recruitment in June 2022. The material collected Meso-ORIGINS will be used for multiomic characterisation of the biology associated with mesothelioma evolution, development of a range of pre-clinical models of pre-invasive and early-stage disease, and for high-throughput drug screening and target-drug validation. This information will be complementary to expected data emerging from 2 observational studies in the US, which are focused exclusively on patients with germline BAP1 mutation (NCT03830229 and NCT044310), which is associated with mesothelioma and other cancers (116). Germline BAP1 status will be recorded in all patients recruited to Meso-ORIGINS alongside asbestos exposure histories designed to

capture likely fibre type exposure (117). The goal of these efforts is development of new, early intervention therapies, ready for human trials. Additional information is available at www.predict-meso.com, including opportunities for collaboration and access tissues and data.

DECLARATIONS

Ethics approval and consent to participate

The protocol of this study was approved by the NHS Health Research Authority South Central-Hampshire B Research Ethics Committee (reference 18/SC/0617, on 30/11/2018).

This research was supported by a grant provided by the June Handcock Mesothelioma Research Fund (JH-17-03).

2.6 Reflections on this Paper

The Meso-ORIGINS feasibility study provided critical information for the main Meso-ORIGINS study, which was essential for designing protocol and clarifying the sample size. While the a priori threshold for recruitment based on our pre-amendment protocol was not met, recruitment rate increased following protocol changes which has implications for the main study design. The study highlighted that LAT is often not a feasible option for repeat biopsy (54% cases in the prospective study) or may not be acceptable to some patients meaning other methods of biopsy would need to be considered for the main study such as image guided biopsy. The MPM evolution rate from AAPI in the retrospective element of the study was 42/257 (16%, 95% CI 12.3, 21.4%), histologically confirmed in 36/257 (14%, 95% CI: (10.3-18.8)). This is a notably higher rate of evolution than seen in previous studies, but our cohort were all asbestos exposed. The Meso-ORIGINS main study opened to recruitment in the first site in June 2022, and at the stage of writing is now open to 22 sites across the UK, with 6 further sites in the process of setting up, having recruited 123 benign pleuritis cases and 60 PM cases thus far. From the key results and experience gained from the feasibility study, we refined the target sample size, altered inclusion criteria to include VATs biopsy cases, and included a range of re-biopsy techniques not limited to LAT as originally planned.

CHAPTER 3:

MESOTHELIOMA EVOLUTION FOLLOWING A DIAGNOSIS OF BENIGN PLEURAL INFLAMMATION: A SYSTEMATIC REVIEW AND META-ANALYISS

3 Chapter 3: Mesothelioma Evolution Following a Diagnosis of Benign Pleural Inflammation: A systematic Review and Meta-analysis

3.1 Introduction to this Paper

As outlined in chapter 1, PM is often presaged by a pleural effusion and apparently benign pleural inflammation. This is often referred to as 'non-specific pleuritis' (NSP), and recently there has been a growing number of studies reporting outcomes of NSP cases. However, the outcomes of these studies vary, reporting a wide range of PM evolution rates, and there is no consensus on duration of clinical follow-up recommended. It is also not clear from the current literature whether there are factors that pose a higher risk for future PM evolution.

In the previous chapter 2 I described the results of the Meso-ORIGINS Feasibility study which involved patients with asbestos associated NSP and reported the MPM evolution rate in our cohort. We found that the PM evolution rate was 16% in the retrospective study, which is higher than most previously published studies. It would be of great clinical value to be able to risk-stratify patients with a diagnosis of NSP, enabling clinicians to give patients informed advice regarding the risk of progression to PM. Therefore, this chapter aims to consolidate the current literature and previous studies on patients with benign pleuritis. This chapter describes the first systematic review and meta-analysis of PM evolution following a diagnosis of benign pleural inflammation. The published data from chapter 2 is included in this review and meta-analysis. The review question was 'what proportion of adult patients diagnosed with benign pleural inflammation develop mesothelioma during subsequent clinical follow-up?' and 'are there any study characteristics that give rise to a higher evolution rate?'. The results of this work will assist in clinical management of NSP and inform the design of future PM prevention and early-stage PM clinical trials. This work will be submitted for publication in a journal, and the work was previously published in abstract form and presented as a poster at the British Thoracic Oncology Group (BTOG) Conference 2024. See section 1.3 for details of the previous poster citation and Section 1.4 for Author Contributions.

3.2 Abstract

Introduction

Pleural Mesothelioma (PM) may be presaged by benign pleural inflammation, typically labelled non-specific pleuritis (NSP). NSP is common and presents a clinical challenge given divergent rates of subsequent PM in previous studies. Greater clarity on cohorts at highest risk would inform clinical decision-making and ongoing precision PM prevention research.

Methods

A systematic review and meta-analysis were performed (CRD42021290792). Studies post-2000 were identified via PubMed and conference proceedings. Data extracted included number of NSP cases and subsequent PM evolutions, NSP biopsy type, asbestos exposure, median follow-up, study design and setting. Between-study heterogeneity was described by Q test and I^2(%). A random effects model was constructed for PM evolution rate (%) with subsequent stratification by significant heterogeneity sources. Risk of bias was assessed using the Quality in Prognosis Studies (QUIPS) tool, Funnel plot and rank correlation test.

Results

17/265 identified studies were included, describing 2607 NSP cases and 146 PM evolutions. The summary point estimate of PM evolution was 5.44% (95% CI 3.37-7.51), with significant heterogeneity ($p<0.001$, I^2 82.7%). Asbestos exposure, study setting and NSP biopsy type were significant sources of heterogeneity. Follow-up and study design were non-significant. Higher PM evolution rate was associated with ≥50% asbestos exposure by cohort and high PM incidence settings. Lower evolution rate was associated with surgical NSP biopsies. Most studies demonstrated moderate/high risk of bias. There was evidence of publication bias.

Conclusion

Clinical surveillance following NSP and precision PM prevention trials are likely to be most impactful in asbestos-exposed cohorts and regions with high PM incidence.

3.3 Citation

Mark Neilly*, Katie Ferguson*, Joshua Roche, Matthew Tate, Kevin Blyth, 65 Mesothelioma Evolution following a Diagnosis of Benign Pleural Inflammation: A Systematic Review and Meta-Analysis, Lung Cancer, Volume 190, Supplement 1, 2024,107626, ISSN 0169-5002, https://doi.org/10.1016/j.lungcan.2024.107626.

*Joint first authors

3.4 Author Contribution

KGB conceived the study and agrees to be guarantor for the content reported. KGB, MN, MT and KF contributed to the study design, data collection and analysis. KGB, MN, MT and KF contributed to QUIPS bias assessment. JR contributed to the statistical analysis. All authors contributed to manuscript writing and agreed on the final version. KF and MN were joint first authors on both the manuscript to be submitted for publication and the published abstract in Lung Cancer.

KF: Katie Ferguson
MN: Mark Neilly
JR: Joshua Roche
MT: Matthew Tate
KGB: Kevin G Blyth

3.5 Manuscript

1.1.1 Introduction

Pleural Mesothelioma (PM) is an asbestos-associated thoracic malignancy with a median survival of 12-18 months and few current treatment options(56, 78). Despite recent improvements in diagnostic techniques, most patients present with high tumour volumes(18, 106). This probably reflects the voluminous size of the pleural cavity, which provides enormous capacitance for disease-related effusion, which eventually causes symptomatic presentation in >80% of patients (118). This places a premium on development of new early detection strategies and suitable therapeutic interventions (5).

In some patients, PM is presaged by a pleural effusion and apparently benign pleural inflammation, commonly referred to as 'non-specific pleuritis' (NSP) (3-5). NSP is common, accounting for up to 1/3 of thoracoscopic diagnoses, and it is currently unclear whether it is a genuine precursor to PM, or simply reflects false negative sampling (4). NSP presents a major clinical challenge, since previous studies, which have proliferated recently, report widely divergent rates of PM evolution, with limited consensus regarding the risk in individuals, the factors associated with risk and the minimum follow up period (3-5, 119-132). Detection of NSP also presents a unique translational research opportunity, which is currently being exploited in the CRUK-funded PREDICT-Meso International Accelerator Network. In this program, longitudinal tissue samples from asbestos-associated NSP and subsequent PM evolution are being collected at-scale (5). These tissues are being used for target identification, preclinical model development and drug discovery, with plans thereafter for trials of precision PM prevention in the highest risk pre-PM cases and early-stage invasive disease (5). We performed the first systematic review and meta-analysis focused on establishing the true proportion of patients who will develop PM following a diagnosis of NSP, whilst aiming to identify study characteristics which give rise to a higher-than-average PM evolution rate. The results reported here will assist in clinical management of NSP and inform the design of future precision PM prevention and early-stage PM therapeutic trials.

1.1.2 Methods

3.5.1.1 Design and Registration

We performed a systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (133). The protocol was registered (PROSPERO: CRD42021290792) and is included in the Supplementary Appendix.

3.5.1.2 Systematic Review

Search Strategy

We systematically searched PubMed using combinations of the following terms: benign pleuritis, idiopathic pleuritis, non-specific pleuritis, fibrinous pleuritis, mesothelioma, outcome, follow-up (Table 3.1). The literature search was first performed by KF and KGB in December 2021. Eligibility was assessed independently and agreed by consensus prior to full review and data extraction. The search was updated by MN and MT in September 2023 using the same methodology.

Table 3.1 Combinations of search terms used in the literature search

'fibrinous pleuritis' AND 'mesothelioma'
'fibrinous pleuritis' AND 'follow up'
'fibrinous pleuritis' AND 'outcome'
'idiopathic pleuritis' AND 'mesothelioma'
'idiopathic pleuritis' AND 'follow up'
'idiopathic pleuritis' AND 'outcome'
'benign pleuritis' AND 'mesothelioma'
'benign pleuritis' AND 'follow up'
'benign pleuritis' AND 'outcome'
'non-specific pleuritis' AND 'mesothelioma'
'non-specific pleuritis' AND 'follow up'
'non-specific pleuritis' AND 'outcome'

Study Selection Criteria

Human studies published or presented in English after 1st January 2000 were eligible, including registry and prospective cohort studies, and retrospective case series of ≥30 patients. Review articles were excluded as were case reports, duplicate reports and studies focused on novel diagnostic markers or patients with active malignancy.

Article Review and Data Extraction

Eligible articles were reviewed independently by two experienced reviewers (KF, KGB), who extracted: publication year, number of NSP cases, number of subsequent PM evolutions, proportion of asbestos exposure in the cohort (if recorded), biopsy technique(s) used for the initial (NSP) and subsequent (PM) diagnosis, median follow-up (in months), study setting (country and region), study design (retrospective or prospective). Study authors were contacted for clarification when required. Extracted data was compared to ensure accuracy before recording. The same methods were used by reviewers (MN, MT) following the updated search.

3.5.1.3 Meta-analysis

Design and Statistical Methods

PM evolution rate was computed for all studies, as the proportion of NSP cases in whom PM was subsequently diagnosed during study follow-up. 95% confidence intervals (CIs (Brown-Wilson)) and standard errors were computed using GraphPad Prism v10 (San Diego, USA). A random effects meta-analysis model was constructed with PM Evolution Rate as the primary endpoint. Results were summarised using Forest plots. A random effects model was chosen due to expected between-study heterogeneity, which was summarised by Q test and I^2. Q test $p<0.1$ and I^2 >50% were considered statistically significant. The following potential clinical sources of heterogeneity were interrogated and if heterogeneity improved or resolved (Q test $p\geq0.1$ and I^2 <50%) sub-group comparisons were considered significant: (a) baseline asbestos exposure ≥50% v <50% or not recorded (b) surgical v non-surgical diagnosis of NSP (c) high PM incidence setting v other settings. For the latter, the high incidence subgroup

comprised all UK studies plus a single study conducted in Central Anatolia, Turkey (Metintas 2012). The UK currently has the highest global incidence of PM due to extensive historical use of asbestos in heavy industry, while Central Anatolia has a very high incidence due to environmental exposure to naturally occurring asbestos and incorporation of asbestos into domestic construction materials. Potential methodological sources were also interrogated including (a) retrospective v prospective design (b) median study follow-up <24 months v ≥24 months. All meta-analyses were performed in JASP version 0.18.1 (Amsterdam, Netherlands.

3.5.1.4 Risk of Bias Assessment

Methodological quality and risk of bias was assessed independently by two reviewers using the Quality In Prognostic Studies (QUIPS) tool (134). Within each domain, methodology and reporting were scored as low, moderate, or high risk. Discussion was used to reach a consensus where required, input from a third reviewer for discordant assessments.

3.5.1.5 Publication Bias Assessment

Risk of publication bias was assessed by Funnel plot and rank correlation test for Funnel asymmetry.

3.5.2 Results

3.5.2.1 Study Selection

Outcomes from the systematic review are summarised in the PRISMA flow diagram (Figure 3.1). 265 potentially eligible studies were identified. 17 were eligible, describing 2607 NSP cases and 146 subsequent PM evolutions (3-5, 119-132).

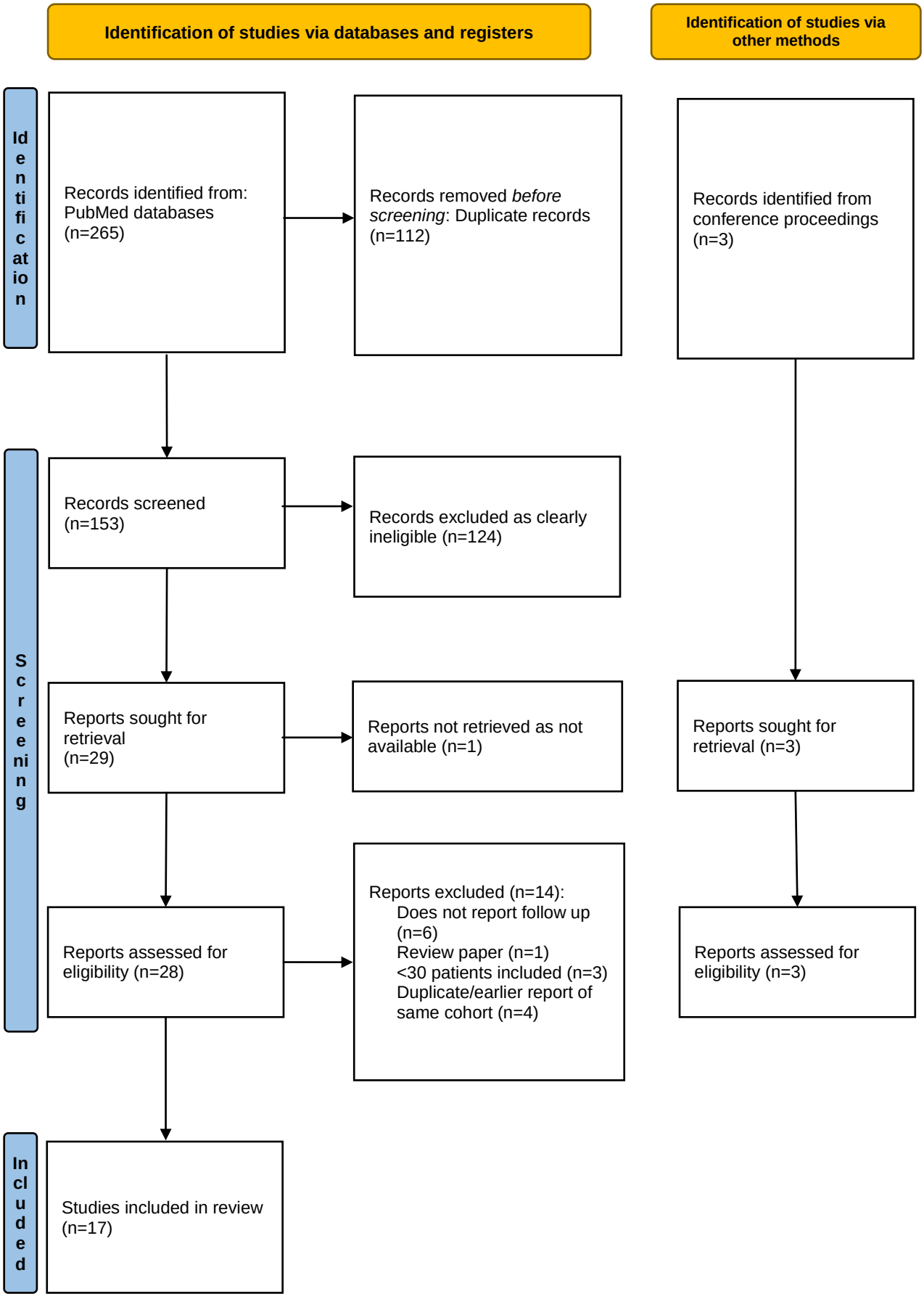


Figure 3.1 PRISMA flow diagram summarising results of the systematic review

3.5.2.2 Characteristics of Studies

Study characteristics are summarised in Table 3.2. 16/17 were retrospective, 1/17 (Ferguson 2023) was a mixed methods design comprising retrospective and prospective studies. This was split into Ferguson 2023 (P) and Ferguson 2023 (R) for analysis.

Table 3.2 Study characteristics

Author & Year	Study Design	NSP (n)	PM Evo (n)	PM Evo Rate (%)	PM Evo Rate 95% CI	PM Evo Rate SE	NSP Biopsy Type(s)	Asb Exp (%)	Median Follow Up (m)	Study Setting	Author & Year	Study Design
Nusair 2002	Retro	31	2	6.5	1.1-20.7	5.4	Non-surgical	N/R	6.4	Israel	Nusair 2002	Retro
Janssen 2004	Retro	208	10	4.8	2.6-8.6	1.5	Non-surgical	N/R	9	Netherlands	Janssen 2004	Retro
Davies 2010	Retro	42	5	11.9	5.2-25.0	5.5	Non-surgical	50	21	UK, Oxford	Davies 2010	Retro
Metintas 2012	Retro	101	16	15.8	10-24.2	3.8	Non-surgical	N/C	24	Turkey, Anatolia	Metintas 2012	Retro
DePew 2014	Retro	64	3	4.7	1.3-12.9	3.1	Surgical	N/R	60	USA, Minnesota	DePew 2014	Retro
Gunloglu 2015	Retro	53	2	3.8	0.7-12.8	3.2	Surgical	N/R	24	Turkey, Istanbul	Gunloglu 2015	Retro
Kyskan 2017	Retro	83	6	7.2	3.4-14.9	3.1	Non-surgical	N/R	24	Canada	Kyskan 2017	Retro
Arkin 2019	Retro	119	2	1.7	0.3-5.9	1.4	Surgical	N/R	29	Turkey, Istanbul	Arkin 2019	Retro
Lin 2019	Retro	213	13	6.1	3.6-10.2	1.7	Non-surgical	N/R	40	UK, Cambridge	Lin 2019	Retro
Reuter 2019	Retro	547	13	2.4	1.4-4.1	0.7	Surgical	N/R	36	Denmark	Reuter 2019	Retro
Karapathiou 2020	Retro	259	3	1.2	0.2-3.3	0.7	Mixed	N/R	47	France, St Etienne	Karapathiou 2020	Retro

Agca 2020	Retro	98	2	2.0	0.4-7.1	1.7	Surgical	N/R	63	Turkey, Istanbul	Agca 2020	Retro
Aujayeb 2020	Retro	105	10	9.5	5.3-16.6	3.0	Non-surgical	N/R	16.7	UK, North England	Aujayeb 2020	Retro
Yu 2021	Retro	154	6	3.9	1.8-8.2	1.7	Non-surgical	N/R	61.5	China	Yu 2021	Retro
Deschuy-teneer 2022	Retro	59	1	1.7	0.1-9.0	2.4	Non-surgical	N/R	N/R	Belgium	Deschuy-teneer 2022	Retro
Sundara-lingam 2023	Retro	175	6	3.4	1.6-7.3	1.5	Mixed	30	18	International	Sundara-lingam 2023	Retro
Ferguson 2023 (P)	Prosp	39	4	10.3	4.1-23.6	5.5	Non-surgical	100	6	UK, Scotland	Ferguson 2023 (P)	Prosp
Ferguson 2023 (R)	Retro	257	42	16.3	12.3-21.4	2.4	Mixed	100	24	UK, Scotland & England	Ferguson 2023 (R)	Retro

NSP:Non-specific Pleuritis; PM Evo: Pleural Mesothelioma Evolution; CI: Confidence Interval; SE Standard Error; Asb Exp: Asbestos Exposure; M: Months; Retro: Retrospective; Prosp: Prospective; N/R: Nor Recorded; N/C: Not computable

3/17 studies reported the proportion of asbestos-exposed participants at baseline (Davies 2010, Sundaralingam 2023, Ferguson 2023). Metintas 2012 described asbestos exposure as 'common', but the data reported precluded reliable classification </≥ 50%. Since a high prevalence of exposure would be expected in this cohort (Central Anatolia, Turkey), it was not felt appropriate to classify this study as '<50% or not recorded'. It was therefore excluded from stratification analyses based on reported asbestos exposure.

5/17 studies used only surgical techniques for diagnosis of NSP (Video-assisted Thoracoscopic Surgery (VATS) in all 5 +/- open biopsy in 2/5). 3 additional studies included some surgically diagnosed NSP cases (Karapathiou 2020, Sundaralingam 2023, Ferguson 2023). Of the 12/17 studies that included non-surgical biopsy techniques for NSP diagnosis, only Local Anaesthetic Thoracoscopy (LAT) was used in 11/12, 1/12 used LAT and image-guided biopsies and 1/12 (Nusair 2002) used only Abram's biopsy.

3.5.2.3 Meta-analysis

Figure 3.2 presents a Forest plot summarising PM evolution in the 17 studies (18 cohorts). The summary point estimate for PM evolution was 5.44% (95% CI 3.37-7.51), with significant between-study heterogeneity ($p<0.001$, I^2 82.7%).

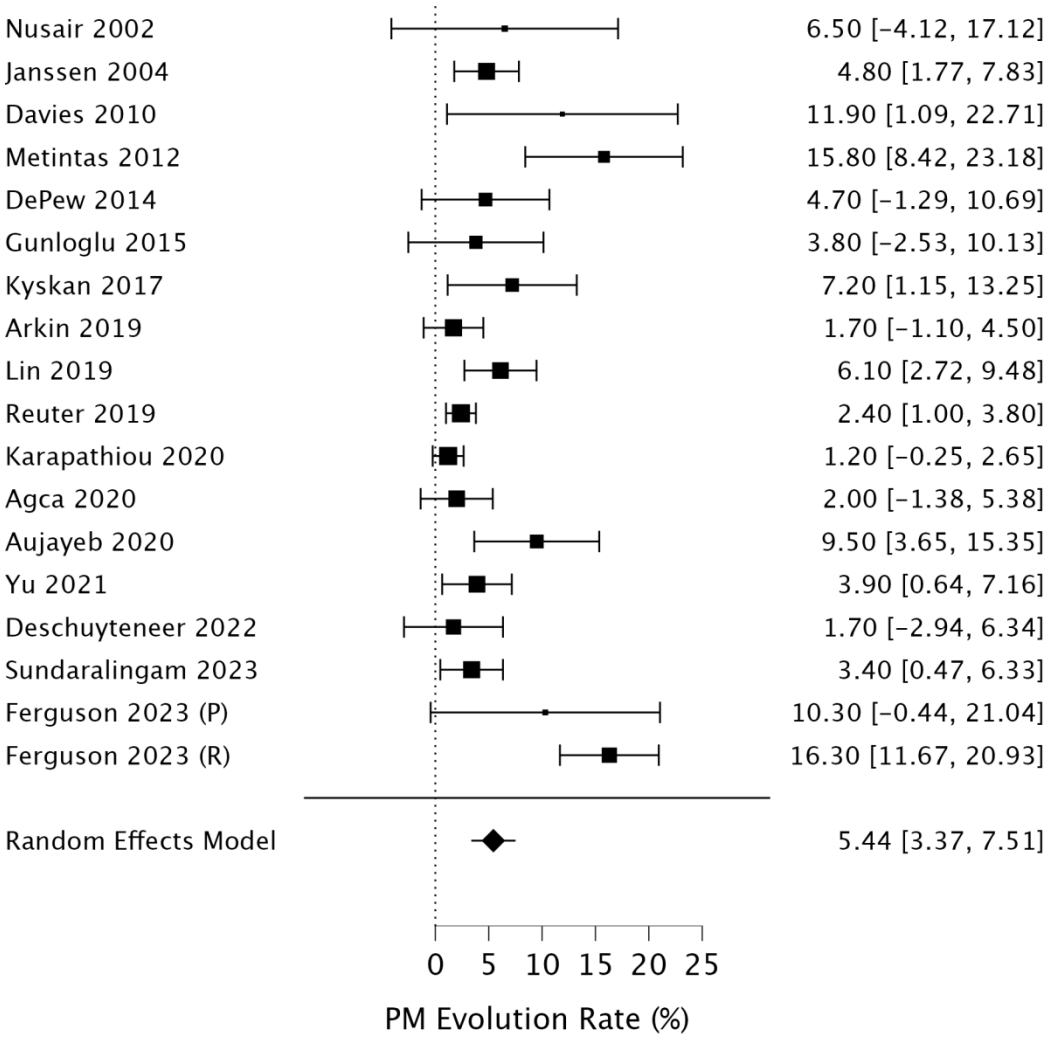


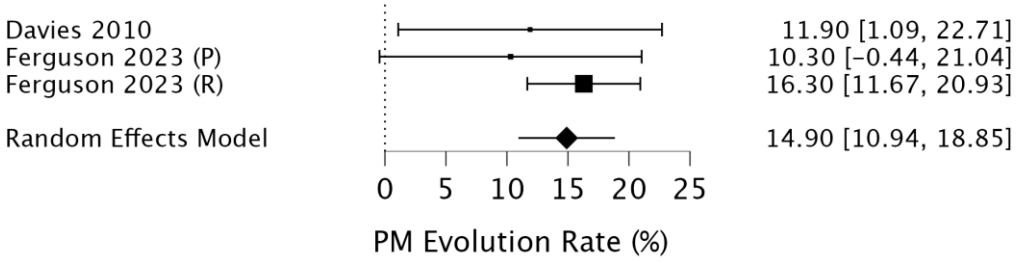
Figure 3.2 Forest plot summarising PM evolution in the 17 studies

3.5.2.4 Clinical Stratification

Asbestos Exposure

PM evolution rate was higher in studies reporting asbestos exposure in ≥ 50% cases than in studies reporting exposure in <50% participants or not reporting this, with non-overlapping CIs (14.90% (95% CI 10.94-18.85) v 3.27% (95% CI 2.16-4.39), respectively), see Figure 3.3 A and 3.3 B. In the high asbestos exposure studies, there was no visible or statistically significant heterogeneity (I^2 0%, Q test p=0.509). In the other studies, between-study heterogeneity was visibly reduced and not statistically significant (I^2 38%, p=0.1). As described in the methods section, these analyses excluded Metintas 2012, in which PM evolution rate was 15.8% (95% CI 10.0-24.2). A high prevalence of asbestos exposure would be expected in this cohort given the setting (Central Anatolia, Turkey).

A: Asbestos Exposure in ≥ 50% NSP cases



B: Asbestos Exposure in < 50% NSP cases or not recorded

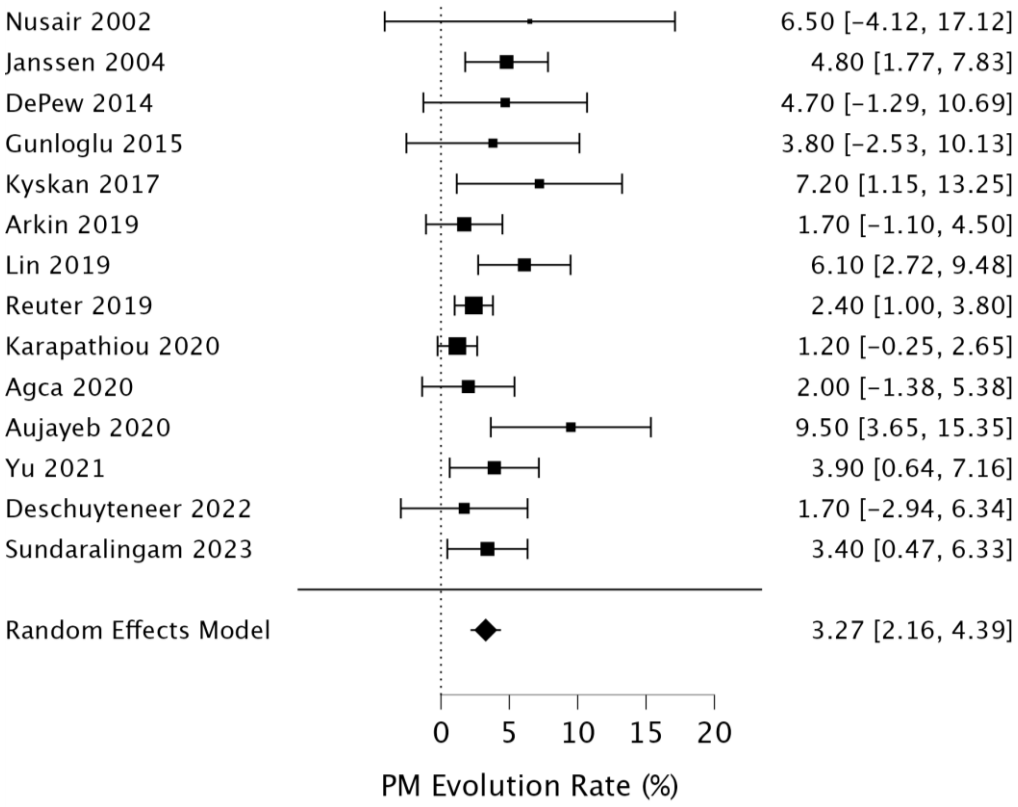
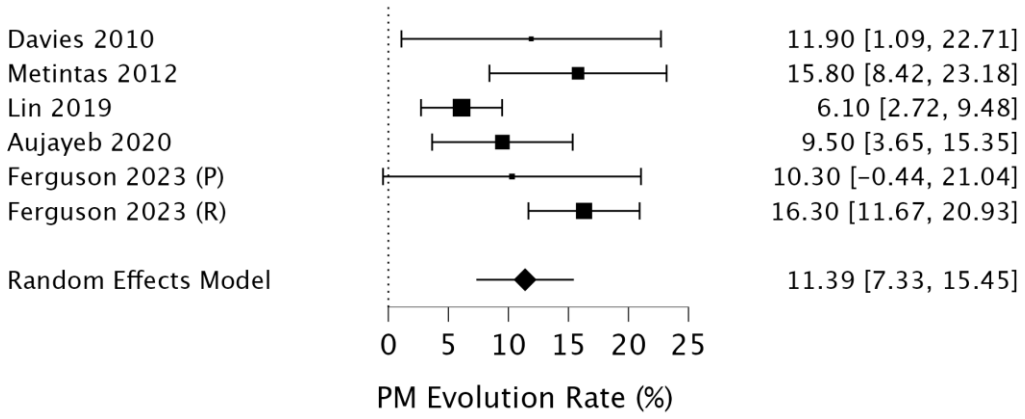


Figure 3.3 Subgroup Analysis by asbestos exposure: ≥50% of NSP cases (A), <50% of NSP cases or not recorded (B)

Study Setting

PM evolution rate was higher in studies from high PM incidence regions (4 UK studies plus Metintas 2012) than studies from other settings, with non-overlapping CIs (11.39% (95% CI 7.33-15.45) v 2.59% (95% CI 1.67-3.51), respectively), see Figure 3A and 3B. In the high incidence subgroup, heterogeneity was visibly reduced but remained statistically significant (I^2 61%, Q test p=0.012). There was no statistically significant heterogeneity between studies from other settings (I^2 14%, p=0.492).

A: Studies conducted in High Mesothelioma Incidence Settings



B: Studies conducted in other settings

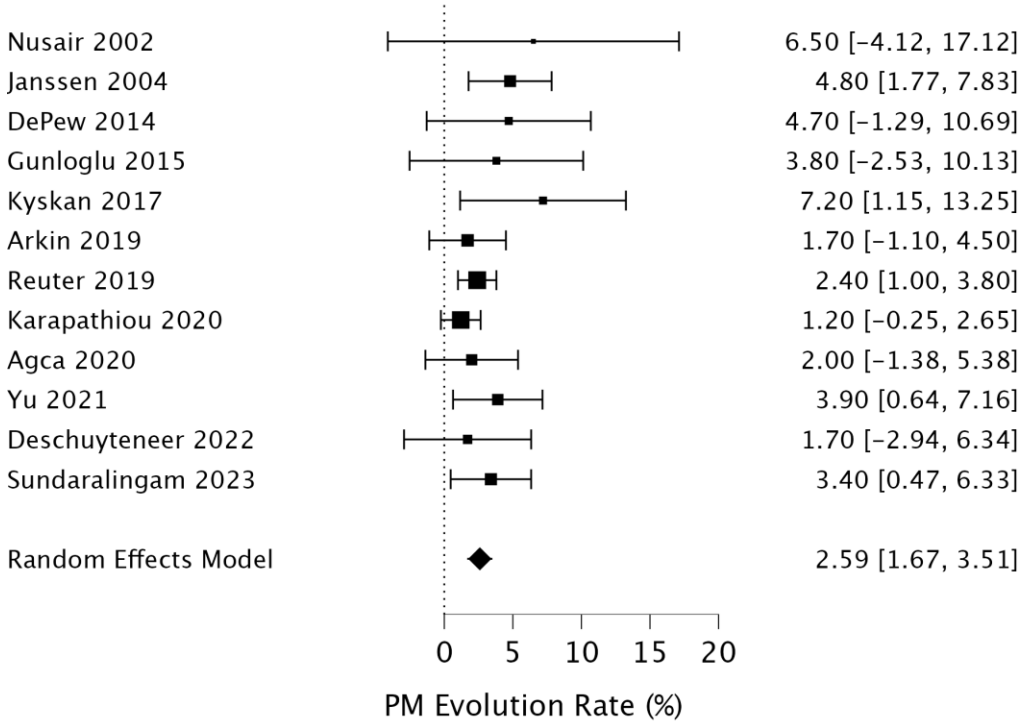
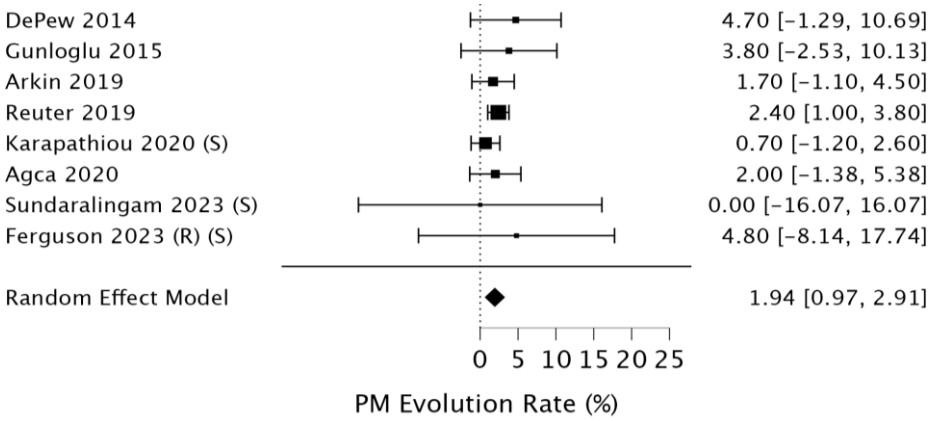


Figure 3.4 Subgroup Analysis by mesothelioma incidence settings: high (A) and other settings (B)

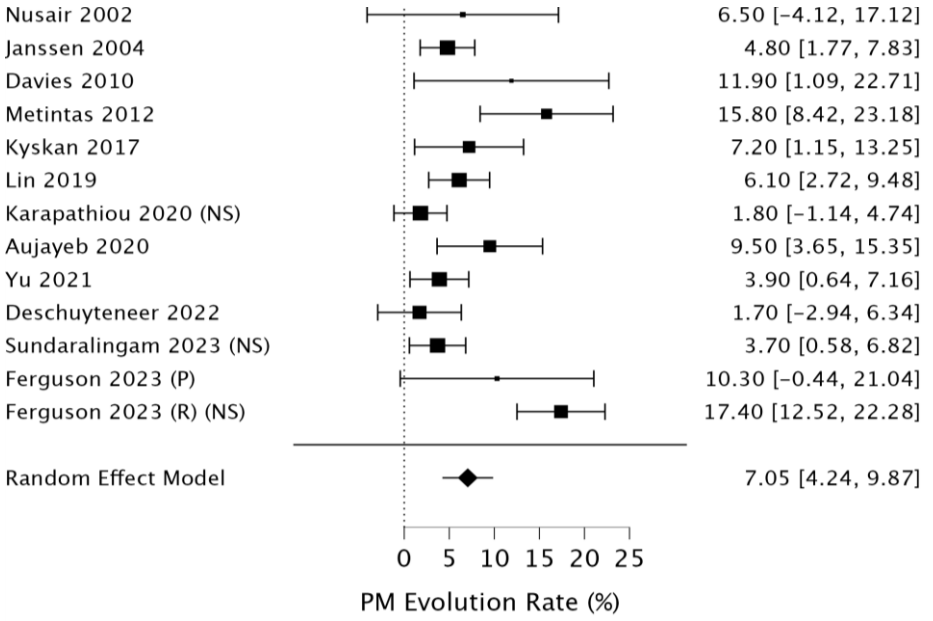
Surgical v Non-surgical NSP Diagnosis

Data regarding the surgically diagnosed NSP cases in Karapathiou 2020, Sundaralingam 2023 and Ferguson 2023 (R) were extracted and combined with the 5 studies that used only surgical biopsies. As illustrated in Figure 3.5 A and 3.5 B, PM evolution rate was lower in surgically diagnosed cases with non-overlapping CIs (1.94% (95% CI 0.97-2.91) v 7.05% (95% CI 4.24-9.87), respectively). In the surgically diagnosed sub-group there was no visible or statistically significant heterogeneity (I^2 0%, Q test p=0.838). Significant residual heterogeneity remained in the non-surgical studies (I^2 79%, p<0.001), which were therefore filtered further to remove the 2 studies (Nusair 2002, Ferguson 2023 (R) (NS)) in which image-guided and/or Abram's biopsies were used. In this LAT-only sub-group, heterogeneity was reduced (I^2 55%, p=0.02), but still significant. PM(5.48% (95% CI 3.46-7.50) v 1.94% (95% CI 0.97-2.91), see Figure 3.5 C.

A: Surgical Diagnosis of NSP



B: Non-surgical Diagnosis of NSP (all modalities)



C: Non-surgical Diagnosis of NSP (LAT only)

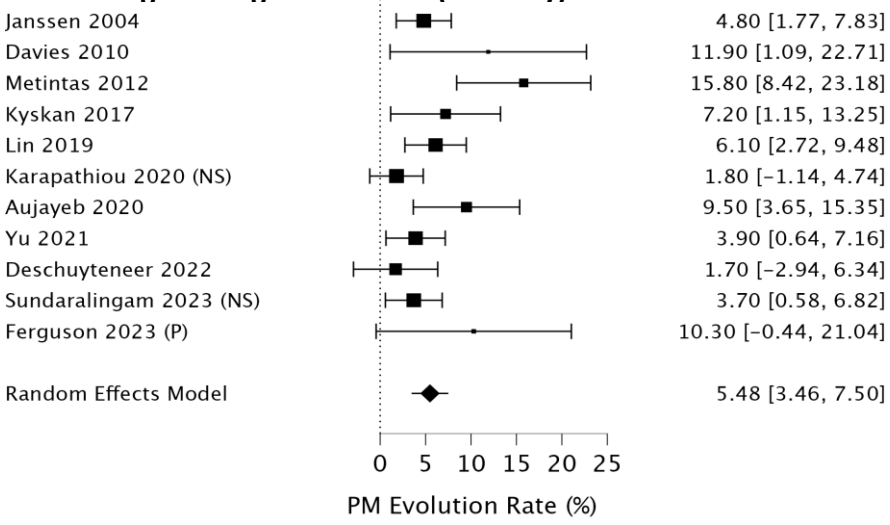


Figure 3.5 Subgroup Analysis by method of diagnosis of NSP: surgical diagnosis (A), non-surgical diagnosis (B), LAT only (C)

3.5.2.5 Methodological Stratification

Retrospective v Prospective Design

PM evolution rate in the single prospective study (Ferguson 2023 (P)) was 10.3% (95% CI 4.1-23.6). This was higher than the summary point estimate from the remaining retrospective studies (5.31% (95% CI 3.21-7.42) but with overlapping CIs (Figure 3.6). Significant residual heterogeneity remained in the retrospective subgroup (I^2 84%, p<0.001).

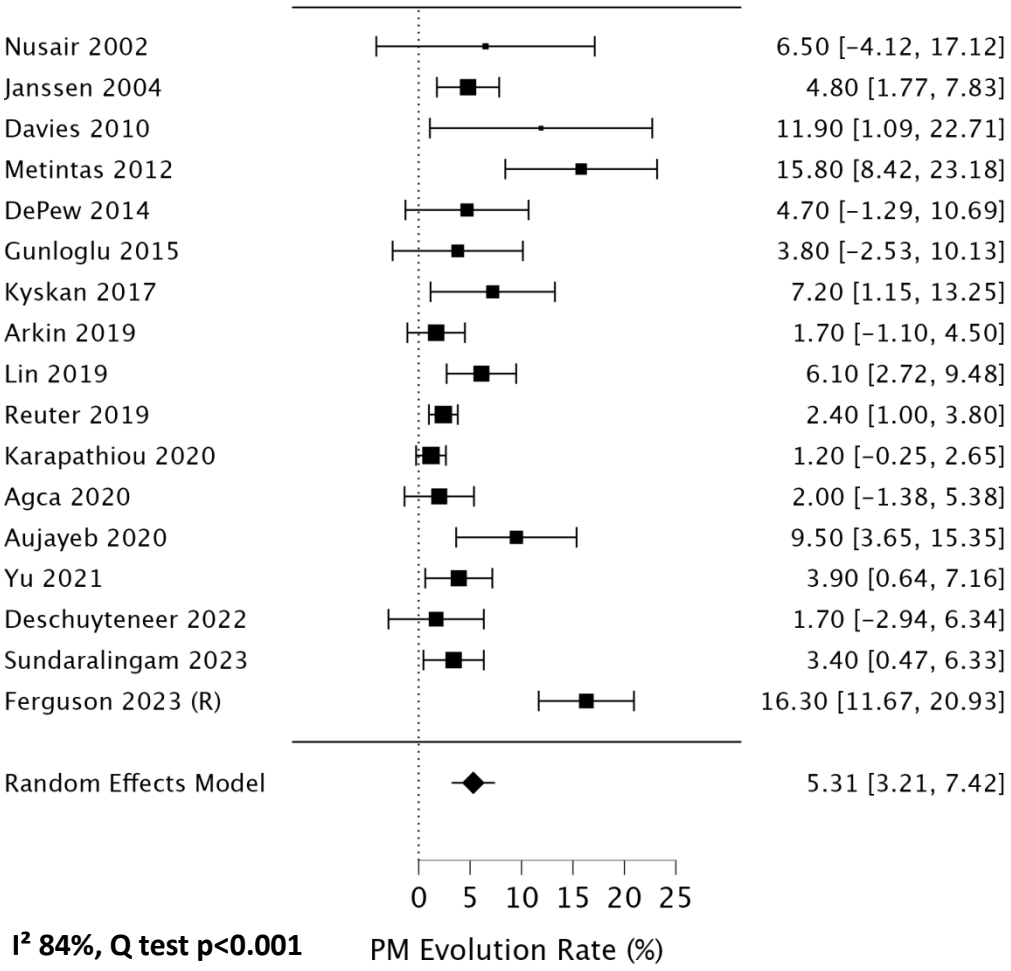


Figure 3.6 Subgroup Analysis by Methodological Stratification

Length of Follow-Up

Studies involving <24 months follow-up reported a similar PM evolution rate to those reporting ≥24 months (4.84% (95% CI 2.82-6.87) v 5.43% (95% CI 2.49-8.37), respectively, see Figure 3.7). There was no statistically significant heterogeneity between studies reporting <24 months (I^2 14%, p=0.253), but significant residual heterogeneity between studies reporting ≥ 24 months (I^2 90%, p<0.001).

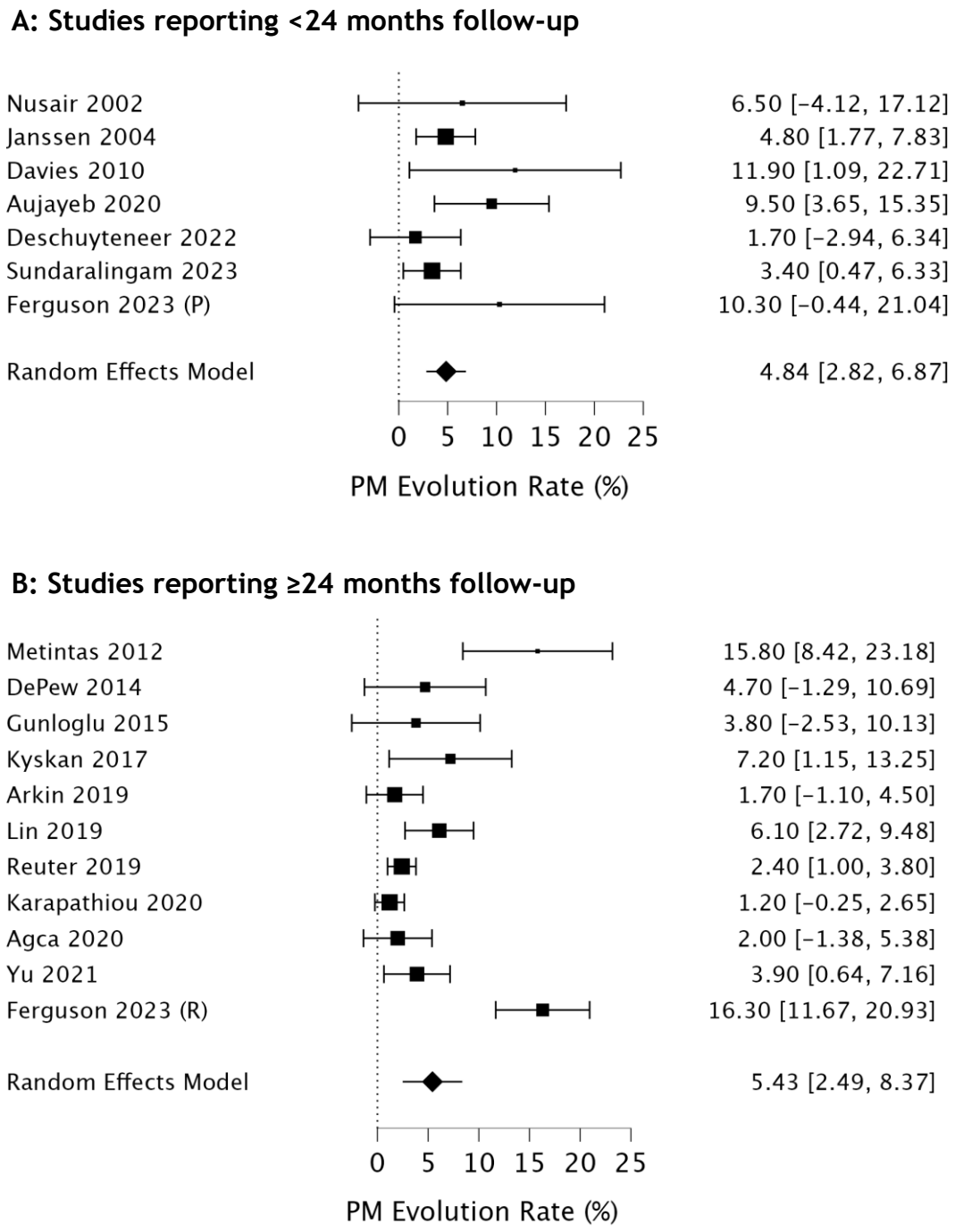
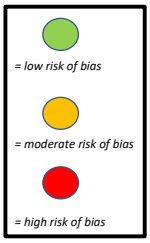


Figure 3.7 Subgroup Analysis by Length of Follow-Up: <24 months follow-up (A), ≥24 months follow-up (B)

3.5.2.6 Risk of Bias Assessment

A summary of the QUIPS assessment is provided in Figure 3.8.



	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting
Nusair 2002	high	low	high	moderate	high	high
Janssen 2004	moderate	low	moderate	moderate	high	high
Davies 2010	low	low	low	low	low	low
Metintas 2012	moderate	low	low	low	low	moderate
DePew 2014	low	high	moderate	moderate	high	high
Gunloglu 2015	high	moderate	moderate	low	high	moderate
Kyskan 2017	low	high	moderate	moderate	high	high
Arkin 2019	moderate	high	moderate	moderate	high	high
Lin 2019	high	high	moderate	high	high	high
Reuter 2019	moderate	high	high	high	high	high
Karapathiou 2020	moderate	high	high	moderate	high	high
Agca 2020	moderate	low	low	moderate	high	moderate
Aujayeb 2020	moderate	low	low	moderate	high	high
Yu 2021	moderate	moderate	moderate	low	high	high
Deschuyteneer 2022	low	moderate	moderate	moderate	high	high
Sundaralingam 2023	low	low	low	moderate	low	moderate
Ferguson 2023 (P)	low	low	low	high	low	low
Ferguson 2023 (R)	moderate	low	low	moderate	low	low

Figure 3.8 QUIPS assessment summary

Measurement Bias regarding Diagnosis of NSP

We found moderate/high risk regarding the NSP diagnosis in 11/18 studies. Several studies did not adequately report exclusion of pleural infection and/or transudative effusions, others used non-specific diagnostic terms, e.g., 'other benign disease'.

Measurement Bias regarding Diagnosis of PM Evolution

There was moderate/high risk in 14/18 studies regarding diagnosis of subsequent PM evolution. Several studies used vague definitions, lacking key histological descriptors (e.g., invasion) and many omitted the immunohistochemical techniques used. Some studies failed to define the re-biopsy technique used or deployed diagnostic methods not recommended in international guidelines (e.g., fluid cytology). High risk of measurement bias was also attributed if inappropriate methods were used to conclude that PM evolution had not occurred, e.g. <12-month follow-up, exclusion of post-mortem PM diagnoses.

Study Confounding

Prior asbestos exposure was only reported in 3/17 studies in baseline characteristics (Davies 2010, Sundaralingam 2023, Ferguson 2023). We also considered biopsy method an important confounder and attributed lower risk to studies based on highly sensitive tools (e.g. LAT or VATS), with high risk attributed to Abram's biopsy, which was only used in one study (Nusair 2002).

Statistical Methods and Reporting

We considered use of a logistic regression model ideal, since this would permit correction of PM evolution rate for confounding factors. However, this method was only used in one study (Ferguson 2023), with most reporting univariate analyses or simple proportions. High risk of reporting bias was also attributed if confidence intervals were absent. 15/18 studies were rated as moderate/high risk.

3.5.2.7 Publication Bias

A Funnel plot demonstrated evidence of publication bias with marked asymmetry (see Figure 3.9), supported by a statistically significant Funnel rank correlation test (p=0.005).

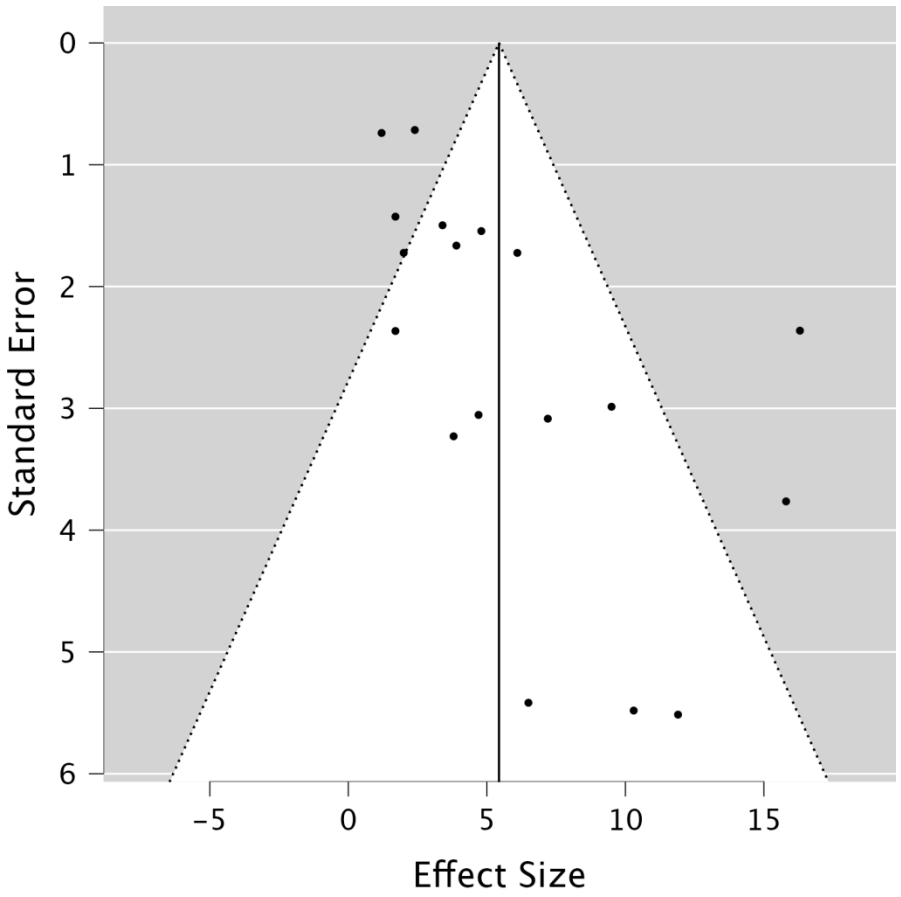


Figure 3.9 Funnel plot to assess publication bias

3.5.3 Discussion

To our knowledge, this is the first systematic review and meta-analysis of PM evolution rate following a diagnosis of NSP. We identified 265 potentially eligible studies published since 2000 and included 17 (comprising 18 study cohorts), describing 2605 NSP cases and 146 subsequent PM evolutions (3-5, 119-132). In a random effects model, the summary point estimate of PM Evolution was 5.44% (95% CI 3.37-7.51), with significant between-study heterogeneity ($p<0.001$, I^2 82.7%). Following stratification by potential clinical and methodological sources of heterogeneity, asbestos exposure, study setting and NSP biopsy type proved to be important. Study design and median follow-up were not significant heterogeneity sources. Stratified model outputs resulted in improved homogeneity and revealed higher PM evolution rate in studies reporting asbestos exposure in ≥50% participants and studies conducted in high PM incidence regions (UK and Central Anatolia, Turkey). PM evolution rate was lower in studies based exclusively on surgical diagnosis of NSP.

Factors Associated with PM Evolution Risk

The relative increase in PM evolution rate was greatest in studies reporting asbestos exposure in ≥50% participants (14.90% (95% CI 10.94-18.85) v 3.27% (95% CI 2.16-4.39), respectively, see Figure 3.3 A and B). This is consistent with the established pathophysiology of PM, which is almost exclusively caused by prior asbestos inhalation (135, 136). For this analysis, we grouped studies into those reporting that ≥50% participants had been exposed to asbestos (3/18) v those reporting less frequent exposure or not reporting exposure at all. The latter criterion applied to 14/18 studies and this missing baseline data is an important limitation and source of bias. However, PM evolution rate was also considerably higher in studies conducted in high PM incidence regions (11.39% (95% CI 7.33-15.45) v 2.59% (95% CI 1.67-3.51), see Figure 3.4 A and B). Study setting data was available for all studies, but this analysis required an arbitrary definition of high incidence. PM incidence varies at a national level with endemic areas typically found in regions with historical asbestos exposure (56). Regional data was not available for our study series and we therefore define high incidence as any UK site plus the Central Anatolia region of Turkey. This reflects the UK currently having the highest global incidence of PM (137, 138) and the estimated 80-799-fold increased relative risk in males and females in Central Anatolia, compared to other similar nations (129). Taken together, the increased PM evolution risks observed for asbestos exposure by cohort and PM incidence strongly suggest that prior asbestos exposure is the dominant risk factor for PM evolution following NSP.

The lower PM evolution rate observed in surgically diagnosed NSP (1.94% (95% CI 0.97-2.91) v 7.05% (95% CI 4.24-9.87), see Figure 3.5 A and B was more modest than the effects observed for asbestos exposure and study setting. Nevertheless, the CIs for this comparison were non-overlapping, even when limited to LAT-only studies (see Figure 3.5 C). These results may reflect more comprehensive sampling of the pleural space by VATS (used in all 5 surgical studies) or open biopsy (used in 2/5). 11/12 of the non-surgical studies used LAT, which should offer comparable biopsy quality and quantity, but 2/12 included image-guided samples, which will generally deliver fewer and smaller samples and one was based solely on Abram's biopsy, which is no longer recommended in suspected

PM (118). However, we interpret these data with some caution, since asbestos exposure was an important confounder to this analysis, with only 1/8 surgical studies conducted in high incidence PM settings, compared with 6/10 non-surgical studies.

Bias and Limitations

Most of the studies included were at moderate/high risk of various biases. Selection bias was frequently due to incomplete reporting of baseline characteristics including asbestos exposure. Attrition bias was frequently due to short follow-up or incomplete reporting. Although follow-up was not a statistically significant heterogeneity source in our analyses, NSP surveillance is generally recommended for 24 months, with the risk of PM thought to be highest in the first 6 months. This is supported by Ferguson 2023 and Aujayeb 2020, in which the median time to repeat PM biopsy was 3.5 and 6.8 months, respectively (3-5, 119-121). In the largest study included here (Reuter 2019), the number needed to follow-up (NNF) to identify one malignancy was 18 in follow-up year 1, rising to 260 from years 1 to 3 (131). Measurement bias related to the NSP diagnosis was commonly due to use of non-specific terminology that made it difficult to reliably extract NSP cases from larger cohorts. This reflects the fact that NSP is a diagnosis of exclusion, and most studies were not exclusively focused on NSP, with many reporting broad pleural service outcomes.

Study reporting and statistical methods were an additional and important source of bias. The absence of multivariable regression models for baseline predictors of PM evolution from all but one study (Ferguson 2023) precluded meta-analysis of odds ratios (OR) for demographics, imaging findings or symptoms[6]. By univariate analyses, malignant CT features were associated with risk in 4/17 studies (Metintas 2012, Kyskan 2017, Sundaralingam 2023, Ferguson 2023) and in Ferguson 2023, malignant CT and age were independent predictors of PM evolution(4, 5, 127, 129).

Our analyses also revealed evidence of publication bias (see Figure 3.9), with no small studies reporting low PM evolution rates and more large studies reporting low rates. The former may reflect caution by authors/editors, given the uncertainty involved in such estimates. The latter may reflect the geographically

heterogeneous nature of asbestos exposure and associated PM incidence. Even in high incidence nations like the UK, high incidence hotspots will be mixed in with large areas of lower incidence. Larger studies may therefore tend towards lower event rates. Independent patient data meta-analysis could address this but was not possible with the data available here.

Clinical and Research Implications

Our results indicate that PM Evolution risk is considerably higher (10-15%) in asbestos exposed NSP than in non-exposed individuals, in which the risk is likely <5%. This information could be integrated into patient-centred discussions regarding risk and follow-up, and the design of future precision PM prevention clinical trials. The reduced risk associated with surgical biopsies should be interpreted with caution, but favours thoracoscopic sampling in suspected PM, where NSP is a possible diagnostic outcome.

3.5.4 Conclusion

In this first systematic review and meta-analysis of PM evolution risk following diagnosis of NSP, higher risk was associated with asbestos exposure and high PM incidence settings. Lower PM evolution was associated with surgical NSP biopsies. Most studies demonstrated moderate/high risk of bias, limiting the clinical utility of these findings. Clinical surveillance and precision PM prevention trials are likely to be most impactful in asbestos-exposed cohorts and regions with high PM incidence.

3.6 Reflections on this Paper

These data suggest that asbestos exposure is the most important factor in PM evolution risk. This will assist in the design of future precision prevention trials and in the selections of recruiting centres for Meso-ORIGINS. This learning has been incorporated by preferential selection of sites in areas of high incidence (e.g. North East, North West), and preferencing sites with access to thoracoscopy which permits full thickness, multi region sampling under direct inspection of the pleural space.

CHAPTER 4: PROGNOSTIC UTILITY OF CDKN2A AND BAP1 TESTING IN PLEURAL MESOTHELIOMA AND ASBESTOS-ASSOCIATED BENIGN PLEURAL INFLAMMATION

4 Chapter 4: Prognostic utility of CDKN2A and BAP1 testing in pleural mesothelioma and asbestos-associated benign pleural inflammation

4.1 Introduction to this paper

Chapters 2 and 3 describe studies on patients with benign pleural inflammation with methods used to provide a PM evolution rate, and identify factors making PM evolution more likely, through a retrospective cohort study, a prospective feasibility study, and a systematic review and meta-analysis. This chapter describes a multi-centre retrospective cohort study examining the frequency and prognostic impact of molecular markers of loss of function events in AAPI and PM. Recent studies report encouraging potential prognostic utility based on CDKN2A/BAP1 status, but there is an increasing appreciation that these genomic events may precede PM development and may also be present in patients with benign pleurisy.

We used at a cohort of archival AAPI cases, with at least two years of clinical follow-up with no PM evolution, identified during a period where tests including BAP1 and MTAP were not in routine clinical use. BAP1 and MTAP IHC were performed on archival tumour biopsies retrieved for the study. We also collected a cohort of histologically confirmed PM cases with both BAP1 and CDKN2A/MTAP tests performed as routine clinical care. We describe the frequency of these molecular events in both cohorts, and whether BAP1/CDKN2A status impacts overall survival.

A comprehensive understanding of the utility of these ancillary tests will aid clinical decision making and influence early PM detection or identify druggable targets at the pre-PM stage.

4.2 Abstract

Introduction

Pleural Mesothelioma (PM) is associated with loss of function in tumour suppressor genes, including CDKN2A and BAP1. CDKN2A loss can be identified using fluorescence in-situ hybridization (FISH) or loss of MTAP expression on immunohistochemistry (IHC). BAP1 loss can be detected by IHC. Previous studies report 100% PM specificity for these markers and independent prognostic value. We tested the prognostic utility of CKDN2A/BAP1 status when testing was performed in diagnostically challenging cases and compared event frequency with a cohort of patients with Asbestos Associated Pleural Inflammation (AAPI) and no PM evolution over 2-year follow-up.

Methods

A multicentre retrospective cohort study was performed. PM cases were identified from the Scottish Mesothelioma Network (SMN) and North-West of England (NWE) regional mesothelioma centre databases. AAPI cases were identified from the recently reported Meso-ORIGINS Feasibility Study (ISRCTN12840870). Eligible PM cases had a histological diagnosis and testing performed for both CDKN2A (by FISH or inferred by MTAP IHC) and BAP1 (by IHC). Test request and reporting was via specialist PM pathologists during diagnostic work-up. Eligible AAPI cases had an asbestos exposure history or compatible radiology, e.g. pleural plaques, compatible histology on any previous pleural biopsy and follow-up until death or ≥2 years. CDKN2A and BAP1 testing were performed on archival tumour biopsies retrieved for the study; results were scored by an expert PM pathologist. Baseline clinical data were collected for both cohorts using electronic records, including histological subtype and stage in PM, and any subsequent diagnosis of PM in AAPI. Overall survival was calculated to death from any case. Univariate survival analysis by Kaplan-Meier +/- multivariate Cox regression was performed.

Results

155 PM cases were included (142 SMN, 13 NWE). CDKN2A and BAP1 loss were observed in in 63.2% and 57.4%, respectively. Stage I prevalence was high (60.7%). PM median OS was 12.4 months (95% CI: 11.2-15.9). Adverse OS was

associated with non-epithelioid histology, higher performance status and later stage. CKDN2A/BAP1 status was not associated with OS, including by histological subtype or when both genes were lost. Among 42 eligible AAPI cases, CDKN2A and BAP1 loss were observed in 1/42 (2%) and 2/39 (5%), respectively. 28/42 cases died during follow-up (≥2 years); 7/28 (25%) had post-mortem examinations. No PM evolutions were observed.

Conclusion

We did not observe any association between OS and CDKN2A/BAP1 status in 155 PM patients, who were generally early-stage. CDKN2A and BAP1 loss were observed at low, but non-zero frequencies in 42 patients with AAPI and no subsequent PM evolution. In isolation, CDKN2A and BAP1 loss may not be strong predictors in in early-stage PM and these events may be present in patients without invasive disease. These data emphasise the importance of multi-omic testing using longitudinal tissue samples to study PM evolution.

4.3 Author contribution

The design was conceived by KF and KGB. KF, JP, MT, CD, ES, RM, RB, AWT, RB, JH, KP, ME contributed to patient recruitment and data collection. NH and FR performed the IHC in the study, Statistical analyses were performed by KGB and KF. KF and KGB prepared the manuscript.

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4.4 Manuscript

4.4.1 Introduction

Pleural Mesothelioma (PM) is an incurable thoracic malignancy with heterogeneous outcomes. A prompt diagnosis and accurate prognostication are important in all patients, facilitating access to life-extending therapy and clinical trials and/or effective palliation (139). Reliable survival prediction is notoriously difficult in PM and better prognostic biomarkers are needed. Tissue features are obvious prognostic biomarker candidates, including several new molecular markers of loss of function events in the PM tumour genome. Mutations and or structural changes in the tumour suppressor genes, CDKN2A and BAP1 are observed in 41-68% and 36-57% of PM patients, based on large international cohort studies (86, 140). CDKN2A is co-located on chromosome 9 with MTAP and co-deletion at both loci is common (99). CDKN2A loss can therefore be detected directly using FISH or inferred from loss of MTAP protein expression on IHC. BAP1 loss has been studied primarily using IHC, and a combination of these assays are now embedded in diagnostic pathways in many centres.

Recent studies report encouraging potential prognostic utility based on CDKN2A/BAP1 status, with inferior survival associated with CDKN2A loss (97) and superior survival associated with BAP1 loss (141, 142). However, clinical confidence in outcome predictions based on CDKN2A/BAP1 is affected by an increasing appreciation that these genomic events may precede development of PM and may also be present in patients with asbestos-related benign pleurisy who do not develop invasive disease. In asbestos-induced mouse models of PM, a series of genomic events, including hypermethylation of CDKN2A precedes loss of function, which precedes invasive disease (143). In humans, this has recently been codified as mesothelioma-in-situ, which is a non-invasive histological lesion defined by a single mesothelial layer, without sub-pleural invasion but in which CDKN2A/BAP1 loss is present. The limited MIS case series published report evolution to invasive PM in up to 60% of patients over 5 years (144). In patients with non-specific pleuritis, an umbrella term that encompasses a broader range of benign pleural histologies, the risk of PM evolution was recently reported in a

systemic review and meta-analysis to be 5.44% (95% CI 3.37-7.51) in 2607 patients, derived from 17 studies (145). In this analysis, the risk of PM evolution was considerably higher in studies performed in areas of high PM incidence and in cohorts where ≥50% participants reported asbestos exposure. The purpose of this multi-centre retrospective cohort study was to examine the prognostic impact of CDKN2A and BAP1 loss in patients with invasive PM in whom both assays had been performed in diagnostically challenging cases, and in patients with asbestos-associated benign pleural inflammation (AAPI), in whom CDKN2A/BAP1 status was determined using archival pleural biopsies.

4.4.2 Methods

4.4.2.1 Study Design, Setting and Approvals

A retrospective, multicentre cohort study was performed. Two cohorts were created:
(1) PM cases were identified from Scottish Mesothelioma Network (SMN) and North-West of England (NWE) regional mesothelioma centre. The SMN database covered 2019-2024. The NWE database covered 2021-2024. (2) APPI cases were identified from the recently reported Meso-ORIGINS Feasibility Study (ISRCTN12840870) database (5). This was a multicentre study conducted in four UK pleural centres. Only Glasgow cases were included as archival pleural biopsies were available for CDKN2A/BAP1 testing. Ethical approval was not required for the PM cohort; all data are prospectively recorded for service evaluation and audit purposes. The APPI cohort was analysed under existing REC approval for the Meso-ORIGINS feasibility study (Ref 18/SC/0617).

4.4.2.2 Cohort Selection Criteria

PM cases were selected based on the following inclusion criteria: (1) histological diagnosis of PM (2) testing performed for both CDKN2A (directly by FISH, or inferred by MTAP IHC) and BAP1 (by IHC) (3) ≥12 months follow-up data. Cases were excluded if any of the following applied: (1) non-histological diagnosis (e.g. fluid cytology only) (2) non-pleural disease (e.g. peritoneal) (3) failed or uninterpretable CDKN2A/BAP1 test result.

AAPI cases were selected based on the eligibility criteria deployed in the Meso-ORIGINS feasibility study. Inclusion criteria were (1) a history of asbestos exposure or compatible radiology, e.g., pleural plaques (2) compatible histological findings on any previous pleural biopsy (e.g., benign fibrinous pleurisy, non-specific pleuritis, atypical mesothelial proliferation). Exclusion criteria were (1) <2 years follow-up (2) histological or cytological diagnosis of PM or any secondary pleural malignancy (3) diagnosis of pleural infection, empyema or granulomatous pleuritis.

4.4.2.3 Data Collection

Data regarding PM cases was extracted from prospectively populated databases maintained by the Scottish National Network and the North-West of England regional mesothelioma centre. Extracted data items included age, gender, asbestos exposure, performance status, biopsy method, stage, histological subtype, date of death (or censor if alive) and results of CDKN2A and BAP1 testing.

Data collection methods for AAPI have previously been reported (5). Using electronic health records, data pertaining to the date of the index AAPI diagnosis were extracted including asbestos exposure history, age, gender, CT and CXR findings. CT findings were codified into benign or malignant, based on previously reported descriptors (58), and by the presence of pleural plaques. CXR findings were used to classify effusion size as small or large (<50/≥50% hemithorax opacification). Date of death, date of or last follow-up if alive were recorded. Any subsequent diagnosis of PM was extracted from the national cancer registry.

4.4.2.4 CDKN2A and BAP1 testing

In PM cases, CDKN2A and BAP1 testing was performed by routine NHS pathology labs using standardised protocols. CDKN2A testing involved either direct testing for homozygous deletion of the CDKN2A/p16 gene by FISH or MTAP IHC as an indirect surrogate. The NHS pathology laboratories involved determined which assay was used and only results were available for the current study. BAP1 testing was based on IHC in all PM cases. In benign cases, only MTAP and BAP1 IHC were performed. For these study assays, immunostaining was performed on

4-*μm*-thick FFPE sections using Leica Bond autostainers. 1:100 and 1:400 dilutions were used for MTAP (M01 clone 2G4, Abnova, Taiwan) and BAP1 (C-4, Santa Cruz Biotechnology, USA), respectively. Both assays were previously validated and approved for routine clinical used within NHS Greater Glasgow & Clyde.

4.4.2.5 Sample size and statistical analysis

This was an exploratory study and a sample size calculation was not performed. Simple descriptive statistics were used to report the frequency of CDKN2A/BAP1 loss in diagnostic biopsies. Univariate survival analyses based on CDKN2A and BAP1 status used Kaplan-Meier methods and the log rank test, with a p-value <0.05 considered statistically significant. Overall survival (OS) time was calculated from date of first suspicious CT scan in PM cases and date of pleural biopsy in AAPI cases, to death from any cause. For OS analyses, the PM cohort was dichotomised based on the presence of CKDN2A and BAP1 loss; these comparisons were also performed in histological subgroups (i.e. epithelioid cases with CDKN2A loss v no loss, non-epithelioid cases with CDKN2A loss v no loss, epithelioid case with BAP1 loss v no loss, non-epithelioid cases with BAP1 loss v no loss). PM cases were also partitioned based on the presence of both CKDN2A and BAP1 loss v loss of neither. Similar analyses were planned for benign cases if there were sufficient subsequent PM diagnoses or deaths during follow-up. Multivariate cox regression modelling was planned if univariate survival analyses indicated potential prognostic effects of CDKN2A or BAP1 status. All analyses were performed using GraphPad Prism v10 (San Diego, USA).

4.4.3 Results

4.4.3.1 Baseline Features and CDKN2A/BAP1 Results

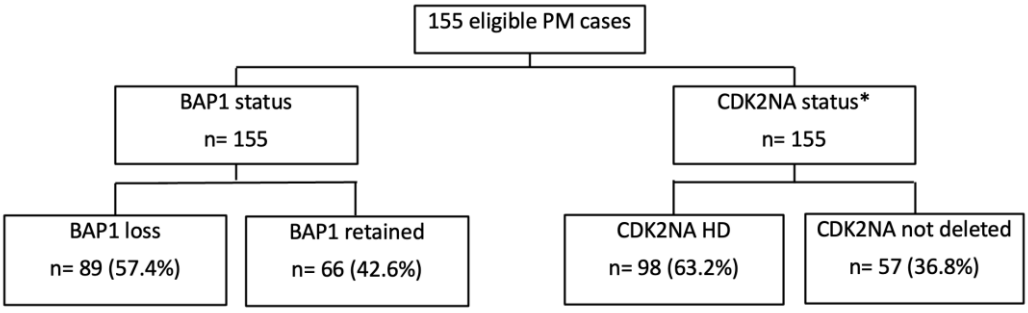
PM cases

155 PM cases met the eligibility criteria and were included in the analysis (142 from SMN, 13 from NWE). This represented a minority of cases in which both CDKN2A and BAP1 testing was used as an ancillary diagnostic. For context, during the study period, 955 PM cases were diagnosed by the SMN (2019-2024), meaning the 142 cases from that site, in whom both tests were performed constituted 15% of all cases (142/955). Clinical characteristics of the 155 PM

cases included are summarised in Table 4.1. As summarised in Figure 4.1, 98 (63.2%) PM cases demonstrated CDKN2A loss; 89/155 (57.4%) demonstrated BAP1 loss.

Table 4.1 Baseline characteristics of the Pleural Mesothelioma cohort (n=155)

Age, median (range)	76 (45-90)
Male, No. (%)	128 (82.6)
Asbestos exposure	
Yes	103 (66.5)
No	25 (16.1)
Unknown	27 (17.4)
MPM Subtype, n (%)	
Epithelioid	96 (61.9)
Non-epithelioid	59 (38.1)
Sarcomatoid	21 (13.6)
Biphasic	24 (15.5)
Desmoplastic	4 (2.9)
Not Otherwise Specified	10 (6.5)
Biopsy Method, n (%)	
Abram's	1 (0.7)
LAT	40 (25.8)
VATS	64 (41.3)
Image-guided	47 (30.3)
Stage, n (%)	
I	94 (60.7)
II	13 (8.4)
III	32 (20.7)
IV	13 (8.4)
Not staged	3 (1.9)



*CDKN2A status was either directly tested by FISH (n=29/155) or inferred indirectly based on loss of MTAP expression on IHC (n=73/155)

Figure 4.1 CDKN2A and BAP1 testing results in 155 patients with Pleural Mesothelioma

AAPI cases

52 AAPI cases were identified as potentially eligible for inclusion. As summarised in Figure 4.2, 10/52 were excluded leaving 42 for inclusion in the analyses. Baseline characteristics are summarised in Table 2. As summarised in Figure 4.2, 1/42 (2%) cases demonstrated CDKN2A loss (inferred by MTAP IHC); 2/39 (5%) demonstrated BAP1 loss. These abnormalities were observed in two individuals, one of whom had both CDKN2A and BAP1 loss, the other had BAP1 loss only.

Table 4.2 Baseline Characteristics of the Asbestos-associated Pleural Inflammation (AAPI) cohort (n=42)

Age, median (range)	78 (45-83)
Male, No. (%)	41/42 (97.6%)
Effusion right sided, No. (%)	22/42 (52.4%)
Effusion bilateral, No. (%)	3/42 (7.1%)
Effusion <50% thorax, No. (%)	33/42 (78.6%)
Pleural Plaques on CT, No. (%)	32/42 (76.2%)
Benign CT according to report, No. (%)	37/42 (88%)

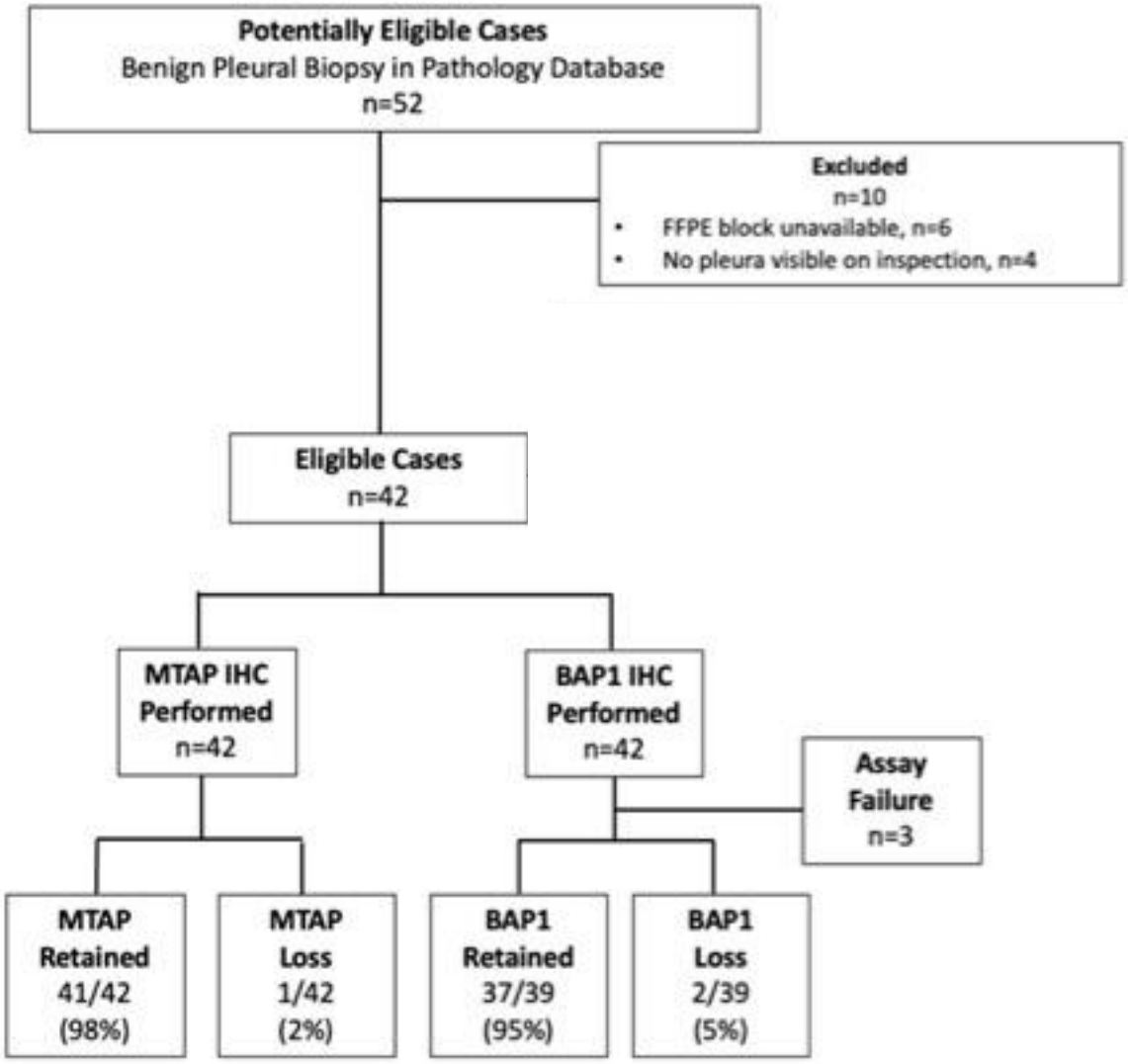


Figure 4.2 CDKN2A and BAP1 testing results in 42 patients with Asbestos-associated Pleural Inflammation (AAPI)

4.4.3.2 Survival Analyses

PM cases

Median survival in the 155 PM cases was 12.4 months (95% CI 11.2-15.9). Results of univariate survival analyses for CDKN2A and BAP1 status, in addition to baseline variables with known prognostic influence are summarised in Table 4.3. As illustrated in Figure 4.3, neither CDKN2A nor BAP1 status were associated with OS. Worse survival was associated with non-epithelioid histology, higher performance status and higher stage.

Table 4.3 Univariate Overall Survival Analysis in 155 patients with PM. Baseline features significantly associated with OS are highlighted in bold (p-value <0.05)

Variable	**Hazard Ratio (95% CI)**	**p-value**
CDKN2A status		
Loss v No loss	1.08 (1.75-1.55)	0.8410
BAP1 status		
Loss v No loss	1.1 (0.7-1.57)	0.6122
Histology		
Non-Epithelioid v Epithelioid	1.58 (1.09-2.3)	**0.0102**
Age		
>75 v ≤75 years old	1.32 (0.92-1.87)	0.1256
Performance status		
2-4 v 0-1	2.06 (1.17-3.61)	**0.0009**
Stage		
Stage 2-4 v Stage 1	1.68 (1.14-2.48)	**0.0039**

More granular analyses failed to identify associations between CDKN2A/BAP1 status and OS, including analyses restricted to histological subtypes (see Figure 4.4: Epithelioid only; Figure 4.5: Sarcomatoid only) and based on loss of both genes (see Figure 4.6). Given the negative univariate OS analysis, multivariate Cox regression modelling was not performed.

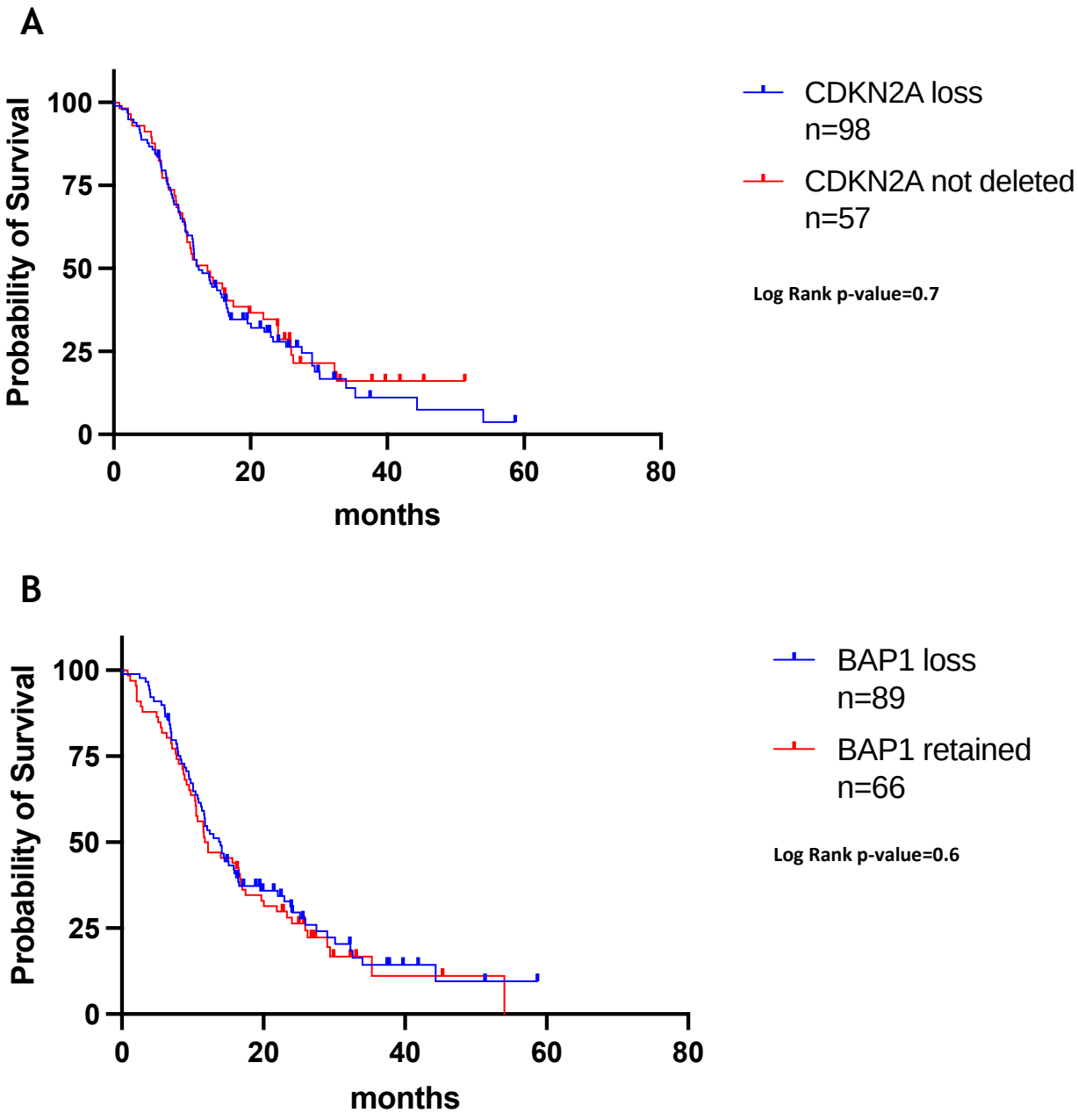


Figure 4.3 Kaplan-Meier survival curves summarising univariate overall survival in 155 PM patients based on A) CDKN2A status and B) BAP1 status.

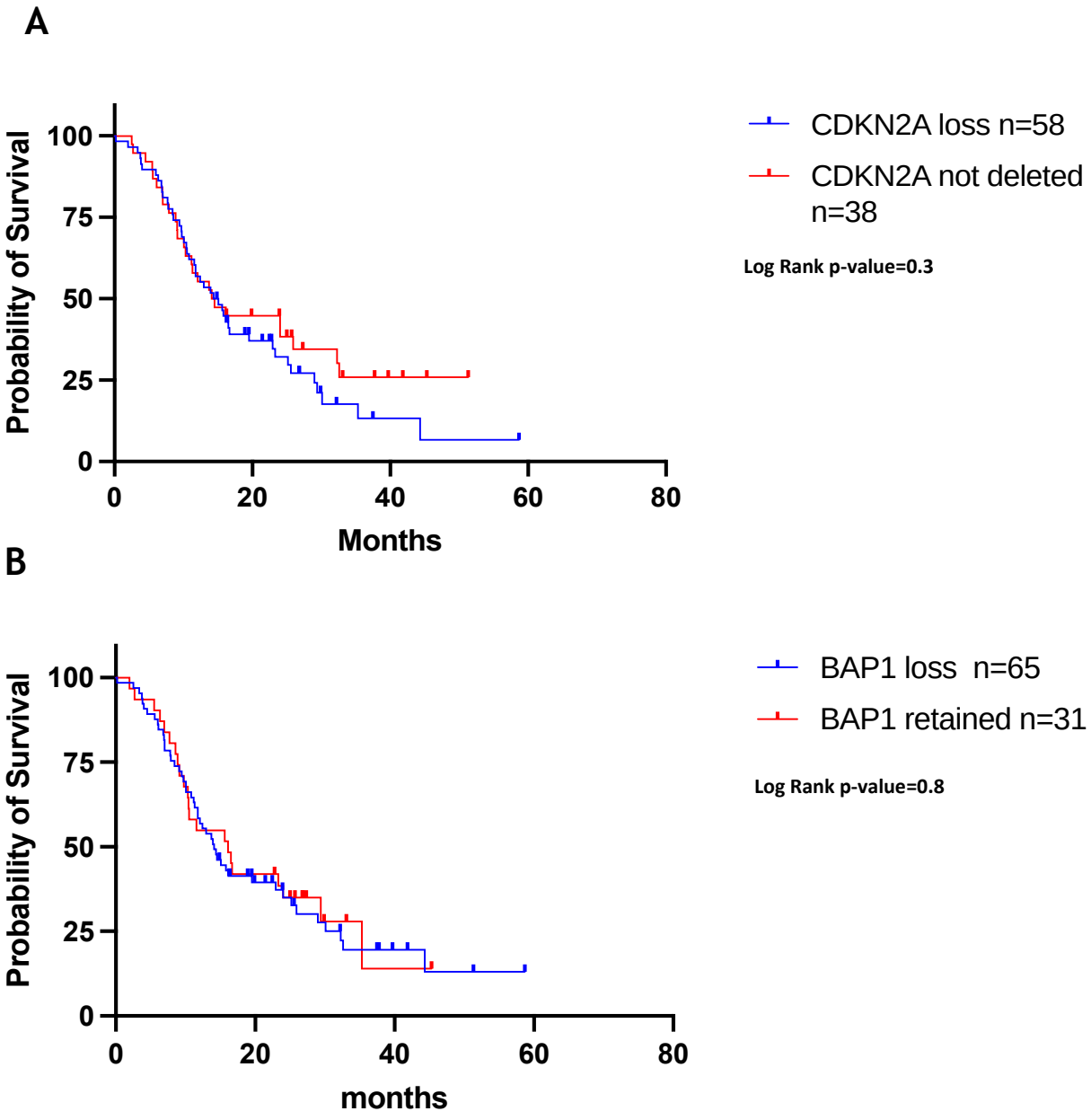


Figure 4.4 Kaplan-Meier survival curves summarising univariate overall survival in 96 epithelioid subtype PM patients based on A) CDKN2A status and B) BAP1 status

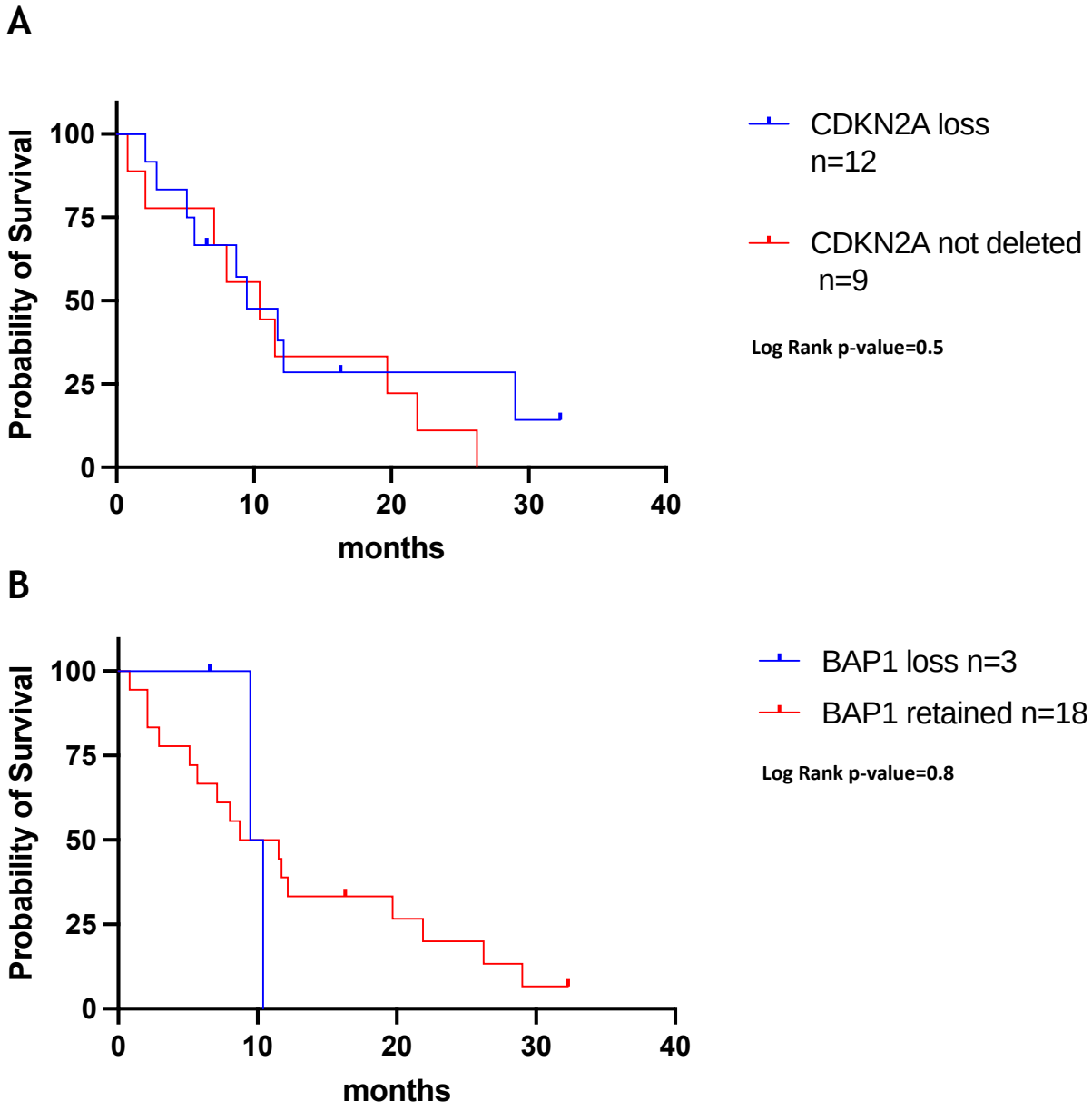


Figure 4.5 Kaplan-Meier survival curves summarising univariate overall survival in 21 sarcomatoid subtype PM patients based on A) CDKN2A status and B) BAP1 status

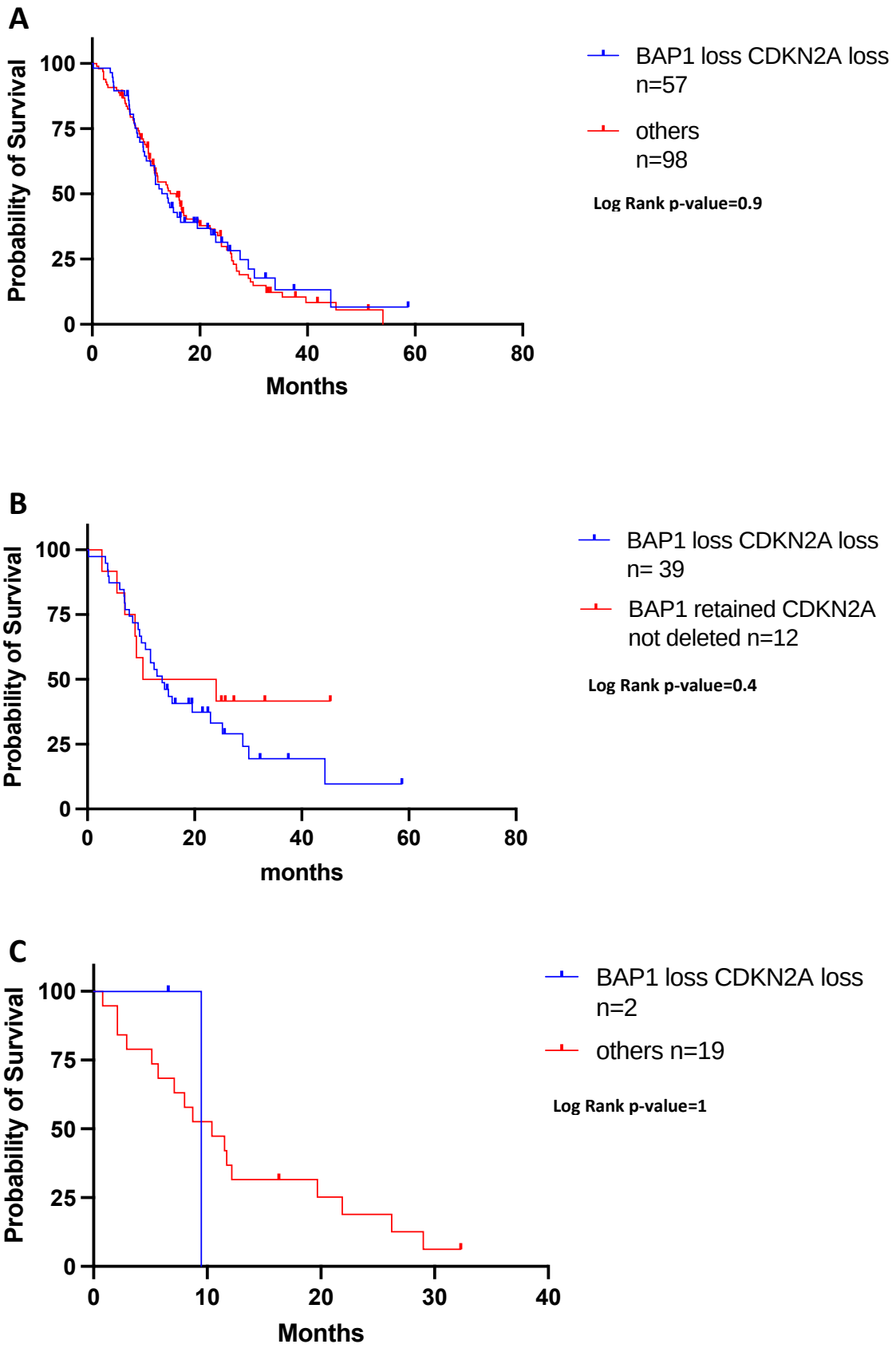


Figure 4.6 Kaplan-Meier survival curves summarising univariate overall survival in A) 155 PM patients of all histological subtypes B) 96 epithelioid subtype PM patients and C) 21 sarcomatoid subtype PM patients based on loss of both CDKN2A and BAP1 genes

AAPI cases

Median survival in the 42 AAPI cases was 50.4 months (95% CI 32.2-93.9). 28/42 cases died during the follow-up period. No PM diagnoses were recorded pre-mortem in these cases. 7/28 deceased (25%) cases had post-mortem examinations. PM was not identified in any of these examinations. The low frequency of CDKN2A and BAP1 loss prevented any meaningful survival analysis by CDKN2A/BAP1 status.

The AAPI patient with combined CDKN2A and BAP1 loss was a 79-year-old male retired roofer, with a history of prior occupational asbestos exposure. His baseline CT revealed pleural plaques and was reported as 'suspicious for malignancy'. He had a small unilateral left-sided pleural effusion and negative effusion cytology. VATS pleural biopsies were reported as dense hyalinised connective tissue only. He died 3 months after VATS without any new pleural diagnosis emerging. He was not referred for post-mortem examination.

The AAPI patient with BAP1 loss was a 72-year-old male retired joiner with a prior history of occupational asbestos exposure. His baseline CT showed pleural plaques and was reported as 'suspicious for malignancy'. He had large bilateral effusions at presentation, from which fluid cytology was negative. VATS pleural biopsies revealed benign fibrinous pleurisy, and several follow-up CT scans showed no progressive pleural thickening. He died 2 ½ years after VATS. A post-mortem examination revealed hypertensive heart disease but no PM.

4.4.4 Discussion

In the current study, information on combined CDKN2A and BAP1 status was collected for 155 patients with PM and 42 patients with apparently benign AAPI. The PM cases were diagnosed in expert PM centres, and all benefited from specialist MDT review, including histological review by experienced pathologists. The AAPI cases were all followed up for at least 2 years, or until death, but the low frequency of CDKN2A and BAP1 loss found in this cohort precluded any meaningful survival analysis in this group. Contrary to previous studies(141, 142), we observed no association between CDKN2A or BAP1 status and OS in the PM cohort. This included more granular analyses restricted to histological subtypes and based on loss of both genes. Concordant with multiple previous studies, we

did observe associations between adverse prognosis and non-epithelioid histology, higher performance status and higher stage.

In AAPI cases we observed a small but non-zero frequency of CKDN2A or BAP1 (5% and 2% respectively). These cases were also reviewed though a specialist MDT and had expert histopathology input and prolonged follow-up. This is discordant with previous studies that have reported 100% specificity for BAP1 and MTAP IHC in differentiating PM from benign pleuritis (90, 146). The two cases with CDKN2A/BAP1 loss in our dataset were not classified as mesothelioma-in-situ (MIS) at diagnosis in 2012 and 2014. This is because neither assay was being used routinely at that time and BAP1/CDKN2A status is required to make this diagnosis (144). MIS is a relatively new syndrome with considerable uncertainty regarding its true nature and the risk of PM evolution. One of the two AAPI cases with molecular events (the 79-year-old with both CDKN2A and BAP1 loss) died within 3 months of VATS biopsy and did not have a post-mortem examination. It is possible this patient had underlying PM, but this was not confirmed. The evolution of benign pleuritis is currently being evaluated in detail using paired sequential biopsies collected via the Meso-ORIGINS study, within the PREDICT-Meso accelerator. This study is currently recruiting in 22 sites across the UK.

In earlier PM studies, CDKN2A (97) and BAP1 loss (141, 142) have been associated with adverse and better OS, respectively. The PM cases studied here were a relatively small subset of all PM cases diagnosed at the study centres, comprising only 15% of the diagnoses made in Scotland. This likely reflects the use of CDKN2A and BAP1 as ancillary, rather than routine tools, during the study period at both study centres. BAP1 IHC and CDKN2A FISH became available in Scotland during 2017, with MTAP IHC added in 2020. In NWE, CKDN2A FISH and BAP1 IHC became available in 2012 and 2021 respectively, with MTAP not yet available. The decision to use these ancillary tests was at the discretion of the reporting pathologist, typically after initial ‘routine’ IHC and MDT discussion. This may have pre-selected a more challenging diagnostic group – a thesis that would be supported by the high incidence of early-stage disease in the current study; 60.7% were stage I. Unfortunately, none of the previous studies that reported prognostic associations reported stage (97, 141, 142), precluding comparison of

stage in the cohorts used. Nevertheless, selection bias may explain some of the discordance between our findings. Reassuringly, we did observe other expected OS associations in our cohort, including with histological subtype, stage and PS (147-150), see Table 4.3. The PM cohort here was also smaller than the cohorts in which positive prognostic associations were previously reported (n=206 (97), n=229 (7) and n=114 and n=234 (8)), which may have increased the chance of type II statistical error. A future larger study, such as Meso-ORIGINS will be able to address this issue given the expected samples size of this cohort is currently set at 500.

Results from the recent OncoCast-PM study are supportive of our observations that the prognostic effects of CDKN2A and BAP1 are questionable. This study used machine learning to develop a prognostic tool (OncoCast-MPM) that combined clinical variables, pathological features, and molecular profiling using NGS. 268 cases (64% with stage I-IIIA disease) were classified into high and low risk (151). By this method, no single variable or gene alteration drove risk differentiation. Indeed, 12% of patients in the low-risk group had CDKN2A loss (151), a marker of adverse survival in previous CDKN2A-focused studies (97) and 21% of patients in the high-risk group had BAP1 loss (151), a marker of better survival in previous BAP1-focused studies (141, 142) . A different study, reported by Osmanbeyoglu et al, used TCGA (The Cancer Genome Atlas Program) and MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) genome data to investigate the combinatorial effect of TS loss (BAP1, CDKN2A and NF2) on subsequent survival. This revealed that tumours with BAP1 loss were associated with improved OS relative to those with CDKN2A and/or NF2 alterations, independent of BAP1 status (152). This and other studies emphasise the complexity of the PM tumour genome and the important effect of non-genomic features, including immune system engagement in determining outcomes. These factors were not assessable in the current study but will be assessed in the PREDICT-Meso accelerator. This additional insight is critical for more accurate prognostication and the targeting of suitable therapy.

Future Studies

The retrospective design of the current study did not permit precise extraction of the triggers for CDKN2A and BAP1 testing, nor the level of confidence of

scepticism of the test data by the treating teams. As stated earlier, during the study period the assays in question were new and were being used as ancillary tests in difficult cases. This is reflected in the high prevalence of stage I disease, where differentiation from APPI is difficult since both may present with a pleural effusion and minimal pleural thickening. Since the study period, CDKN2A and BAP1 testing are becoming 'routine' in many centres. This change has coincided with, and may even by driving, increasing awareness of MIS as a key differential diagnosis in samples exhibiting loss of expression. This distinction requires expert histopathologist input, particularly since the widely reported 100% sensitivity of these markers risks an increase in false positive PM diagnosis. In addition to the ongoing Meso-ORIGINS study within PREDICT-Meso, we are therefore also planning a UK survey of current CDKN2A and BAP1 test use and understanding this information will help inform the case and design of a national policy for molecular testing in PM.

4.4.5 Conclusion

In this multicentre retrospective study, we did not observe any significant association between OS and CDKN2A/BAP1 status in 155 PM patients. The PM cases studied were generally of early stage, which may have influenced this result. CDKN2A and BAP1 loss were observed at low, but non-zero frequencies (5% and 2% respectively) in a cohort of 42 patients with AAPI and no subsequent PM evolution over ≥2 years of clinical follow-up. These data suggest that CDKN2A/BAP1 status, in isolation, are not strong predictors of survival in earlier stages of PM, and loss of function events may be present in patients without invasive disease at low frequency. These findings emphasise the importance of longitudinal tissue sampling spanning the period preceding PM development and use of multi-omic testing to define the various biological processes driving PM evolution and adverse survival once PM is established. This work is being conducted in the PREDICT-Meso international accelerator network.

4.5 Reflections on this paper

This data suggests that ancillary tests in PM (CDKN2A and BAP1) may not be strong predictors of survival in PM, especially in early stage disease. We also reported a low, but important, frequency of loss of function events in our

robustly benign cohort. This learning will be explored further in the PREDICT-Meso international accelerator network, and through a UK survey of current CDKN2A and BAP1 to help inform the case and design of a national policy for molecular testing in PM.

CHAPTER 5: SUMMARY

5 Summary

5.1 Summary of thesis

The diagnosis of PM, and its differentiation from benign pleuritis, can be challenging. Furthermore, patients with biopsy-proven benign pleural inflammation can evolve to mesothelioma at a later stage. A better understanding of early mesothelioma biology and the processes that drive or permit PM evolution is required for the development of more effective therapies. This thesis set out to expand on the current understanding of benign asbestos associated pleural inflammation, and its evolution to mesothelioma.

5.1.1 The Meso-ORIGINS Feasibility Study

In this multicentre, mixed methods feasibility study, we tested several critical elements of an initial design for Meso-ORIGINS, which will perform biological surveillance of a large cohort of patients with AAPI and collect matched benign-PM tissue pairs in the minority who evolve into PM.

296 AAPI patients (39 prospective, 257 retrospective) were recruited/selected for the study. A prospective recruitment target was set a priori at 27 histological AAPI cases (or 14 in any 6-months). 21/39 (54%) patients recruited had a histological diagnosis of AAPI at registration, meaning the a priori threshold for recruitment feasibility based on the original design was not met. However, post-amendment recruitment (allowing recruitment of prevalent (not just incident) cases and patients diagnosed using techniques other than LAT) was higher than it would have otherwise been and surgically diagnosed cases were notably under-represented.

Repeat LAT was technically feasible and acceptable in 13/28 (46%) and 24/36 (67%) cases with complete follow-up data. Mesothelioma evolution was confirmed histologically in 36/257 retrospective cases (14%(95%CI 10.3-18.8) and associated with malignant CT features (OR 4.78(95% CI 2.36-9.86) and age (OR 1.06(95% CI 1.02-1.12).

This feasibility study has shown that our initial eligibility criteria were too narrow. Meso-ORIGINS will recruit a broader cohort, including prevalent cases, any biopsy type, and patients with malignant CT features. A range of re-biopsy techniques will be allowed, accounting for technical and patient factors. The sample size has been reduced to 500.

5.1.2 Mesothelioma Evolution following a diagnosis of Benign Pleural Inflammation: A systematic review and meta-analysis

To our knowledge, this is the first systematic review and meta-analysis of PM evolution rate following a diagnosis of NSP. We assessed the current published literature to establish the true proportion of patients who will develop PM following a diagnosis of NSP, whilst aiming to identify study characteristics which give rise to a higher-than-average PM evolution rate. We identified 265 potentially eligible studies published since 2000 and included 17 (comprising 18 study cohorts), describing 2605 NSP cases and 146 subsequent PM evolutions. In a random effects model, the summary point estimate of PM evolution was 5.44% (95% CI 3.37-7.51), with significant heterogeneity ($p<0.001$, I^2 82.7%). Asbestos exposure, study setting and NSP biopsy type were significant sources of heterogeneity. Median follow-up and study design were non-significant. Higher PM evolution rate was associated with ≥50% asbestos exposure by cohort and high PM incidence study settings. Lower evolution rate was associated with surgical NSP biopsies. Most studies demonstrated moderate/high risk of bias using the QUIPS methodology assessing a range of potential areas of bias. There was also evidence of publication bias shown by marked asymmetry on a Funnel plot, which was supported by a statistically significant Funnel rank correlation test ($p=0.005$).

These results indicate that clinical surveillance following NSP and precision PM prevention trials are likely to be most impactful in asbestos-exposed cohorts and regions with high PM incidence. The reduced risk associated with surgical biopsies described in our study should be interpreted with caution, but favours thoracoscopic sampling in suspected PM, where NSP is a possible diagnostic outcome.

5.1.3 Prognostic utility of CDKN2A and BAP1 testing in pleural mesothelioma and asbestos associated benign pleural inflammation

This multicentre retrospective study collected data on combined CDKN2A and BAP1 status for 155 patients with PM and 42 patients with apparently benign AAPI. In the PM group, CDKN2A and BAP1 loss were observed in in 63.2% and 57.4%, respectively. Stage I prevalence was high (60.7%) and median OS was 12.4 months (95% CI: 11.2-15.9). Adverse OS was associated with non-epithelioid histology, higher performance status and later stage, in keeping with multiple previous studies. CKDN2A/BAP1 status was not associated with OS, including by histological subtype or when both genes were lost, contrary to previous studies. Among 42 eligible AAPI cases, CDKN2A and BAP1 loss were observed in 1/42 (2%) and 2/39 (5%), respectively, which is discordant with previous studies that have reported 100% specificity for BAP1 and MTAP in differentiating PM from benign pleuritis. 28/42 (66%) AAPI cases died during prolonged follow-up (minimum 2 years), with no PM evolutions observed, including in 7 cases with post-mortem examinations. The low frequency of CDKN2A and BAP1 loss in the AAPI cohort prevented any meaningful survival analysis by CDKN2A/BAP1 status.

These data suggest that CDKN2A/BAP1 status, in isolation, are not strong predictors of survival in earlier stages of PM, and loss of function events may be present at low frequency in patients without invasive disease. These findings emphasise the importance of longitudinal tissue sampling spanning the period preceding PM development and use of multi-omic testing to define the various biological processes driving PM evolution and adverse survival once PM is established. This work is being conducted in the PREDICT-Meso international accelerator network.

5.2 Future work

The work presented in this thesis has ultimately informed the design of the Meso-ORIGINS study, with refinement of the study eligibility criteria and prompting significant changes to the re-biopsy strategy, site selection and the sample size estimate. The study, which forms a major part of the PREDICT-Meso

International Accelerator, opened to recruitment in June 2022. The material collected Meso-ORIGINS will be used for multi-omic characterisation of the biology associated with mesothelioma evolution, development of a range of pre-clinical models of pre-invasive and early-stage disease, and for high-throughput drug screening and target-drug validation. The goal of these efforts is development of new, early intervention therapies, ready for human trials. Alongside the PREDICT-Meso Accelerator work, we are planning a UK survey of current CDKN2A and BAP1 test use and understanding this information will help inform the case and design of a national policy for molecular testing in PM.

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