The development of novel biomarker techniques for the early detection of colorectal anastomotic breakdown

by

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Overall thesis abstract (Summary)

Anastomotic leak (AL) following colorectal surgery leads to significant morbidity, mortality and poorer oncological outcomes. Diagnosis of AL is frequently delayed as current methods of detection are not 100% sensitive or specific. Recent work has illustrated that local biomarkers from the perianastomotic environment may have the ability to detect AL early. A literature review undertaken as part of this project identified lactate and pH as the most promising biomarkers. Advances in biosensor technology means that the development of a degradable or removal biosensor for AL represents an exciting possibility. With the ultimate aim of developing such a sensor, this study aimed to determine the stability of lactate and pH in peritoneal fluid, further assess their usefulness as biomarkers of AL and other complications after surgery and compare their performance to that of commonly used blood biomarkers in detecting AL and other complications following colorectal surgery. Peritoneal fluid lactate was found to be stable over 24 hours, except if the fluid had a high WCC. For pH, clinically significant changes were detected after 1 hour. As there were no ALs in the study it was not possible to determine the usefulness of lactate and pH in relation to this complication. Although the difference was not statistically significant, pH generally rose over the first 3 days post-operatively in patients making an uncomplicated recovery. Lactate did not appear to be useful in predicting post-operative complications but it was thought that this was related to the method by which lactate was measured and that further study of lactate was merited. Blood biomarkers were superior to perianastomotic lactate and pH at predicting post-operative complications but they lacked specificity and significant differences were only apparent at 5 days following surgery. Overall, the work has contributed to our understanding of the stability of lactate and pH in peritoneal fluid, highlighted trends in pH in post-operative patients that merit further investigation and has illustrated the limitations of blood biomarkers. In addition, it has provided insight into how further studies in the area should be conducted and which questions need to be addressed next in order to pursue the eventual aim of developing a biosensor for the early detection of AL.

Table of contents

List of figures and tables

List of publications

Full paper

Wright EC, Connolly P, Vella M, Moug S. Peritoneal fluid biomarkers in the detection of colorectal anastomotic leaks: a systematic review. Int J of Colorectal Dis 2017; 32(7):935-945.

Poster presentations

The detection of anastomotic leak following colorectal surgery: The clinical applicability of perianastomotic lactate and pH.
ASGBI Annual Conference, Liverpool, May 2018

Towards a biosensor for colorectal anastomotic leak: determining the stability of peritoneal fluid biomarkers.
NHS Research Scotland Annual Conference, Perth, October 2017.

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Finally, my thanks to family and friends who have continually taken an interest in my work and provided support and encouragement throughout.

Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution. The contribution of other authors to this work has been explicitly indicated below.

Chapter 1 (General Introduction) includes work arising from the publication entitled, 'Peritoneal fluid biomarkers in the detection of colorectal anastomotic leaks: a systematic review', International Journal of Colorectal Diseases 2017; Volume 32, Issue 7, pages 935-945. The review article was written by the candidate. Prior to publication the manuscript was reviewed and edited by Mr Mark Vella (Consultant Colorectal Surgeon) and the candidate's supervisors Professor Patricia Connolly and Ms Susan Moug.

Abbreviations

Full list of abbreviations

AAA	Abdominal aortic aneurysm
ACPGBI	Association of Coloproctology of Great Britain and Ireland
AF	Atrial fibrillation
AL	Anastomotic leak
ALD	Alcoholic liver disease
APACHE	Acute Physiology and Chronic Health Evaluation
APR	Abdominoperineal resection
ASA	American Society of Anaesthesiologists
ATP	Adenosine triphosphate
AUC	Area under curve
BMI	Body mass index
°C	Degrees Celsius
COSHH	Control of Substances Hazardous to Health
CRC	Colorectal cancer
CRP	C-reactive protein
CT	Computed tomography
DD	Diverticular disease
ERAS	Enhanced recovery after surgery
FAP	Familial adenomatous polyposis
FOB	Faecal occult blood
GI	Gastrointestinal
HIV	Human immunodeficiency virus
HNPCC	Hereditary nonpolyposis colorectal cancer
H_2O	Water
H_2O_2	Hydrogen peroxide
IBD	Inflammatory bowel disease
ICU	Intensive Care Unit
IL-1b	Interleukin 1b
IL-6	Interleukin 6
IL-10	Interleukin 10
IPAA	Ileal pouch-anal anastomosis

IQR	Interquartile range
JPS	Juvenile polyposis syndrome
LDH	Lactate dehydrogenase
LF ICG	Laser fluorescence angiography
LP ratio	Lactate/pyruvate ratio
LPS	Lipopolysaccharide binding protein
LP2	Lactate Pro 2
LRTI	Lower respiratory tract infection
MBP	Mechanical bowel preparation
MDT	Multi-disciplinary team
MHP	Metaplastic hyperplastic polyposis
mL	Millilitre
mM	Millimole
mm^3	Millimetres cubed
mmol/l	Millimole per litre
MMPs	Matrix metalloproteinases
MMR	Mismatch repair
MRI	Magnetic resonance imaging
NELA	National Emergency Laparotomy Audit
nm	Nanometre
NHS GGC	NHS Greater Glasgow & Clyde
NICE	National Institute for Clinical Excellence
N&V	Nausea and vomiting
O_2	Oxygen
PET	Positron emission tomography
PIS	Patient information sheet
PJS	Peutz-Jeghers Syndrome
$pC0_2$	Partial pressure of carbon dioxide
PCT	Procalcitonin
POD	Post-operative day
$p0_2$	Partial pressure of oxygen
POSSUM	Physiological and Operative Severity Score
PPV	Positive predictive value
RAH	Royal Alexandra Hospital
RBCs	Red blood cells

RCT	Randomised controlled trial
RT-PCR	Real-time polymerase chain reaction
SBO	Small bowel obstruction
SBP	Spontaneous bacterial peritonitis
SDD	Selective decontamination of the digestive tract
SOP	Standard Operating Procedure
SPSS	Statistical Package for the Social Sciences
$St0_2$	Tissue oxygen saturation
SVT	Supraventricular tachycardia
TGF-B	Tissue growth factor B
TIMPs	Tissue inhibitors of metalloproteinase
TNFa	Tumour necrosis factor alpha
TOOS	N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine
UC	Ulcerative colitis
UK	United Kingdom
UTI	Urinary tract infection
WCC	White cell count
µL	Microlitre

Chapter 1 - General introduction

1 Chapter 1 - General introduction

General surgery is one of the largest surgical specialties, 31% of all United Kingdom (UK) surgeons are general surgeons (England). Colorectal surgery is the largest sub-specialty within general surgery, in the UK there are 850 colorectal surgeons registered with The Association of Coloproctology of Great Britain and Ireland (personal communication). Colorectal surgery is required for a variety of benign and malignant conditions. Frequently it involves removal of a diseased segment of bowel. In this situation surgeons will consider performing an anastomosis to re-join the cut ends of bowel in order to optimize the patients' quality of life. This section discusses, in depth, the definition of an anastomosis, conditions where an anastomosis may be required, possible complications of an anastomosis and the current methods to predict and detect these complications.

1.1 Healing in the gastrointestinal (GI) tract

1.1.1 The anatomical structure of the gastrointestinal tract

The intraperitoneal gastrointestinal tract consists of 4 layers: mucosa, submucosa, muscularis propria and serosa (see figure 1.3); the extraperitoneal areas, e.g. lower third of rectum, lack the serosa. The mucosa consists of epithelium, lamina propria (loose connective tissue containing collagen) and a muscularis mucosa (thin layer of smooth muscle cells). Migration and hyperplasia of epiltheilal cells can seal a mucosal breach within 3 days.

The submucosa consists of collagenous and elastic fibres together with a submucosal plexus of nerve fibres, blood vessels and lymphatics (Thornton and Barbul, 1997). The composition of types of collagen is different in the bowel compared to other soft tissues. In addition to type I and III collagen, bowel also contains type V collagen. The relative proportions of collagen in the submucosa are type I 68%, type III 20% and 12% is type V collagen (Thornton and Barbul, 1997). In 1887 Halsted discovered that most of the tensile strength of the bowel comes from the submucosa (Halsted, 1887). This principle of this is still applied today as hand sewn bowel anastomoses are generally performed by incorporating serosa and submucosa within the anastomotic suture line (a sero-submucosal

stitch). Experimental studies have shown that sutures through the mucosal layer do not add to the strength of the anastomosis and therefore serosubmucosal bites, rather than full-thickness bites, are taken through the bowel wall (Egorov et al., 2002, Tera and Aberg, 1976).

The muscularis propria consists mostly of smooth muscle cells and collagen. The serosa is a thin layer of connective tissue covering the muscularis propria. Sound apposition of the serosa during formation of an anastomosis reduces the risk of anastomotic leak (DiZerega, 1989, Getzen, 1966, LaCalle et al., 1982).

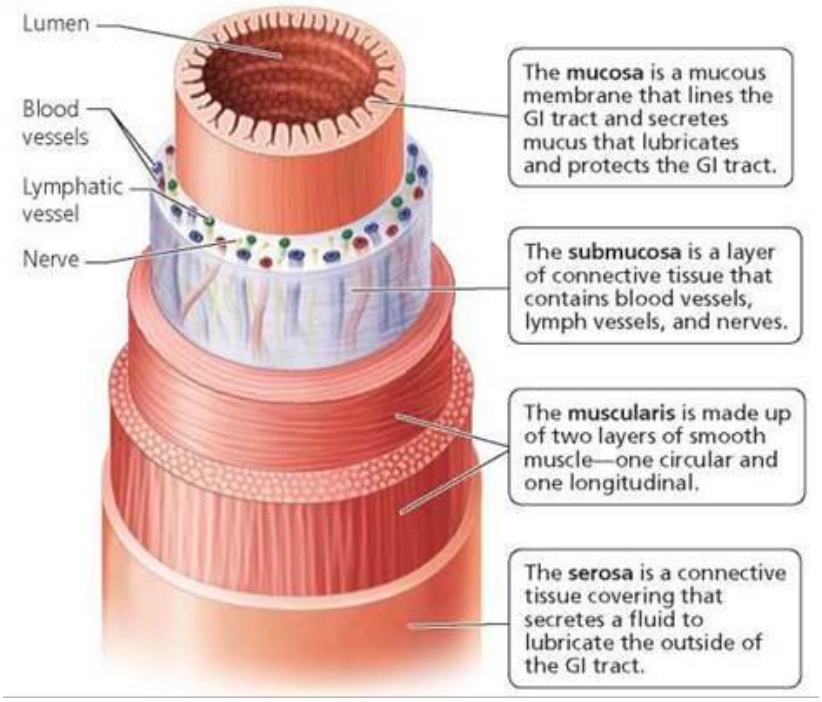

FIGURE 1.1 LAYERS OF THE GASTROINTESTINAL TRACT (SCHOOLBAG.INFO, 2016)

1.1.2 General wound healing

Wound healing is a series of steps designed to re-establish an immune barrier and then repair the injured tissue. The classic phases of wound healing are divided into three phases: the inflammatory or 'lag' phase, proliferative phase and remodelling phase (see Figure 1.4).

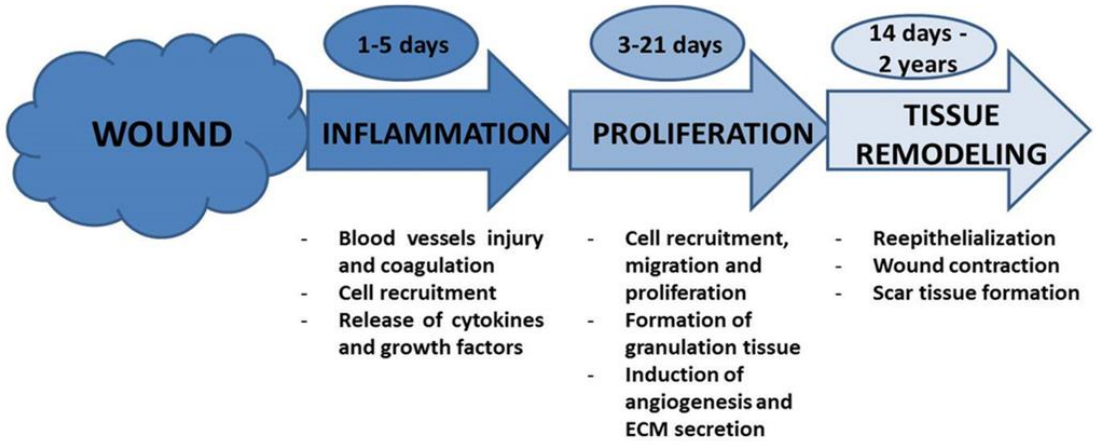

FIGURE 1.2 PHASES OF WOUND HEALING (ANDREU ET AL., 2015)

The classic phases of wound healing have been studied extensively. In 2006 Thompson et al provided a succinct overview of the three stages (Thompson et al., 2006);

1. In the inflammatory phase, haemostasis is secured by platelet migration to the site of injury to form a fibrin-based clot. Increased vessel permeability allows inflammatory cells to migrate into the area. Initially the main cell type is neutrophils but they are rapidly superseded by macrophages which are the dominant cell type by day 2-3. They synthesise and release tissue growth factors essential to the progression of tissue repair.
2. The start of the proliferative phase is marked by the arrival of fibroblasts, they are the major cell type by day 4. Fibroblasts replace the provisional matrix with collagen-rich granulation tissue. Normal soft tissue is made up of 80% type I collagen and 20% type III collagen. The new granulation tissue laid down at the outset of healing contains a higher level of type III collagen – 30-40%. Ferrous iron, molecular oxygen, alpha-ketoglutarate and vitamin C are required as cofactors for hydroxylation necessary for collagen synthesis. During this proliferative phase angiogenesis also occurs in order to ensure adequate oxygen and nutrient supply to the healing tissues.
3. Remodelling is the final phase in wound healing. This takes place over several weeks to months. The granulation tissue laid down initially is

remodelled. The number of macrophages and fibroblasts decreases and the percentage of type III collagen falls to 20%.

1.1.3 Healing of an anastomosis

Healing of a gastrointestinal anastomosis proceeds via the three phases outlined above – inflammatory (lag), proliferative and remodelling phases but there are some important differences between healing in the GI tract and skin (see table 1.6) (Thompson et al., 2006). The appearance of granulation tissue in the anastomosis marks the beginning of the proliferative phase. The collagen in the wound undergoes both lysis and synthesis. In 1887 Halsted demonstrated that the submucosa provides the majority of the strength to the GI tract (Halsted, 1887). Consequently, the strength of the anastomosis is derived from the collagen in the submucosal layer of the bowel wall. Unlike skin, initially collagenase activity predominates which results in a reduction in collagen and therefore a decrease in anastomotic strength (Martens et al., 1992). For the first 1 - 3 days (the inflammatory phase) the anastomosis will rely upon the sutures or staples used to create it for its strength. This is followed by the proliferative phase which is characterized by a switch from collagen degradation to collagen deposition. This leads to a rapid increase in anastomotic strength (Thompson et al., 2006). This initial dip in wound tensile strength is demonstrated in the curves shown in figure 1.5. Any factor which delays or prevents the transition to the proliferative phase can result in a failure of the anastomosis to heal.

TABLE 1.1 DIFFERENCES BETWEEN HEALING IN SKIN AND THE GI TRACT (THOMPSON ET AL., 2006)

	GI tract	**Skin**
Collagen		
• Subtypes	1, 3, 5	1, 3
• Production	Smooth muscle cells and fibroblasts	Fibroblasts only
• Regulation	Tissue growth factor B (TGF-B)	TGF-B, dexamethasone, interleukin-1B
Wound strength		
• Rate of healing	Rapid (weeks)	Prolonged (months)
• Components	Additional strength due to serosa	No serosa equivalent
Wound environment		
• Shear stress	Increased due to peristalsis and bulk transit	Minimal
• Bacteria	Aerobic and anaerobic, may affect anastomotic healing	Aerobic, rarely causes problems
• Vascular perfusion	Downregulated if hypovolaemic	Constant
Collagenase activity	Increased in the first 3 days; causes transient decrease in anastomotic strength	Not significant

Wounds in the GI tract gain strength more rapidly than cutaneous wounds (Martens and Hendriks, 1991). In addition, the rate of healing varies within the GI tract. Ileum has less collagen degradation and more collagen formation compared to colon. Small bowel anastomoses approach the strength of normal bowel by 4 weeks but in the colon the anastomosis only approaches 75% strength at 4 months (Martens and Hendriks, 1991, Mast, 1997).

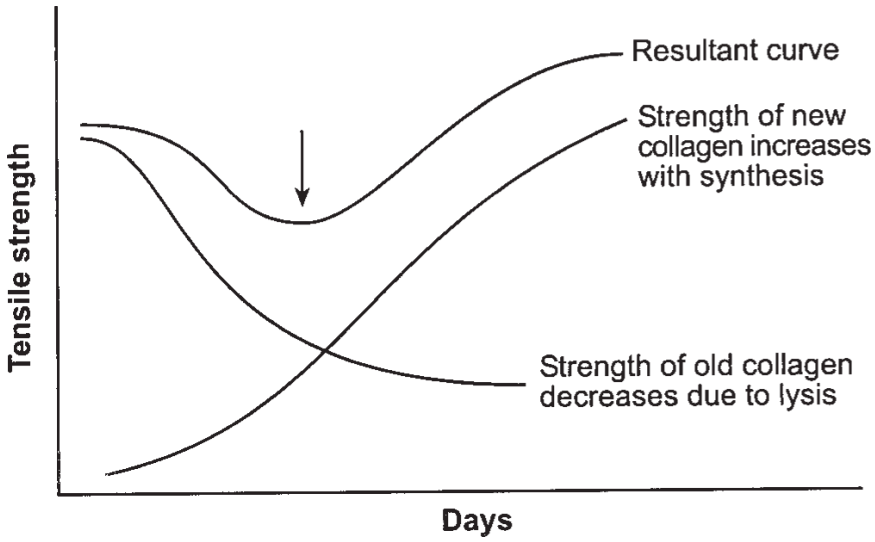

FIGURE 1.3 WOUND-HEALING IN GASTROINTESTINAL TRACT IS FINE BALANCE DURING 'LAG' PHASE BETWEEN COLLAGEN SYNTHESIS AND COLLAGENOLYSIS. LINE LABELED 'RESULTANT CURVE' SHOWS THIS BALANCE. WEAK TIME PERIOD DEPICTED ON GRAPH (ARROW) CAN BE PROLONGED OR EXACERBATED BY LOCAL OR SYSTEMIC FACTORS THAT UPSET EQUILIBRIUM (THOMPSON ET AL., 2006)

A wound will fail to heal if there is a disruption of one of the 3 phases of wound repair. Thompson et al described a range of local and systemic factors that can adversely impact on wound healing (see table 1.7). Before surgery, the medical team should aim to correct malnutrition. The energy required for wound repair necessitates adequate calorific intake. Vitamins and minerals such as vitamins A, C and B6, and zinc, iron and copper are required for collagen synthesis (Dubay and Franz, 2003). Nutritional deficiencies of these elements will impair anastomotic healing. Glycaemic control should be optimized and other problems such as jaundice or infection should be treated.

Intra-operatively, good surgical technique providing sound apposition of the bowel and avoiding excess tension is required. Tissue hypoperfusion should be avoided. This is dependent upon vascular anatomy, vasomotor control and arterial tissue oxygen pressure (pO_2). The vascular anatomy local to the anastomosis should be disrupted as little as possible to ensure that the area receives a good blood supply. Systemic hypotension in the post-operative period

must be avoided because in the setting of hypovolaemia the body downregulates intestinal perfusion and diverts blood to essential organs such as the brain and heart. Mature collagen formation fails if pO_2 falls below 40mmHg (Shandall et al., 1985).

Strict haemostasis should be ensured to minimise the need for blood transfusion in the intra or post-operative period. In animal models it has been shown that blood transfusion has a negative impact on wound healing. It is thought that transfusions impair the migration and function of macrophages in the wound and thus delay the first phase of healing – the inflammatory phase (Tadros et al., 1992).

TABLE 1.2 LOCAL AND SYSTEMIC FACTORS WITH ADVERSE EFFECTS ON GASTROINTESTINAL HEALING (THOMPSON ET AL., 2006)

Local	**Systemic**
Tissue hypoperfusion	Malnutrition
Anastomotic tension	Blood transfusion
Poor apposition of wound edges	Hypovolaemia
Local infection	Medication (e.g. cisplatin)
Radiation injury	Immunodeficiency
Distal obstruction	Poorly controlled diabetes
	Jaundice
	Obesity

1.2 The colorectal anastomosis

1.2.1 Definition of a colorectal anastomosis

Formation of a colorectal anastomosis is a procedure to restore continuity, i.e. creating a join, between two cut ends of bowel (see Figure 1.1). The indications for this procedure are wide-ranging but can be broadly split into two categories; procedures where a portion of diseased bowel has been removed or to bypass a

diseased segment which cannot be removed. The vast majority of operations fall into the first category. Examples of situations where a diseased section of bowel is removed include: malignancy, diverticular disease, bowel ischaemia, benign polyps, traumatic perforations, complications of inflammatory bowel disease (e.g. toxic megacolon, perforation, stricture), infections (e.g. TB causing stricturing), complications of radiation enteritis (e.g. stricture, perforation), catastrophic lower gastrointestinal bleeding and chronic constipation. Examples of situations where diseased bowel may need to be bypassed are: locally advanced tumour too large to be resected or metastatic disease causing bowel obstruction.

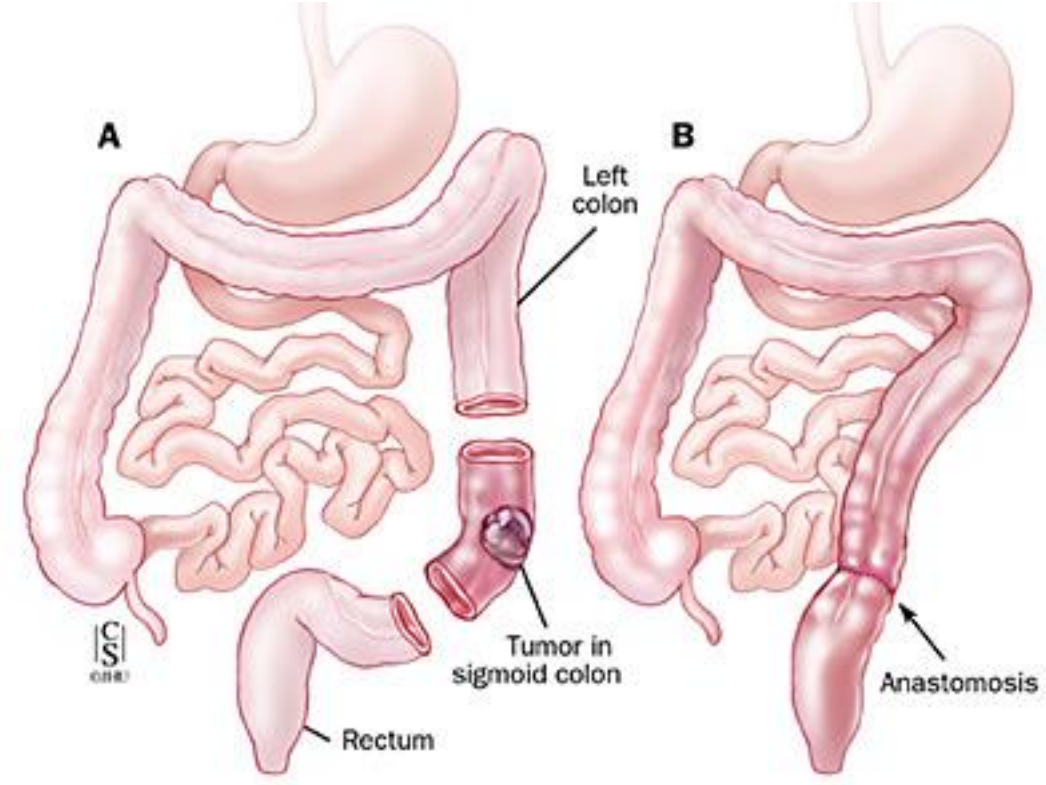

FIGURE 1.4 AN EXAMPLE OF A COLORECTAL ANASTOMOSIS – A SEGMENT OF BOWEL CONTAINING A TUMOUR IS REMOVED AND CREATING AN ANASTOMOSIS RESTORES CONTINUITY OF THE CUT ENDS OF BOWEL (MEDICINE, 2016)

1.2.2 Common conditions requiring formation of a colorectal anastomosis

1.2.2.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) is generally the term used to describe the two most common chronic inflammatory conditions of the bowel – Crohn's disease and Ulcerative Colitis (UC), which affect more than 300,000 people in the United Kingdom (UK, 2016a). There are other less common types of IBD that can only be seen microscopically – collagenous and lymphocytic colitis (Palmer KR, 2006). Sometimes it is difficult to differentiate between the types of IBD on histology and there can be substantial inter-observer variability between pathological reporting. Histopathological features can overlap leading to a diagnosis of "indeterminant colitis" in 5-10% of patients with just colonic disease (Thompson-Fawcett MW, 2014). The clinical picture and evolution of symptoms may ultimately support one diagnosis over another, e.g. subsequent small bowel involvement favouring a diagnosis of Crohn's disease.

Although the incidence of IBD varies throughout the world, it is more common in the developed than the developing world. The incidence of IBD increased substantially over the 20th century in developed countries with Europe now having an annual incidence of UC and Crohn's of 24.3 per 100,000 and 12.7 per 100,000 respectively (Ananthakrishnan, 2015). The Asia-Pacific Crohn's and Colitis Study noted an increasing incidence of IBD in so-called emerging nations such as Korea and China which have seen a more recent growth of urbanisation and development. Although still substantially lower than in Europe, the incidence rates of UC and Crohn's in the study were 0.76 per 100,000 and 0.54 per 100,000 respectively (Ng et al., 2013). In Africa IBD remains a rare disease but case series do suggest an increasing incidence (Archampong and Nkrumah, 2013). The onset of IBD is usually in early adulthood (Palmer KR, 2006). Crohn's disease and UC typically follow a relapsing and remitting course. Whilst UC only involves the colon and rectum, Crohn's disease can affect any part of the gastrointestinal tract from the mouth to the anus.

The pathogenesis of IBD appears to be a response to an environmental trigger in a genetically susceptible individual, this may explain the variation in incidence in the developing versus developed world. There also seems to be a "north – south gradient" with studies in both the USA (Schultz and Butt, 2012) and Europe (Shivananda et al., 1996) noting higher incidence at more northern latitudes. One hypothesis is that increased exposure to vitamin D at southern latitudes is

protective. Table 1.1 illustrates a range of known genetic and environmental risk factors for IBD.

TABLE 1.3 GENETIC AND ENVIRONMENTAL FACTORS ASSOCIATED WITH IBD (PALMER KR, 2006)

Genetic factors
• Ashkenazi Jewish descent • Affected first degree relative • Associated with autoimmune thyroiditis and systemic lupus erythematosus (SLE) • Linkage with mutations in CARD 15/NOD-2 gene on chromosome 16 (IBD-1 locus) • HLA-DR103 associated with severe UC • UC and Crohn's patients with HLA-B27 commonly develop ankylosing spondylitis
Environmental factors
• UC more common in non-smokers and ex-smokers • Associated with low residue, high refined sugar diet • Appendicectomy is potentially protective against UC

Crohn's disease tends to present with abdominal pain, diarrhoea and weight loss. Other features such as fever and malaise may also be present. If the colon is predominately affected, then the presentation may be with bloody diarrhoea. UC presents with diarrhoea that frequently contains blood and/or mucus. Although there are many similarities in the presentation and symptoms of Crohn's and UC there are some key differences in the pattern of disease, macroscopic and microscopic features, risk of colorectal cancer and histological features. Table 1.2 provides a comparison of the two conditions.

TABLE 1.4 FEATURES OF CROHN'S DISEASE AND UC (MG, 2007)

	Crohn's disease	**Ulcerative colitis**
Incidence	5-7/100,000 and rising	10/100,000 and stable
Extent	May involve entire gastrointestinal tract	Limited to large bowel
Rectal involvement	Variable	Almost invariable
Disease continuity	Discontinuous (skip lesions)	Continuous
Depth of inflammation	Transmural	Mucosal
Macroscopic appearance of mucosa	Cobblestone, discrete deep ulcers and fissures	Multiple small ulcers, pseudopolyps
Histological features	Transmural inflammation, granulomas (50%)	Crypt abscesses, submucosal chronic inflammatory cell infiltrate, crypt architectural distortion, goblet cell depletion, no granulomas
Presence of perianal disease	75% of cases with large bowel disease; 25% of cases with small bowel disease	25% of cases*
Frequency of fistula	10-20% of cases	Uncommon
Colorectal cancer risk	Elevated risk (relative risk = 2.5) in colonic disease	25% risk over 30 years in pancolitis
Relationship with smoking	Increased risk, greater disease severity, increased risk of relapse and need for surgery	Protective, first attack may be preceded by smoking cessation within 6 months

**This includes haemorrhoids, fissures, skin tags, abscesses and fistulae. The incidence of complex perianal disease with fistulae and abscesses in UC is closer to 5% (Zabana et al., 2011)*

IBD is frequently first managed with medical therapies. Steroids may be used to settle an acute flare. In the longer term anti-inflammatory drugs such as 5-aminosalicylic acid agents (e.g. mesalazine, olsalazine) and immunosuppressants such as methotrexate and ciclosporin can be used. Biological drugs such as infliximab and adalimumab are now considered for use in in refractory cases (Danese et al., 2015).

At some point 80% of people with Crohn's disease will require surgery, in UC the corresponding figure is around 10-30% (Cosnes et al., 2011). In the elective setting, surgery may be indicated for: subacute obstructions, strictures, perianal sepsis and fistulae, disease refractory to medical management, complications of medical management (e.g. osteoporosis), concerns about the long term risks of immunosuppression or the onset of malignancy. Emergency surgery is often needed for fulminant colitis unresponsive to maximal medical management (especially when rescue therapy such as anti-TNFs are failing), toxic megacolon, perforation or obstruction (MG, 2007). In Crohn's disease, bowel resection should be as limited as possible in order to maintain gut length as 40% of patients go on to require further surgery (Coffey et al., 2016). A study looking at resection margins for Crohn's disease found that extensive resection margins are unnecessary (Fazio et al., 1996). The 2017 European Crohn's and Colitis Organisation (ECCO) Consensus on Surgery continue to recommend a limited resection when surgery is required (Bemelman et al., 2017). However, in a recent study of 64 patients undergoing ileocaecal resection for Crohn's disease, excision of the mesentery along with the affected segment of bowel resulted in a lower reoperation rate (2.9% vs. 40%) (Coffey et al., 2018). If these results can be replicated in larger cohorts then it may lead to a change in practice. In UC removal of the colon and rectum is essentially curative. In the acute setting in patients with fulminant colitis; the operation of choice is a subtotal colectomy with a long rectal stump, this can provide options for future restorative surgery (Carter et al., 2004). This also provides an opportunity for histopathological analysis to confirm the diagnosis. Up to 13% of patients having an emergency resection for colitis may be found to have either indeterminate colitis or Crohn's disease rather than UC (Hyman et al., 2005), meaning further surgery to remove the rectum may be avoided. In the elective setting, a proctocolectomy with end ileostomy represents a cure and patients will not require on going disease

surveillance. However, there is considerable stoma-associated morbidity such as stenosis, herniation and skin excoriation. Requirement for stoma revision surgery can be as high as 24% (Carlstedt et al., 1987). As a consequence, a restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) has gained in popularity. Despite the need for on going disease surveillance at the resection margin in the anal transition zone and the fact that 24-48% experience pouchitis (Fazio et al., 1995, Meagher et al., 1998), patients report an improved quality of life and 98% would have the surgery again or recommend it to someone else with UC (Delaney et al., 2003).

1.2.2.2 Colorectal cancer

Colorectal cancer is the 4th most common cancer in the UK after breast, lung and prostate. Around 41,900 cases are diagnosed in the UK each year (UK, 2016c). Two thirds of cancers occur in the colon and one third in the rectum (Steele, 2014). Risk factors for colorectal cancer include: increasing age, male gender, family history, 'Westernised diet', inflammatory bowel disease, genetic factors, and pre-existing adenomatous polyps.

The majority of colorectal cancers occur sporadically but genetic susceptibility contributes about 15-20% (Lynch and De la Chapelle, 1999) to the overall incidence of colorectal cancer. Mostly this relates to individuals with an ill-defined increased risk due to a strong family history of colorectal cancer, however 5-10% are due to detectable genetic abnormalities (Lynch and De la Chapelle, 1999). The three main categories of genetic susceptibility are: hereditary nonpolyposis colorectal cancer (HNPCC), dominant polyposis syndromes (familial adenomatous polyposis, (FAP); Peutz-Jeghers Syndrome, (PJS); juvenile polyposis syndrome, (JPS)) and recessive inheritance syndromes such as metaplastic hyperplastic polyposis (MHP).

HNPCC (Lynch syndrome) is the most common autosomal dominant syndrome and accounts for 3-5% of all colorectal cancers. The underlying genetic defect is a mutation in one of the mismatch repair (MMR) genes MLH1, MSH2, MSH6 or PMS2 (Vasen et al., 2013). It is characterized by a small number of adenomas but with increased potential for transformation into malignancy, with 70% of males and

35% of females developing colorectal cancer over their lifetime. There is also an increased risk of other cancers: endometrial, gastric, upper urinary tract and small intestinal cancers. New guidelines released by the National Institute for Clinical Excellence (NICE) in 2017 recommend that all patients diagnosed with a colorectal cancer should be offered testing for Lynch syndrome at the time of their diagnosis (NICE, 2017). This can be done using immunohistochemistry for mismatch repair proteins or microsatellite instability testing to identify tumours with deficient DNA mismatch repair. The results of these tests guide further sequential testing for Lynch syndrome.

FAP is another autosomal dominant disorder that confers increased risk of CRC. The mutation is in the APC gene on the long arm of chromosome 5. The incidence of FAP is 1 in 6670 live births and the population prevalence is 1 in 13528 (Dunlop, 2007). The syndrome is characterized by the presence of > 100 adenomatous polyps within the colon. These usually develop in the teenage years/early adulthood and affected individuals will almost certainly develop colorectal cancer by the third or fourth decade. In such cases, prophylactic colectomy is performed whilst the patients are young and therefore FAP now accounts for less than 0.2% of colorectal cancers in the UK (Dunlop, 2007). Those with FAP also have widespread duodenal polyps and there is a risk of malignant change peri-ampullary polyps becoming malignant. Patients are also at risk of intra-abdominal desmoid tumours and females < 35 years have an increased risk of papillary thyroid cancer.

Whether sporadic or genetic, CRC can present with a wide range of symptoms including intermittent rectal bleeding, blood and mucus mixed with stool, altered bowel habit, abdominal pain, iron deficiency anaemia and in the case of low rectal cancers, tenesmus. Around 15% of patients present with obstruction and 3% have a perforation at presentation (Dunlop, 2007). In the fourth UK National Emergency Laparotomy Audit (NELA), the operative finding was listed as “colorectal cancer” in 8.1% of cases (Team, 2018). This will include those operated on as an emergency for a perforated or obstructed colorectal cancer.

With the advent of the national bowel screening programme some cancers are now picked up in asymptomatic individuals – in England 1778 cancers were

picked up from the first 1 million bowel screening tests that were returned (Logan et al., 2012). In Scotland screening started in 2007. All individuals between 50 – 74 years receive a stool testing kit testing kit every 2 years. Around 2% have an abnormal test (Mackay et al., 2014) and are invited for colonoscopy. Of these, 50% do not have a polyp or a cancer, 40% have a polyp and 10% have a cancer (UK, 2016b). Results from a single centre in Scotland showed that over a 4 year period, following the introduction of bowel screening, 17% of the cancers they treated were identified via the bowel screening programme. These cancers were diagnosed in younger people (median 65.5 years for screen-detected vs. 71.6 years "other referrals"), were at a less advanced stage and had significantly better survival outcomes (Mackay et al., 2014). Between 2007 – 2017 participants received a faecal occult blood (FOB) testing kit. In 2017 there was a transition to the faecal immunochemical test (FIT) (Scotland, 2017). The FIT test requires only one stool sample which makes it simpler and more user friendly than the old FOB test which required samples over 3 days. This makes the test more acceptable and has been shown to increase participation in the bowel screening programme (Chambers et al., 2016).

The management and prognosis of a CRC is determined by the stage of the cancer and whether it is in the colon or rectum. Following detection of a cancer, all patients will undergo a staging computed tomography (CT) scan of the chest, abdomen and pelvis. In the absence of widespread metastatic disease surgery is the main treatment for colon cancer. In rectal cancer, a key determinant of staging is the assessment of the extramural tumour spread in relation to the mesorectal fascia and sphincter complex. Magnetic resonance imaging (MRI) provides the most accurate imaging of soft tissues in this area (Jhaveri and Hosseini-Nik, 2015), therefore all patients with a rectal cancer have an MRI pelvis in addition to staging CT. In rectal cancer, patients with resectable disease and no metastases will be offered surgery. Those patients with a rectal cancer that is margin-threatening (tumour on MRI is ≤ 1mm from the circumferential resection margin) or low in the rectum (< 5cm from anal verge) will be considered for neoadjuvant chemo-radiotherapy prior to potentially curative surgery as this has been shown to reduce local recurrence and increase long term survival (Poston et al., 2011, Glimelius et al., 2013). Following

chemoradiotherapy some patients are judged to have had a clinical complete response with no evidence of residual tumour on MRI or clinical examination. In such patients a “watch and wait approach” may be adopted. A recent systematic review found that at 2 years 15.7% of patients had evidence of local tumour regrowth, of these patients 95.4% were able to go on to have salvage therapies such as surgery or further oncological treatment (Dossa et al., 2017). In the future, this may mean that fewer patients require major complex surgery for rectal cancer thus reducing the number of patients having a colorectal anastomosis and therefore reducing the number of patients exposed to the risk of AL and its deleterious consequences. However, this approach has still only been studied in a relatively small number of patients and prospective studies are on-going to confirm the long-term safety of a “watch and wait approach”.

Surgery for a CRC involves resection of the affected segment of bowel along with excision of the colonic mesentery, ligation of the arterial supply at its origin and excision of loco-regional lymph nodes. For tumours of the colon a 5cm disease free margin is recommended, and in the rectum, a 2cm margin but if the tumour is < 5cm from the anal verge then a 1cm is considered acceptable (Nelson et al., 2001). The cut ends of bowel then have to be re-joined (formation of an anastomosis) or if they are not anastomosed then a stoma is brought out onto the skin. For rectal cancers, the mesorectum (which carries blood vessels and lymph nodes) is excised along with the diseased segment of rectum. Figure 1.2 shows the operations carried out depending on the site of the tumour.

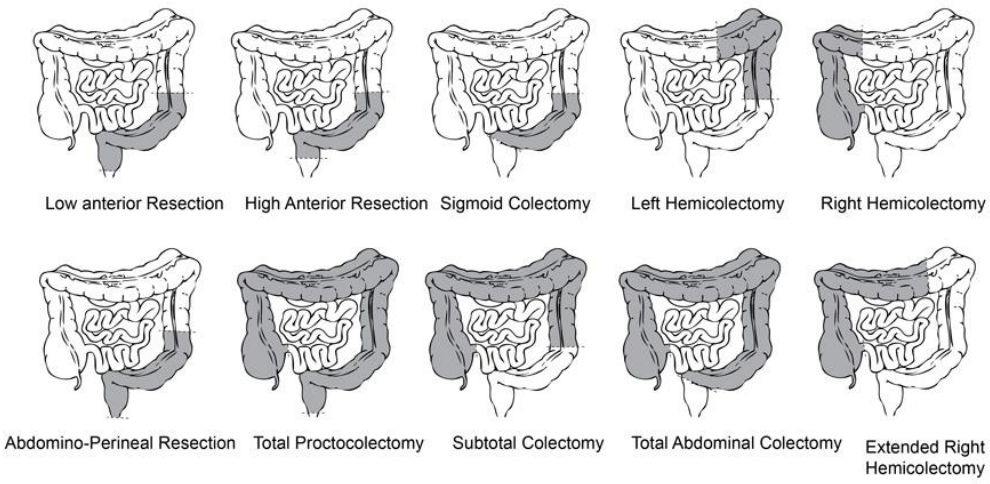

FIGURE 1.5 COMMON COLORECTAL RESECTIONS (FRY RD, 2012). THE SHADED AREA INDICATES THE SEGMENT OF BOWEL REMOVED WITH EACH PROCEDURE.

Once the cancer has been removed it can be accurately staged. In the UK this is done using either the TNM staging system shown in table 1.3 or the Duke's staging system shown in table 1.4.

TABLE 1.5 TNM STAGING OF COLORECTAL CANCER (UICC TNM STAGING 8TH EDITION) (GOSPODAROWICZ ET AL., 2017)

T (Tumour)

- **Tx** Primary tumour cannot be assessed
- **T0** No evidence of primary tumour
- **Tis** Carcinoma in situ: invasion of the lamina propria
- **T1** Cancer invades the submucosa
- **T2** Cancer invades into muscularis propria
- **T3** Cancer invades through muscularis propria and into subserosa or adjacent non-peritonealised tissues
- **T4** Cancer perforates the visceral peritoneum or directly invades adjacent organs
- **T4a** Tumour perforates visceral peritoneum
- **T4b** Tumour directly invades other organs or structures

N (Nodes)

- **Nx** The regional lymph nodes cannot be assessed
- **N0** No regional lymph nodes involved
- **N1** Metastases in 1-3 pericolic or perirectal lymph nodes
- **N1a** Metastasis in 1 regional lymph node
- **N1b** Metastasis in 2 -3 regional lymph nodes
- **N1c** Tumour deposits (s), i.e. satellites, in the subserosa, or in non-peritonealised pericolic or perirectal soft tissue *without* regional lymph node metastasis
- **N2** Metastasis in 4 or more pericolic or perirectal lymph nodes
- **N2a** Metastasis on 4 – 6 regional lymph nodes
- **N2b** Metastasis in 7 or more regional lymph nodes

M (Metastases)

- **Mx** The presence of distal metastases cannot be assessed
- **Mo** No distant metastases
- **M1** Distant metastases
- M1a Metastasis confined to one organ (liver, lung, ovary, non-regional lymph node(s) without peritoneal metastasis
- M1b Metastasis in more than one organ
- M1c Metastasis to the peritoneum with or without other organ involvement

TABLE 1.6 DUKE'S STAGING FOR COLORECTAL CANCER

Duke's stage	Description
A	Spread into, but not beyond, muscularis propria
B	Spread through full thickness of the bowel wall
C	Spread to involve lymph nodes
D	Distant metastases

The TNM classification is used to group CRCs into numerical stages. These stages guide decisions regarding adjuvant therapy and serve as a guide to prognosis (table 1.5). The NICE guidelines state that those with stage 1 disease do not require adjuvant therapy. Chemotherapy should be offered to those with stage 3 CRC who are fit enough to receive it. In stage 2 disease adjuvant chemotherapy may be offered if the tumour has high risk features (e.g. poorly differentiated, high grade, presence of venous invasion). Patients who present with stage 4 disease should have the resectability of their primary and metastatic disease assessed by site-specific multi-disciplinary teams (MDTs), e.g. liver metastases by a hepato-biliary MDT or lung metastases by a lung MDT. Such cases are considered by the MDTs on an individual basis with consideration given to chemotherapy, hepatic or lung surgery and colorectal surgery; the order of these interventions will be decided upon by the individual MDT. In the case of irresectable disease, palliative chemotherapy or radiotherapy can be considered but the main focus in these patients is symptom control and quality of life (Poston et al., 2011).

TABLE 1.7 NUMERICAL STAGING OF CRC AND PROGNOSIS (UK, 2016D)

Stage	Description	Prognosis (% 5 year survival)
0 (carcinoma in situ)	Cancer cells contained within the inner lining of the bowel	Men: 95% Women: almost 100%
1	T1 N0 M0 or T2 N0 M0	
2a	T3 N0 M0	Men: 80% Women: 90%
2b	T4 N0 M0	
3a	T1 N1 M0 or T2 N1 M0	Men & women: 65%
3b	T3 N1 M0 or T4 N0 M0	
3c	Any T N2 M0	
4	Any T Any N M1	Men: 5% Women: 10% (but if resection of liver metastases possible then can be 25-40%)

1.2.2.3 Diverticular disease

Diverticular disease (DD) is a benign acquired condition of the large bowel that is very common in developed countries with more than 60% of those over 70 years having features of DD on imaging or colonoscopy (Dunlop, 2007). It is associated with a low fibre diet and DD is rare in populations with a high fibre diet (Winter, 2014). The sigmoid colon is the most commonly affected site in the western world, likely due to the increased intraluminal pressures generated here when a low-fibre diet is consumed. Pulsion diverticulae develop between the mesenteric and antimesenteric taeniae and are due to herniation of mucosa through the circular muscle at the sites of penetration of blood vessels (Dunlop, 2007). DD is frequently asymptomatic and picked up as an incidental finding but it can cause lower abdominal/left iliac fossa pain, altered bowel habit (usually constipation) and colonic bleeding.

The exact pathogenesis of acute diverticulitis is unclear. Classically it was thought that diverticulitis was caused by local trauma to the mucosa due to impaction of inspissated faeces in a diverticulum which then produced stasis and a local inflammatory response. More recently an "ischaemic hypothesis" has been proposed. It has been suggested that prolonged or recurrent contractile spikes due to alterations in the muscle of the diverticular segment result in compression of vascular structures in the neck of the diverticulum. This causes localised ischaemia and subsequent micro-perforation of the diverticulum which in turn sets up a localised inflammatory response (Zullo, 2018). Further work is required to determine which of these hypotheses, or indeed whether a combination of the two, cause acute diverticulitis.

Patients with acute diverticulitis often present with left iliac fossa pain and a low grade pyrexia. Diverticulitis can range in severity from uncomplicated inflammatory diverticulitis to complicated diverticulitis (with abscess formation or perforation). For decades the Hinchey classification, based on surgical findings, has been used to classify the severity of diverticulitis into 4 grades: stage 1, pericolic abscess confined by the mesocolon; stage 2, pelvic abscess distant from the area of inflammation; stage 3, generalised peritonitis resulting from abscess rupture; stage 4, faecal peritonitis resulting from the perforation of colonic diverticulum (Hinchey et al., 1978). With the increased availability of CT scanning this has now become the investigation of choice in cases of suspected diverticulitis (Ambrosetti et al., 2000). In 2016 the World Society of Emergency Surgery Consensus Conference created guidelines for the management of acute diverticulitis (Sartelli et al., 2016). They suggest that cases of mild inflammation, not complicated by systemic upset or localised complication (e.g. abscess or perforation), can managed symptomatically without antibiotics. If systemic upset is present then antibiotics should be give. In cases of diverticulitis complicated by a localised abscess, IV antibiotics may be sufficient but if the abscess is > 4-5cm then percutaneous drainage should be performed. For patients with perforated diverticulitis and generalised peritonitis they recommend surgery. Depending on the clinical condition of the patient and the expertise of the operating surgeon, either resection of the affected segment with primary anastomosis or a Hartmann's procedure (resection of the affected segment with the proximal end of bowel being brought to the surface and over-

sewing of the rectal stump) can be performed. They do not recommend laparoscopic lavage and drainage in patients with generalised peritonitis. Where a Hartmann's procedure is performed only 30% of these colostomies are reversed (MG, 2007). The reversal procedure itself is often challenging and post operative complications are common with one study finding that patients undergoing a reversal of Hartmann's procedure were 2.1 times more likely to experience a complication than those undergoing an elective resection and primary anastomosis (Aydin et al., 2005).

1.2.3 Creation of an anastomosis

As described below anastomoses can be formed in a variety of ways, however, some general principles apply to all methods: the cut ends of bowel should be handled carefully, the size discrepancy between the ends should be minimised to allow good apposition and excess tension at the anastomosis must be avoided.

Traditionally anastomoses were hand-sewn. This can be either a single or double layer technique. Although more time consuming to perform, the double layer technique was thought to offer a more secure anastomosis, however randomized controlled trials and meta-analyses have failed to demonstrate a significant difference in outcome between single and double layer techniques (Burch et al., 2000, Shikata et al., 2006). A recent multi-centre randomised control trial of single vs. double layer anastomotic techniques for colonic anastomoses closed early due to poor recruitment. However, of the 252 patients recruited (129 single layer anastomosis; 123 double layer anastomosis) there was no significant difference in the anastomotic leak (AL) rate but the single layer technique was significantly quicker to perform (Herrle et al., 2016). A systematic review also failed to show superiority of one technique over the other but also commented that the single layer technique was quicker and easier to perform (Slieker et al., 2013). With the advent of reliable stapling devices in the 1970s and 1980s stapled anastomoses have grown in popularity. They are easy to use and less time-consuming than hand-sewn anastomoses. A plethora of studies have compared hand-sewn versus stapled anastomoses and these have been the subject of recent systematic Cochrane reviews. In the first Nuetzling et al

(Neutzling et al., 2012) looked at 1233 patients across 9 studies. Stapled colorectal anastomoses were performed in 622 patients and 611 had hand-sewn anastomoses, no significant difference in outcome was found between the two groups. However, in the case of right-sided ileo-colic anastomoses a Cochrane review found there were significantly fewer anastomotic leaks in the stapled group (Choy et al., 2011). However, contrasting results were found in an international cohort study published in 2017 by the European Society of Coloproctology (Battersby et al., 2017). They looked at stapled versus hand sewn anastomoses for right hemicolectomy or ileocaecal resection in both the elective and emergency setting in 3208 patients. A higher rate of AL was identified in the group who underwent a stapled anastomosis.

1.3 Complications of colorectal surgery

Potential complications of colorectal surgery can include: anastomotic stricture (narrowing of the join), bleeding, prolonged ileus, wound infection and anastomotic leak (AL).

1.3.1 Anastomotic stricture

A stricture is a delayed complication that develops over weeks to months after the creation of anastomosis. The incidence is approximately 2-5% (Del Gaudio et al., 1993, Schaefer et al., 1993, Laxamana et al., 1995, Fingerhut et al., 1995). The exact mechanism of stricture formation is unclear but post-operative AL, pelvic sepsis, radiotherapy and ischaemia at the anastomosis have all been implicated (Orsay et al., 1995, Senagore et al., 1990, Påhlman et al., 1989, Chung et al., 1988). It is more common in low, stapled joins e.g. in the rectum. It usually presents with difficulty in defecation, partial or sometimes complete intestinal obstruction. In the past it was treated surgically but now endoscopic dilatation is firmly established as the first line treatment of choice (Belverde et al., 2012).

1.3.2 Bleeding

Significant bleeding from a newly created colorectal anastomosis is uncommon but can cause significant morbidity and mortality. In a series of 2181 patients undergoing anterior resection for rectal cancer with a stapled anastomosis 6 patients (0.3%) had bleeding requiring intervention (Lou et al., 2014). All required blood transfusion but the bleeding was controlled with colonoscopic techniques such as clipping and electrocautery. However, occasionally uncontrolled bleeding may necessitate a return to theatre.

1.3.3 Prolonged ileus

Post-operative ileus is a transient impairment of bowel motility and can occur after any type of intra-abdominal surgery. It presents with nausea, vomiting, stomach cramps, lack of passage of flatus and stool, bowel distension and reduced/absent bowel sounds. The aetiology is multifactorial involving inhibitory neural reflexes and inflammatory mediators from the site of surgery. The duration of ileus is affected by the segment of the gastrointestinal tract that has been operated on. The longest duration of ileus is encountered after operations on the colon compared to operations on the stomach or small intestine (Holte K, 2000). Laparoscopic surgery, use of local anaesthetic and thoracic epidurals, opioid-sparing analgesia and the use of chewing gum and caffeine (Chan MKY, 2007) in the post-operative period have previously been shown to reduce the duration of ileus. However, a recent systematic review concluded that, although interventions such as chewing gum and caffeine were safe many of the trials had a high risk of bias. They concluded that, whilst the aetiology of postoperative ileus remains unclear, minimally invasive surgery, protocol-driven recovery programmes and attempts to avoid major inflammatory events represent the best chance of reducing the incidence of ileus (Chapman et al., 2018).

1.3.4 Wound infection

Wound infection, or surgical site infection (SSI), can occur after any surgical procedure. They are common, accounting for up to 14 – 16% of all hospital acquired infections and increase the length of stay of the patient (Smith et al., 2004). Superficial wound infections may be managed with removal of sutures or clips from the wound to allow pus to drain. Deeper infections often require systemic antibiotics and/or negative pressure dressings and occasionally results in full thickness wound dehiscence requiring a return to theatre with a potential prolonged recovery.

1.4 Anastomotic leak

1.4.1 The clinical definition of an anastomotic leak

Anastomotic leak (AL) is one of the most challenging complications following colorectal surgery. The preferred definition of AL, as outlined by the UK Surgical Infection Study Group is, "…a leak of luminal contents from a surgical join between two hollow viscera" (Peel and Taylor, 1991). ALs are typically diagnosed at around day 6 - 8 post surgery (Kanellos et al., 2004, Alves et al., 1999). The reported incidence of AL is variable ranging from 1-30% (Kingham and Pachter, 2009). Some of this variation is due to the impact of anatomical location of the anastomosis - low extraperitoneal joins have a much higher leak rate than right-sided colonic anastomoses (Platell et al., 2007).

1.4.2 Patient and clinical consequences of an anastomotic leak

When a leak occurs, bowel contents are able to leak into the normally sterile abdominal cavity, this can lead to significant morbidity and mortality. The economic burden of AL is estimated to be 1.1 – 3.5 million pounds per year in England (Ashraf et al., 2013) and $24,129 per person in the USA (Hammond et al., 2014). Mortality can be as high as 33% following a leak due to overwhelming sepsis and multiorgan failure in addition to requiring an ITU stay (Moher et al., 2009). Many patients will require emergency surgery and formation of a stoma. It may be possible to subsequently reverse the stoma but in many cases it

becomes permanent (Erb et al., 2014) . Even when it is possible to reverse the stoma, it has been shown that patients who suffered an AL have impaired long-term anorectal function (e.g. frequency, urgency and faecal incontinence) compared to those who did not suffer a leak (Nesbakken et al., 2001). AL has also be shown to lead to poorer long term cancer-specific survival (Mirnezami et al., 2011). Early detection of a leak is crucial in attempting to reduce the resultant morbidity and mortality.

1.4.3 Clinical and surgical management of an anastomotic leak

Management of a leak depends on the severity of the leak and the clinical condition of the patient. The Association of Coloproctology of Great Britain and Ireland (ACGBI) has recently issued guidance regarding the management of AL ((ACGBI), 2016) (table 1.8). All those with a leak should receive oxygen, intravenous fluid, antibiotics and be managed in an environment (e.g. ward, high dependency, intensive care) appropriate to their overall condition. A small contained leak in a stable patient without radiological evidence of anastomotic discontinuity may be amenable to treatment with intravenous antibiotics and/or radiological drainage or may necessitate a return to theatre for washout and/or defunctioning. Defunctioning can involve bringing out a proximal end or loop of bowel whilst leaving the original anastomosis intact or suture repairing the anastomotic defect to allow it to heal. This temporary stoma may be able to be reversed at a later date but sometimes this is not possible and it becomes a permanent stoma with a potential impact on quality of life for that patient. If there is radiological evidence of multiple collections or anastomotic discontinuity then radiological drainage will not be likely to succeed. Those who do not respond to this management or have severe sepsis or septic shock should return to theatre for washout, resection of the anastomosis and creation of an end stoma.

TABLE 1.8 ACGBI RECOMMENDATION FOR MANAGEMENT OF AL ((ACGBI), 2016)

Grade	Description	Management	Source Control
1	No sepsis	Conservative	None
2	Sepsis/contained leak/abscess	Drainage needed	Radiological drainage. If unavailable, laparoscopy/laparotomy washout, drain and repair AL with proximal defunctioning stoma or resect anastomosis and create stoma
3	Sepsis, ileus/ Single quadrant peritonitis	Resuscitate and operate	Laparoscopy/laparotomy washout, drain and proximal defunctioning stoma or resect anastomosis and create stoma
4	Severe sepsis/ more than single quadrant peritonitis	Resuscitate and operate	Laparoscopy/laparotomy washout, resect anastomosis and create stoma
5	Septic shock/ generalized peritonitis	Resuscitate and operate	Laparoscopy/laparotomy washout, resect anastomosis and create stoma

The recommended time limits for intervention relate to the severity of sepsis and recommendations have been made by the Royal College of Surgeons of England:

- Those without evidence of organ dysfunction should have a surgical or radiological intervention to achieve source control as soon as possible but at least within 18 hours;
- If organ dysfunction is present intervention should take place within 6 hours;

- In cases of septic shock intervention should be immediate, or at least within 3 hours (England, 2011).

1.4.4 Difficulties in the standardized reporting of anastomotic leaks and implications for research

Research and reporting of anastomotic leaks is hampered by the lack of a single universally accepted definition of what constitutes an anastomotic leak. Although in 1991 the UK Surgical Infection Study Group proposed the following definition, "…a leak of luminal contents from a surgical join between two hollow viscera" (Peel and Taylor, 1991), a 2001 review of 97 studies looking at the definition and measurement of anastomotic leakage after gastrointestinal surgery found 56 different definitions (Bruce et al., 2001). This goes someway to explaining the wide variation seen in the reporting of leak rates. It also makes comparison of existing research into AL difficult.

In 2010 the International Study Group on Rectal Cancer defined AL as, "a communication between the intra- and extraluminal compartments due to a defect of the integrity of the intestinal wall at the anastomosis between the colon and the rectum or the colon and the anus". They went onto to classify 3 grades of anastomotic leakage (A-C). Type A (radiological leakage) is usually asymptomatic and requires no active intervention. Type B includes leaks treated by percutaneous or transanal drainage and antibiotics. Type C is a leak requiring laparotomy (which may involve washout and/or stoma formation) (Rahbari et al., 2010). Use of this grading system would allow future research to be interpreted and compared with greater clarity.

1.5 Methods of predicting anastomotic leakage and their limitations

There has been considerable interest in trying to predict which patients may develop an AL to minimise the complications listed already. A range of pre-operative and intra-operative risk factors have been identified which confer an increased risk of anastomotic leak.

1.5.1 Pre-operative patient and pathological risk factors

These can be divided into modifiable and non-modifiable risk factors.

a. Non-modifiable risk factors

Gender

Males have been consistently found to have an increased risk of AL (Boccola et al., 2011, Lipska et al., 2006). In a study of 956 patients undergoing a TME with primary anastomosis for rectal cancer, 10.6% of male patients suffered a leak versus only 5.1% in females, the difference was statistically significant (Zhou et al., 2018). The reason for the difference between males and females is likely to be multifactorial and, as yet, not fully elucidated. The fact that the male pelvis is narrower and more technically challenging to operate in may account for some of the difference (Law et al., 2000). The effect of hormones on the intestinal microcirculation is also of interest. Ba et al found that androgens play an inhibitory role in small intestinal epithelial function (Ba et al., 2004), potentially impairing healing of the anastomosis. Recent work in animal models of colonic anastomoses has also demonstrated a significantly lower level of soluble collagen levels on post-operative day 3 in male rats compared to females (Kjaer et al., 2018). More work is needed to establish if these findings also apply to humans and, if so, to develop strategies to mitigate the effects. Overall, Zhou et al recommend that consideration should be given to creation of a temporary covering stoma in any male patient having a low anastomosis, especially if there is any concern about the anastomosis at the time of surgery (Zhou et al., 2018).

Age

The impact of increasing age on rates of AL is not entirely clear. A large study of 1391 patients undergoing anterior resections found that increasing age was associated with a higher rate of AL (Jung et al., 2008). Damhuis et al also found that mortality and operative morbidity increased with age in patients undergoing surgery for colorectal cancer (Damhuis et al., 1996). In contrast, Matthiessen et al looked at 432 anterior resections and found that advanced age was not an independent risk factor for AL (Matthiessen et al., 2004). Arenal et al also found that age > 70 was not associated with increased risk (Arenal et al., 1999). Despite the lack of clear evidence as to whether age is an independent risk factor for AL the literature is clear that good operative outcomes can be achieved in older patients with colorectal cancer and surgery/primary anastomosis should not be denied on the basis of age alone (Edna and Bjerkeset, 1997, Barrier et al., 2003, Houry et al., 1994).

Fitness/co-morbidities

The American Society of Anaesthesiologists (ASA) grading system is used to classify patients' general fitness prior to surgery (see table 1.9). An ASA score of 3 or more is associated with increased risk of AL (Buchs et al., 2008, Mäkelä et al., 2003, Choi et al., 2006). In addition, co-morbidities such as vascular, pulmonary and renal disease increase the risk of AL (McDermott et al., 2015).

TABLE 1.9 ASA GRADING SYSTEM

ASA grade	Description
I	Normal healthy person
II	Mild systemic disease
III	Severe systemic disease
IV	Severe systemic disease that is a constant threat to life
V	A moribund person not expected to survive without surgery
VI	A declared brain-dead person whose organs are being removed for donor purposes

Emergency surgery

Creation of an anastomosis during an emergency operation carries a higher risk of AL compared to during an elective procedure (Boccola et al., 2011, Platell et al., 2007). However, emergency surgery is not an absolute contraindication to creation of an anastomosis. It has been shown in studies, including a randomised controlled trial (RCT), that in the case of perforated diverticular disease primary anastomosis with covering ileostomy is a safe and successful option (Constantinides et al., 2007, Oberkofler et al., 2012). Emergency surgery is often challenging with patients being more "unwell" than those operated on electively, for example those with a perforation are often septic requiring inotropic circulatory support and frequently experience greater blood loss. It is the accumulation of these risk factors, rather than just emergency surgery itself, that is likely to increase the risk of AL after emergency surgery. It is therefore the case that emergency surgery is a relative, rather than absolute, risk for AL.

Features of the tumour

Increasing size of the tumour and TNM stage of the cancer are risk factors for AL (Boccola et al., 2011, Eberl et al., 2008, Warschkow et al., 2011). In addition, the presence of metastatic disease at the time of surgery increases risk (Richards et al., 2012). This may be a consequence of poor nutrition with advanced disease but there is also evidence that those with advanced or metastatic disease have a cancer-associated systemic inflammatory response

which has been shown to result in an increased risk of post-operative infective complications (Richards et al., 2012, Moyes et al., 2009).

b. Modifiable risk factors

Smoking

Current (Bertelsen et al., 2010, Bisgård et al., 2013, Richards et al., 2012) and previous heavy smoking (Kruschewski et al., 2007) (greater than 40 pack year history) are independent risk factors for AL. The reasons for the association are likely to be complex but it is postulated that smoking may alter/impair blood flow to the mucosa and therefore have a detrimental effect on the healing of the anastomosis (Richards et al., 2012).

Alcohol

In a study of 333 patients undergoing colonic or rectal resections with anastomosis (> 15.3% leak rate), those who consumed excess alcohol (>21 units a week) compared with those who abstained had a relative risk of an AL of 7.18 (Sørensen et al., 1999).

Obesity

Raised body mass index (BMI) (Benoist et al., 2000, Senagore et al., 2003), and in particular increased waist circumference, is an independent risk factor for AL (Kartheuser et al., 2013).

Neoadjuvant therapy

It was previously thought that neoadjuvant radiotherapy +/- chemotherapy increased the risk of AL (Warschkow et al., 2011, Park et al., 2013) but recent RCTs have not supported this (Sebag-Montefiore et al., 2009, Marijnen et al., 2002, Chang et al., 2014). However, a history of previous pelvic radiotherapy, e.g. for prostate or cervical cancer, is a risk factor for AL (Smith et al., 1985).

Nutrition

Malnutrition, preoperative weight loss and electrolyte disturbances have been shown to increase risk of AL in patients undergoing right hemicolectomies (Veyrie et al., 2007) and rectal resections (Kang et al., 2013).

Non-steroidal anti-inflammatory drugs (NSAIDs)

As part of a multi-modal analgesia approach in enhanced recovery programmes after colorectal surgery the role of NSAIDs has been controversial. A large study by Gorissen et al in 2012 found that use non-selective NSAIDs and non-selective cyclo-oxygenase 2 (COX-2) inhibitors was associated with a higher rate of AL (Gorissen et al., 2012). However, in a meta-analysis of 6 studies published the following year the AL rate was not significantly higher in those who received NSAIDs in the post-operative period (Burton et al., 2013). Since then, 3 further meta-analyses have been published (Smith et al., 2016, Huang et al., 2018, Modasi et al., 2019). All have shown that the use of NSAIDs is associated with an increased risk of AL. However, when sub-divided into non-selective NSAIDs (e.g. diclofeac) and selective COX-2 inhibitors (e.g. celecoxib) only the non-selective NSAIDs are consistently associated with increased risk of AL. Based on the current available evidence, the use of a non-selective NSAID is not to be recommended in the post-operative period, COX-2 inhibitors may be safe but larger RCTs are required to clarify their safety following colorectal surgery.

Immunosuppressant and immune-modulator drugs

Patients on a prolonged course of corticosteroids have an increased risk of AL (Slieker et al., 2012). The monoclonal antibody infliximab has been show to delay wound healing but not to increase the risk of AL (Canedo et al., 2011, Krane et al., 2013). Mycophenolate (Zeeh et al., 2001), tacrolimus (Schäffer et al., 1998) and cyclosporin (Petri et al., 1998) have also been found to increase the risk of AL in experimental studies. One of the new chemotherapy agents, bevacizumab (a vascular endothelial growth inhibitor), works by reducing neovascularization and healing therefore increasing the risk of AL. Consequently the manufacturer recommends stopping it 28 days prior to surgery and waiting at least 28 days before restarting it (Inc, 2015).

Mechanical bowel preparation (MBP)

MBP was traditionally used to prepare the bowel for surgery as it was thought that clearing the bowel would reduce infection and AL rates. MBP also aids insertion of stapling devices and provides better views during intra-operative endoscopy. However, MBP is unpleasant with up to 40% of patients taking it

reporting discomfort (Bretagnol et al., 2010). The GRECCAR III RCT found that whilst in rectal resections MBP reduced the rate of infectious complications it did not lower the risk of AL (Bretagnol et al., 2010). Meta-analyses and systematic reviews looking at MBP for all types of colorectal resections have found that its use does not reduce the risk of AL (Slim et al., 2009, Guenaga et al., 2009, Cao et al., 2012). Consequently, the ACGBI advises that MBP is not essential for colorectal surgery ((ACGBI), 2016).

Antibiotics

Perioperative (within 30-60 minutes before surgery) parenteral antibiotics are given as standard during elective colorectal surgery (Nelson et al., 2009). Recently studies have explored the use of oral antibiotics pre-operatively for selective decontamination of the digestive tract (SDD). Different protocols exist ranging from single to multiple antibiotics and use of antibiotics for 1 or 2 days pre-operatively to continuation of treatment post-operatively. A meta-analysis of 8 studies found that SDD combined with parenteral antibiotics reduced rates of post-operative infection and AL (Roos et al., 2013). More recently, a large trial has shown that the combination of mechanical bowel preparation with pre-operative antibiotics reduced the rate of post-operative wound infection and AL (Scarborough et al., 2015). This finding may mean that over the next few years more surgeons return to using MBP but also combine it with antibiotics.

1.5.2 Intra-operative risk factors

The fundamental basis of creating a healthy anastomosis that heals without complication is good surgical technique with sound apposition of two healthy ends of bowel. The ends must not be under excess tension and must have a good blood supply. However, there are a myriad other intra-operative variables that have been shown to influence the rate of AL.

Duration of surgery

Prolonged operating times have been found to be an independent risk factor for AL. However, it may be the case that prolonged operating time of over 3-4 hours is a surrogate for complex or challenging surgery (Mäkelä et al., 2003, McDermott et al., 2015). More extensive dissection may have been required, or time taken to control bleeding which may have necessitated blood transfusion or brief use of vasopressor or inotropic agents.

Fluid therapy

Several studies have indicated that a policy of restrictive fluid replacement reduced general complications after colorectal surgery (rate of AL was not specifically addressed) (Brandstrup et al., 2003, Nisanevich et al., 2005). However, a systematic review and meta-analysis looking at the effect of perioperative fluid restriction on complications after colorectal surgery found no significant difference in rates of post-operative complications (Boland et al., 2013). There has been growing interest in the use of oesophageal Doppler monitoring of cardiac output to guide individual goal-directed fluid therapy. Two meta-analyses found that the use of oesophageal Doppler monitoring reduced complications after major abdominal surgery (Abbas and Hill, 2008, Walsh et al., 2008). Although the role of oesophageal monitoring has not been studied in direct relation to AL, the use of oesophageal Doppler monitoring to guide intra-operative fluid therapy is now recommend by the NICE guidelines in major abdominal surgery (Ghosh et al., 2011).

Inotropes

The use of inotropes increases the risk of AL. Zakrison et al (Zakrison et al., 2007), found that their use increased the risk 3-fold and the risk was greater if multiple agents were used. This increased risk was independent of the general fitness and operative morbidity of the patient as judged by the Acute Physiology and Chronic Health Evaluation (APACHE) and Physiological and Operative Severity Score (POSSUM) scores respectively. The mechanism is thought to be

vasoconstriction leading to impaired splanchnic perfusion thus a reduced blood supply to the anastomosis.

Blood loss and blood transfusion

Increased perioperative blood loss (Bertelsen et al., 2010), blood loss of > 100ml (Leichtle et al., 2012) and the requirement for multiple transfusions (Mäkelä et al., 2003) are associated with an increased risk of AL.

Anastomosis technique

Anastomoses are either hand sewn or created using a stapling device. For colorectal anastomoses a Cochrane review found no difference in AL rates with either method (Neutzling et al., 2012). In the case of right-sided surgery with ileocolic anastomoses the Cochrane review favoured stapled anastomoses (Choy et al., 2011).

Level of anastomosis

Lower, left sided anastomoses, particularly of the rectum, have a higher risk of AL compared to right-sided anastomoses (Lipska et al., 2006, Alves et al., 2002). In one series the leak rates were 2.2% for right hemicolectomy and 7.4% for anterior resection of the rectum (Lipska et al., 2006). Furthermore, the lower in the rectum the anastomosis is the higher the leak rate. Those < 5cm from the anal verge are particularly at risk (Rullier et al., 1998).

Diverting stomas

As low colorectal anastomoses are known to be at risk of AL many surgeons choose to create a diverting stoma, either end ileostomy, loop ileostomy or loop colostomy in an attempt to protect the anastomosis and reduce the impact of peritoneal soiling following a leak. Four RCTs (Graffner et al., 1983, Pakkastie et al., 1997, Pimentel et al., 2003, Matthiessen et al., 2007a), including one with over 200 patients, have demonstrated reduced leaks after formation of a diverting stoma. When their results were pooled in a meta-analysis (Hüser et al.,

2008), creation of a diverting stoma was supported. A Cochrane review in 2010 (Montedori et al., 2010) added further support to this and added that although not all studies have found a significantly reduced rate of AL with diverting stoma, patients with one who have a leak have less morbidity and need for repeat surgical intervention. It appears that an ileostomy rather than colostomy is superior, in part due to reduced infective complications (Chen et al., 2013, Rondelli et al., 2009).

Placement of drains

Commonly drains have been left in situ following colorectal anastomotic surgery with the aim of reducing the risk of complications and as an aid to identifying complications such as AL. Two systemic review and meta-analyses looked at the role of drainage following colorectal surgery. Patients had a variety of operations from right hemicolectomy to anterior resection. They found that insertion of a drain did not significantly decrease the risk of AL (Karliczek et al., 2006, Petrowsky et al., 2004). Consequently, many surgeons now chose not to leave a drain. This is particularly the case in the context of enhanced recovery programmes which advocate the omission of drains and the early removal of IV lines and urinary catheters in order to promote mobility and an early return to "normal" function (Varadhan et al., 2010). However, in a recent meta-analysis Rondelli and colleagues (Rondelli et al., 2014) looked at the role of drains in extraperitoneal anastomoses e.g. low anterior resection where the risk of AL is known to be greater than in other intraperitoneal anastomoses. There were 8 studies including 3 RCTs. They found that patients who had a drain where significantly less likely to develop an anastomotic leak and less likely to require reintervention. Overall it would seem that drains do not have an adverse effect on outcome and there is evidence to support their routine usage in low colorectal anastomoses. Despite this, placement of a drain following a low pelvic anastomosis is still not routine practice in all surgical units and is a the discretion of the operating surgeon.

Air-leak testing

Performing an air-leak test can highlight deficiencies in the anastomosis at the time of surgery and allows the surgeon to take steps to correct this either by reinforcing the anastomosis, redoing it or considering creation of a stoma. Briefly, one surgeon manually occludes the bowel proximal to the newly created anastomosis, saline is poured into the abdominal cavity around the anastomosis and an assistant injects air via syringe into the colon via the anus. Bubbles of air seen in the fluid indicate a breach in the anastomosis as air is able to move from the colon into the saline. This test is relatively simple and does not add significantly to the length of the operation. Data from a RCT (Beard et al., 1990) and other studies (Ricciardi et al., 2009, Ivanov et al., 2011) have consistently found that performing an intra-operative air-leak test reduced the risk of clinically apparent anastomotic leaks, in the RCT AL was reduced from 14% to 4%.

Intra-operative endoscopy

Several studies have shown that the use of intra-operative endoscopy (Li et al., 2009, Shamiyeh et al., 2012, Carlo and Valerio Corazza, 2012) to assess the anastomosis may be useful in reducing the risk of anastomotic complications. However, there have yet to be any RCTs in this area and many surgeons remain wary of inserting a sigmoidoscope or colonscope through newly anastomosed bowel for fear of physically damaging the new join.

Laparoscopic versus open surgery

A multi-centre randomized controlled trial (the CLASICC trial) found no significant difference in the rate of AL in open versus laparoscopic surgery for colorectal cancer. Leak rates were 3% and 4% respectively for open and laparoscopic colonic resections and in rectal resections the rates were 7% and 8% respectively (Guillou et al., 2005). The COLOR trial also found no difference in AL in open versus laparoscopic colonic resections and found that laparoscopic resections required less analgesics and were discharged home earlier than those having open resections (Group, 2005).

Interest is now growing in the use of robotic surgery for colorectal cancer resections, in particular for rectal resections. The ROLARR trial found no difference in the rate of conversion to open surgery between robotic and laparoscopic colorectal surgery (Jayne et al., 2017). However, many of the surgeons in the trial were still on the "learning-curve" for robotic surgery and this may have impacted the results (Corrigan et al., 2018). A more recent systematic review and meta-analysis of 5 RCTs in robotic vs. minimally invasive surgery for rectal cancer concluded that robotic surgery resulted in fewer conversions to open surgery (Prete et al., 2018). The experience and availability of robotic surgery is likely to increase in the years to come and it will be possible to determine if it can impact on the incidence of AL.

1.6 Current methods of detecting of an anastomotic leak following surgery and their limitations

Currently detection of an anastomotic leak in the post-operative period requires a high index of suspicion taking into account known pre- and intra-operative risk factors and is aided by clinical assessment, measurement of objective clinical signs and blood tests. When a leak is strongly suspected radiological studies, most often CT scans, are performed in an attempt to confirm the diagnosis.

1.6.1 Clinical assessment and objective clinical signs

Heart rate, blood pressure, respiratory rate, oxygen saturation level, temperature and white cell count (WCC) are objective parameters measured routinely in all post-operative patients. Any deviation of these parameters from normal values can indicate the development of complications such as sepsis. Given the heterogeneity of presentation of AL any clinical deterioration in a patient may serve as an early indication of the development of AL. Erb et al (Erb et al., 2014) carried out a study looking at 452 patients undergoing colorectal surgery with formation of an anastomosis in a single centre over a 2 year period.

A total of 271 complications occurred in 141 patients, including 19 anastomotic leaks (4.2%). Over half the patients with an uncomplicated recovery (311 patients) experienced tachycardia and tachypnoea at least once a day during their post-operative stay. Hypotension, pyrexia and a leucocytosis were also common. The positive predictive value (PPV) for any abnormal sign or WCC ranged from only 4-11%. Therefore, the development of abnormal vital signs following colorectal surgery with formation of an anastomosis can almost be considered routine and therefore alone they are poor predictors of AL.

1.6.2 Blood tests

C-reactive protein (CRP)

CRP is an acute phase reactant produced in the liver in response to pro-inflammatory cytokines such as interluekin-6. It actives the complement pathway and acts as a stimulus to phagocytosis of foreign and damaged cells (Pepys and Hirschfield, 2003, Mold et al., 1999). It has a half-life of 19 hours and is known to be a good marker of acute inflammation and is used post-operatively as a marker to help predict/identify infective complications (Bianchi et al., 2004, Warschkow et al., 2012). It has been extensively investigated in the context of AL and there have been 2 recent meta-analyses. In the first a cut-off CRP of 135 on post-operative day 4 had a negative predictive value of 89% for infective complications (Warschkow et al., 2012). In the second meta-analysis of 2483 patients across 7 studies (including 3 RCTs) cut-off CRP levels of 172, 124 and 144 on post-operative days 3, 4, and 5 respectively had a negative predictive value of 97% for anastomotic leak. However, PPV was 21-23% (Singh et al., 2014). It therefore seems that CRP has limited power to predict AL but may have a role as a “rule out test” providing reassurance to the surgeon that AL is unlikely.

Procalcitonin

Procalcitonin (PCT) is produced by C cells in the thyroid gland and is involved in activation of neutrophils (Garcia-Granero et al., 2013). PCT is elevated in bacterial, fungal and parasitical infection but not in viral infection or non-specific inflammation (Meisner et al., 2001). It has also been validated as an early marker of sepsis (Assicot et al., 1993). This makes it a particularly attractive marker of AL as it is more specific for bacterial infection and sepsis than CRP. In a study of 205 patients Garcia-Granero et al (Garcia-Granero et al., 2013) had 17 patients who had an AL, of these 11 were classified as a "major leak" meaning they required either radiological or surgical intervention. Serum PCT was not predictive of AL in general but an elevated PCT at post-operative days 3-5 was predictive of "major leak". Performance on POD 5 was best with reported sensitivity of 100%, 72% specificity, 100% negative predictive value (NPV) but only 17% PPV.

Komen et al (Komen et al., 2014a) looked at levels of CRP, PCT and lipopolysaccharide binding protein (LPS) in drain fluid of 243 patients. Whilst PCT was significantly higher in patients who developed a leak it was not found to be an independent predictor during subsequent multivariate analysis.

In another smaller study of 84 patients (Reisinger et al., 2014) with 8 anastomotic leaks on median day 6 (range 3-10) PCT appeared to be an earlier predictor of AL than CRP. Mean PCT was higher in those with AL on post-operative days 2-4 whereas mean CRP only reached statistical significance on POD 4. They found that using a formula to create a combined score using PCT and CRP yielded the best results with sensitivity and specificity on POD 3 of 100% and 89% respectively. However, this was a relatively small study and values for PPV and NPV are not quoted. Clearly PCT may have some utility as a marker of AL but there are only a few studies with small numbers and heterogeneity of definitions of AL and there has yet to be a meta-analysis performed.

1.6.3 Imaging

In cases where there is diagnostic doubt regarding the integrity of an anastomosis most clinicians will undertake imaging in an attempt to confirm or refute the presence of a leak. Currently the most commonly used modality is CT. Where oral or rectal contrast has been used extravasation of contrast out with the bowel indicates a leak. Collections of air and fluid around the anastomosis also indicate a high probability of a leak especially if the surgery was performed more than 5 days previously. Reports on the sensitivity and specificity are variable but there is a consensus of opinion that CT alone is not a reliable test for AL (Hirst et al., 2014, McDermott et al., 2015). A 2013 meta-analysis of 8 studies involving 2715 patients where 221 CT scans were performed found that the sensitivity of CT was only 68% (Kornmann et al., 2013) . The addition of oral or rectal contrast may be beneficial. Three of the studies just looked at rectal resections and all used rectal contrast, the sensitivity of CT across these studies was 92%. (Nesbakken et al., 2005, Eckmann et al., 2004, Kanellos et al., 2004). In the first few postoperative days interpretation of CT is particularly challenging as patients with and without AL may have similar features such as air-fluid collections (DuBrow et al., 1995, Matthiessen et al., 2008).

One study has explored the possibility of using Positron emission tomography (PET) scanning to detect a leak. Teeuwen et al showed that in normal uncomplicated recovery following colorectal surgery there is low uptake of F-18 fluorodeoxyglucose (Teeuwen et al., 2012). Therefore, PET scanning may be of use in detecting deviations from the normal post-operative course possibly indicative of a leak but more work needs to be done to determine its usefulness in the detection of AL.

1.7 New/novel technologies to prevent or detect anastomotic leaks

1.7.1 Intra-operative assessment of perfusion at the anastomosis

Several studies have looked at assessment of the microperfusion of the anastomosis via measurement of blood flow or tissue perfusion. A recent systematic review by Nachiappan et al has assessed the current advances in this area (Nachiappan et al., 2014). Doppler assessment of blood flow has found an association between reduced blood flow and increased risk of AL (Ambrosetti et al., 1994, Hallböök et al., 1996, Vignali et al., 2000, Seike et al., 2007, Boyle et al., 2000). Two studies have measured tissue oxygen tension at the perianastomotic region. Measurements were made before and after creation of the anastomosis. In a study of 50 patients, Sheridan et al found a significant association between reduced oxygen tension and risk of anastomotic leak (Sheridan et al., 1987). Hall et al found an association between reduced oxygen tension and AL but it was not statistically significant (Hall et al., 1995). These studies illustrated that there may be some potential benefit in the technique but it is not readily available which may explain why no studies have looked at this again since 1995.

Visible light oxygen spectroscopy has also shown good potential. Karliczek et al used a hand held probe to measure tissue oxygen saturation ($St0_2$) at the proposed site of anastomosis and again at the same site following anastomosis. No intraoperative actions were taken based on the results. Out of 77 patients there were 14 leaks. They found that reduced $St0_2$ at the perianastomotic site was associated with AL, specifically a rise in $St0_2$ was seen in the group of patient who did not have a leak (Karliczek et al., 2010). Hirano et al also looked at perianastomotic $St0_2$ using near infrared oxygen spectroscopy. They found that the 18/20 patients with no post-operative complications had a > 66% $St0_2$ rise whereas the 2 patients with complications had a reduction in $St0_2$ (Hirano et al., 2006).

Kudszus et al used indocyanine green with laser fluorescence angiography (LF ICG) to assess the perianastomotic site. In their study with 402 patients, 201 had assessment with LF ICG and the other 201 did not. In the LF ICG group, 28

patients had their operative management altered on the basis of the measurement. This essentially halved the number of anastomotic leaks with 7/201 in the LF ICG group suffering a leak compared to 15/201 in the control group (Kudszus et al., 2010). Several other studies have proved that measurement of tissue perfusion with indocyanine green is feasible and has the potential to be useful in anastomotic surgery (Jafari et al., 2013, Sherwinter, 2012, Sherwinter et al., 2013). The usefulness of this promising technology will be assessed in a multinational RCT ("The InAct Study", the protocol for which was published in August 2018. Over 3 years 880 patients undergoing either high or low anterior resection for adenocarcinoma will be recruited. The primary outcome measure will be clinical AL at 90 days (Armstrong et al., 2018).

1.7.2 Devices

Over the years, attempts have been made to create mechanical devices to prevent, or reduce the consequences of AL. In a recent review, these devices were classified as transanal decompressive devices, intracolonic devices and biodegradable devices (Morks et al., 2011).

Transanal decompressive devices keep the anal sphincter open and thus reduce intraluminal pressure and consequently reduce the pressure on the anastomosis. The results have been mixed. In a 2001 study of 50 patients undergoing low anterior resection, Sterk et al found that the rate of AL was not significantly different in those who received a transanal stent compared to those who received a loop colostomy (Sterk et al., 2001). In contrast, a large trial of transanal stents was stopped early due to increased complication rates in the stent group (Bülow et al., 2006).

Several intraluminal devices have been trialled in animals with promising results (Morks et al., 2011). Intraluminal devices do not prevent the formation of an AL but they prevent faeces contacting the anastomotic site and therefore prevent leakage of faeces into the peritoneal cavity. In the 1980s a device called the "Coloshield", which is a latex, rubber or silicon tube sutured to the submucosa proximal to the anastomosis which is naturally expelled several days after surgery, was developed for use in humans (Ravo, 1997). There were some

promising results but also some complications related to the device and it therefore did not achieve widespread acceptance. Another group used a latex condom which was sutured in place and naturally expelled around 10 days after surgery. In a group of 10 patients having a low anterior resection the condom was shown to be safe, practical and none of the patients went on to have an AL (Yoon et al., 1994). However, details about the patients in the study were lacking. As a result, intraluminal devices have not been adopted into common practice.

Recently attention has focused on biodegradable devices. A Dutch group has developed a device called a "C-seal". It is a thin-walled tube like a soft sheet or a condom with tapes at either end. The tapes are glued to the anvil of the circular stapler. This allows it to be fixed just proximal to the anastomosis. It degrades after about 10 days. In a pilot study of 15 patients undergoing stapled low anterior resections the device was safe and there were no ALs (Kolkert et al., 2011). However, in an RCT of the C-seal involving 402 patients undergoing colorectal anastomosis ≤15cm from the anal verge, those with the C-seal were found to have a higher rate of AL. Technical failures and complications of inserting the device were observed. Some of this may have been attributable to a lack of experience of surgeons inserting the device but overall the results mean that the C-seal is unlikely to become commonly used (Bakker et al., 2017).

Overall, much of the work looking at intraluminal devices to prevent/reduce the consequences of anastomotic leak involves pilot studies in animals or small numbers of human subjects. However, it appears some of the work has had promising results and merits further investigation by clinicians and engineers working together.

1.7.3 Electrical resistance

Recently DeArmond et al (DeArmond et al., 2010) have used animal models of an upper gastrointestinal leak to detect anastomotic leak using changes in electrical resistance. In a pilot study using 8 rats with a surgically created gastrotomy they were able to show that when passing hypertonic saline solution

through the stomach there were electrical resistance changes in the rats with the gastrotomy. They have followed this up by comparing the technique to the performance of barium fluoroscopy in the detection of an anastomotic leak. Detection of electrical resistance had 100% sensitivity and specificity and was superior to barium fluoroscopy (DeArmond et al., 2013). However, this was a small study with only 10 rats. Similar studies have not been carried out in animal models of colorectal anastomotic leak or in humans. It would be interesting to see further research in this area as the technique has the advantages of being available at the bedside, giving rapid results and not being affected by the underlying pathological disease state.

1.7.4 Measurement of peritoneal fluid biomarkers

1.7.4.1 Definition of a peritoneal fluid biomarker

A 'biomarker' is defined as, "…an objectively measured characteristic, which is an indicator of a physiological or pathogenic process, or a pharmacological response to a therapeutic intervention" (Colburn et al., 2001). There has been interest in trying to identify a biomarker of AL in the immediate environment of the anastomosis by sampling peritoneal fluid. These local biomarkers reflect conditions in the milieu of the anastomosis and have the potential to detect AL earlier and with greater specificity than systemic observations and blood parameters. In 2008 Komen et al suggested criteria for biomarkers of AL in peritoneal fluid (Komen et al., 2008) (table 1.10).

TABLE 1.10 SUGGESTED CRITERIA FOR A BIOMARKER OF AL.

- Significant change in biomarker concentration in anastomotic leakage,
- Stability of the biomarker in the peritoneal environment and drain fluid,
- Biomarker level not influenced by the primary disease,
- Biomarker with sufficient sensitivity and specificity for anastomotic leakage,
- Biomarker allows for easy, fast and cheap real-time testing.

1.7.4.2 Biomarkers of ischaemia

Seven studies have looked at biomarkers of ischaemia measured either by microdialysis or in peritoneal drain fluid (table 1.11). The principle of this approach is that ischaemia at the anastomosis or in nearby bowel (just proximal or distal to the anastomosis) increases the risk of a leak (Thornton and Barbul, 1997, Locke et al., 1984). In particular, studies have used lactate and pH as markers of ischaemia.

1.7.4.1.1 Lactate

1.7.4.1.1.1 Discovery of lactate

Lactate was first discovered and described in sour milk in 1780 (Oberkofler et al., 2012). It takes its name from lact-, the latin for milk. In 1808 a Swedish chemist, Jons Jakon Berelius, found lactic acid in fluid extracted from meat (Constantinides et al., 2007, Platell et al., 2007). The first description of lactic acid as a pathological finding was in 1843 by Johann Joseph Scherer. He identified lactic acid in the blood of young women who had died of puerperal fever (Ricos C, 1999). Several years later in 1891 Araki and Zillessen demonstrated the relationship between tissue hypoxia and lactic acid. They showed that if they interrupted the oxygen supply to muscles in mammals and

birds lactic acid was formed and the levels of lactic acid increased (T, (1891) , T, 1891, T, 1892b, T, 1892a, H, 1891).

1.7.4.1.1.2 Lactate metabolism

Lactate is a metabolite formed during the cellular production of energy (adenosine triphosphate, ATP). Glycolysis is the first step in energy production and occurs in the cytoplasm of a cell. Glucose is broken down into two molecules of pyruvate and two molecules of ATP (figure 1.6). In the presence of oxygen, energy production proceeds via the aerobic pathway whereby pyruvate enters the Krebs' cycle, a series of reactions takes place which removes carbon dioxide and generates hydrogen ions (figure 1.7). These high energy electrons then pass into the electron transfer chain on the inner membrane of the mitochondria. This is the final pathway in the production of energy and provides 34 molecules of ATP. In the absence of oxygen, pyruvate does not pass into the mitochondria and instead undergoes fermentation. The enzyme lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to lactate (figure 1.8). The purpose of this is to oxidise the electron carriers so that they can participate in glycolysis again - NADH is oxidized to NAD+ which is then re-used in glycolysis.

The rate of glycolysis can increase much more rapidly than oxidative phosphorylation, so briefly in times of cellular stress, glycolysis can produce more ATP than oxidative phosphorylation. Excess pyruvate rapidly accumulates and it is converted into lactate. ATP is also generated via this route in the absence of oxygen (anaerobic respiration), this again leads to an excess production of lactate. Therefore, elevated lactate can serve as a marker of a hypoxic environment. This relationship between tissue hypoxia and lactate has been confirmed by several studies (Cain, 1965, Zhang and Vincent, 1993). The balance of aerobic/anaerobic metabolism can be measured by the lactate/pyruvate ratio (LP ratio) with an increased ratio also indicating an ischaemic environment. Lactate and other markers of ischaemia can be measured in peripheral blood but they lack specificity in detecting AL (Corke and Glenister, 2001).

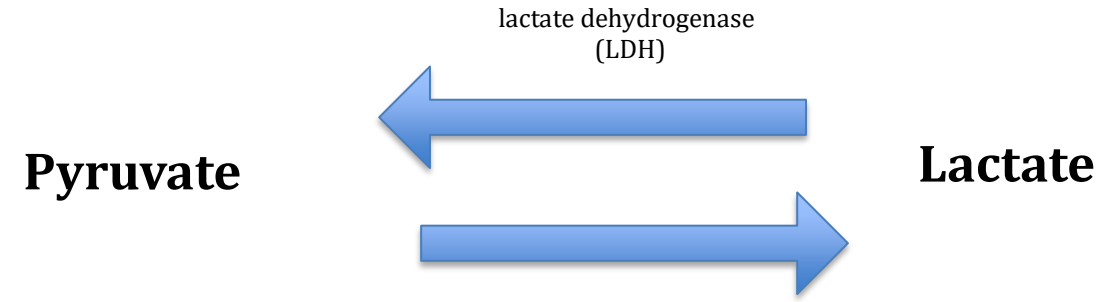

FIGURE 1.6 INTER-CONVERSION OF PYRUVATE TO LACTATE BY THE ENZYME LDH

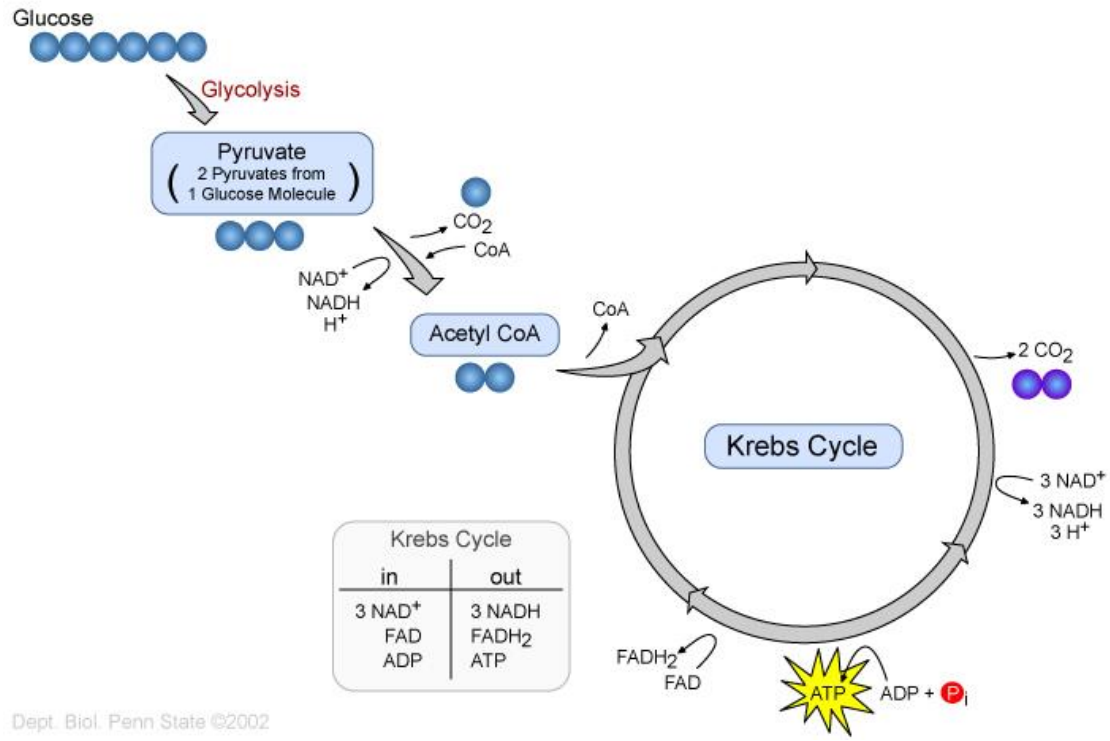

FIGURE 1.7 THE KREB'S CYCLE (STATE, 2002)

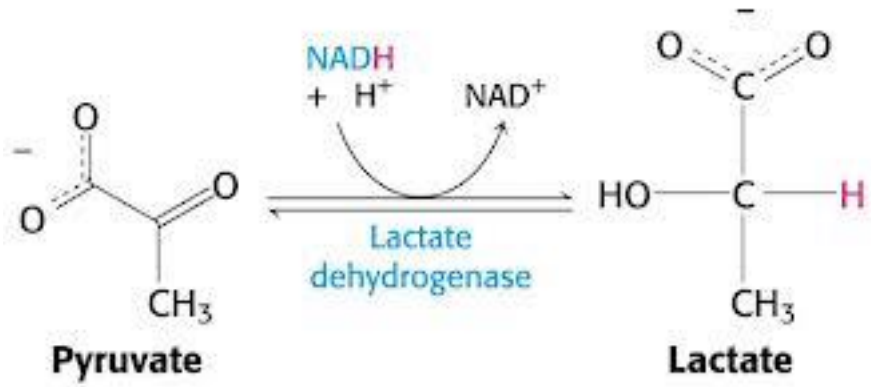

FIGURE 1.8. FERMENTATION OF PYRUVATE

TABLE 1.11 SUMMARY OF STUDIES LOOKING AT BIOMARKERS OF ISCHAEMIA

Author, year	Biomarkers studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Method of biomarker measurement	Frequency and duration of biomarker measurement	Main results
Millan, 2006(Millan et al., 2006)	pH	Prospective	All patients had CT or contrast imaging on POD 6. Divided into subclinical and clinical but analysed together.	90	10	Anterior resections for colorectal cancer	Tonometry catheter placed just superior to the anastomosis.	Samples taken at 24 & 48hrs after surgery	pH at 24 hrs, but not 48 hrs, significantly lower in those with AL
Matthiessen, 2007(Matthiessen et al., 2007b)	LP ratio, glucose	Prospective	Peritonitis caused by leakage, pelvic abscess or discharge of faeces from drain or a fistula. Confirmed by imaging or DRE.	23	7	Anterior resections for colorectal cancer	Measurement via microdialysis	Samples every 2 hours until POD 2 and then every 6 hours until POD 6.	LP ratio significantly increased on POD5 & 6 in those with AL. Glucose not significant.

Pedersen, 2009 (Pedersen et al., 2009)	Glucose, lactate, pyruvate, glycerol, LP ratio	Prospective	Peritonitis or abscess with "proven dehiscence" – no mention of how it was proven. Fistulas NOT counted as AL.	50	4	Low anterior resections for cancer	Measurement via microdialysis	Samples every 4 hours until discharge or POD 10	3 patients had a late leak and lactate levels and LP ratio was significantly increased prior to onset of symptoms. In one case of early leak increased lactate coincided with onset of symptoms
Horer, 2011(Horer et al., 2011)	Glycerol, lactate, pyruvate, glucose, LP ratio	Prospective	No definition given. AL included in "major complication" category.	60	16*	Various gastrointestinal and intra-abdominal vascular surgeries	Measurement via microdialysis	Every 2 hrs until 48 hrs post surgery	LP ratio was significantly elevated and glycerol levels significantly lower in those with AL Lactate alone - no significant difference
Yang, 2013(Yang et al., 2013)	pH	Prospective	Pelvic abscess, faecal/purulent discharge from drain, fistulas, peritonitis confirmed	753	57	Anterior resections for cancer	Peritoneal drain sample	Daily until POD 12	pH < 6.978 on POD 3 significantly associated with AL, sensitivity 98.7% and specificity 94.7%

			radiologically and all needed additional surgical treatment.						
Bini, 2014(Bini et al., 2014)	Lactate	Prospective	No definition given. AL included in group requiring "reintervention".	88	31 *	Various gastrointestinal operations	Measurement from peritoneal drain on POD 4 in those meeting the criteria: late passage of flatus, pain, pyrexia & raised white cell count	Once on POD 4	Peritoneal lactate level >9.1mmol and peritoneal: serum lactate level ratio > 4.5 significantly associated with requiring reintervention
Daams, 2014(Daams et al., 2014)	Lactate, pyruvate, glucose, glycerol	Prospective	Clinical suspicion of intra-abdominal complication was investigated by CT to diagnose AL. All AL were	24	3	Left sided colonic resections for cancer or diverticular disease	Measurements via microdialysis	Every 4 hours until POD 5	Mean intra-peritoneal lactate levels, but not LP ratio, was significantly higher in those with AL

			confirmed at reoperation or endoscopy.						

**End point was "patient requiring reintervention", of the 31 in this group 11 had an AL*

1.7.4.1.1.3 Studies using lactate as a marker of ischaemia

Several of the studies used microdialysis (Matthiessen et al., 2007b, Pedersen et al., 2009, Horer et al., 2011, Daams et al., 2014) to measure lactate. This technique has been previously used to study tissue ischaemia (Kanthan et al., 1995) and involves inserting very small probes with dialysis membranes into the operative field at the end of a procedure. Fluid then flows through the circuit constantly allowing measurement of various analytes including lactate and pyruvate. Trials in animal models of intestinal ischaemia showed that increased lactate and LP ratios were reliable markers of local ischaemia (Klaus et al., 2002, Sommer and Larsen, 2004).

Matthiessen et al (Matthiessen et al., 2007b) measured LP ratio and glucose for 6 days following anterior resections in 23 patients. Four "early leaks" on post-operative days (PODs) 2-14 and 3 "late leaks" on PODs 18-22 were analysed together as a "leak group". LP ratios on PODs 5 & 6 were significantly higher in those with AL, glucose was not significantly different. However, as the numbers were low it was not possible to determine cut-off levels for LP ratio to indicate the likelihood of a leak. Pedersen et al (Pedersen et al., 2009) looked at 45 low anterior resections. In one AL on POD 5 lactate levels were significantly increased 18 hours before the onset of symptoms and LP ratio became significantly elevated at the onset of symptoms. Three other patients developed a leak at day 10 or later. LP ratio and lactate levels were significantly increased compared to controls throughout the first 5 days following surgery (Pedersen et al., 2009). Daams et al (Daams et al., 2014) measured lactate, pyruvate, glycerol and glucose every 4 hours for 96 hours in 24 patients undergoing left sided colorectal anastomoses. In 3 patients with ALs median intraperitoneal lactate levels, but not LP ratio were significantly higher. There appeared to be spikes in lactate prior to POD 3 in the leak group but cut-off values were not described. It is important to note that 2 of the leaks occurred early at PODs 4 and 5, the third was said to have occurred at POD 97 but the three were grouped together. Horer et al (Horer et al., 2011) had similar findings, they noted that increased LP ratio and decreased glycerol levels were associated with "major intra-abdominal complications". However, this was in a varied cohort of abdominal surgeries including colorectal anastomosis, abdominal aortic aneurysm (AAA) repair, gastric procedures and cholecystectomy.

Bini et al (Bini et al., 2014) looked at lactate via peritoneal drain fluid on POD 4 in patients who met all 4 of the following criteria: pyrexia (>38.3), raised white cell count (>12), delayed passage of flatus (>72 hours) and abdominal pain. A peritoneal/serum lactate level > 4.5 or peritoneal lactate level > 9.1 were significantly associated with postoperative complications requiring intervention (ALs were included in this group). However, this selection bias with no control group limits the interpretation of these findings.

1.7.4.1.2 pH

1.7.4.1.2.1 The definition of pH

pH is a numerical scale from 0 - 14 that specifies the acidity or alkalinity of a solution. pH 7 is neutral, solutions with a pH < 7 are acidic and those with pH > 7 are alkaline. pH is measure of the concentration of hydrogen ions $\{H^+\}$ and can be calculated as follows:

$$pH = - \log \{H_3O^+\}$$

1.7.4.1.2.2 pH as a marker of ischaemia

In a hypoxic environment mitochondria cannot produce sufficient ATP. The rate of glycolysis is therefore increased to provide extra ATP. Whilst increased glycolysis helps to compensate for reduced ATP from oxidative phosphorylation it cannot bind the additional hydrogen cations that result from ATP hydrolysis. This leads to an increased hydrogen cation concentration (thus a reduction in pH) (Hochachka and Mommsen, 1983). Therefore a low pH is a marker of a hypoxic environment.

Adequate perfusion and oxygenation are essential for healing of an anastomosis (Thornton and Barbul, 1997). In the 1960s it was shown that intramucosal pH correlated with blood supply to the mucosa (Bergofsky, 1964) – a low pH being indicative of poor perfusion. Measurement of pH via tonometry, a non-invasive method of measuring intramucosal pH, was found to be a good prognostic indicator of the viability of the gastroesophageal anastomosis following thoracic oesophagectomy (Tarui et al., 1999). This has lead to pH being investigated as a potential biomarker for colorectal AL.

1.7.4.1.2.3 Studies using pH as a marker of ischaemia

Two studies have investigated pH as a biomarker. In the first, a catheter was sited intraluminally just above the anastomosis and pH measured via tonometry (a non-invasive method to measure intramucosal pH in hollow organs). All patients were imaged on POD 6 and "clinical" and "subclinical" leaks were analysed together in a "leak group". The "leak group" had a significantly lower pH. A pH < 7.28 yielded a specificity of 98.3% but sensitivity was only 28.1% (Millan et al., 2006). Yang et al (Yang et al., 2013) measured the pH of fluid collected from peritoneal drains up to POD 12 following rectal surgery in 753 patients. There were 57 leaks (7.6%) on PODs 6-12 requiring surgical intervention, subclinical leaks were not included. pH was significantly lower in patients who leaked. pH < 6.978 on POD 3 showed excellent sensitivity (98.7%) and specificity (94.7%). However, no confidence intervals or ranges were provided so it is difficult to know how much overlap there is between groups. The authors highlight the decline in pH was notable prior to the detection of a leak in all of the patients. Whilst the results of the large study by Yang et al would suggest that POD 3 drain pH is an extremely useful test for AL, it has not gained widespread acceptance as a test. This is perhaps because it remains a single centre study and surgeons are reticent about changing practice based on one study alone. It is therefore important to attempt to replicate this study to validate or refute the findings.

Overall, of the biomarkers of ischaemia, lactate and pH emerge as the best candidates for further research. Most studies measured lactate by microdialysis (Matthiessen et al., 2007b, Pedersen et al., 2009, Daams et al., 2014, Horer et al., 2011) which is expensive, labour-intensive and technically challenging (e.g. 9 (20% of cases) technical failures in (Daams et al., 2014)). In contrast, measurement of lactate from peritoneal drain fluid may provide a quick, easy and inexpensive alternative. The stability of lactate and pH in peritoneal drainage fluid needs to be addressed. The stability of these biomarkers in peritoneal fluid has not been explored but it has been shown that lactate levels rise in blood if there is a delay in analysis (Calatayud and Tenias, 2003). It would therefore be pertinent to address this issue in peritoneal fluid.

1.7.4.3 Biomarkers of bacterial infection

This approach is based upon the principle that if the colorectal anastomosis breaks down bacteria which are normally contained within the bowel lumen can spill into the peritoneal cavity. Four studies have explored whether detection of intra-peritoneal bacteria and its quantitative assessment could aid early detection of AL (table 1.12).

TABLE 1.12 SUMMARY OF STUDIES LOOKING AT BACTERIAL BIOMARKERS

Author, year	Bacterial biomarker studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Method of biomarker measurement	Duration and frequency of measurement	Main results
Junger, 1996 (Junger et al., 1995)	Lipopolysaccharide (LPS)	Prospective	"Clinical signs of a leak"	22	3	"Colorectal anastomoses"	Sample from peritoneal drain fluid.	Daily until POD 8	LPS significantly increased in those with AL
Komen, 2009(Komen et al., 2009)	E coli, e faecalis	Prospective	Not defined	9	0	Various colorectal resections from ileocaecal resections – low anterior resection; benign & malignant	Sample from peritoneal drain fluid. Fluid sent for bacterial culture and for RT-PCR to detect bacteria.	Morning daily sample until POD 5	RT-PCR results fully concordant with culture results but there were 4 false positives.
Fouda, 2011(Fouda et al., 2011)	E coli, Klebsiella, pseudomonas, bacteroides	Prospective	Gas, pus, or faecal discharge from the	56	8	Low anterior resections for malignancy	Sample from peritoneal drain fluid.	Samples on POD 1, 3 & 5	E coli, Klebsiella, pseudomonas & bacteroides

			drain, faecal discharge from the operative wound, pelvic abscess, peritonitis, and rectovaginal fistula. Confirmed by radiological contrast study, CT scan or digital rectal palpation.						cultured significantly more often from patients with AL
Komen, 2014(Komen et al., 2014b)	E coli, e faecalis	Prospective	A clinical state requiring reintervention confirmed by	243	19	Left sided colorectal resections; benign and malignant	Sample from peritoneal drain fluid. Fluid sent for	Morning daily sample until POD 5	E coli concentration significantly increased on POD 4 & 5 and

			radiological studies, operative findings or faecal discharge from the drain.				RT-PCR to detect bacteria.		E faecalis on POD 2,3 &4 in those with AL. Best diagnostic odds ratio was e faecalis on POD 3.

Fouda et al (Fouda et al., 2011) looked at intra-peritoneal bacterial colonization in peritoneal drain fluid samples from 56 low anterior resections on POD 1, 3 and 5. Eight ALs were "detected clinically" and confirmed on imaging. E Coli, Bacteroides and Pseudomonas were cultured significantly more often on POD 1, 3 and 5; and Klebsiella on PODs 3 and 5 in patients with AL. However, specificity was low as there were several false positives with all 4 bacteria detected in patients who did not develop AL.

The time delay in growing cultures limits its applicability and usefulness as a rapidly analysable biomarker of AL. Komen et al addressed this in a pilot study of 17 patients using real-time polymerase chain reaction (RT-PCR). Peritoneal drain fluid was sampled daily up to POD 5 using E Coli and Enterococcus Faecalis as indicator organisms. RT-PCR results were fully concordant with microbiological cultures. However, the technique lacked specificity as there were 4 false positive results (Komen et al., 2009). The authors progressed to a multicentre study of 243 patients undergoing elective left sided colonic anastomosis. There were 19 leaks (7.8%) on mean POD 6 (2-26 days). E Coli concentration was significantly increased on PODs 4 & 5 and E Faecalis on PODs 2, 3, & 4 in those with AL. E Faecalis on POD 3 achieved the best results with a sensitivity and negative predictive value of 92.9% and 98.7% respectively, the diagnostic odds ratio was 31.6 but specificity and positive predictive value were only 70.9% and 30.2% respectively (Komen et al., 2014b). Despite the large number of false positives the study demonstrated that the absence of E Faecalis on day 3 could potentially exclude AL.

In 1996 Junger et al (Junger et al., 1995) looked at peritoneal drain lipopolysaccharide (LPS) levels (a component of the outer wall of gram-negative bacteria) in 22 patients. LPS levels were significantly elevated in 3 patients with AL. However, 2 of these patients had surgery for perforated sigmoid diverticulitis so levels may have been elevated due to pre-existing bacterial contamination. LPS has not been explored again.

Overall, bacterial biomarkers for AL may have some utility in acting as a "rule-out" test for AL. Whilst RT-PCR for bacteria offers fast, real-time testing it is not

commonly performed in most hospital laboratories which may limit its usefulness.

1.7.4.4 Biomarkers of inflammation (cytokines)

Seven studies have explored the measurement of peritoneal cytokines to detect AL (table 1.13). Cytokines are glycoproteins involved in regulating inflammation and are thought to be the main mediators of the systemic inflammatory response to sepsis (Dinarello, 1984, Hesse et al., 1988, Wong and Clark, 1988, Castell et al., 1989). Peritoneal cytokine levels are elevated compared to serum cytokine levels after major abdominal surgery and in peritonitis (Herwig et al., 2002, Jansson et al., 2004, Wiik et al., 2001).

TABLE 1.13 SUMMARY OF STUDIES LOOKING AT CYTOKINES

Author, year	Biomarkers studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Method of measurement	Frequency and duration of measurement of biomarkers	Main results
Herwig, 2002(Herwig et al., 2002)	IL-1, IL-6, TNFa	Prospective	Clinical diagnosis confirmed by endoscopy, contrast radiology and finally confirmed at laparotomy	24	12	Right hemicolectomy to anterior resections for malignancy, inflammatory bowel disease, diverticulosis and trauma	Peritoneal drain fluid sample taken daily within 2 hours of emptying drainage bag	Daily until POD 4-9	IL-6 & TNFa from POD 1 and IL-1 from POD 3 significantly increased in those with AL
Bertram, 2003(Bertram et al., 2009)	IL-6, TNFa	Prospective	No definition provided but states AL was confirmed at relaparotomy	25	3	Right hemicolectomies to anterior resections for adenomas and cancers	Peritoneal drain fluid sample taken daily at 8am	Daily until POD 7	IL-6 and TNFa not helpful in predicting AL
Matthiessen,	IL-6, IL-10,	Prospective	Peritonitis	23	7	Anterior	Peritoneal drain	Peritoneal	IL-6, IL-10 significantly

2007(Matthiessen et al., 2007b)	TNFa		caused by leakage, pelvic abscess or discharge of faeces from drain or a fistula. Confirmed by imaging or DRE.			resections for colorectal cancer	fluid sample	drain sample every 6 hours until 42 hours after surgery	increased from POD 1&2 in those with AL TNFa only significant on POD 1
Ugras, 2008(Ugras et al., 2008)	IL-6, IL-10, TNFa	Prospective	"Clinical signs (gas, pus, faeces from pelvic drain; pelvic abscess, peritonitis, pus from rectum or rectovaginal fistula) along with biological tests and abdominal CT findings".	34	4	Right hemicolectomies to low anterior resections for colorectal cancer	Peritoneal drain fluid samples	Daily from peritoneal drains until POD 5	IL-6, IL-10, TNFa all significantly increased in those with AL on all days. Those without AL had decreasing cytokine levels.

Fouda, 2011(Fouda et al., 2011)	IL-6, IL-10, TNFa	Prospective	Gas, pus or faecal discharge from the drain, faecal discharge from the wound, pelvic abscess, peritonitis and rectovaginal confirmed by CT, contrast scan or rectal palpation.	56	8	Low anterior resections for rectal cancer	Peritoneal drain fluid sample	Peritoneal drain samples on PODs 1,3 & 5	IL-6 & IL-10 significantly increased on POD 1,3&5 and TNFa significantly increased on POD 3 & 5 in those with AL
Yamamoto, 2011(Yamamoto et al., 2011)	IL-1, IL-6, TNFa	Prospective	Post-operative peritonitis with leak thereafter confirmed by contrast study, ultrasound or CT scan.	100	8	Anterior resections & AP resections for colorectal cancer	Peritoneal drain fluid sample taken within 2 hours of emptying the drain	Peritoneal drain samples on PODs 1,2 & 3	IL-1, IL-6 and TNFa significantly increased on POD 3 in those with AL. Cytokine levels significantly increased over the days in those with AL and fell in those without AL.
Alonso,	IL-6	Prospective	Clinical	60	30	Right	Baseline IL-6 level	Peritoneal	IL-6 significantly

2015(Alonso et al., 2015)			suspicion of a leak was confirmed by radiological study or findings at reoperation. Intra-abdominal abscesses were not classified as AL but abscess and ALs were analysed together as an "infective complications" group.			hemicolectomies to anterior resections for colorectal cancer	measured by lavaging abdominal cavity immediately after laparotomy or creation of pneumoperitoneum Post-operative samples taken from peritoneal drain fluid	drain sample at 48 hrs and 4 days post surgery	increased at both time points in those with AL

There is wide heterogeneity in the type of operations performed, underlying pathology and definitions of AL. In addition, most involved small cohorts and measurements of peritoneal cytokines have been taken at varying times and frequencies from every 4 hours for 2 days (Matthiessen et al., 2007b) to once daily for 7 days (Bertram et al., 2009).

Tumour necrosis factor alpha (TNFa)

Herwig et al (Herwig et al., 2002), Ugras et al (Ugras et al., 2008) and Matthiessen et al (Matthiessen et al., 2007b) found that TNFa was significantly higher on POD 1 in patients who developed an AL. However, no cut-off value could be established to give an estimate of risk and confidence intervals were overlapping in each group. Fouda et al (Fouda et al., 2011) and Yamamoto et al (Yamamoto et al., 2011), which contained the largest sample of patients (n=100), found TNFa did not reach statistical significance until POD 3. Both also noted that TNFa decreased significantly from POD 1 to 3 in patients who did not develop a leak(Fouda et al., 2011, Yamamoto et al., 2011). In contrast, Bertram et al (Bertram et al., 2009), who used similar methodology, looked at TNFa levels for 7 days in 25 patients and found that TNFa was not helpful in predicting AL.

Interleukin -6 (IL-6)

Ugras et al (Ugras et al., 2008), Herwig et al (Herwig et al., 2002), Matthiesen et al (Matthiessen et al., 2007b) and Fouda et al (Fouda et al., 2011) all found IL-6 to be significantly higher in patients who developed an AL from POD 1, however Yamamoto et al (Yamamoto et al., 2011) demonstrated no significant difference until POD 3 and Bertram et al (Bertram et al., 2009) found no difference in IL-6 levels at anytime in the first 7 days post-op.

A more recent case-control study by Alonso et al (Alonso et al., 2015) looked at IL-6 levels on PODs 2 and 4 in 60 patients after various colorectal resections. 30 leaks and intra-abdominal abscesses were grouped into an “infection” category and compared to 30 controls with an uncomplicated recovery. IL-6 was significantly higher on both days in the “infection group”. They did not state how or when the leaks were detected.

Other cytokines

Of other cytokines studied, the pro-inflammatory interleukin 1b (IL-1b) was found to be significantly elevated on POD 3 (Herwig et al., 2002, Yamamoto et al., 2011) and the anti-inflammatory interleukin 10 (IL-10) on POD 1 in patients who developed an AL (Fouda et al., 2011, Matthiessen et al., 2007b, Ugras et al., 2008).

Overall, despite some conflicting results, a meta-analysis (which did not include Alonso et al (Alonso et al., 2015)) found that IL-6 and TNFa were significantly raised from POD 1 and 2 respectively in those who developed a leak (Cini et al., 2013). Studies with larger sample sizes and better standardization of definitions and sampling protocols are required to clarify their role.

1.7.4.5 Biomarkers of wound repair (matrix metalloproteinases; MMPs)

MMPs are zinc dependent enzymes responsible for tissue turnover by extracellular matrix degradation. Tissue inhibitors of metalloproteinase (TIMPs) are the natural inhibitors of MMPs. The balance of MMPs and TIMPs is involved in physiological and pathological processes including inflammation (Verma and Hansch, 2007). Three papers, with wide variation in study population, sampling frequency and end points have explored peritoneal MMPs and TIMPs (table 1.14).

TABLE 1.14 SUMMARY OF STUDIES LOOKING AT BIOMARKERS OF WOUND REPAIR

Author, year	Biomarkers studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Methods of biomarker measurement	Frequency and duration of biomarker measurement	Main results
Baker, 2003(Baker and Leaper, 2003)	MMP-1, 2,3,8,9 TIMP-1,2	Prospective	Not specified – AL grouped with "major complications"	58	NS*	Right hemicolectomies to AP resections for colorectal cancer	Sample from peritoneal drain	Daily until POD 5-8	MMP-2 (POD 3), MMP-2 (POD 6), MMP-9 (POD 6 & 7) positively correlated with complications TIMP-2 (POD 2&3), TIMP-1 (POD 7) negatively correlated with complications
Pasternak, 2009(Pasternak et al., 2010)	MMP-1,2,3,7,8,9,13 TIMP-1,2	Prospective	Clinical diagnosis including fistulas confirmed by CT, contrast study or DRE	29	10	Low anterior resections for colorectal cancer	Sample from peritoneal drain	Once at 4 hours post-operation	MMP-8 & 9 significantly increased in those with AL

Kostic, 2015(Kostic et al., 2015)	MMP-9	Prospective	Clinical diagnosis – finding of pus/faeces in drain, pelvic abscess, peritonitis or fistula	150	15	Left sided colorectal resections for cancer	Sample from peritoneal drain. MMP-9 measured via ELISA.	On PODs 1, 3, 5 & 7	MMP-9 not significantly different in those with AL

**NS – not specified*

Pasternak et al (Pasternak et al., 2010) measured MMP-1, 2, 3, 7, 8, 9 and 13 once at 4 hours after low anterior resections in 30 patients. Five early leaks between PODs 2 – 13 and 5 late leaks between PODs 13 -41 were analysed together. Only MMP-8 and 9 at 4 hours post surgery were significantly elevated in the AL group.

Baker et al (Baker and Leaper, 2003) measured MMP-1-3, 8, 9 and TIMP-1 and 2 for 7 days after various colorectal anastomoses in 58 patients. The end point was "major complications" which included AL. Levels of MMP-2 (day 3) MMP-2 (day 6) and MMP-9 (days 6, 7) were positively correlated with complications whereas TIMP-2 (days 2, 3) and TIMP-1 (day 7) were negatively correlated. Only MMP-9 was significantly elevated in both studies. However, Kostic et al (Kostic et al., 2015), who looked specifically at MMP-9 on PODs 1, 3, 5 and 7 in 150 patients undergoing elective left sided colorectal resections (with 15 clinically detected ALs), found that levels were not significantly different to those without a leak. The inconsistent results for MMPs limit their usefulness in future research.

1.7.5.6 Summary of peritoneal biomarkers

Peritoneal cytokines, lactate and pH have the potential to identify AL early. The consistency of the results for lactate and pH, alongside the fact they are simple, quick and inexpensive to test, makes them the strongest targets. In addition to clarifying their stability in peritoneal fluid, in the future studies should aim to use a homogenous study population with a standardized AL definition. The wide variability in the definition and measurement of AL is not unique to this group of studies. A 2001 review of 97 studies identified 56 different definitions of AL (Bruce et al., 2001). Since then the International Study Group on Rectal Cancer have set out a definition of AL and graded its severity (A-C) depending on the impact on clinical management (Rahbari et al., 2010). Adoption of this definition and grading system in future work would be useful. Ultimately, it is to be hoped that incorporation of a biomarker sensor into a peritoneal drain or biodegradable implantable device could provide early recognition of AL leading to early

initiation of intervention and significant reductions in patient morbidity and mortality.

1.8 Statement of project aims

The aims of this project are as follows:

1. To characterise the stability of the biomarkers lactate and pH in peritoneal fluid from a selected control group (Chapters 4 & 5)
2. A prospective analysis of the characterisation of post-operative daily peritoneal drain fluid biomarkers (lactate and pH) in detecting anastomotic leak in patients undergoing colorectal resection (Chapter 6)
3. A comparison of peritoneal fluid biomarkers in the detection of colorectal anastomotic leak to blood biomarkers (Chapter 7)

Chapter 2 – Materials and methods

2 Chapter 2 – Materials and methods

2.1 Materials

This section lists and describes the materials used for the testing of lactate and pH from clinical and non-clinical samples in chapters 3 – 6. When reading chapters 3 – 6 refer back to this section.

2.1.1 Virkon

The multi-purpose disinfectant Virkon was purchased from Antec International, (Sudbury, Suffolk, UK).

2.1.2 Ethanol

Ethanol solution was purchased from Sigma-Aldrich (UK).

2.1.3 Deionised water

Deionised water was purified using an Elga Purelab system (Elga Process Water , UK).

2.1.4 Sodium l-lactate

Sodium l-lactate was purchased from Sigma-Aldrich (UK).

2.1.5 Sterile water

'Water for injection BP' was purchased from Braun, Germany. This is sterile water and was used for the dilution of peritoneal drain fluid samples.

2.1.6 Blue nitrile gloves

Blue nitrile gloves were purchased from Robinson Healthcare (Worksop, UK).

2.1.7 Face shield

A “Sphere” face shield was purchased from Bollé Safety (Villeurbanne, France). It was worn when handling clinical samples in the laboratory.

2.1.8 UN3373 packaging kit

To comply with guidelines for the transport of biological substances (category UN 3373), a UN 3373 packaging kit was purchased from Dakla Pack (Chiswick, UK). The kit consisted of a cardboard outer box, 500ml green leak proof container, plastic shock absorbers and absorbing tube holders (see figure 2.1).

FIGURE 2.1 UN 3373 PACKAGING KIT

2.1.9 Lactate Pro 2

The ‘Lactate Pro 2’ handheld lactate test meter and ‘Lactate Pro 2 Test Strips” manufactured by Arkay (Japan) were purchased from HaB International Ltd (Southam, UK).

2.1.10 Omega pH meter

The ‘pH pocket tester, PHH-7011’ manufactured by Omega was purchased from ‘Omega Engineering Ltd” (Manchester, UK). In addition, pH 4, 7 and 10 buffer solutions for calibration of the meter were purchased from the same manufacturer.

2.1.11 Clinical chemistry analyser

The ‘Labsystems Multiskan Ascent’ (Thermo Electron Corporation, USA) was used to measure absorbance in order to determine the lactate level of standard solutions and of peritoneal drain fluid samples in chapters 3 and 6 respectively.

2.1.12 Colorimetric lactate assay

L-lactate colorimetric assay kits were purchased from Randox Laboratories, County Antrim, UK.

2.1.13 Mettler Toledo pH meter

The “Mettler Toledo S220 Seven Compact “ pH meter (Mettler Toledo, USA) was used to measure the pH standard solutions and of peritoneal fluid samples in chapters 3, 5 and 6.

2.1.14 pH standard solutions

pH 4 and 7 tablets from Fischer Scientific (Loughborough, UK) and pH 10 buffer solution (Arcos Organics, UK) were used to calibrate the “Mettler Toledo S220 Seven Compact” used in chapters in chapter 3 and 6.

2.2 General methods

This section describes the background and methodology for the testing of lactate and pH from clinical and non-clinical samples in chapters 3 – 6. When reading chapters 3 -6 refer back to this section. Any additional methods will be defined and explained in each relevant chapter.

2.2.1 Measuring lactate

2.2.1.1 Methods with the Lactate Pro 2

This section provides information about the hand-held lactate meter, the Lactate Pro 2, and why it was chosen for use in this project.

2.2.1.1.1 Background information and product specification

The Lactate Pro 2 is a hand-held portable analyser designed to quantitatively measure the lactate level in human fresh capillary whole blood (see figure 2.2). Product specifications are shown in table 2.1. A sample of blood is added to the test strip and the reagent strip fills by capillary action. The sensor used is amperometric. The lactate in the sample reacts with potassium ferricyanide and lactate oxidase to form potassium ferrocyanide and pyruvate. A voltage is applied and ferrocyanide is oxidised, electrons are released producing a current. This current is measured amperometrically and is directly proportional to the lactate level of the sample.

FIGURE 2.2 THE HANDHELD LACTATE PRO 2

TABLE 2.1 LACTATE PRO 2 PRODUCT SPECIFICATION (INCLUDE PHOTOGRAPHS OF METER AND TEST STRIPS)

Manufacturer	Arkray KDK, Japan
Method of measurement	Amperometric reagent
Minimum sample size	0.3 µl
Analysis time	15 seconds
Operating environment	5 – 40 °C
Measurement range	0.5 – 25.0 mmol/L
Calibration	Automatic

2.2.1.1.2 Uses of the Lactate Pro 2

The Lactate Pro 2, is primarily used by athletes to evaluate training performance and prescribe training intensities and has been validated for this purpose (Bonaventura et al., 2015). The Lactate Pro (Arkray, Japan), the predecessor of the Lactate Pro 2, has also been validated for use in hospital emergency

department triage areas to identify those with an elevated lactate who would benefit from early treatment of sepsis (Goyal et al., 2010, Gaieski et al., 2013). The manufacturer only provides validation data for the measurement of lactate from blood, however, studies in veterinary medicine have shown that portable, hand-held lactate analysers such as the Lactate Pro can be used to measure lactate levels in other fluids such as peritoneal fluid. The Lactate Pro has been validated against a standard bench-top blood gas analyser and also a gold standard laboratory colorimetric enzymatic assay for the measurement of peritoneal fluid from horses (Nieto et al., 2015).

2.2.1.1.2 Lactate Pro 2 testing procedure

Testing the lactate level of a solution with the Lactate Pro 2 is carried out as follows:

1. Insert a test strip into the Lactate Pro 2
2. Touch the tip of the testing strip against the solution
3. Let the test strip draw up solution until the check window is filled with solution
4. After 15 seconds the test result appears on the screen.

2.2.1.2 Colorimetric lactate assay

A colorimetric assay is a gold standard method of analysing lactate. The assay and equipment required to perform this analysis is kept at the Wolfson Centre, University of Strathclyde. It was used to test the lactate concentration of non-clinical samples in chapter 3 and a subset of clinical samples in chapter 6.

The L-lactate assay (Randox Laboratories) was used to determine the lactate concentration of peritoneal fluid via a colorimetric method on a 'Labsystems Multiskan Plate Reader'. The concentration of L-lactate in the sample is determined according to the following reaction:

$$\mathrm{L-Lactate} + \mathrm{O_2} \xrightarrow{\text{Lactate oxidase}} \text{pyruvate} + \mathrm{H_2\,O_2}$$

$$\mathrm{H_2O_2} + 4-\text{aminoantipyrine} + \mathrm{TOOS} \xrightarrow{\text{Peroxidase}} \text{purple product} + 4\mathrm{H_2O}$$

(TOOS = N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine)

Serial dilutions of a 20mmol sodium lactate solution were created to make concentrations of 10 mmol, 5 mmol, 2.5 mmol and 1.25 mmol. 2.5µl of calibration solution, along with 2.5µl of peritoneal fluid samples and a blank solution (2.5µl deionized water), were added to a 96 well plate reader as shown in Figure 2.3. Next 250µl of the enzyme reagent was added to each well contained a sample, calibration solution or blank. The plate reader was then placed in the Multiskan Plate Reader. Settings for the plate reader were: incubation temperature 37°C, low speed shake for 5 minutes and the absorbance 550nm (as per Randox assay instructions). The results were saved into a Excel file and a calibration curve was calculated. From this an equation is generated which allows the lactate level of the samples to be determined (see figure 2.4).

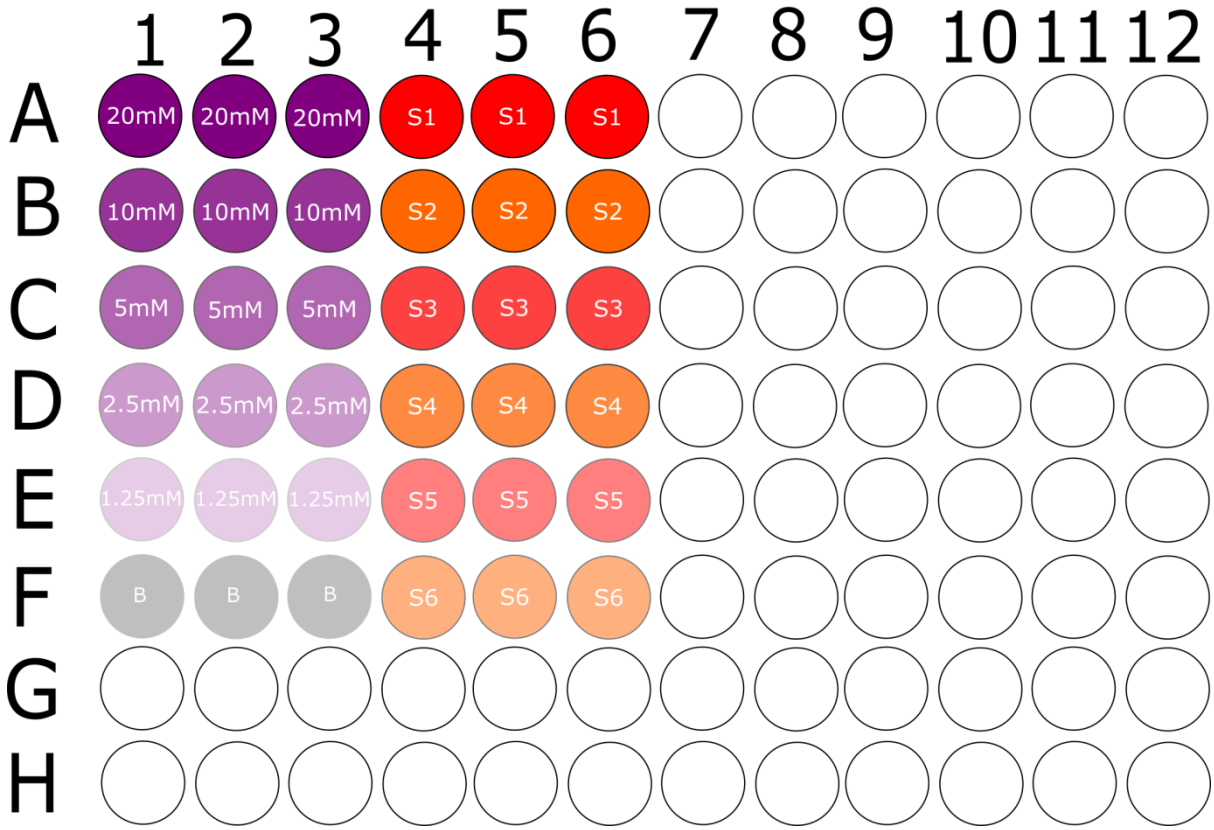

FIGURE 2.3 SET UP OF 96 WELL PLATE READER FOR L-LACTATE ASSAY.

(KEY: B=BLANK SAMPLE, S=SAMPLE REPEAT (E.G. S1=SAMPLE 1 REPEAT), PURPLE WELLS= CALIBRATION SOLUTIONS)

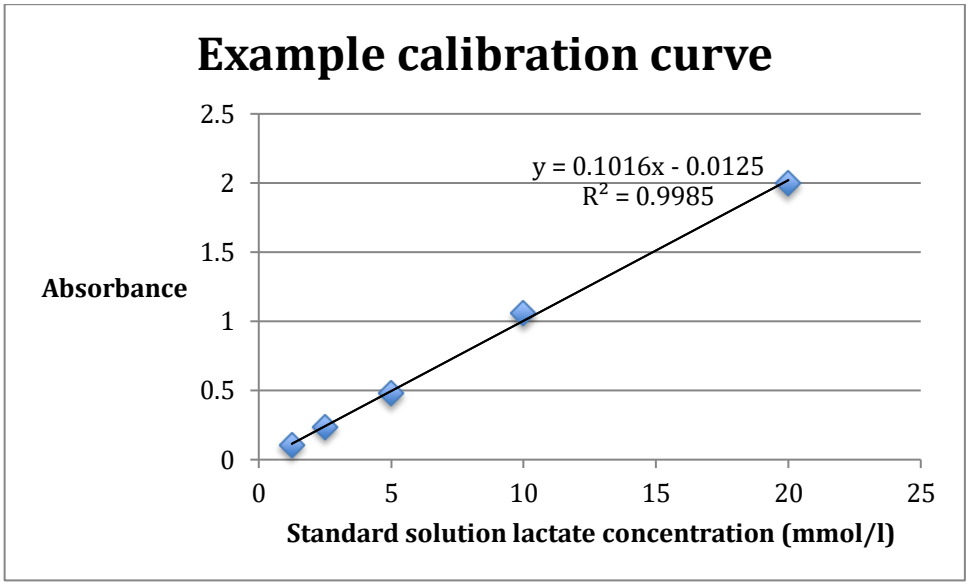

FIGURE 2.4 EXAMPLE CALIBRATION CURVE WITH EQUATION AND CORRELATION DISPLAYED ON CHART

2.2.2 Measuring pH

2.2.2.1 Omega PH-7011 Meter

This section provides information about the hand-held pH meter, the Omega PH-7011 used in chapters 3, 5 and 6.

The Omega PH-7011 meter (Omega Engineering, UK) is a handheld potentiometric electronic pH meter (see figure 2.5). Potentiometric pH meters work by measuring the voltage between 2 electrodes (the glass electrode and the reference electrode) and then convert the result into a pH value. Commonly the 2 electrodes are contained together in a combination electrode. The glass electrode (pH electrode) has a voltage that varies with the pH of the solution being measured. This electrode is a hydrogen ion sensitive glass bulb (often referred to as the glass electrode) with a millivolt output that varies with the relative change in hydrogen ion concentration inside and outside the glass bulb. The reference electrode output does not vary with differing hydrogen ion concentrations and gives a constant output. The potential between the 2 electrodes is therefore a measure of the number of hydrogen ions in the solution

which can then be converted into a pH value.

The Omega meter was chosen because it was portable, light-weight, easy to use and featured temperature compensation (temperature is known to affect the measurement of pH).

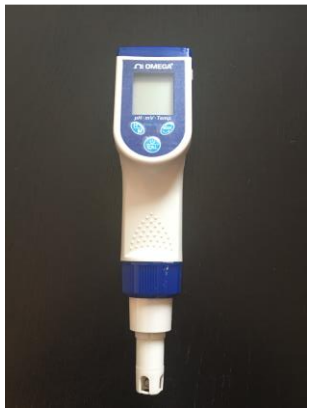

FIGURE 2.5 THE OMEGA PH-7011 METER

2.2.2.1.1 Omega PH-7011 Meter testing procedure

Before testing of a solution the meter must be calibrated. Calibration, testing and storage of the meter is carried out as follows:

1. Remove the protective cap and unscrew the soaking cap from the meter and rinse the electrode with clean water and wipe it dry.
2. Switch on the meter.
3. Dip the electrode into the buffer solution pH 7. Stir gently and wait until the display stabilises.
4. Press and hold the power/calibration button to enter calibration mode, the CAL icon appears and flashes 7.00
5. Hold electrode in the pH 7 solution until the display stops flashing and indicates “SA”, then “End”.
6. Rinse the electrode again in clean water and wipe dry.
7. Dip the electrode into buffer solution pH 4. Stir gently and wait until the display stabilises.

8. Press and hold the power/calibration button to enter calibration mode, the CAL icon appears and flashes 4.00
9. Hold the electrode in the pH 4 buffer solution until the display stops flashing and indicates "%" (percentage of slope), then "SA", then "End".
10. If the percentage of the slope (PTS) is between 70-130% calibration has been successful. A percentage < 70% or >130% indicates that the electrode needs to be replaced.
11. Rinse the electrode in clean water and wipe dry.
12. Dip the electrode into the sample solution to be measured. Stir gently and wait until a stable reading is obtained.
13. Once the pH of the solution has been recorded the electrode should be rinsed in clean water, wiped dry and then placed in in the protective cap containing storage solution.

The pH reading takes time "to settle" and does not beep or flash to indicate that a reading is complete. It was therefore decided to hold the meter in the sample for 2 minutes and use the reading displayed at 2 minutes as the pH of the solution. This time was chosen because on trialling the meter it was noted that the reading at 2 minutes was steady and keeping the meter submerged for longer only resulted in small changes of 0.01 every 30 seconds – 1 minute.

2.2.2.2 Mettler Toledo pH meter

The Mettler Toledo pH meter is a gold standard piece of laboratory equipment kept at the Wolfson Centre, University of Strathclyde, for measuring pH. It was used to test the pH of non-clinical samples in chapter 3 and a subset of clinical samples in chapter 6.

2.2.2.1.2 Mettler Toledo pH meter calibration and testing procedure

In accordance with the manufacturers instructions the meter must be calibrated with pH 4, 7 and 10 buffer solutions prior to use. The electrode has to be cleansed with deionised water between being placed in each buffer solution. Once calibration has been successfully completed the electrode is rinsed in deionised water then placed in the solution to be tested. The electrode is held in place until the meter indicates that the reading is complete and gives the pH value. The electrode is then rinsed in deionised water and placed in a pH 4

buffer solution for storage. See figure 2.6 for an image of the meter.

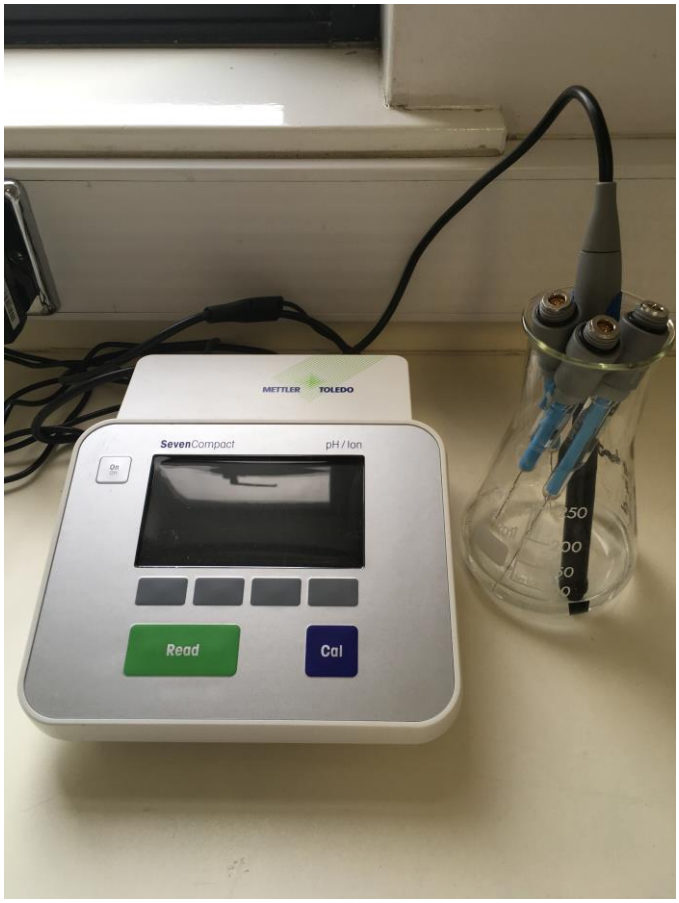

FIGURE 2.6 THE METTLER TOLEDO PH METER

Chapter 3 - The validation of handheld lactate and pH meters with standard laboratory solutions

3 Chapter 3 – The validation of handheld lactate and pH meters with standard laboratory solutions

3.1 Introduction

Previous studies that have looked at lactate and pH in human peritoneal fluid have used either microdialysis (Matthiessen et al., 2007b, Pedersen et al., 2009, Horer et al., 2011, Daams et al., 2014) or a blood gas analyser (ABL700 Blood Gas Analyser, Radiometer, Copenhagen, Denmark) (Bini et al., 2014) to measure lactate, whilst for pH tonometry catheters (Millan et al., 2006) and a bench top pH analyser (pH meter pp-15, Sartorius Ltd, Germany) (Yang et al., 2013) have been used. Initially the researcher had hoped to use the hospital blood gas analyser (GEM Premier 4000, Instrumentation Laboratory, Bedford, MA, USA) to measure lactate and pH for the work in chapters 3 -6. It offered the advantages of being readily available, simple to use and had been successfully used in a previous veterinary study for analysing peritoneal drain fluid samples. Via personal communication with the manufacturer it was confirmed that, although reference ranges for peritoneal fluid samples had not been created, running a peritoneal fluid sample through the machine was possible and would not damage it. Unfortunately the hospital laboratory was concerned that a peritoneal fluid sample may contaminate the machine and therefore did not grant permission for its use. Consequently the use of commercial and established handheld meters was investigated.

The use of handheld meters was investigated. Lactate is used in the sporting world as a guide to training and performance (I, 1986), this has lead to the development of a range of handheld lactate meters that can be used at the track side to give instant results from finger-prick blood samples. These meters are quick and simple to use. Use of these meters has also been described in the veterinary world (Nieto et al., 2015). Peritoneal fluid lactate is used in the diagnosis and management of suspected horse colic. A recent study has shown that handheld monitors such as the Lactate Pro, Lactate Plus and Lactate Scout

are reliable measures of horse peritoneal fluid lactate in the field when compared to standard bench top analysers and a gold standard laboratory assay (Nieto et al., 2015). In the study by Nieto et al(Nieto et al., 2015), the Lactate Pro performed best in the analysis of horse peritoneal fluid lactate. The Lactate Pro has been superseded by the Lactate Pro 2 (Arkray, Japan); this was selected as our handheld lactate meter. The Lactate Pro 2 (LP2) is designed primarily for whole blood samples in the range 0.5 – 25.0 mmol/L and so provision was made for calibration of this device for peritoneal fluid.

pH is measured in a range of situations – including medical scenarios, soil analysis and home beer brewing. As such there are a wide range of handheld pH meters available on the open market. The Omega PH-7011 (Omega Engineering, UK) was selected as it was easily portable and automatically compensated for temperature.

To date, neither the LP2 nor the Omega pH meter have been used to measure lactate or pH in human peritoneal fluid.

3.2 Aims

The aim of this chapter was to assess the intra-analyser variability of the Lactate Pro 2 and Omega PH-7011 meters and to compare their performance to that of a laboratory gold standard method of measurement of lactate and pH.

3.3 Methods

3.3.1 Validation of the Lactate Pro 2

3.3.1.1 Intra-analyser variability of the Lactate Pro 2 – testing standard solutions

To test the intra-analyser variability of the Lactate Pro 2 standard solutions of "high", "medium" and "low" lactate concentrations were created. A 20mmol solution of sodium lactate was prepared and then serially diluted with distilled water (by the method outlined in section 2.2.2) to produce lactate solutions of concentration 10mmol, 5mmol and 2.5mmol. Each of these solutions was then measured 10 times with the Lactate Pro 2 using a single batch of test strips (A173B01L) to determine variability. The standard deviation, mean and coefficient of variation were then calculated. A coefficient of variation is a statistical test that has ben used to assess the variability of quantitative assays and intra-analyser performance (Reed et al., 2002). There are no absolute agreed cut-offs for an acceptable level of variation but a low percentage variation indicates low variability (Spiegel, 1961) and indicates that a test is reliable. In addition, the impact of different batches of Lactate Pro 2 test strips was analysed by testing with 2 different batches (serial numbers A173B01L and J163B01L). A paired T-test performed to look for differences between different batches of strips. Data was analysed using 'Statistical Package for the Social Sciences' (SPSS) version 22. $P < 0.05$ was considered statistically significant.

3.3.1.2 Comparison of the Lactate Pro 2 to laboratory gold standard – testing standard solutions

A 20mmol solution of sodium lactate was prepared and then serially diluted with distilled water (by the method outlined in section 2.2.2) to produce lactate solutions of concentration 20mmol, 10mmol, 5mmol, 2.5mmol and 1.25mmol. Each solution was measured in triplicate with the Lactate Pro 2 and in triplicate by a colorimetric method on the 'Labsystems Multiskan Plate Reader' using an L-lactate assay (method as described in section 2.2.2). A scatter graph was drawn to compare the results with the 2 analysers and a Pearson correlation coefficient

was calculated to look for agreement between the 2 meters. The graphs were drawn and Pearson correlation coefficient calculated using Excel, 2011.

3.3.2 Validation of the Omega PH-7011 Meter

3.3.2.1 Intra-analyser variability of the Omega PH-7011 Meter

A standard pH 7 buffer solution was prepared by adding one pH 7 tablet (Fischer Scientific general purpose buffer tablets) to 100ml distilled water. Prior to use the Omega PH-7011 meter was calibrated via the method outlined in section 2.2.3.2. A total of 10 serial measurements were made of this pH 7 solution using the Omega PH-7011 Meter. The standard deviation, mean and coefficient of variation were then calculated. Data was analysed using SPSS version 22. $P < 0.05$ was considered statistically significant.

3.3.2.2 Comparison of the Omega PH-7011 Meter to the laboratory gold-standard Mettler Toledo pH meter – standard solutions

The pH 4, 7 and 10 buffer solutions were prepared as per section 2.2.4.1. They were measured in triplicate on the Omega PH-7011 Meter (as per the method in section 2.2.3.2) and on the Mettler Toledo pH Meter (as per the method described in section 2.2.4.1). A scatter graph was drawn to compare the results with the 2 analysers and a Pearson correlation coefficient was calculated to look for agreement between the 2 meters. The graphs were drawn and Pearson correlation coefficient calculated using Excel, 2011.

3.4 Results

3.4.1 Validation of the Lactate Pro 2

3.4.1.1 Intra-analyser variability and accuracy of the Lactate Pro 2

Table 3.1 shows the results for the repeated measurement of low, medium and high concentrations of sodium lactate with the Lactate Pro 2 (LP2) meter. It was noted that the LP2 meter was not accurate in measurement of the standard solutions as it consistently over-estimated the lactate level of the standard solutions by just over double the concentration of the standard solution.

However, variations in readings made by the LP2 was minimal. The coefficient of variation for low, medium and high concentrations were 5.97%, 3.29% and 2.03% respectively. These low values indicate that the Lactate Pro 2 produces consistent measurements of lactate at a range of concentrations. As shown in figure 3.1 there was good correlation (R=0.99) between measurements on the Lactate Pro 2 versus the colorimetric assay, the linear equation was:

$$Y = 0.4227x - 0.0282,$$

This can be used to calculate the true lactate concentration based on the measurements made on the Lactate Pro 2.

TABLE 3.1 INTRA-ANALYSER VARIABILITY AT 10MMOL, 5MMOL AND 2.5MMOL CONCENTRATION OF SODIUM LACTATE

	Repeat number												
Lactate conc (mmol/l)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**SD**	**Mean**	**Coefficient of variation (%)**
10	24.6	23.1	23.4	23.2	23.1	23.6	23.5	23.7	22.9	23.3	0.48	23.4	2.03
5	12.6	12.5	13.8	12.6	12.7	12.3	12.7	12.5	12.6	12.4	0.42	12.7	3.29
2.5	5.8	5.7	6.3	5.2	5.4	5.4	5.2	5.4	5.5	5.4	0.33	5.5	5.97

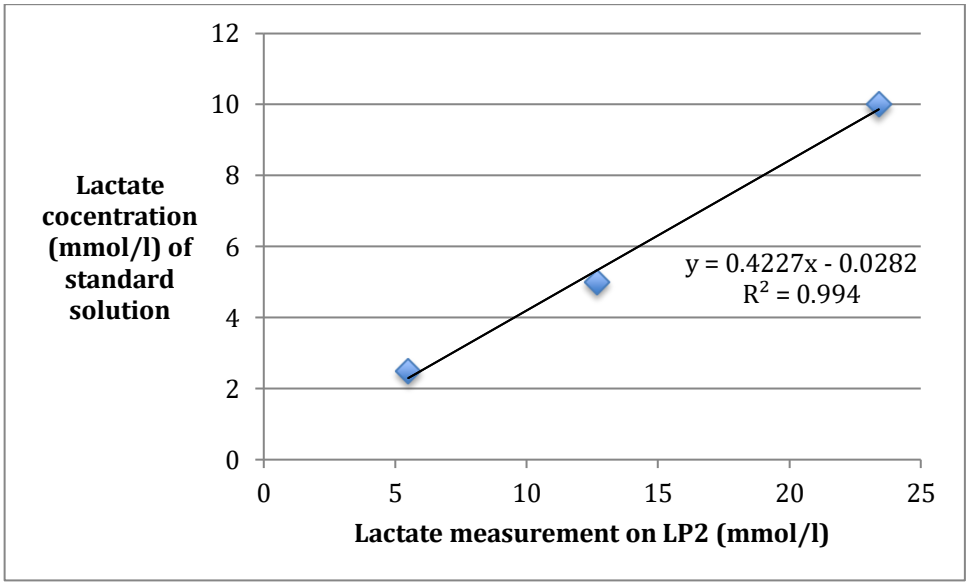

FIGURE 3.1 CORRELATION BETWEEN LACTATE CONCENTRATION MEASURED BY LP2 METER AND COLORIMETRIC ASSAY

Table 3.2 displays the serial measurements made of low and medium concentrations of sodium lactate with 2 different batches of test strips – A173B01L and J163B01L. For a medium concentration (5 mmol) the A73B01L group had a mean of 12.7 (SD 0.42), the J163B01L group had a mean of 12.2 (SD 0.44); there was no significant difference between the 2 groups (p = 0.12). At lower concentration (2.5mmol) the A73B01L group had a mean of 5.5 (SD 0.33) and the J163B01L group had a mean of 5.4 (SD 0.18), the difference between the groups was not statistically significant (P = 0.15).

TABLE 3.2 COMPARISON OF DIFFERENT BATCHES OF TEST STRIP AT 5MMOL & 2.5MMOL CONCENTRATION OF SODIUM LACTATE

		Repeat number												
Serial number	**Lactate conc (mmol/l)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**SD**	**Mean**	**P value**
A173B01L	5	12.6	12.5	13.8	12.6	12.7	12.3	12.7	12.5	12.6	12.4	0.42	12.7	
J163B01L	5	6.5	11.5	12.1	12.6	11.9	12.3	12.4	12.3	11.9	13.0	0.44*	12.2*	0.12
A173B01L	2.5	5.8	5.7	6.3	5.2	5.4	5.4	5.2	5.4	5.5	5.4	0.33	5.5	
J163B01L	2.5	5.7	5.5	5.6	5.4	5.2	5.1	5.3	5.5	5.3	5.4	0.18	5.4	0.15

3.4.2 Validation of the Omega PH-7011 Meter

3.4.2.1 Intra-analyser variability of the Omega PH-7011 Meter

Table 3.3 displays the results of the serial measurements made on the Omega PH-7011 Meter. It displayed low intra-analyser variability with excellent repeatability of measurements. The coefficient of variation was 0.47% which is very low.

TABLE 3.3 RESULTS OF OMEGA PH-7011 METER SERIAL MEASUREMENTS OF PH 7 SOLUTION

	Repeat number												
pH meter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**SD**	**Mean**	**Coefficient of variation (%)**
Omega	7.27	7.33	7.35	7.35	7.34	7.36	7.37	7.38	7.38	7.39	0.03	7.35	0.47

3.4.2.2 Comparison of the Omega PH-7011 Meter to the laboratory gold-standard Mettler Toledo pH meter – standard solutions

Table 3.4 shows the measurements made on the Omega and Mettler pH meters of standard buffer solutions of pH 4, 7 and 10. The readings made on the Omega pH meter were slightly lower than on the Mettler Toledo Meter. However, as shown in figure 3.2 there is good correlation (R = 0.99) between the results made on the two meters, the linear equation was:

$$Y = 0.9757x + 0.2687.$$

This can be used to calculate the true pH based on the measurement made on the Omega PH-7011 Meter.

TABLE 3.4 MEASUREMENTS OF PH 4, 7 AND 10 BUFFER SOLUTIONS ON THE OMEGA AND METTLER PH METERS

	Omega PH-7011 Meter readings				**Mettler Toledo Meter readings**			
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**
pH 4	3.87	3.85	3.84	3.85	3.99	4.03	4.02	4.01
pH 7	6.91	6.96	6.97	6.95	7.07	7.09	7.08	7.08
pH 10	9.98	10.01	10.03	10.01	10.04	10.00	10.03	10.02

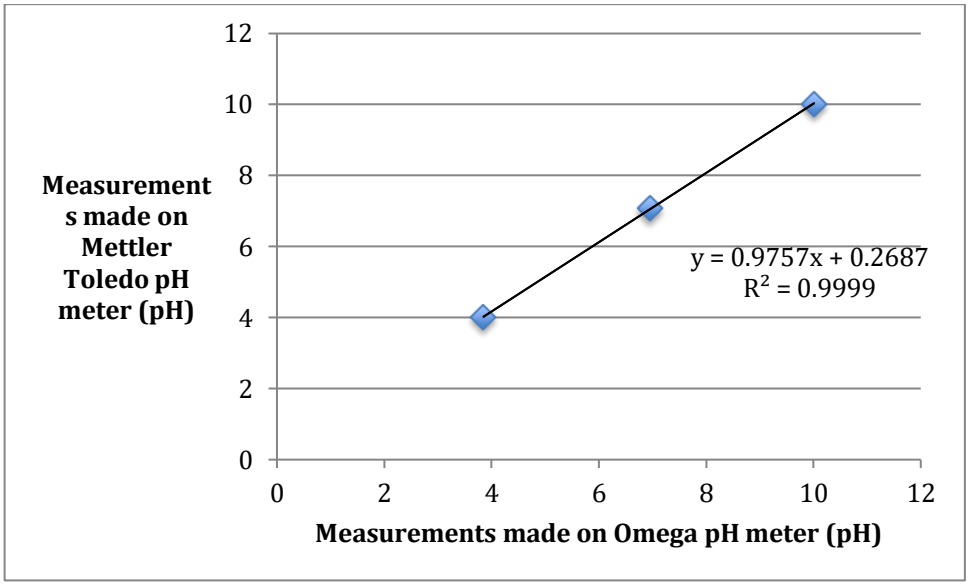

FIGURE 3.2 CORRELATION BETWEEN PH MEASUREMENTS OF STANDARD BUFFER SOLUTIONS MADE ON THE OMEGA AND METTLER TOLEDO PH METERS

3.5 Discussion

The LP2 and Omega pH meters had low intra-analyser variability and provided reliable measurements of lactate and pH respectively. However, both meters lacked accuracy when compared to laboratory gold standard meters.

The LP2 consistently over-estimated the lactate level of a standard solution by a factor of just over 2. The results were consistent and it was shown that they had good correlation with the results of the colorimetric assay (R = 0.99). The linear equation generated can thus be used to calculate the true lactate level of a sample based on the measurement made by the LP2. The LP2 is an ampometric meter and uses lactate oxidase to react with the sample. The laboratory gold standard is a colorimetric assay that also uses lactate oxidase to react with the sample. Therefore the discrepancy in the results between the LP2 and the colorimetric assay is not due to the use of different reagents. The LP2 was designed to measure lactate concentration of whole blood samples. Previous studies have validated its performance against bench top lab blood gas analysers in measuring samples of whole blood (Tanner et al., 2010, Pyne et al., 2000). They have found it be reliable and accurate across a range of lactate

concentrations. It was not tested against laboratory solutions of sodium lactate. It may therefore be that the specific design of the LP2 to measure unfiltered whole blood samples accounts for the differences seen when testing the laboratory standard solutions. Despite its lack of accuracy, the LP2 does provide reliable results with low levels of intra-analyser variability making it a valid choice for assessing change in lactate levels in a clinical setting. This study was performed in order to assess the potential for the LP2 to be used to measure lactate from human peritoneal fluid samples in the work carried out in chapters 4 and 6. There is no documentation in the literature of the LP2 having been used in this manner before. It has however been successfully used to measure the lactate level in other in-vivo settings (e.g. peritoneal fluid from horses). In the proposed work in chapters 3 and 6 no clinical decisions would be made based on the lactate measurements. Trends in lactate concentration and changes over time rather than absolute cut-off levels were anticipated to be more important. Consequently, the finding that the LP2 could provide reliable results was sufficient to consider it a satisfactory substitute for measurement on a standard laboratory analyser.

The Omega pH meter slightly under-estimated the pH of standard buffer solutions compared to the Mettler Toledo Meter. However, like the LP2, it had low intra-analyser variability and good correlation with the Metter Toledo meter ($R = 0.99$). Both pH meters featured temperature compensation and measured pH by the same method (see sections 2.2.3.1 & 2.2.4.1). The Omega pH meter and probe were stored in storage solution as per the manufacturers instructions and calibration was completed successfully before use. During calibration a slope percentage is calculated and a value outwith 70 – 130% indicates that the electrode has expired. Prior to testing the slope percentage fell within these limits indicating satisfactory condition of the electrode. It is therefore difficult to explain the difference between the results of the Omega and Mettler Toledo pH meters. However, as mentioned above this work was undertaken to assess the potential of the Omega pH meter to be used for the measurement of human peritoneal fluid, clinical decisions would not be based on the results and trends in pH and changes over time were considered more important than absolute cut-off levels. This is similar to the approach taken to the interpretation of post-operative blood tests, for example, a declining CRP and WCC, rather than a drop

to below a set cut-off value, in a patient who has received treatment for an infective complication would be interpreted as a good response to treatment.

In conclusion, based on the findings of this chapter the handheld meters, LP2 and Omega PH-7011, were judged to be valid analysers for the measurement of lactate and pH from peritoneal fluid. The linear equations generated by their comparisons to laboratory gold-standard meters in section 3.4 should be used to adjust the results obtained.

Chapter 4 - The stability of lactate in peritoneal fluid

4 Chapter 4 – The stability of lactate in peritoneal fluid

4.1 Introduction

Several of the studies that have attempted to find a local, intra-abdominal biomarker of anastomotic leak have looked at biomarkers of intestinal ischaemia (Matthiessen et al., 2007b, Pedersen et al., 2009, Horer et al., 2011, Bini et al., 2014, Daams et al., 2014). The confirmed relationship between lactate and tissue hypoxia has lead to lactate being a key target for much of this research. The majority have used microdialysis (Matthiessen et al., 2007b),(Pedersen et al., 2009),(Horer et al., 2011),(Daams et al., 2014) which provides a real-time measurement of lactate. However, this technique is expensive and technically challenging. Bini et al explored lactate by measuring the lactate level of drain fluid (Bini et al., 2014). They describe obtaining a sample of abdominal fluid from the drainage bag and then immediately analysing it using an ABL700 blood gas analyser (Radiometer, Copenhagen, Denmark). This represents a simpler, cheaper and non-invasive method by which to measure lactate as a biomarker of intestinal ischaemia/AL.

When measuring substances from fluid in a peritoneal drain it is necessary to consider the stability of the substance in the fluid. In clinical practice the fluid collected in a drainage bag is emptied at set intervals, commonly once per day just before the clinical ward round allowing drain volume over a 24 hour period to be calculated and interpreted clinically. This means that fluid often sits in a drainage bag for several hours. This may modify biomarkers in drain fluid as occurs with a blood sample.

When a whole blood sample is drawn glycolysis continues, in part due to the fact that erythrocytes do not have mitochondria (Astles R, 1994). This means that over time the lactate level of the sample will increase. Noordally et al found that when blood samples were stored at room temperature lactate levels increased from 2.36mol/l +/- 1.68mmol/l to 2.52mmol/l +/- 1.74mmol/l over 10 minutes. Whilst this was a statistically significant increase, the clinical

significance of this is debatable (Noordally and Vincent, 1999). Calatayud et al (Calatayud and Tenias, 2003) found that by 15 minutes at room temperature lactate levels increased by > 0.2mmol/l which they considered to be clinically significant. The rate of increase in lactate was significantly associated with the white cell count (WCC) of the sample (the higher the WCC then quicker the rate of rise). They found that when the samples were stored on ice there was no significant change in lactate over 15 minutes. Astles et al (Astles R, 1994) demonstrated that the rise in lactate could be mitigated by the addition of the antiglycolytic agents sodium fluoride and potassium oxalate; their addition also prevented the increase seen with samples which had a leucocytosis. The results of these studies has lead to the general guidance that when measuring the lactate level in blood the analysis should be performed within 15 minutes or if a prolonged delay is expected then the sample should be placed in a tube with fluoride oxalate.

To date, no studies have explored the effect of time on the lactate level of peritoneal drain fluid. This is an important question if we are to attempt to use lactate from drain fluid samples to aid detection of AL. Fluid in the drain is likely to have a different cellular makeup compared to whole blood. There will be fewer red blood cells and the fluid may be diluted by the remnants of saline wash used at the end of the operation to irrigate the abdomen. Fewer red blood cells may mean less glycolysis and therefore a less pronounced change in lactate levels over time. In recently published work, a group aiming to develop a lactate sensor for anastomotic leak found wide variability in lactate concentrations in peritoneal fluid. They specifically cited the stability of lactate in peritoneal fluid as a potential causative factor and recommended it be explored (Hirst, 2014).

4.2 Chapter 4 aim

The aim of the study was to investigate the effect of time on lactate levels in peritoneal fluid from the abdominal cavity.

4.3 Use of ascitic fluid as surrogate of drain fluid

Gaining a fresh sample of peritoneal fluid in a post-operative patient presents difficulties. For example, sampling the first 10mls that enter a drainage bag will not yield a truly fresh sample as it will have taken a variable amount of time to travel down the drainage tubing into the bag which can typically be 20cm or more in length. The speed of this will be affected by the position of the patient, position of the drain, diameter of the drainage tubing, the viscosity of the fluid and the speed at which it flows through the tube. The alternative would be to obtain a sample of free fluid under radiological guidance. However, this involves an invasive procedure under local anaesthetic in which a needle is inserted into the abdominal cavity under ultrasound (US) or CT guidance. Targeting a small pool of fluid comes with the risk of damaging surrounding structures such as bladder, bowel and blood vessels within the abdomen. A procedure under CT guidance would also expose the patient to ionising radiation. The patient would also experience some discomfort during the procedure. In addition, it is not possible to use healthy subjects as a control because there is normally only about 100mls of fluid in the peritoneal cavity which would be extremely challenging to sample even under a radiologically guided procedure.

Patients with ascites (a term for the pathological accumulation of free fluid within the peritoneal cavity due to a wide range of causes (see table 4.1) (Chapman RW, 2006) can frequently have up to 10 – 20 litres of excess fluid within their abdominal cavity. This can cause symptoms such as discomfort and respiratory difficulties. Consequently, many of these patients require elective placement of a peritoneal drain as a therapeutic procedure to drain the excess fluid. Insertion of a drain is typically performed at the bedside with local anaesthetic and does not require exposure to ionizing radiation. This group of patients would therefore represent a surrogate sample group. Whilst the fluid may have a slightly different composition to that in a post-operative abdomen, obtaining a sample of fluid at the time of insertion of a peritoneal drain represented a practical means of obtaining a fresh sample of fluid from the peritoneal cavity for immediate analysis without the need for an additional/unnecessary procedure to the patient. Between 10 – 30% of patients

admitted to hospital with ascites due to cirrhosis have infected ascites (spontaneous bacterial peritonitis, SBP) (Piddock et al., 2000). This is detected by a high WCC in a sample of their peritoneal fluid. Peritoneal fluid in the abdomen following colorectal surgery frequently contains bacteria (Komen et al., 2009), this is likely due to overspill from the colon when it is divided and then anastomosed. Therefore, a significant proportion of the group of patients having elective drainage of ascites will potentially have peritoneal fluid similar to patients in the post-operative setting, making them a useful surrogate group.

TABLE 4.1 CAUSES OF ASCITES

Common causes	**Other causes**
Malignant disease (hepatic or peritoneal)	Infection (e.g. spontaneous bacterial peritonitis, TB)
Cardiac failure	Hypoproteinaemia (e.g. nephrotic syndrome)
Hepatic cirrhosis	Hepatic venous occlusion (e.g. Budd-Chiari syndrome)
	Pancreatitis
	Lymphatic obstruction
	Rare causes (e.g. Meigs' syndrome, Vasculitis, renal dialysis, hypothyroidism)

4.4 Methods

Ethical approval was granted by the Wales REC 7 NHS Ethics Committee (reference 16/WA/0142, see appendix 1) and approval from the NHS Greater Glasgow & Clyde (NHS GGC) Research and Development Department (reference GN16SG091, see appendix 2) in May 2016 to perform serial lactate and pH measurements on peritoneal fluid obtained from patients who were admitted to the Gastroenterology Ward at The Royal Alexandra Hospital (RAH), Paisley, for drainage of excess peritoneal fluid (ascites) over an 8 month period (July 2016 – March 2017). The full study protocol is available in appendix 3.

Patients were eligible if they were over 18 years old and had capacity to consent. Patients known to have blood borne viruses (HIV, hepatitis B, hepatitis C) were excluded. The clinical team looking after the patient (liver nurse specialist and doctors from the gastroenterology department) informed the researcher about potential patients who met the criteria. The researcher then screened their medical record to confirm eligibility. A patient information sheet (PIS) (appendix 4) was provided, if they were willing to participate written consent (appendix 5) was obtained. The researcher then attended the ward when the clinical team inserted the peritoneal drain. Routinely a small sample is sent to the lab for cell count and culture and then several litres are drain over a 24 hour period and discarded. At the time the drain was inserted 20mls of fluid was collected in a 20ml syringe and given to the researcher. This was placed into a universal white top container and stored at room temperature. The Lactate Pro 2 was then used to measure lactate levels within 5 minutes, at 1 hour, 2 hours and 24 hours after the sample was taken (as per the method in section 2.2.1.4). The linear equation generated in section 3.4:

$$Y = 0.4227x - 0.0282,$$

was applied to calculate the true lactate concentration. Once the experiment was complete the sample of peritoneal fluid was discarded via the hospital clinical waste stream. Patient demographics, underlying diagnosis and blood and peritoneal fluid tests taken by the clinical team on the day of drain insertion were recorded and stored under an anonymous study number.

Statistical analysis

Statistical analysis was carried out on SPSS (version 22). Median and interquartile ranges were calculated for lactate levels and a Sign test used to test for differences in lactate over time from paired samples. $P < 0.05$ was considered significant.

Clinical significance

There is no standard consensus in the literature about what represents a clinically significant change in peritoneal lactate. In the paper by Calatayud et al (Calatayud and Tenias, 2003), which looked at the effect of time on the lactate concentration of whole blood, they considered a change of > 0.2mmol/l to be clinically significant. In the absence of other evidence in the published

literature, a pragmatic decision was taken to use the same cutoff for clinical significance in the present study.

4.5 Results

Over an 8 month period a total of 20 patients were identified, screened and recruited to the study. The majority of patients included in the study were male (90%), the median age was 59 (46 – 69 years) and the most common underlying diagnosis was alcoholic liver disease (ALD) (see table 4.2). Three of the 20 patients (numbers 8, 9 and 17) had a fluid WCC count > 250 indicative of spontaneous bacterial peritonitis (SBP). However, only 2 (patients 8 and 17) had a positive bacterial culture, both growing the bacteria E. coli in cultures of their peritoneal fluid.

TABLE 4.2 PATIENT CHARACTERISTICS

Characteristic	**Number (%)**
Sex	
Male	18 (90)
Female	2 (10)
Age	59 (46 – 69)*
Underlying diagnosis	
Alcoholic liver disease (ALD)	17 (85)
Malignancy	2 (10)
ALD & malignancy	1 (5)
Peritoneal fluid WCC	
WCC < 250	17 (85)
WCC > 250**	3 (15)
Fluid bacterial culture result	
Negative	18 (90%)
Positive	2 (10%)

** Age represented as median and interquartile range*

***WCC > 250 indicative of spontaneous bacterial peritonitis (SBP)*

Table 4.3 shows the results for the lactate analysis from the 20 patients. Figure 4.1 illustrates the trend in lactate levels over time in patients who had all 4 data points collected. The change in lactate over time was small. The median change at 1 hour was 0 mmol/l. At 2 and 24 hours the median change was 0.1 mmol/l (see table 4.4). The change at 1 and 2 hours was considered statistically significant ($P < 0.05$) but it was not a clinically significant change (i.e. not > 0.2 mmol/l). The change in lactate at 24 hours was not statistically or clinically significant. No patient had a significant decline in lactate over time. Although the change in lactate concentration over time was small there are two outliers – patients 8 and 17 who had larger increases in lactate at 24 hours (see table 4.3 and figure 4.1). These were the two patients with positive bacterial cultures from their peritoneal drain fluid.

TABLE 4.3 LACTATE OVER TIME RESULTS

						Lactate (mmol/l)			
Patient no.	**Sex**	**Age**	**Diagnosis**	**Fluid WCC (cells/mm^3)**	**Bacterial culture**	**5 mins**	**1hr**	**2hrs**	**24hrs**
1	M	57	Malignancy	0	Negative	3.2	*	2.9	3.1
2	M	70	Malignancy	86	Negative	2.3	*	2.3	2.6
3	M	46	ALD	114	Negative	3.0	2.9	2.9	*
4	M	46	ALD	88	Negative	3.1	3.0	3.1	3.0
5	M	65	ALD	100	Negative	2.7	2.7	2.6	2.6
6	M	46	ALD	5	Negative	4.1	3.8	3.9	3.5
7	M	46	ALD	92	Negative	*	3.9	4.2	4.1
8	M	64	ALD	2768	Positive	2.8	2.7	2.9	4.3
9	M	59	ALD	332	Negative	1.5	1.4	*	1.5
10	F	76	ALD	110	Negative	1.3	1.3	1.3	1.2
11	F	76	ALD	0	Negative	1.2	1.0	0.9	0.9
12	M	65	ALD	88	Negative	1.6	1.6	1.5	1.5
13	M	59	ALD	128	Negative	1.7	1.7	1.6	1.7
14	M	46	ALD	50	Negative	5.8	5.6	5.7	5.8
15	M	59	ALD	180	Negative	1.7	1.7	1.7	*
16	M	71	ALD	106	Negative	1.5	1.5	1.4	1.5
17	M	70	ALD & malignancy	5020	Positive	3.5	3.5	3.5	5.1
18	M	44	ALD	74	Negative	1.8	1.8	1.7	1.8
19	M	57	ALD	126	Negative	1.2	1.2	1.2	1.2
20	M	60	ALD	56	Negative	1.5	1.5	1.4	1.4

** missing data due to protocol deviations; shaded data = those with positive bacterial cultures*

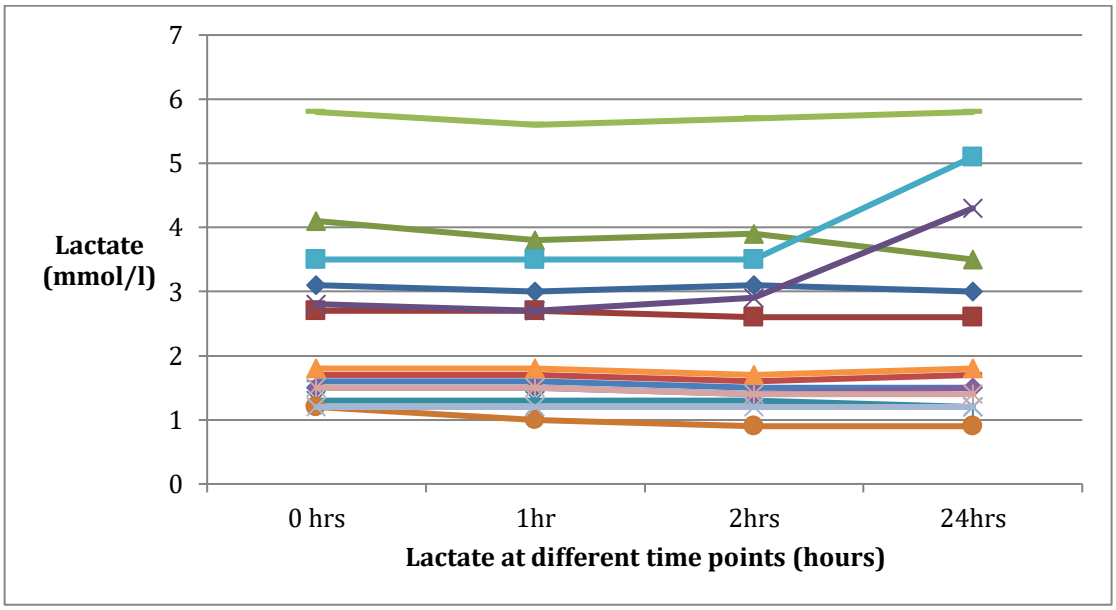

FIGURE 4.1 CHANGE IN LACTATE OVER TIME IN EACH PATIENT WITH COMPLETE DATA SET. EACH LINE REPRESENTS AN INDIVIDUAL PATIENT; THE PURPLE AND BLUE LINES WITH AN UPWARD TREND REPRESENT PATIENTS 8 AND 17 RESPECTIVELY.

TABLE 4.4 THE MEDIAN CHANGE IN LACTATE LEVELS WITH TIME

	Lactate level (mmol/l)		
Timing of sample (hours)	**Median (IQ range)**	**Median change (IQ range)**	**P value (Sign test)**
0	1.8 (1.5 - 3.1)		
1	1.8 (1.5 - 3.1)	0.0 (0 - 0.1)	0.008
2	2.3 (1.4 - 3.1)	0.1 (0 - 0.01)	0.021
24	2.2 (1.4 - 3.7)	0.1 (0 - 0.3)	0.077

4.6 Discussion

At room temperature lactate levels show a small change over time in peritoneal fluid. The median change is not clinically significant within the first 24 hours and statistically the changes are considered significant at 1 and 2 hours but not at 24 hours. This is at odds with the clinical picture. It is likely that these statistical results are the consequence of the small sample size and therefore more emphasis should be placed on the clinical significance (this has been discussed with a statistician, personal correspondence).

The finding that lactate levels in peritoneal fluid rise slightly over time is in keeping with the previous work in whole blood (Noordally and Vincent, 1999, Calatayud and Tenias, 2003, Astles R, 1994). However, the rise occurs more slowly in peritoneal fluid as clinically significant changes occurred at 15 minutes in whole blood. This is likely due to the different cellular make up of the fluid, with peritoneal fluid containing fewer red blood cells (RBCs) and white blood cells (WBCs). The reference range for RBCs in whole blood from the local hospital lab is 4.5 – 6.5 x 10^{12}/L for males and 3.8 – 5.8 x 10^{12}/L for females whereas peritoneal fluid usually contains few RBCs, with < 100/µl being considered normal. As previously mentioned RBCs lack mitochondria so generate energy via glycolysis thus generating lactate, less glycolysis may help explain the slower rise in lactate levels in peritoneal fluid over time. WBCs will also act as reservoirs for glycolysis. The normal WBC in blood in adult humans is 4.0 – 11.0 x 10^{9}/L. Again the WBC of peritoneal fluid is lower with < 250 cells/mm^3 (which equates to 0.25 x 10^{9}/L) being considered normal. Calatayud et al(Calatayud and Tenias, 2003) observed that a higher WBC count in blood was associated with a quicker rise in lactate. Our findings are in keeping with this as the 2 patients who had the greatest change in lactate over 24 hours – patient 8, 2.8 mmol/l to 4.3 mmol/l, and patient 17, 3.5 mmol/l to 5.1 mmol/l are the only patients with a peritoneal fluid WCC > 1000/mm^3 and the only 2 to have positive fluid cultures growing E. Coli. Although the sample size is small, the findings in these two patients in particular highlight that peritoneal fluid behaves differently in the presence of infection, even though at the time neither patient had clinical signs of sepsis such as abdominal pain or pyrexia. This lends support to the theory that local measurable changes which reflect the overall disease state occur early in peritoneal and therefore analysis of the peritoneal environment

following creation of an anastomosis may provide an earlier warning of an AL than clinical assessment alone.

A limitation of this study is the use of peritoneal fluid from patients with ascites as a surrogate for peritoneal fluid from the post-operative abdomen. It is possible that the peritoneal fluid present post-operatively may have a different cellular composition, e.g. higher levels of RBCs, WBCs and the presence of bacteria. During the operation there will inevitably be some bleeding and it is unlikely that the saline wash used at the end of the operation will remove all the blood. If the post-operative fluid were therefore to contain a higher concentration of RBCs than ascitic fluid then it could be postulated that lactate levels will be less stable and rise more quickly. A study by Komen et al(Komen et al., 2014a) looking at the presence of bacteria in post-operative drain samples as a marker of AL found that E. Coli and E. Faecalis were frequently cultured from patients who did and did not develop AL. This indicates that bacteria will commonly be found in peritoneal fluid in the post-operative environment. This would likely have an impact on the stability of lactate – as demonstrated by the finding in our study that the 2 patients with E. Coli cultured from their fluid had a large increase in lactate levels at 24 hours whereas those who were bacterial culture negative had a median change of < 0.1 mmol/l. As a future piece of work it would be useful to compare the RBC count, WBC count and bacterial culture results of samples of peritoneal drain fluid to those in our study population.

A further limitation is the sample size. Over the duration of the study there were certainly more than 20 eligible patients. However, the ethics committee stipulated that patients had to be identified by the clinical team and the researcher then informed, the researcher was not permitted to screen admissions to the gastroenterology ward. Owing to admissions out of hours, weekends and frequent changes of medical staff on the gastroenterology ward eligible patients were frequently not highlighted to the researcher. It is not possible to ascertain how many eligible patients were excluded.

Despite these limitations, the study still has unique and useful findings. It is the

first to explore the stability of lactate in peritoneal fluid finding that over time, unless the fluid has a high WBC concentration, the lactate concentration remains fairly constant and the minor changes that do occur are not clinically significant. In those with a high WBC and positive bacterial cultures (patients' 8 and 17) by 24 hours lactate concentration had markedly increased and this increase was considered clinically significant. Given the likelihood that bacteria will be found in the peritoneal fluid of patients following colorectal surgery this has important implications for the interpretation and exploration of lactate as a local biomarker of anastomotic leak. The one study that had looked at lactate measured from a peritoneal drain made no mention of the timing of sample collection(Bini et al., 2014). On the basis of the findings in this study it is clear that the timing of sample collection could have impacted on the results. Future studies looking at lactate from peritoneal drain fluid samples as a marker of AL should aim to collect as fresh a sample as possible. A suggested strategy would be to empty the drain and then obtain a sample from the drain within 2 hours and measure lactate immediately. Within 2 hours one would expect enough fluid to gain a sufficient sample and our findings illustrate that, even in the presence of a high WBC count and positive bacterial cultures, clinically relevant changes in lactate concentration are unlikely to have occurred within this timeframe.

Chapter 5 - The stability of pH in peritoneal fluid

5 Chapter 5 – The stability of pH in peritoneal fluid

5.1 Introduction

As outlined previously (section 1.8.4.2.2.1) pH is a marker of hypoxia. Its exploration in relation to assessment of the viability of gastroesophageal anastomoses has lead to it being explored as a biomarker for colorectal anastomotic leak (Millan et al., 2006, Yang et al., 2013).

As concluded in chapter 4, analysis of fluid from a peritoneal drain following surgery is a simple and non-invasive means of assessing the milieu of a colorectal anastomosis. Yang et al (Yang et al., 2013) also took advantage of this method and measured the pH from pelvic drain fluid in the days following anterior resection for rectal cancer. They found that a pH lower than 6.978 on post-operative day 3 had a sensitivity of 98.7% and specificity of 94.7% for predicting AL. This is a potentially exciting finding. However, the exact timing of the drain fluid sampling is not stated and this may have important implications for the clinical applicability of the results. Simmen et al (Simmen and Blaser, 1993) measured the pH from peritoneal fluid obtained at the time of laparotomy or post-operatively via a passive drainage system. They found that a 2 hour delay in analysis of the sample resulted in an average change in pH of 0.5%. No comment was made as to the statistical or clinical significance of this in their study but it does provide evidence that pH may change over time in a peritoneal fluid sample. No other studies have addressed this issue in peritoneal fluid but it has been studied in pleural fluid analysis where pH has been found to change over time. pH is commonly measured in pleural fluid samples with an acidic pH considered a marker of infection(Maskell and Butland, 2003). Rahman et al (Rahman et al., 2008) defined a change in pH of 0.05 as being clinically significant. In 92 samples of pleural fluid they found a clinically significant change in pH in 13% at 1 hour, 26% at 4 hours and in 68% of samples at 24 hours. The difference was statistically significant at 4 and 24 hours. These results highlight the importance of investigating the stability of pH in peritoneal fluid. If significant changes occur over time then there are important implications for

protocols for fluid sampling, handling and interpretation of results. Furthermore, in working towards creation of a pH biosensor for AL an understanding of its stability in peritoneal fluid is required to inform the design of this technology.

5.2 Aims

The aim of the study was to determine the effect of time on pH levels of peritoneal fluid from the abdominal cavity.

5.3 Methods

This study was carried out in conjunction with the study in chapter 4 looking at the effect of time on lactate levels. The use of ascitic fluid as a surrogate for peritoneal fluid from post-operative patients is described in section 4.3.

pH levels were concurrently measured from the same peritoneal fluid samples. The ethical and R&D approvals and method of patient identification, sample collection and disposal for this study are identical to those described in section 4.4.

pH specific methods:
The pH measurements were made on the Omega PHH-7011 meter. The meter was calibrated daily with pH 7 and 4 buffer solutions and measurements made as per the protocol in section 2.2.3. The linear equation generated in section 3.4:

$$Y = 0.9757x + 0.2687,$$

was applied to calculate the true pH level.

Statistical analysis
Statistical analysis was carried out on SPSS (version 22). Median and interquartile ranges were calculated for pH values and a Sign test used to test for differences in pH levels over time from paired samples. $P < 0.05$ was considered significant.

Clinical significance

There is not a universally agreed definition of a "clinically significant change in pH". In looking at pleural fluid Rahman et al(Rahman et al., 2008) considered a change of 0.05 to be significant. However, in a trial published in 'The Lancet' Gutierrez et al used gastric pH monitoring as a guide to management of patients in the Intensive Care Unit (ICU) and used 0.10 as a cut-off for a clinically significant change (Gutierrez et al., 1992). At pH 7.00 a change of 0.05 and 0.10 represent changes of 0.73% and 1.43% respectively. Both represent very small changes, a less than 1% change is perhaps too small to be reliably detected so in this study it was decided to use 0.10 as a marker of clinical significance.

5.4 Results

A total of 20 eligible patients were recruited to the study. The demographics of the patients and their pH results are displayed in table 5.1. The majority were male (90%) and had a diagnosis of alcoholic liver disease (ALD) (90%). Three of the 20 patients (numbers 8, 9 and 17) had a fluid WCC count > 250 which is indicative of spontaneous bacterial peritonitis (SBP). However, only 2 of them (patients 8 and 17) had a positive bacterial culture growing E. coli in their peritoneal fluid.

Table 5.1 includes the results for the pH analysis from the 20 patients. Patient 8 did not have a 24 hour pH recorded as the meter developed an error and could not be calibrated, this also precluded measurement of pH in patients 9 – 12 until the issue was resolved. Protocol errors resulted in pH measurements being missed at 1 hour in patients 1 and 2 and at 24 hours in patients 3 and 15. One patient was excluded – patient 6 who had an initial pH of 9.17, this is likely a measurement error. Figure 5.1 shows the change in pH over time in patients who had a full data set collected. The overall median pH at the time of drain insertion was 7.66 (IQR 7.56 – 7.73) and at 1 hour, 2 hours and 24 hours was 7.75 (IQR 7.69 – 7.83), 7.81 (IQR 7.69 – 7.88) and 7.98 (IQR 7.85 – 8.11) respectively. pH rose steadily over time in patients with negative bacterial cultures. However, in the 2 patients with a high WCC and positive bacterial cultures this was not the case. Patients 8 and 17 showed a decrease in their pH over time. In patient 8 pH

at 1 (7.52) and 2 hours (7.57) was lower than the starting pH of 7.92 (24 hour pH was not recorded). In patient 17 the initial pH was 7.58; there was a small rise to 7.65 at hours 1 and 2 and then it fell slightly to 7.63 at 24 hours. The median overall change in pH at 1 hour is 0.11, at 2 hours is 0.17 and at 24 hours is 0.37 (table 5.2). These changes are all considered to be statistically and clinically significant.

TABLE 5.1 PH RESULTS

Patient no.	Sex	Age	Diagnosis	Fluid WCC (cells/mm^3)	Bacterial culture	pH 0	pH 60 min	pH 120 min	pH 24hrs
1	M	57	Malignancy	0	Negative	7.52	*	7.66	7.98
2	M	70	Malignancy	86	Negative	7.47	*	7.69	7.91
3	M	46	ALD	114	Negative	7.45	7.71	7.84	*
4	M	46	ALD	88	Negative	7.70	7.76	7.95	8.13
5	M	65	ALD	100	Negative	7.67	7.68	7.81	7.85
6	M	46	ALD	5	Negative	9.22	7.82	8.07	8.21
7	M	46	ALD	92	Negative	*	7.56	7.81	7.71
8	M	64	ALD	2768	Positive	7.92	7.52	7.57	*
9	M	59	ALD	332	Negative	*	*	*	*
10	F	76	ALD	110	Negative	*	*	*	*
11	F	76	ALD	0	Negative	*	*	*	*
12	M	65	ALD	88	Negative	*	*	*	*
13	M	59	ALD	128	Negative	7.61	7.76	7.86	7.98
14	M	46	ALD	50	Negative	7.70	7.88	7.95	8.15
15	M	59	ALD	180	Negative	7.72	7.83	7.87	*
16	M	71	ALD	106	Negative	7.88	8.00	8.05	8.28

17	M	70	ALD & malignancy	5020	Positive	7.58	7.65	7.65	7.63
18	M	44	ALD	74	Negative	7.75	7.83	7.88	8.05
19	M	57	ALD	126	Negative	7.61	7.68	7.71	7.87
20	M	60	ALD	56	Negative	7.65	7.75	7.80	7.98

**missing data*

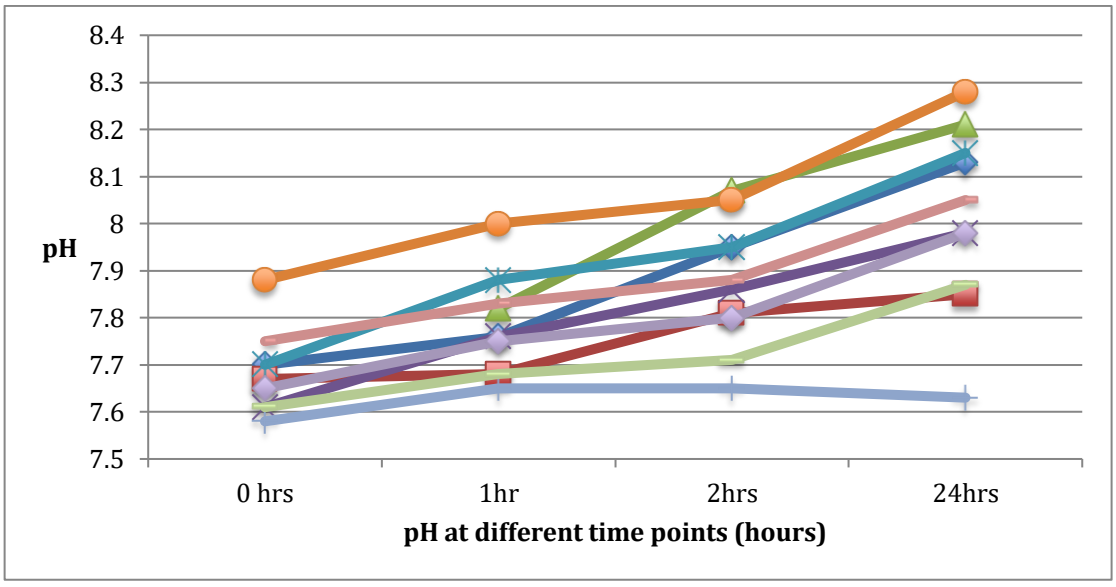

FIGURE 5.1 CHANGE IN PH OVER TIME FOR PATIENTS WITH COMPLETE DATA SETS; EACH COLOURED LINE REPRESENTS AN INDIVIDUAL PATIENT.

TABLE 5.2 THE MEDIAN CHANGE IN PH LEVELS WITH TIME

	pH		
Timing of sample (hours)	**Median (IQ range)**	**Median change (IQ range)**	**P value (Sign test)**
0	7.66 (7.56 – 7.73)		
1	7.75 (7.69 – 7.83)	0.11 (0.08 – 0.18)	0.006
2	7.81 (7.69 – 7.88)	0.17 (0.14 – 0.24)	0.002
24	7.98 (7.85 – 8.11)	0.37 (0.26 – 0.44)	0.001

5.5 Discussion

The median changes of 0.11 at 1 hour, 0.17 at 2 hours and 0.37 at 24 hours represent a 1.4%, 2.2% and 4.8% change from the baseline respectively. These changes were all statistically significant. However, as previously discussed there is no universally agreed cut-off for a clinically significant change in pH in the literature. It has not been investigated in peritoneal fluid but based on work in pleural and gastric fluid we considered a change of 0.1 to be clinically significant. Although by this definition the median change at 1 hour of 0.11 is technically clinically significant it represents a small (1.4%) change from the baseline and overall it was thought that this may not prove to be truly clinically significant.

In our study pH generally increased over the 24 hour period. However, as in chapter 4, patients 8 and 17, who grew E. Coli in their peritoneal fluid, behaved differently. They did not show a continual increase in pH over time. This is most likely due to the high levels of bacteria in their fluid causing a high level of metabolic activity which lowers the pH. This will counteract the factors discussed later in this section which generally led to a rise in pH over time in our samples. In pleural fluid, a low pH is considered a marker of infection. The

finding that pH, along with lactate in chapter 4, behaved differently in these two patients adds further support to the theory that investigation of peritoneal fluid surrounding an anastomosis may yield early clues as to the health of the anastomosis.

On immediate testing of the fluid sample the median pH was 7.66 (IQ range 7.56 – 7.73). No standard accepted reference range for peritoneal fluid pH exist. This is largely due to the difficulty in sampling "normal" peritoneal fluid in healthy individuals. A study by Noh et al (Noh, 2003) attempted to answer this question. They measured the pH of fluid in the subhepatic space on opening the abdomen of patients undergoing surgery for non-serosal invasive gastric adenocarcinoma without know ascites or metastatic disease, the assumption being that this would represent "normal peritoneal fluid". They sampled 134 patients and found the mean pH to be 7.73 with a range of 7.46 - 8.10. In another study, Simmen et al (Simmen et al., 1994) measured pH in samples from peritoneal fluid drains of patients making an uncomplicated recovery from abdominal surgery and recorded a median pH of 7.49 with range 7.04 – 7.94. Our findings are concordant with these two previous studies.

The stability of pH in peritoneal fluid has only been mentioned briefly once before in the literature. Simmens et al commented that a delay in analysis of pH from peritoneal fluid taken intra-operatively or post-operatively from a peritoneal drain resulted in a change of 0.5%. They do not display the data, provide ranges or state whether this was an increase or decrease in pH. However, as outlined in section 4.3 stability of pH in pleural fluid has been studied with the finding that pH generally increased over time. The mean difference at 1, 4 and 24 hours was 0.01, 0.03 and 0.05 respectively. This is a smaller difference than in our study (0.11, 0.17 and 0.37 at 1, 2 and 24 hours respectively). One possible explanation for this difference is the exposure of our samples to air. Samples were collected in a 20ml syringe which had had the air expelled but the samples were then stored in white topped universal container. Whilst the container was air tight the container was not completely full and therefore would have contained air. In addition, when the samples were retested the containers were reopened thus providing additional exposure to air. Simmens et al looked at the impact of exposure of the pleural fluid samples to

air and found that air exposure lead to a mean increase in pH of 0.08 (95% C.I. 0.06 – 0.09). They also measured partial pressure of O_2 ($p0_2$) and partial pressure of CO_2 (pCO_2) and found that the change in pH with the presence of air was correlated to a change in CO_2. They suggested that the mechanism for a rise in pH with exposure to air might be that the gradient between the partial pressure of CO_2 in pleural fluid and air (much higher partial pressure in pleural fluid) would result in a rapid diffusion of CO_2 through the fluid-air interface, thus raising the pH of the fluid. This may explain why our samples had a larger increase in pH over time. In future work it would be useful to measure the PO_2 and PCO_2 levels of the samples in an effort to elucidate the mechanism by which pH rises over time.

A limitation of this study is the small sample size owing to missing data. Unfortunately prior to measuring the 24 hour pH from patient 8 the meter developed an error – it could not be calibrated. This also precluded measurement of pH in patients 9 – 12. It transpired that the fault developed because the probe was not being kept in enough storage fluid. Once this was identified and resolved pH measurements were resumed for the remainder of patients recruited. The 1 hour pH measurements were missed in patients 1 and 2 and the 24 hour measurements missed in patients 3 and 15 due to protocol errors. The researcher was reliant upon members of the gastroenterology team. One person was also trained to measure samples. However, on occasion due to ward pressures it was not possible to complete measurements at the correct time thus resulting in several missing results.

This study is the first to document the stability of pH in peritoneal fluid over a 24 hour period. On the basis of these results, future work looking at the pH of peritoneal fluid samples should aim to measure the peritoneal fluid within 1 hour of sampling and to minimise exposure of the sample to air.

Chapter 6 - The serial measurement of lactate and pH from peritoneal drain fluid as a marker of colorectal anastomotic leak

6 Chapter 6 – The serial measurement of lactate and pH from peritoneal drain fluid as a marker of colorectal anastomotic leak

6.1 Introduction

As outlined in chapter 1, anastomotic leak (AL) following colorectal surgery leads to significant morbidity, mortality and poorer oncological outcomes. A range of pre-, intra- and post-operative risk factors have been identified but as yet there is no reliable test to accurately detect or predict AL. Recent work exploring the utility of biomarkers from the perianastomotic environment was appraised in chapter 1 and in a literature review published by the researcher (Wright et al., 2017). Lactate and pH emerged as the most promising biomarkers of anastomotic leak. Studies exploring lactate and pH have consisted of small, heterogeneous cohorts, used variable definitions of AL, different end points and widely differing measurement techniques and protocols.

Several studies used microdialysis to measure lactate (Matthiessen et al., 2007b, Pedersen et al., 2009, Horer et al., 2011, Daams et al., 2014). This can be technically difficult and connection of a patient to a microdialysis circuit may make mobilisation in the early post-operative period challenging. 'Enhanced recovery after surgery' (ERAS) protocols, a key component of which is early mobilisation, are considered the standard of care following colorectal surgery (Varadhan et al., 2010). This challenges the applicability of microdialysis as a means by which to monitor the anastomotic environment and detect AL. The use of peritoneal drains following colorectal surgery used to be routine but this is now debated. A Cochrane review found no reduction in the rate of AL when a drain was placed (Karliczek et al., 2006). There was some suggestion that drains may have a role in infra-peritoneal rectal anastomoses, but further trials are needed to clarify this. Despite this, many surgeons still place pelvic or abdominal drains on an individual basis allowing researchers the potential to serially measure lactate and pH in peritoneal fluid.

The future may lie in the use of biosensor technology. Screen-printed sensors can be created at a low cost and are capable of measuring samples with volumes as small as several microlitres (Ahmed et al., 2016). Creation of such a sensor for lactate/pH is a realistic aim. This could be incorporated into a tiny removable piece of tubing. Furthermore, wireless technology creates the possibility of producing a biodegradable implant that could be fixed around the anastomosis and remotely signal information about the perianastomotic environment. Such a device may be several years away but the first stage in its conception and development is to determine what biomarker the sensor should aim to measure. As discussed lactate and pH appear to show the greatest potential. The work of Yang et al in relation to pH is potentially exciting but requires confirmation. For lactate, given the limitations of the studies exploring it, further work is also necessary to confirm its utility in relation to the detection of AL in a larger cohort of patients using a standardised definition of AL. Clarification of the utility of lactate and pH will help to direct development of a biosensor device for the detection of AL. Measurement of lactate and pH via peritoneal drain fluid, whilst not likely to be a long term recommendation, offers a simple and convenient way to measure these biomarkers for the purposes of confirming their clinical utility prior to biosensor development.

The work in chapters 3 and 4 demonstrate that lactate and pH are stable over short period of time in peritoneal fluid. The finding of a clinically significant rise in lactate over a 24 hours period in patients who were found to have SBP provides further evidence that measurement of local biomarkers may provide useful insights into the milieu of the peritoneal cavity.

6.2 Aims

The primary aim of the study was to determine if the serial measurement of lactate or pH from intra-abdominal drains in the post-operative period could predict colorectal anastomotic leak. The secondary outcome was the ability of lactate or pH to predict other complications following colorectal surgery, this

has not previously been explored and the potential to identify other intra-abdominal complications early is an exciting prospect.

6.3 Methods

Ethical approval was granted by the NHS Leicester Central Research Ethics Committee (reference 16/EM/0394, see appendix 6) in September 2016 to measure lactate and pH levels in peritoneal drain fluid in patients following colorectal surgery. The study was also approved by the NHS Greater Glasgow & Clyde Research and Development Department (reference GN16GA471, see appendix 7), September 2016. The full study protocol is available in appendix 8.

All patients attending the Royal Alexandra Hospital (RAH) over a 12.5 month period from October 15th 2016 to October 31st 2017 for elective colorectal surgery, for both benign and malignant disease, with the pre-operative intention of creation of an anastomosis or leaving a stapled off segment of bowel within the abdominal cavity (e.g. a stapled off rectal stump in a Hartmann's procedure) were eligible. Potential patients were identified by the researcher via the weekly colorectal team multidisciplinary team meeting (MDT) and via consultant electronic operation lists. Ultimately the decision regarding the creation of an anastomosis or decision to leave a stapled off segment of bowel within the abdomen was made by the individual consultant responsible for the patient's surgery. If intra-operative findings or events meant that the decision whether or not to perform an anastomosis changed, patients were eligible and included in the study based on the pre-operative planned operation on an intention to treat basis. Patients were excluded if they were under 18 years of age or were known to have a blood-borne virus (e.g. HIV, hepatitis B, hepatitis C). Patients were given an information sheet (appendix 9) prior to surgery and signed a consent form (appendix 10) before their operation. Placement of a peritoneal drain, and the subsequent timing of its removal, was at the discretion of the operating surgeon. If a peritoneal drain was inserted fluid was taken daily as per the drain sampling protocol from post-operative day 1 until removal of the drain.

Drain sampling protocol

Drains were routinely emptied on the ward at 6am each day. A fresh sample was then taken within 2 hours for measurement of lactate and pH. This time frame was chosen based on the findings in this thesis. Obtaining a peritoneal fluid sample and storing it at room temperature in a universal white topped container was thought to be analogous to a sample of fluid sitting in a drainage bag outwith the abdominal cavity. In chapter 5 it was recommended that pH was sampled and analysed within an hour. The pH meter required a minimum of 3mls for analysis. Following drain emptying it was observed that within 1 hour the volume of fluid that had drained into the bag was frequently insufficient for pH analysis, however by 2 hours the volume was sufficient. As demonstrated in section 4.5 lactate levels would be expected to be stable within 2 hours. Therefore, to ensure a sufficient volume of fluid was available for analysis a pragmatic decision was taken to obtain samples for pH and lactate analysis within 2 hours of the drain being emptied each day.

Lactate was measured with the Lactate Pro 2 and pH with the Omega PHH-7011 meter (see sections 2.2.1.4 and 2.2.3.2 for protocols for testing using the handheld meters). The linear equations generated in section 3.4.1.1 for correcting lactate and pH measurements were applied to determine absolute peritoneal values.

Validation of the handheld lactate and pH meters for the measurement of human peritoneal drain fluid

In chapter 3 the 'Lactate Pro 2' (LP2) and the 'Omega PHH-7011' meters were validated against laboratory meters in measuring lactate and pH from standard solutions. Previous work demonstrated that the LP2 had been successfully used to measure lactate from the peritoneal fluid of horses (Nieto et al., 2015). The Omega pH meter had not previously been used in a clinical context. Given that this was the first time that both meters were to be used to measure human peritoneal fluid it was decided that a subset of peritoneal fluid samples should be tested in duplicate on the handheld meters and on laboratory gold standard meters to ensure external validation.

A total of 9 samples of human peritoneal drain fluid were transferred to Strathclyde University for analysis. Following measurement of lactate and pH on handheld meters 10mls of peritoneal drain fluid was placed in a universal white-topped container. This was then placed in a protective secondary pack and then an outer rigid pack in line with the guidelines for the transport of category B biological substances (Agents, 2005). The specimen was then immediately transported from The Royal Alexandra Hospital to the biomedical engineering laboratory in The Wolfson Centre at the University of Strathclyde. Upon arrival at the lab the sample was removed from its protective container. It was then divided into 2 equal measures. One was used to measure lactate on a colorimetric assay on a 'Labsystems Multiskan Plate Reader' using an L-lactate assay (via the method outlined in section 2.2.2). The other half of the sample was then used to measure pH on the Mettler Toledo Meter in triplicate via the methods outlined in sections 2.2.4.1. Once testing was complete the samples were disposed of in line with University Guidance for the handling and disposal of clinical samples via the University clinical waste stream (see appendices 11 – 13 for the Standard Operating Procedures (SOPs) and Control of Substances Hazardous to Health (COSHH) assessments and protocols that applied to the testing and handling of the clinical samples).

Data collection

Patient demographics, underlying diagnosis and results of routine post-operative blood tests (e.g. WCC, CRP, albumin) performed by the clinical team were recorded. Patients were followed for 30 days post-operatively; complications and 30 day mortality were recorded.

Definition of anastomotic leak

Anastomotic leak was defined and graded as per the 'International Study Group on Rectal Cancer' as, "a defect of the intestinal wall at the anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments. Grade A anastomotic leakage results in no change in patients' management, whereas grade B leakage requires active therapeutic intervention but is manageable

without re-laparotomy. Grade C anastomotic leakage requires re-laparotomy" (Rahbari et al., 2010). If clinically an AL was suspected, a CT scan was performed. These were reported by a Consultant Radiologist. The following are recognised features of an AL on CT: the presence of perianastomotic air, perianastomotic collection and a defect in staple-line integrity. Where a left sided anastomoses has been created the use of rectal contrast increases the diagnostic confidence (Kaur et al., 2014).

Definition of a post-operative complication

The definition of Dindo et al of a post-operative complication as, "…any deviation from the normal postoperative course", was used (Dindo et al., 2004). Dindo describes a classification of complications - 'The Clavien-Dindo Classification' which grades complications from 1 – 5 based on increasing severity. Complications of all grades were recorded.

Statistical analysis

(i) Prediction of AL and post-operative complications

Data are presented as median and interquartile range. A Mann-Whitney test was used to identify significant differences in the lactate and pH of those who did and did not develop AL or other complications. A chi squared test was used to look for significant differences in rate of complications based on whether patients had a drain inserted or not. P values of < 0.05 were considered statistically significant. Analysis was performed on SPSS version 24 for Mac.

(ii) Validation of the handheld lactate and pH meters for measuring human peritoneal drain fluid

A scatter graph was drawn to compare the results with the handheld meters vs. the standard laboratory meters and a Pearson correlation coefficient was calculated to look for agreement. The graphs were drawn and Pearson correlation coefficient calculated using Excel, 2011.

A power calculation was not performed. The study was a preliminary, exploratory study to determine if the work of a previous large study of peritoneal fluid pH could be replicated, and to determine if lactate measured directly from the perianastomotic peritoneal fluid via a drain had any correlation to AL. The aim was to guide and direct future work towards the develop a biosensor for anastomotic leak rather than to determine exact cut-off values of lactate and pH to use for clinical decision making. The time-frame available for the study was limited and it was anticipated that fewer than 100 patients could be recruited. However, it was felt even with a smaller cohort meaningful trends could be identified to guide future biosensor development.

6.4 Results

A total of 66 patients were recruited (see figure 6.1). One patient was excluded because following recruitment they were deemed to be unfit to proceed with surgery. A further 7 were excluded as they ultimately underwent an abdominoperineal resection (APR) and therefore did not have an anastomosis or a rectal stump in situ. In some cases the decision was taken intra-operatively and in others the plan was changed after consent for the study had been obtained. As shown in table 6.1, the majority of patients were female (51.7%), aged < 65 (51.7%), with a BMI > 25 (58.6%), ASA grade II (63.8%), had an underlying diagnosis of cancer (55.2%), had an open operation (72.4%) and had a left sided resection (65.5%). The majority of those with cancer did not have neoadjuvant chemotherapy (93.5%), 15.5% had a covering stoma and 6.9% had an end stoma. Most patients (55.2%) had a drain inserted and were discharged in under 7 days (60.3%). The 30 day mortality and complication rates were 1.7% and 36.2% respectively (see table 6.1). There were no anastomotic leaks.

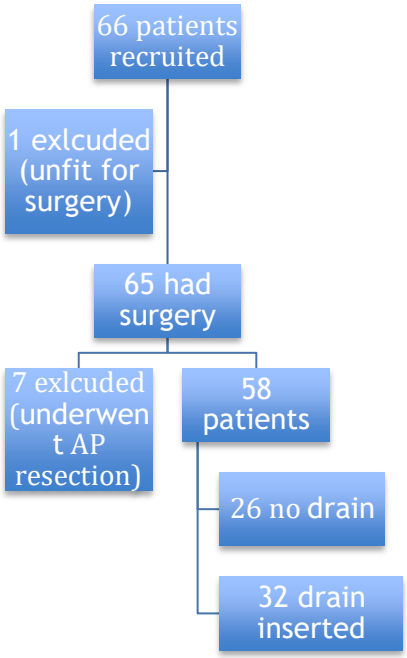

FIGURE 6.1 CONSORT DIAGRAM OF RECRUITMENT

TABLE 6.1 PATIENT DEMOGRAPHICS – ALL PATIENTS (N=58)

Variable	Number (%)
Age	
< 65	30 (51.7)
≥65	28 (48.3)
Gender	
Male	28 (48.3)
Female	30 (51.7)
BMI	
< 25	24 (41.4)
25-29	17 (29.3)
≥30	17 (29.3)
ASA grade	
I	7 (12.1)
II	37 (63.8)
III	13 (22.4)
IV	1 (1.7)
Diagnosis	
Cancer	32 (55.2)
IBD	11 (19)
Diverticular disease	9 (15.5)
FAP	2 (3.4)
Dysplastic polyp	4 (6.9)
Open v lap	
Open	42 (72.4)
Lap	16 (27.6)
Operation	
Right hemicolectomy	15 (25.9)
Anterior resection	23 (39.7)
Hartmanns	2 (3.4)
Reversal of Hartmanns	2 (3.4)
Sigmoid colectomy	1 (1.7)
Reversal ileostomy	3 (5.2)
Ileoanal pouch	1 (1.7)

Subtotal colectomy & end ileostomy	1 (1.7)
Subtotal colectomy & ileorectal anastomosis	1 (1.7)
Completion proctectomy & end ileostomy	1 (1.7)
Completion proctectomy & ileoanal pouch	1 (1.7)
Left hemicolectomy	3 (5.2)
Ileocolic resection	1 (1.7)
Resection ileocolic anastomosis	1 (1.7)
Total colectomy and ileorectal anastomosis	1 (1.7)
En bloc ileocolic and rectosigmoid resection	1 (1.7)
Stoma formation	
No	45 (77.6)
Yes – covering stoma	9 (15.5)
Yes – end stoma	4 (6.9)
Neoadjuvant treatment	
No	30 (51.7)
Yes	2 (3.4)
N/A	26 (44.8)
30 day mortality	
No	57 (98.3)
Yes	1 (1.7)
30 day complication	
No	37 (63.8)
Yes	21 (36.2)
Drain inserted	
No	26 (44.8)
Yes	32 (55.2)
Day of discharge	
< POD 7	35 (60.3)
≥ POD 7	23 (39.7)

The demographics of the patients who did and did not have a drain inserted is shown in table 6.2. Although the difference was not statistically significant, patients were more likely to have a drain inserted if they had left-sided surgery compared to those having right-sided surgery (63.2% vs. 40%, P = 0.092).

TABLE 6.2. DEMOGRAPHICS OF PATIENTS WITH/WITHOUT A DRAIN

	No drain (n=26)	Drain (n=32)	
Variable	**Number (%)**		**P value**
Age			
< 65	11 (36.7)	19 (63.3)	
≥65	15 (53.6)	13 (46.4)	0.291
Gender			
Male	12 (42.9)	16 (57.1)	
Female	14 (46.7)	16 (53.3)	0.798
BMI			
< 25	13 (54.2)	11 (45.8)	
25-29	9 (52.9)	8 (47.1)	
≥30	4 (23.5)	13 (76.5)	0.110
ASA grade			
I	2 (28.6)	5 (71.4)	
II	18 (48.6)	19 (51.4)	
III	6 (46.2)	7 (53.8)	
IV	0 (0)	1 (100)	0.618
Diagnosis			
Cancer	17 (53.1)	15 (46.9)	
IBD	3 (27.3)	8 (72.7)	
Diverticular disease	3 (33.3)	6 (66.7)	
FAP	0 (0)	2 (100)	
Dysplastic polyp	3 (75.0)	1 (25.0)	0.211
Open v lap			
Open	18 (42.9)	24 (57.1)	
Lap	8 (50)	8 (50)	0.625
Operation			
Right hemicolectomy	12 (80)	3 (20)	
Anterior resection	9 (39.1)	14 (60.9)	
Hartmanns	0 (0)	2 (100)	
Reversal of Hartmanns	1 (50)	1 (50)	
Sigmoid colectomy	1 (100)	0 (0)	
Reversal ileostomy	0 (0)	3 (100)	

Ileoanal pouch	0 (0)	1 (100)	
Subtotal colectomy & end ileostomy	(0)	1 (100)	
Subtotal colectomy & ileorectal anastomosis	0 (0)	1 (100)	
Completion proctectomy & end ileostomy	0 (0)	1 (100)	
Completion proctectomy & ileoanal pouch	0 (0)	1 (100)	
Left hemicolectomy	2 (66.7)	1 (33.3)	
Ileocolic resection	0 (0)	1 (100)	
Resection ileocolic anastomosis	0 (0)	1 (100)	
Total colectomy and ileorectal anastomosis	(0)	1 (100)	
En bloc ileocolic and rectosigmoid resection	1 (100)	0 (0)	
Covering stoma			
No	24 (50)	24 (50)	
Yes	2 (20)	8 (80)	0.083
Neoadjuvant treatment			
No	17 (56.7)	13 (43.3)	
Yes	0 (0)	2 (100)	
N/A	9 (34.6)	17 (65.4)	0.110
30 day mortality			
No	26 (45.6)	31 (54.4)	
Yes	0 (0)	1 (100)	
30 day complication			
no	17 (45.9)	20 (54.1)	
yes	9 (42.9)	12 (57.1)	0.820

Morbidity and mortality

At 30 days, the majority of patients were still alive (98.3%) and had recovered without any complications (63.8%). One post-operative death occurred from

acute renal failure on post-operative day 1. Table 6.3 displays the complications experienced and whether or not the patients had a drain inserted. There was no significant difference in the incidence of complications between those with and without a drain (P = 0.82). There was no significant difference in the rate of non-infective vs infective complications in those with and without a drain (non-infective: P = 0.199 and infective: P = 0.098). There were a total of 21 complications, 5 infective and 16 non-infective complications. There were no anastomotic leaks.

TABLE 6.3. COMPLICATIONS SUFFERED IN ALL PATIENTS (WITH/WITHOUT DRAIN)

Complication	Drain inserted		Totals	P value
	No	Yes		
None	17	20	37	
Any complication	9	12	21	0.82
Infective complication	4	1	5	0.098
LRTI	1	0	1	
UTI	0	1	1	
Wound infection	2	0	2	
Gram negative sepsis	1	0	1	
Anastomotic leak	0	0	0	
Non-infective complication	5	11	16	0.199
Renal failure	0	1	1	
Ileus	2	5	7	
AF	2	0	2	
N&V	0	1	1	
Heart block & perforated stress ulcer	1	0	1	
Pancreatitis	0	1	1	
Wound dehiscence	0	1	1	
SVT	0	1	1	
Death	0	1	1	

Diagnostic potential of drain lactate & pH

As previously stated the decision to insert a drain and the timing of its removal was at the discretion of the operating surgeon. Of the 32 patients who had a drain inserted, the majority (78.1%) had it removed on POD3 (see table 6.4) meaning that it was possible to measure lactate and pH in 32 patients on POD1, in 29 on POD2 and in 25 on POD3. Table 6.5 shows the actual number of lactate

and pH measurements that were made on POD 1-3 for all patients. Of a total of 172 possible measurements, 154 (89.5%) were made and 18 (10.5%) were missed. The reasons for missing data points were as follows: 12 protocol deviations, 5 due to meter errors and 1 due to insufficient drain sample for analysis. Of the 12 protocol deviations, 6 were due to the drain not being emptied in the morning and the other 6 were due to the researcher being unexpectedly unable to attend to collect the samples (due to illness).

TABLE 6.4. WHEN POST-OPERATIVE DRAINS WERE REMOVED

Day of drain removal (total n = 32)	**Number**
POD 1	3
POD 2	4
POD 3	25

TABLE 6.5. NUMBER OF LACTATE AND PH MEASUREMENTS THAT WERE MADE

POD	**Number of patients with a drain**	**Number of lactate measurements**	**Number of pH measurements**	**Number of measurements missing**
1	32	29	30	5
2	29	24	25	9
3	25	23	23	4
Total		76	78	18

When lactate measurements were made for the first 5 patients the Lactate Pro 2 consistently read them as "high" i.e. greater than 25 mmol/l which was the upper limit of the device. This was unexpected given that when lactate was measured in chapter 4 the readings on the LP2 using peritoneal fluid, prior to applying the correction, were between 2.1 – 13.9 mmol/l. An adjustment was therefore made to the protocol to dilute the samples with distilled water in a 50:50 ratio, 3mls of peritoneal fluid was mixed with 3mls of distilled water. Although this would affect the values obtained for lactate concentration it would still allow a change in concentration to be detected. As previously

mentioned in section 3.5, it is thought that the change, rather than absolute concentration of lactate may be more important in detecting AL and other complications. This modification allowed lactate to be measured on the Lactate Pro 2. The correction generated in section 3.4.1.1 was then applied. This reduced the number of lactate measurements available for analysis to 28 on POD1, 25 on POD2 and 22 on POD3. Measurement of pH was not affected.

Primary outcome – drain lactate and pH as a predictor of anastomotic leak

There were no anastomotic leaks detected within 30 days in any of the 58 patients. It was therefore not possible to assess the primary aim of the study.

Secondary outcome – drain lactate and pH as a predictor of complications

Table 6.6 shows the peritoneal drain fluid lactate levels on PODs 1-3 from all patients. There was wide variability in lactate levels ranging from 0.7 – 10.5mmol/l. In those without complications, the median lactate levels rose from POD 1 (5.4 mmol/l) to POD 3 (7.3 mmol/l) (see table 6.7) but, as shown in table 6.6 and the boxplots in figure 6.2, there was wide variability in the measurements from each patient with some falling and others rising on PODs 2 and 3 compared to POD 1. In those with complications the median lactate level rose from 4.7 mmol/l on POD 1 to 7.6 mmol/l on POD 2 then fell to 5.4 mmol/l on POD 3 but again there is wide variability in the results for individual patients. There was no significant difference in the POD 1-3 lactate between those with and without complications (table 6.7).

TABLE 6.6. LACTATE LEVELS IN ALL PATIENTS

		Lactate (mmol/l)		
Patient Number	**Day drain out**	**POD 1**	**POD 2**	**POD 3**
1	3	8.9	High*	High
2	2	High	8	
3	3	High	High	High
5	3	8	High	High
7	3	High		8.7
8	3	2.1		3.3
12	3	7	8.5	7.2
16	3	5.4	2.1	7.6
17	3	8.2	7.6	0.7
18	3	3	7.4	6.9
21	3	2.3	2.8	4.8
23	3	8.1	10.1	10.2
24	3	3.6	4.7	6.9
27	1	10.1	n/a	n/a
28	3	8.4	9.9	7.3
29	3	5.2	8	9.9
33	3	10.5	9.7	9
36	3	4.3	6.9	6.1
38	3	3.9	3.9	6.8
44	2	3.1	2.9	n/a
45	2	7.5	5.1	n/a
46	1	6.1	n/a	n/a
51	3	5.5	5.6	1.0
55	3	4	8	5.9
56	3	3.9	6.3	5.6
58	3	7.7	10.5	9.1
59	3	5.3	7.8	2.2
62	1	2.8	n/a	n/a
64	3			10.5
66	2	4.6	6.6	n/a

No shading = those without complications; grey shading = those with complications;

*N/a = not applicable, drain already removed, *High = above upper limit of detection of Lactate Pro 2*

TABLE 6.7. MEDIAN AND IQ RANGES OF LACTATE SAMPLED FROM DRAINS IN THOSE WITH AND WITHOUT POST-OPERATIVE COMPLICATIONS

	Lactate (mmol/l)		
	No complication	Complication	P value
	Median (IQR)	Median (IQR)	
POD 1	5.4 (3.9 – 8.1)	4.7 (2.6 – 8.3)	**0.397**
POD 2	6.8 (4.5 – 8.8)	7.6 (4.9 – 8.6)	**0.718**
POD 3	7.3 (6.3 – 9.6)	5.4 (2.4 – 8.2)	**0.115**

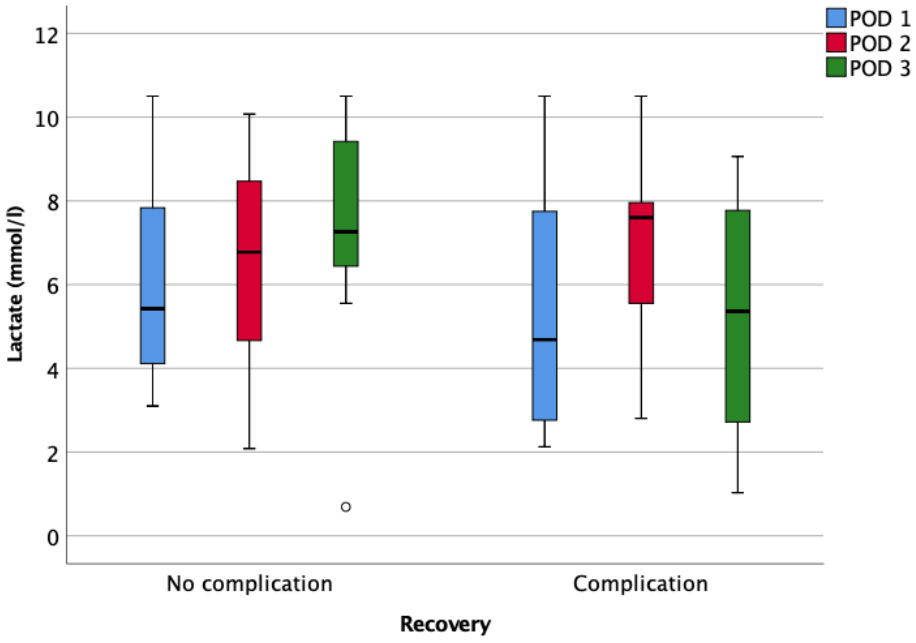

FIGURE 6.2. BOX PLOT OF MEDIAN LACTATE (MMOL/L) WITH IQR IN THOSE WITH AND WITHOUT COMPLICATIONS

Table 6.8 shows the peritoneal drain fluid pH levels on PODs 1-3 from all patients. Although there was variation, in those without complications pH

generally rose each day with the median values on POD 1-3 being 7.71, 7.88 and 8.12 respectively (see table 6.9 and figure 6.3). For those with complications the median pH on POD 1 was lower at 7.61, it rose to 7.70 on POD 2 and, in contrast to those without complications, the pH decreased from POD 2 to POD 3 (7.60). Despite the trend, the differences between pH in those with/without complications was not significantly different on any post-operative day. The median rise in pH from POD 1 to POD 3 was smaller in those with vs. without complications (0.18 and 0.32 respectively) but it was not statistically significant (P = 0.51).

TABLE 6.8. PH LEVELS IN ALL PATIENTS

		pH		
Patient number	**Day drain out**	**POD 1**	**POD 2**	**POD 3**
1	3	6.92	7.99	7.33
2	2	7.01	8.2	n/a
3	3	8.65	6.22	7.08
5	3	6.93	6.74	7.17
7	3	8.37		7.15
8	3	7.97		7.49
12	3	7.66	7.65	7.78
16	3	7.82	8.19	8.39
17	3	7.27	8.1	8.26
18	3	7.65		7.71
21	3	7.7	7.95	8.46
23	3	7.71	6.93	
24	3	7.54	7.46	7.86
27	1	7.21	n/a	n/a
28	3	7.01	7.77	8.19
29	3	7.66	7.5	7.24
33	3	7.41	7.72	7.84
36	3	8.06	8.15	8.21
38	3	8.19	8.09	8.12
44	2	8.7	8.15	
45	2	7.82	8	n/a
46	1	7.88	n/a	n/a
51	3	7.47	7.47	8.39
55	3	7.61	7.67	7.78
56	3	7.68	8.21	8.39
58	3	7.49	7.7	7.48
59	3		7.92	8.1
62	1	8.76	n/a	n/a
64	3	7.52	7.7	7.51
66	2	7.73	7.34	n/a

No shading = those without complications; grey shading = those with complications;

N/a = not applicable, drain already removed

TABLE 6.9. MEDIAN AND IQ RANGES OF PH SAMPLED FROM DRAINS IN THOSE WITH AND WITHOUT POST-OPERATIVE COMPLICATIONS

	pH		
	No complication	Complication	P value
	Median (IQR)	Median (IQR)	
POD 1	7.71 (7.52 – 8.06)	7.61 (7.21 – 7.97)	**0.312**
POD 2	7.88 (7.49 – 8.16)	7.70 (7.47 – 7.95)	**0.263**
POD 3	8.12 (7.65 – 8.32)	7.60 (7.29 – 8.17)	**0.186**
Difference POD 3 – POD 1	0.32 (-0.04 – 0.63)	0.18 (-0.24 – 0.59)	**0.512**

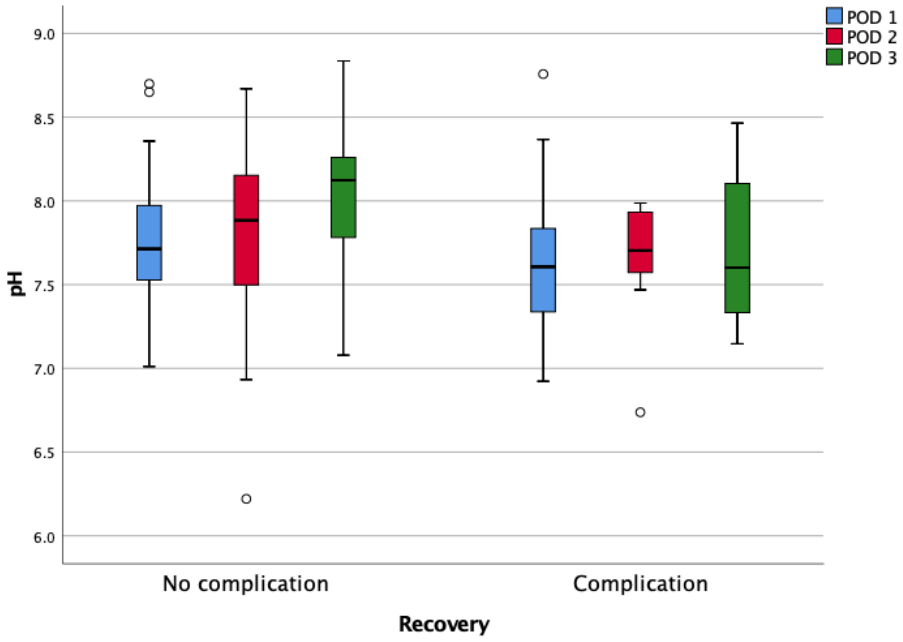

FIGURE 6.3. BOX PLOT OF PH IN THOSE WITH AND WITHOUT COMPLICATIONS

6.4.1 Comparison of the Lactate Pro 2 and colorimetric assay testing clinical samples of peritoneal drain fluid

A total of 9 clinical samples were transported from the Royal Alexandra Hospital to the University of Strathclyde for testing with the colorimetric lactate assay. Table 6.10 displays a comparison of the results of the lactate measurements made for each of the samples with the Lactate Pro 2 and the colorimetric lactate assay. There is little agreement between the results obtained with the different measurement techniques. The scatter graph in figure 6.4 confirms the lack of correlation between the results (R = 0.21).

TABLE 6.10. LACTATE MEASUREMENTS MADE ON THE LP2 AND LAB COLORIMETRIC ASSAY

Clinical sample number	Lactate Pro 2 (mmol/l)	Colorimetric assay (mmol/l)
S044.1	3.1	11.0
S045.1	7.5	27.2
S046.1	6.1	5.1
S044.2	2.9	19.7
S045.2	5.1	16.5
S050.1	5.4	19.8
S056.1	3.9	13.9
S059.1	5.3	25.8
S062.1	2.8	6.6

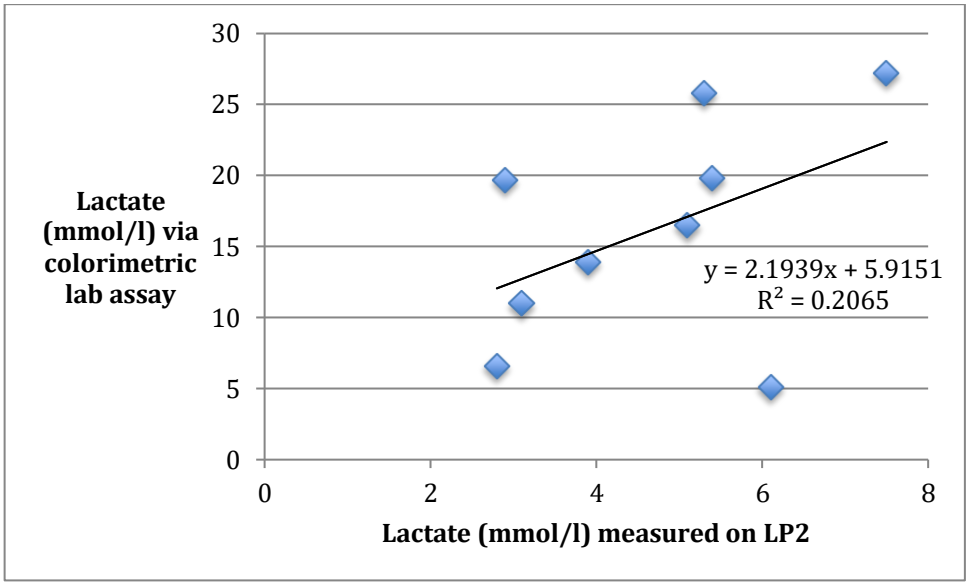

FIGURE 6.4. CORRELATION BETWEEN LACTATE READINGS OF LP2 VS. THE LAB COLORIMETRIC ASSAY

6.4.2 Comparison of the Omega PH-7011 Meter and Mettler Toledo pH meter testing clinical samples of peritoneal drain fluid

A total of 9 clinical samples were transported from the Royal Alexandra Hospital to the University of Strathclyde for testing with the Mettler Toledo pH meter. Table 6.11 displays a comparison of the results of the pH measurements made for each of the samples with the Omega PH-7011 Meter and the Mettler Toledo Meter. As shown in figure 6.5 there is a good correlation between the results of the Omega Meter and the Mettler Toledo Meter (R = 0.97) however, for each sample the reading from the Omega meter is higher indicating that the readings are not accurate.

TABLE 6.11. PH MEASUREMENTS MADE ON OMEGA AND METTLER TOLEDO PH METERS

Clinical sample number	Omega pH meter	Mettler Toledo pH meter
S044.1	8.70	8.4
S045.1	7.82	7.53
S046.1	7.88	7.55
S044.2	8.15	7.78
S045.2	8.00	7.58
S050.1	8.11	7.91
S056.1	7.68	7.40
S059.1	*	7.42
S062.1	8.76	8.48

**size of clinical sample insufficient for measurement with Omega pH meter*

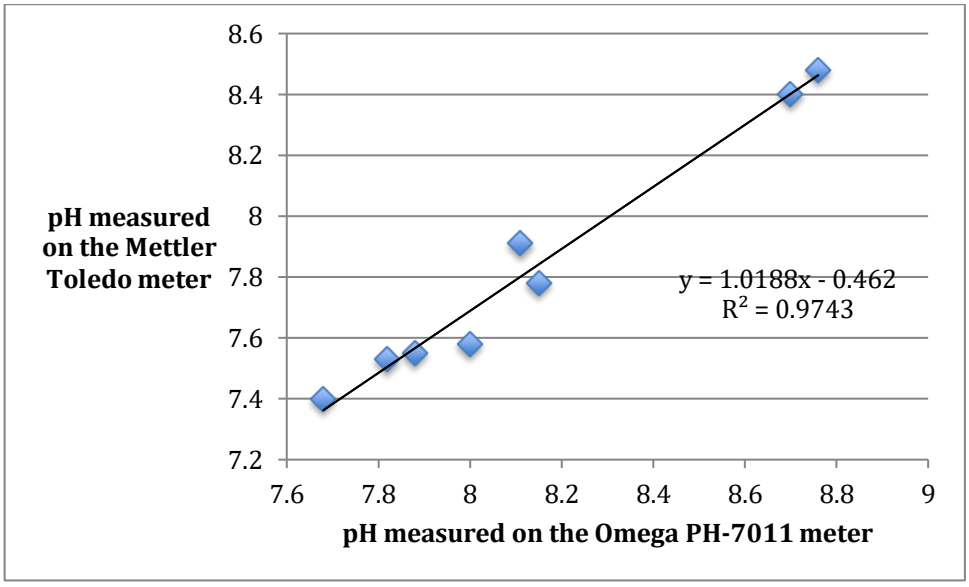

FIGURE 6.5. CORRELATION BETWEEN PH READINGS OF OMEGA VS. METTLER TOLEDO PH METERS

6.5 Discussion

The absence of any ALs in the study meant that it was not possible to confirm or refute the primary aim of the study: the ability of peritoneal fluid lactate and

pH to serve as early markers of AL. A recent systematic review quoted the rate of AL as ranging from 1-19% depending on the anatomical site: enteroenteric 1-2%, ileocolic 1-4%, colocolic 2-3%, ileorectal 3-7%, colorectal/coloanal 5-19% and ileoanal pouch 4-7% (McDermott et al., 2015). In the current study there were a total of 58 patients with 65.5% having left-sided surgery. Based on a perhaps conservative estimated leak rate for the study of 5%, a minimum of 3 ALs would have been expected. Whilst 58 patients does not represent a large cohort it is still unusual that there were no ALs.

The secondary outcome was any complication following surgery. Although there was a trend towards a steady increase in pH in those making an uncomplicated recovery, there was no significant difference in lactate and pH from the peritoneal drain fluid on POD 1-3 in those with and without post-operative complications.

The lactate measurements showed wide variation, both between patients and within individual patients. The median POD 1 lactate level in all patients without complications was 5.4 mmol/l with a large interquartile range (IQR) of 3.9 – 8.1 mmol/l. An example of the variation in results within one individual is patient 016 who made an uncomplicated recovery: POD 1 5.4 mmol/l, POD 2 2.1 mmol/l, POD 3 7.6 mmol/l. Rather than peritoneal drain fluid lactate not being associated with complications, the findings may reflect that either lactate is unstable in peritoneal drain fluid in the immediate post-operative period or that the LP2 meter was cross reacting with something in the fluid and was therefore unable to accurately measure lactate in this setting. It is possible that both of these issues were present simultaneously.

In comparison to the fluid tested from patients in chapters 4 and 5, the post-operative peritoneal drain fluid was more obviously blood-stained but this would not have been anticipated to be problematic because the LP2 was designed and validated to test lactate from whole blood samples. It would be useful to examine the cellular makeup of the drain fluid and measure the concentration of RBCs and WBCs. As demonstrated by Komen et al (Komen et al., 2009), bacteria are also likely to be present. It maybe that on going metabolism by the bacteria influence the stability of lactate levels on a day to day basis. As shown in

chapter 4, when a sample had a high WCC and bacteria were cultured the lactate level increased considerably over 24 hours. It maybe that variation in the number of bacteria present in the drain each day might contribute to the variation in results. The presence of a high level of bacteria may also explain why the lactate levels in the drain fluid were much higher than in the samples obtained from the control group in chapter 4. In future work it would be worthwhile performing bacterial culture on the samples and also to quantify the bacterial load in order to determine its impact and relationship to the lactate concentration of the drain fluid. This would help to determine whether the variable results for lactate were a consequence of instability of lactate in post-operative peritoneal fluid.

As mentioned, the other possible explanation for the variable lactate results would be that the LP2 was cross-reacting with something in the fluid and therefore not able to provide accurate measurements. Such an issue was not anticipated given the successful validation of the LP2 in chapter 3 and the fact it had been used and validated against a blood gas analyser and laboratory colorimetric assay in the veterinary world for measuring the lactate level of peritoneal fluid from horses with suspected colic (Nieto et al., 2015). Furthermore, in chapter 4 it appeared to provide reliable measurements of lactate from peritoneal fluid in patients with ascites. However, it may be the case that the peritoneal fluid from post-operative patients may have a substantially different cellular and/or biochemical make up to that of patients with ascites. In addition, in the study by Nieto et al(Nieto et al., 2015) the peritoneal fluid samples from horses were centrifuged and only the supernatant tested with the handheld lactate meters. This may have removed cellular constituents that would otherwise have cross-reacted with the LP2. Further evidence that the unreliable results were due to an issue with the meter is the finding that there was no correlation between the 9 lactate levels measured on both the LP2 meter and in the laboratory at Strathclyde University on the colorimetric assay (see section 6.4.1). A limitation of this comparison is the fact that there was a delay in analysis on the colorimetric assay. The laboratory was a 30 minute journey from the hospital and once there it took 30 minutes to unpack the samples and set up the assay. This meant samples tested on the colorimetric assay were more than 2 hours old. However, the variation seen

(e.g. sample 044.2 from 2.9mmol/l to 19.7mmol/l and sample 056.1 from 3.9mmol/l to 13.9mmol/l) was much greater than that seen in the in any of the patients over 24 hours in section 4.5, including those with high WBC counts and positive bacterial cultures. Testing of all the samples before and after centrifugation with LP2 and then on a colorimetric lab assay within 2 hours would be a useful next step to determine whether the results were due to the sample being unstable or an effect of the cellular constituents of the sample on the LP2 meter.

Although the pH levels on POD 1-3 were not significantly different in those with and without complications they were more consistent and showed less variability than the lactate results. As mentioned in section 4.7 no normal range exists for peritoneal pH but 2 previous studies attempting to answer this question had quoted means of 7.73 and 7.49 and ranges of 7.46 - 8.10 and 7.04 – 7.94 respectively (Noh, 2003, Simmen et al., 1994). In addition, in chapter 5 the samples of peritoneal fluid yielded a median of 7.66 and IQ range of 7.56 – 7.73. The POD 1-3 medians and IQ ranges (see table 6.9) found in patients in this study are in line with these results. pH has only previously been explored in relation to detection of AL and not complications in general. It is therefore difficult to draw conclusions from this work due to the relatively small sample size. However, the tentative results suggesting that trend in pH may have some utility in prediction of post-operative complications lead the author to propose further work with a larger patient cohort to explore these findings. With regard to the choice of pH meter in future studies, the author would not recommend using the Omega PH-7011 meter. In chapter 3 it was shown that it could produce reliable but not accurate results compared to a laboratory gold standard meter. When 9 samples of peritoneal drain fluid were tested on the handheld Omega meter and then the Mettler Toledo meter in the lab (see section 6.4.1.2), there was correlation between the meters but the Omega meter again lacked accuracy. However, some of this variation may have been due to the delay in analysis on the Mettler Toledo which was a consequence of the transit time and sample preparation time detailed earlier in this section. The Omega meter was considered sufficient to illustrate trends in pH and differences between those with and without complications but the absolute values would have to be interpreted with

caution. The author recommends that future work exploring pH uses a laboratory standard meter to measure all samples rather than the handheld Omega meter.

A major limitation of the study is the small sample size. Over the first 9 months of the study there were a total of 104 eligible patients. 76 were approached, of these 62 agreed to participate. Suitable patients were identified by the researcher via the colorectal MDT and theatre lists. Most patients attended a pre-operative assessment clinic 1 – 2 weeks prior to surgery and it was arranged that clinic staff would hand out the PIS. After a period of time it became apparent that the PIS was frequently not being given to patients thus reducing the number of patients who could be recruited. Therefore the researcher and a colleague attempted, when possible, to attend the pre-assessment clinic to give out the PIS. This improved the rate of patients getting the PIS and boosted recruitment.

Of the 58 patients recruited and included in the study, only 32 patients (55.1%) had a drain inserted. Whilst the literature does not support the routine use of a drain in colonic resections, their role in rectal resections is less clear and the use of a drain in rectal resections may still be advisable (McDermott et al., 2015). Although insertion of a drain was at the discretion of the operating surgeon, the fact that 63.3% had left sided surgery means that one may have expected a slightly higher percentage of patients to have a drain inserted. In planning further work looking at the analysis of post-operative peritoneal drain fluid the low percentage having a drain inserted would need to be taken into account and consideration given to a longer recruitment period or recruiting from more than one site to increase the number of eligible patients over a set period of time. An alternative strategy would be to design a study that requires insertion of a drain into all patients. Use of a very fine bore piece of tubing such as a wound catheter draining into an external bag may represent a reasonable option. This would likely have very little impact on the patient and may be more acceptable to operating surgeons who are reluctant to place a wide bore drain. This would help to increase the sample size.

Overall, it was not possible to assess the utility of lactate and pH from post-operative peritoneal drain fluid in predicting AL. In assessing the secondary

outcome of the development of post-operative complications, the findings from pH, although not significant, are encouraging and require further exploration. In the current study, lactate from peritoneal drain fluid, was not found to be a useful biomarker of post-operative complications. In chapter 4 lactate was found to be stable in peritoneal fluid, and in patients with positive bacterial cultures from their peritoneal fluid it rose over 24 hours to a much greater degree than in those with culture negative peritoneal fluid. Given these findings it is felt likely that the results in this chapter are a consequence of a high level of bacteria in the drain samples and possibly a cross-reaction between the LP2 and constituents of the drain fluid. This study has therefore identified a number of issues that should be elucidated before the utility of lactate in detecting AL and other post-operative complications can be re-explored.

Chapter 7 - Comparison of peritoneal drain fluid biomarkers to blood biomarkers in the detection of colorectal anastomotic leak

7 Chapter 7 – Comparison of peritoneal drain fluid biomarkers to blood biomarkers in the detection of colorectal anastomotic leak

7.1 Introduction

In the early stages the signs and symptoms of AL can be subtle. Prompt detection currently relies upon clinician awareness of the pre- and intra-operative risk factors to which the patient has been exposed and close attention to deviations in clinical observations and in the expected progress of the patient. As described in section 1.7.2 blood biomarkers such as and c-reactive protein (CRP) and procalcitonin (PCT) can provide a useful adjunct. CRP has been much more extensively investigated than PCT. A recent systematic review suggested that high levels of CRP (>150mg/l) or procalcitonin on post-operative day (POD) 3 - 5 should act as an alert for clinicians to monitor the patient closely and consider investigations for signs of AL (McDermott et al., 2015). White cell count (WCC) is also commonly measured in the post-operative period and is a marker of infection/inflammation. It has previously also been identified as a useful marker of AL alongside CRP (Platt et al., 2012) but the results are not consistent (Warschkow et al., 2011). Elevated blood biomarkers, in the presence of concerning clinical features, frequently lead to further investigation, most often a CT scan, to look for evidence of an AL. As described in section 1.7.3 CT scans are not 100% sensitive or specific for AL. This is particularly true in the early post-operative period when one would still expect to see fluid and possibly also some gas in the vicinity of the anastomosis. This is why pursuit of a readily measurable local biomarker of AL is an attractive target as it may help to differentiate between “normal” postoperative appearances versus AL on CT, thus facilitating the appropriate management of the patient in a timely manner.

If technology is to be developed to allow the measurement of local perianastomotic biomarkers of AL such as lactate and pH then these biomarkers

should have a superior performance to that of pre-existing blood biomarkers such as WCC, CRP and procalcitonin which are cheap and simple to analyse and are the current standard of care.

7.2 Aims

The primary aim of the study was to determine if the serial measurement of blood biomarkers – WCC, CRP and PCT in the post-operative period could predict colorectal anastomotic leak. The secondary outcome was the ability of blood WCC, CRP and PCT to predict other complications following colorectal surgery.

7.3 Methods

This study was run in conjunction with the study in chapter 6. The ethical approval, recruitment, inclusion and exclusion criteria are outlined in section 6.3. Patients were recruited over a 12.5 month period from October 15th 2016 – October 31st 2017.

Data collection

Patient demographics and underlying diagnosis were recorded prospectively in a data base. As routine standard of care within the department daily post-operative blood tests including WCC and CRP were taken on each post-operative day until discharge. These results were recorded. In addition, between Oct 15th 2016 – July 31st 2017, each patient had a procalcitonin (PCT) level measured on post-operative days 1, 3 and 5. Patients were followed for 30 days post-operatively. Complications and 30 day mortality were recorded.

Definition of anastomotic leak and post-operative complications

This study used the same definitions of AL and post-operative complications as described in section 6.3.

Statistical analysis

Data are presented as the median and interquartile range. A Mann-Whitney test was used to look for significant differences in median CRP, WCC and PCT levels in those with/without complications. P values of < 0.05 were considered statistically significant. To compare the performance of CRP, WCC and PCT in predicting complications ROC curves and the corresponding area under curve (AUC) were used. All analyses were performed on SPSS version 24 for Mac.

7.4 Results

Between October 2016 – October 2017 a total of 66 patients were recruited. One patient then did not go on to have surgery (due to poor fitness) so was excluded from further analysis. As described in section 6.4 a further 7 patients were excluded because they ultimately underwent an APR. The demographics of these patients are described in section 6.4. At 30 days, the majority of patients were still alive (98.3%) and had recovered without any complications (68.3%). One post-operative death occurred from acute renal failure on post-operative day 1. A total of 58 patients were available for analysis. Figure 7.1 shows the post-operative days on which patients were discharged. The median day of discharge was POD 6 (IQR 4 - 8).

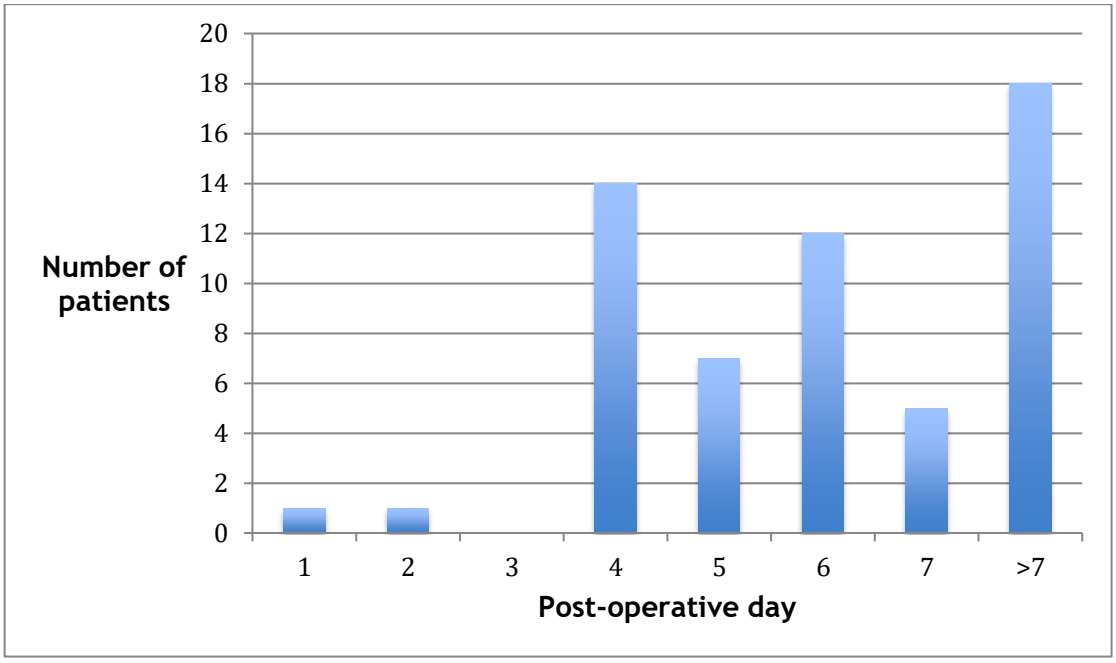

FIGURE 7.1 DAY OF DISCHARGE FOLLOWING SURGERY. *(POD 1 DISCHARGE = PATIENT DEATH ON POD 1)*

There were a total of 21 complications, 5 infective and 16 non-infective complications (see table 7.1). There were no anastomotic leaks.

TABLE 7.1. RECOVERY FOLLOWING COLORECTAL SURGERY

Complication	Totals
None	36
Any complication	21
Infective complication	5
LRTI	1
UTI	1
Wound infection	2
Gram negative sepsis	1
Anastomotic leak	0
Non-infective complication	16
Renal failure	1
Ileus	7
AF	2
N&V	1
Heart block & perforated stress ulcer	1
Pancreatitis	1
Wound dehiscence	1
SVT	1

Primary outcome – blood biomarkers for the prediction of AL

As there were no anastomotic leaks it was not possible to address the primary outcome.

Secondary outcome – blood biomarkers for the prediction of other complications

C-reactive protein (CRP)

The relationship between CRP and complications on PODs 1 - 5 is shown in table 7.2 and boxplots for POD 1 - 5 in figure 7.2. Compared with patients who did not develop a post-operative complication, median CRP on POD 3 - 5 was significantly higher in those who developed a complication (P = 0.0032, P = 0.003 and P = 0.002 respectively). On PODs 1 and 2 there was no significant difference in CRP between those with and without complications.

TABLE 7.2. MEDIAN POST-OPERATIVE CRP (MG/L) IN THOSE WITH/WITHOUT COMPLICATIONS

	POD 1	POD 2	POD 3	POD 4	POD 5
No complication	111* (78-151)	143 (108-186)	101 (78-188)	68 (48-126)	49 (29-102)
Any complication	103 (75-122)	168 (129-202)	151 (111-230)	131 (85-210)	136 (73-184)
P value	0.195	0.150	0.032	0.003	0.002

**values presented as median and IQ range*

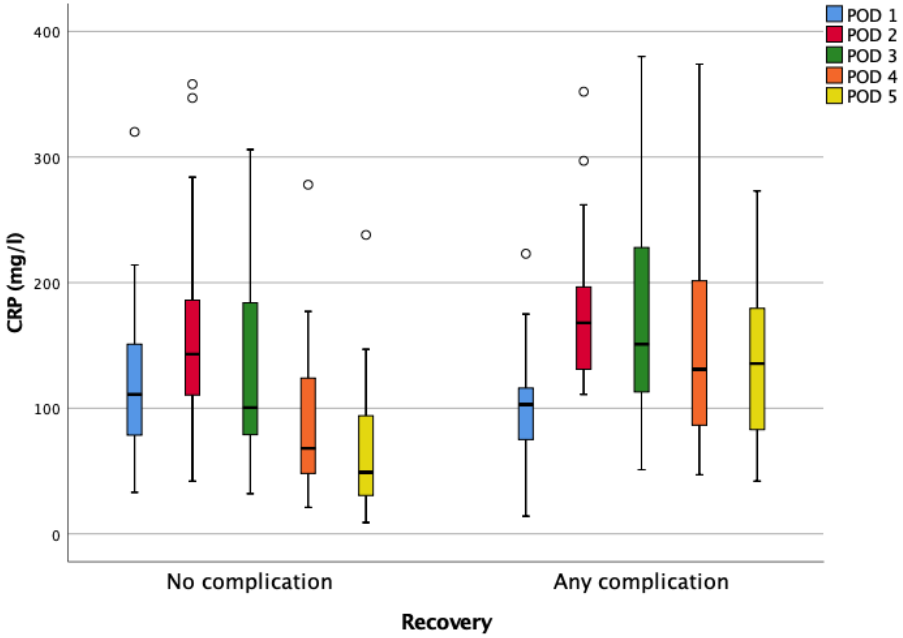

FIGURE 7.2. POD 1 – 5 CRP IN PATIENTS WITHOUT V WITH COMPLICATIONS

To establish a threshold for the relationship between post-operative CRP and complications Receiver Operator Curves were plotted for CRP on PODs 3 -5 (figure 7.3). POD 5 CRP performed best. The area under the curve (AUC) values for PODs 3 – 5 were 0.567 (P = 0.527), 0.683 (P = 0.082) and 0.804 (P = 0.004) respectively. For CRP on POD 5 the optimum threshold was 153mg/l with a sensitivity of 37.5% and specificity of 93.3%.

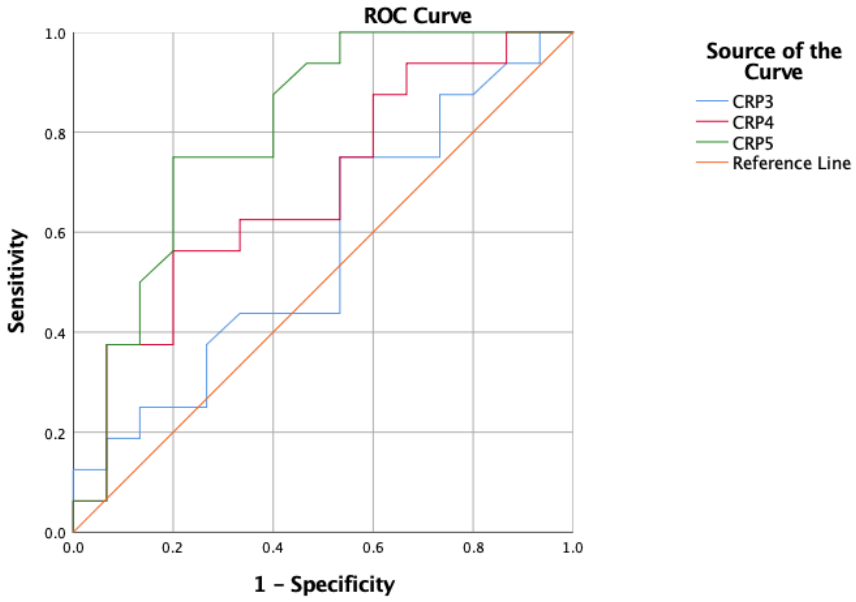

FIGURE 7.3. ROC CURVE FOR CRP ON POD 3-5 AS A PREDICTOR OF ANY COMPLICATION FOLLOWING SURGERY

When complications were divided into non-infective (see table 7.3) and infective complications (see table 7.4), CRP was still useful in discriminating between the groups. In the 16 patients with non-infective complications, CRP was significantly higher on POD 3 – 5 (P = 0.03, P = 0.01 and P = 0.008 respectively). Only 5 patients suffered an infective complication. CRP was only significantly higher on PODs 4 and 5 (P= 0.044 and 0.012 respectively) in those with infective complications versus no complications (see table 7.4).

TABLE 7.3 MEDIAN POST-OPERATIVE CRP (MG/L) IN THOSE WITHOUT COMPLICATIONS V THOSE WITH NON-INFECTIVE COMPLICATIONS

	POD 1	POD 2	POD 3	POD 4	POD 5
No complication	111* (78-151)	143 (108-186)	101 (78-188)	68 (48-126)	49 (29-102)
Non-infective complication	99 (69-114)	171 (130-211)	151 (115-230)	128 (82-203)	126 (58-184)
P value	0.158	0.157	0.030	0.010	0.008

**values presented as median and IQ range*

TABLE 7.4. MEDIAN POST-OPERATIVE CRP (MG/L) IN THOSE WITHOUT COMPLICATIONS V THOSE WITH INFECTIVE COMPLICATIONS

	POD 1	POD 2	POD 3	POD 4	POD 5
No complication	111* (78-151)	143 (108-186)	101 (78-188)	68 (48-126)	49 (29-102)
Infective complication	116 (76-145)	149 (122-223)	141 (88-248)	159 (76-220)	136 (126-190)
P value	0.781	0.551	0.476	0.044	0.012

**values presented as median and IQ range*

White cell count (WCC)

As demonstrated in figure 7.4 there was little difference in the WCC on PODs 1 - 5 in patients making an uncomplicated recovery vs. those who suffered a complication. This was confirmed by the finding that there was no statistically significant difference between the median WCC in any of the groups on POD 1 - 5 (see table 7.5). Subdivision into infective and non-infective complications did not lead to an improvement in the performance of WCC (data not shown).

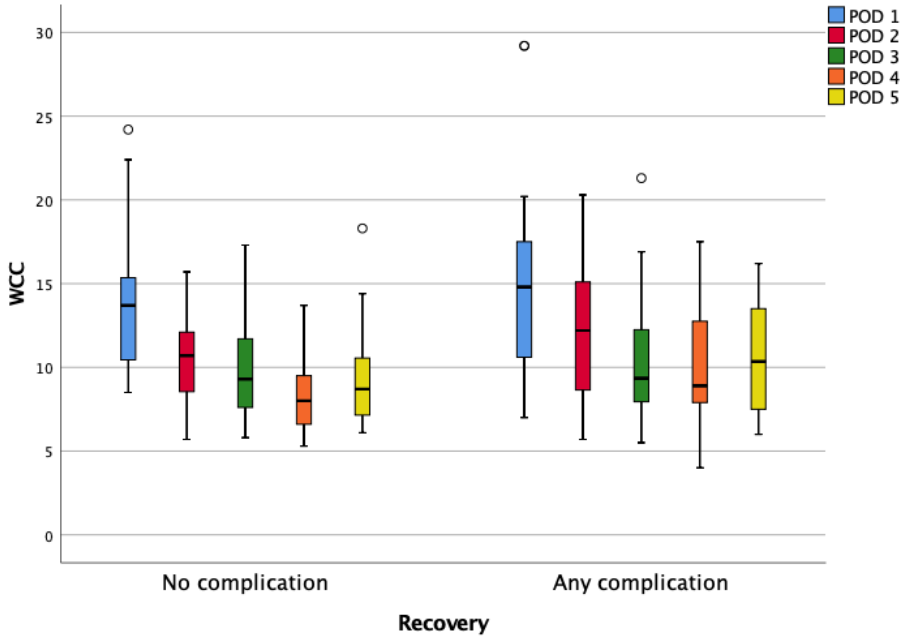

FIGURE 7.4. POD 1 – 5 WCC IN PATIENTS WITHOUT V WITH COMPLICATIONS

TABLE 7.5. RELATIONSHIP BETWEEN MEDIAN POST-OPERATIVE WCC AND COMPLICATIONS

	POD 1	POD 2	POD 3	POD 4	POD 5
No complication	13.7* (10.4-15.4)	10.7 (8.5-12.3)	9.3 (7.6-11.7)	8.0 (6.4-9.7)	8.7 (6.7-10.6)
Any complication	14.8 (10.5-17.8)	12.2 (8.3-15.4)	9.4 (7.7-13.2)	8.9 (7.7-13.1)	10.4 (7.4-13.6)
P value	0.367	0.155	0.747	0.065	0.253

**values presented as median and IQ range*

Procalcitonin (PCT)

As demonstrated in figure 7.5 the PCT levels in those making an uncomplicated recovery vs. those who suffered a complication was similar.

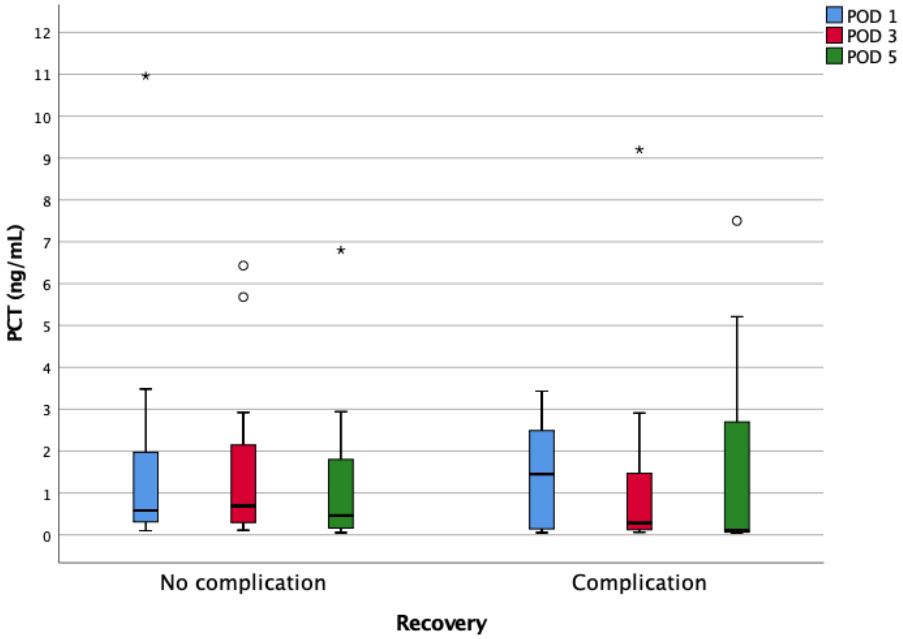

FIGURE 7.5 POD 1, 3 & 5 PCT IN PATIENTS WITHOUT V WITH COMPLICATIONS

As PCT is known to be a marker of bacterial infection, PCT levels in those without complications vs those with infective complications were compared. Only 5 patient suffered an infective complication. One of those patients (unique number 065) had surgery after August 2017 when PCT was no longer being recorded, this meant only 4 patients could be analysed. The POD 1, 3 & 5 PCT results for these patients are shown in table 7.6. Due to logistical difficulties in measuring PCT (discussed further in section 7.5) several PCT measurements were missed. Although several of the patients had high levels of PCT (5.21ng/mL on POD 5 in patient 008; 3.05ng/mL on POD 1 in patient 042 and 3.40ng/mL on POD 4 in patient 047), the results were not significant and the median PCT did not differ between those with infective complications and those without complications (table 7.7). It was not significantly different in the "no complication" versus "any complication" groups (see table 7.7) nor in the "no complication" versus "non-infective complications" groups (data not shown).

TABLE 7.6. POST-OPERATIVE PCT (NG/ML) IN PATIENTS WITH INFECTIVE COMPLICATIONS

Unique patient number	POD 1	POD 3	POD 4	POD 5	Complication	Day of discharge
004	1.47	-	0.11	-	Wound infection	6
008	0.23	0.20		5.21	UTI**	12
042	3.05	0.20			Wound infection	4
047	-	-	3.40	-	LRTI	12
*065***	-	-		-	*Gram negative sepsis*	*18*

*LRTI: lower respiratory tract infection; **UTI: urinary tract infection; ***outwith PCT collection period

TABLE 7.7 RELATIONSHIP BETWEEN MEDIAN POST-OPERATIVE PCT (NG/ML) AND COMPLICATIONS

	No complication	Any complication		Infective complication	
	Median (IQR)	Median (IQR)	P	Median (IQR)	P
POD 1	0.58* (0.28-2.10)	1.45 (0.11-2.91)	0.912	1.47 (0.23-1.47)	0.673
POD 3	0.69 (0.26-2.15)	0.28 (0.11-1.83)	0.155	0.2**	0.104
POD 5	0.46 (0.15-2.03)	0.10 (0.05-5.21)	0.287	5.21***	0.267

**values presented as median and IQ range; **No IQR as n=2; ***No IQR as n=1*

Comparison of peritoneal drain fluid lactate and pH to blood biomarkers

As there were no ALs it was not possible to compare the performance of peritoneal fluid lactate and pH to blood biomarkers as predictors of AL.

In relation to the secondary outcome of "other post-operative complications", peritoneal fluid pH showed a trend towards a steady increase in those making an uncomplicated recovery (see section 6.4) but the difference was not statistically significant. The blood biomarker CRP, but not WCC or PCT, had a superior performance as it was found to be statistically significantly higher from POD 3 onwards in those who developed a post-operative complication (table 7.8).

TABLE 7.8 COMPARISON OF PERITONEAL FLUID BIOMARKERS TO BLOOD BIOMARKERS ON EACH POST-OPERATIVE DAY

	P values for no complications versus complications				
Biomarker	POD 1	POD 2	POD 3	POD 4	POD 5
pH	0.312	0.263	0.186	-	-
Lactate	0.397	0.718	0.115	-	-
CRP	0.195	0.150	**0.032**	**0.003**	**0.002**
WCC	0.367	0.155	0.747	0.065	0.253
PCT	0.912	-	0.155	-	0.287

P < 0.05 = statistically significant

7.5 Discussion

As there were no ALs in the study it was not possible to assess the primary outcome – comparing the performance of blood biomarkers to peritoneal drain fluid lactate and pH in the detection of anastomotic leak. As previously mentioned in section 6.5 this is unfortunate as even with the relatively small sample size of 58 patients a minimum of 3 ALs would have been expected.

In relation to the secondary outcome of the detection of any complication following colorectal surgery, the blood biomarker CRP, but not WCC or PCT,

performed better than peritoneal drain fluid lactate and pH. POD 3 - 5 CRP was significantly higher in those who developed a complication. In particular POD 5 CRP > 153mg/l had a specificity of 93.3%. This is in agreement with a recent systematic review's recommendation that a POD 3 - 5 CRP > 150mg/l merits close observation of the patient for signs of sepsis/AL (McDermott et al., 2015). However, the sensitivity of a POD 5 CRP > 153mg/l was only 37.5%. This highlights that whilst CRP may be a useful "rule out" test for complications/AL and may be a useful component of discharge criteria it is not a good marker of AL.

In a previous study by Platt et al(Platt et al., 2012), WCC on POD 7 was found to be significantly higher in patients who developed an infective complication following elective colorectal surgery. In the context of AL typically being detected between POD 6 – 8 (Kanellos et al., 2004, Alves et al., 2002, Alves et al., 1999), and with the aim of early identification, the usefulness of a POD 7 WCC as a marker of AL or infective complications is questionable. Other studies have not found WCC to be useful (Warschkow et al., 2011). Overall, a recent systematic review concluded that post-operative WCC was not a useful marker of AL(McDermott et al., 2015). This study confirms the lack of usefulness of WCC as a marker of complications in this group of patients.

PCT was not found to be a useful predictor of complications. Given the sample size and specifically the number of infective complications this was unsurprising. PCT is marker of infection and has been found to out-perform CRP and other markers of inflammation such as interleukins and TNF-alpha in differentiating patients with sepsis from those with systemic inflammatory response syndrome (SIRS) (Balcl et al., 2002). As such PCT would not be expected to be significantly elevated in patients with non-infective complications. This was indeed the case in the present study. Of the 58 patients there were 21 complications but only 5 of these were infective, with only 4 being within the timeframe in which PCT was being measured. Of the 5 patients with an infective complication, there should have been a total of 14 PCT samples (one patient was discharged on POD 4) but only 9 were correctly collected, a further 2 were taken on POD 4 with only 2 patients having PCT taken on POD 1, 3 and 5 as per protocol. Although the median POD 5 PCT was higher in the group with infective complications vs.

those with no complications (5.21ng/mL and 0.46ng/mL respectively), with such a small sample size it was not possible to determine if the difference was significant.

Significant logistical challenges were the major limitations in the assessment of PCT as a marker of AL and post-operative complication. In our institution all post-operative blood tests are ordered via a computer system, the requests printed off by the phlebotomists and samples taken accordingly. As PCT was processed in a different lab there was no facility for online requesting and a paper request form had to be completed. The form was completed by the researcher and left on the phlebotomy trolley. The phlebotomy department were aware of the study and the arrangements for measurement of PCT but despite this a substantial number of PCT tests were missed. The PCT samples were analysed in batches so it was several weeks before the issues with missed samples became apparent.

Overall, in the current study CRP was found to be the only useful biomarker of complications and out-performed lactate and pH from peritoneal drain fluid. Due to the small sample size and logistical difficulties encountered it would be useful to study PCT again in a larger cohort of patients to determine if it was a more sensitive marker of infective complications, including AL, than CRP in patients who have had colorectal surgery.

Chapter 8 - Discussion and conclusions

8 Chapter 8 – Discussion and conclusions

Anastomotic leak (AL) following colorectal surgery remains a challenging problem. Recent work has illustrated that local biomarkers of infection, inflammation, bacteria and wound repair from the perianastomotic environment may have the ability to detect AL early and the potential of this technology has been highlighted as a target for further research (McDermott et al., 2015, Hirst et al., 2014). A literature review undertaken as part of this project identified lactate and pH as the biomarkers which had the most consistent and promising results for the early detection of AL. With the availability of biosensor technology that can measure samples as small as several microlitres development of a small, implantable and degradable or removable biosensor for AL represents an exciting possibility. As the first stage in the development of such a sensor this study aimed to further assess the usefulness of lactate and pH as a local biomarker of AL and other complications after colorectal surgery. In addition it aimed to address the issue of the stability of lactate and pH in peritoneal fluid to inform sensor design and also compare the performance of perianastomotic lactate and pH to commonly used, yet limited, blood biomarkers.

This work has demonstrated that lactate and pH in peritoneal fluid can be easily measured using surgically placed intra-abdominal or pelvic drains with minimal upset to the patient. Analysis of these samples found both lactate and pH undergo clinically significant changes over time, supporting early analysis after sampling (within 2 hours). Whilst it was not possible to confirm the usefulness of perianastomotic lactate and pH as biomarkers of AL there was a trend towards an increasing pH level over PODs 1 – 3 being a marker of an uncomplicated recovery following colorectal surgery. The work in chapter 7 confirms the previously well documented finding that an elevated CRP from POD 3 onwards, and in this study particularly a CRP > 153mg/L on POD 5, can be an indication of the development of a complication following colorectal surgery, however it also highlights the poor sensitivity of CRP. WCC was found not be a useful marker of complications and the numbers were too small to comment on the usefulness of PCT as a marker of complications.

In the context of a small sample size and the limitations discussed in sections 4.6, 5.7 and 6.5 it is felt that both perianastomotic lactate and pH merit further investigation in relation to their ability to predict AL early and the feasibility of constructing lactate and pH biosensors that can be used to monitor the perianastomotic environment. The major limitations of this work could be addressed by continuing the work in larger clinical cohorts. Ideally lactate and pH should be measured from a fresh sample of perianastomotic peritoneal fluid, or within 2 hours and should be measured on laboratory standard meters.

Leading on from this project to further explore the utility of lactate and pH as biomarkers of AL one of the first issues to be addressed is to determine what affects the stability of lactate and pH in peritoneal fluid. Interrogation of cellular constituents of samples taken in a variety of settings – from those with non-infective ascites to those with blood-stained post-operative peritoneal fluid containing bacteria should be compared. Measurement of the concentration of RBCs, WBCs, osmolality, partial pressures of oxygen and carbon dioxide, number of bacteria present, the effect of temperature and of exposure of the sample to air should be explored. The results of this work would inform both measurement protocols in future studies and the design of lactate and pH sensors. It would also help to determine whether the variable lactate results obtained in chapter 6 were due to inherent instability of lactate in post-operative peritoneal fluid or due to the LP2 meter cross reacting with other cellular constituents.

Medical biosensor research and technology is a rapidly expanding field with over 200 companies working in the area (Luong et al., 2008). The potential use of this technology is exciting. For example, a group at The University of Strathclyde is exploring the potential to incorporate biosensors into implantable technology to detect re-endothelisation of coronary stents. Current imaging techniques are insufficient to detect this re-endothelialisation at an early stage. Early, in-vitro work has shown that impedance spectroscopy measurement can be used characterise different vascular cell types (Holland et al., 2018). This has the potential to allow non-invasive monitoring of the arterial response to stent placement using the stent as an electrode. Specifically in relation to AL, in a thesis made available in 2018, Hirst et al (Hirst, 2014) developed a point-of-care

amperometric biosensor for lactate as a biomarker of AL and carried out a feasibility study using an animal model of AL and peritoneal drain fluid from a group of patients undergoing colorectal resections. Their results were promising and highlight the growing interest in lactate as a local biomarker of AL. Potentially aspects of the concept and design of the work in these two studies could be translated into the setting of non-invasive monitoring of a colorectal anastomosis.

A key consideration in the development of a biosensor for AL is how to deploy the sensor into the perianastomotic environment, prevent it being dislodged and then safely remove it when it is no longer required. The option of incorporating a tiny real-time sensor into the tip of a drain could be explored, this would allow simple removal but ensuring it remained in the correct position intra-abdominally would be more challenging. Exploration of the feasibility of inserting a sensor transanally would be another alternative. As described in section 1.8.2 transanal devices have previously been created that are either manually removed or naturally expelled after several days. Indeed, transanal devices such as the EndoSPONGE® (a foam sponge which applies controlled pressure) are already in use to manage low extra-peritoneal ALs (Riss et al., 2010). Ultimately it may even be possible to harness current techniques for local management of AL with a biosensor to detect leak so that not only could a device detect a problem early it could also deliver local treatment such as the automatic release of antibiotics or the instigation of pressure therapy in real-time in response to signs of a leak.

Recently medical devices have come under increased scrutiny. An article published in December 2018 has highlighted that the number of reports of malfunctions and injuries sustained from medical devices is on the increase (Godlee, 2018). It is thought that one of the reasons for this may be the introduction of new devices after only testing in animals or very small numbers of humans. This highlights the importance of having a firm scientific basis prior to the development of any new technology. The work carried out in this project represents the first steps in gaining a better understanding of the perianastomotic environment which will provide the foundation for future work. In conjunction with this work a group of biomedical engineers at the University

of Strathclyde are exploring aspects of the modelling and design of an implantable device to detect AL (personal communication). Taking this approach and ensuring that there is thorough exploration of the biological and engineering considerations at any early stage will ensure that any device that is ultimately developed is safe and supported by clinical and scientific data. These important considerations underpin the approach taken in this thesis.

Whilst pursuit of such technology is likely to be expensive and time consuming its potential to impact on patient orientated outcomes may help to drive research. Previously most research topics and studies were selected and designed by clinicians and scientists. More recently, with the knowledge that patients and clinicians often have differing priorities when it comes to research (Chalmers et al., 2014), there has been increasing recognition of the importance of involving patients in setting research priorities and designing new studies. In a recent patient and public consultation about research priorities in bowel disease carried out by the ACPGBI, 71% of patients considered exploring, "the impact of treatment for bowel and anal cancers on quality of life" to be of 'high importance' as a research topic (McNair et al., 2017). In a study of the impact of post-operative complications on long-term quality of life after colorectal cancer surgery, AL was found to adversely impact on quality of life at 3 years with patients reporting significantly poorer outcomes for physical and social functioning, body image, mobility, self care and pain/discomfort (Brown et al., 2014). It is therefore likely that research to pursue a biosensor to detect/treat AL will be looked on favourably by patients and those making funding decisions. A further driver for research in this area is the potential applicability of the technology outwith the context of AL. For example, a lactate or pH sensor incorporated into the tip of a drain could be used in patients with a therapeutic ascitic drain to give early warning of the development of SBP by identifying a rising lactate and falling pH. Similarly incorporation into a chest drain would give warning as to the development of infection within the pleural cavity. Placement in a central venous access catheter may also provide real-time information as to the response to resuscitation of patients with a severe acidosis secondary to sepsis.

Overall, whilst in this work it was not possible to determine the clinical usefulness of perianastomotic lactate and pH as markers of AL, the stability of lactate and pH in peritoneal fluid over time has been characterised, trends identified in the pH of peritoneal fluid of patients making an uncomplicated recovery have been highlighted and the limits of blood biomarkers of AL have been demonstrated. In addition, it has provided insight into how further studies in the area should be conducted and which questions need to be addressed next in order to pursue the eventual aim of developing a biosensor to detect AL early. It is to be hoped that earlier detection of AL would allow prompt management ultimately reducing the morbidity and mortality currently associated with AL.

9 Appendices

9 Appendices

9.1 Appendix 1: Ethical permission for the study – 'The effects of time on pH and lactate levels in ascitic fluid

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

Gwasanaeth Moeseg Ymchwil
Research Ethics Service

Ariennir gan Lywodraeth Cymru
Funded by Welsh Government

WALES REC 7
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St David's Park
Jobswell Road
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SA31 3WY

Tel: 01267 225045
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Miss Emma Wright
Senior Clinical Fellow (Morton Fellow)
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN

11 May 2016

Dear Miss Wright

Study title:	**The effects of time on pH and lactate levels in ascitic fluid**
REC reference:	**16/WA/0142**
IRAS project ID:	**197239**

The Proportionate Review Sub-committee of the Wales REC 7 reviewed the above application on 11 May 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Ms Sue Byng, sue.byng@wales.nhs.uk. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Ethical opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

1) The Protocol should be updated in the section 'Methods' to outline that the clinical staff would assess potential participants in line with the criteria and put forward patients who were suitable. They would explain the study and give patients an information sheet and obtain their agreement verbally to have their details passed to the researcher.
2) The Protocol should be updated in the section 'Methods' to outline that patients would be given as much time as possible to consider participation in the study and that an information sheet would be provided at the earliest opportunity by the clinical staff.

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion").

Summary of discussion at the meeting (if applicable)

Care and protection of research participants; respect for potential and enrolled participants' welfare and dignity

The Sub-Committee understood that the Chief Investigator intended to access patient and potential participants' medical records prior to the actual recruitment and consent process. The Inclusion / Exclusion criteria established seemed to be very clear and concise and therefore the Sub-Committee was unclear why it was necessary for the researcher to have access to medical records prior to obtaining consent.
Miss Wright explained that she would only obtain minimal information just to clarify the patient details to ensure they met the criteria once the clinical nurse specialist had passed her the names of the patients. She needed to know which patient was coming to clinic so that she could organise being there.
The Sub-Committee understood this explanation but reminded Miss Wright there were strict rules regulating access to identifiable patient data by personnel not directly involved in patient care without the specific informed consent of the patient. It was reiterated that the inclusion/exclusion criteria which has been established was clear and concise and the normal approach would involve the clinical staff assessing potential participants in line with the criteria. If the potential participant consents to meet with the researcher she would then have the opportunity to discuss the study with them and provide the PIS and Consent Form.
Miss Wright would ideally like to have had advance sight of the patient records to ensure they met the study criteria but understood the position outlined by the Sub-Committee. She therefore agreed that she would not access the records in advance and would make sure the nurse specialist alerted her to the attendance of a patient meeting the criteria. The nurse specialist would explain the study and gain their verbal approval to meet the Chief Investigator who would discuss the study with the patient, obtain consent and only then look at their records.
The Sub-Committee accepted that obtaining verbal consent to provide their details to the researcher was acceptable given the low risk to the participants in this study.

The Sub-Committee discussed whether it was necessary for the researcher to contact the Scottish equivalent of the Confidentiality Advisory Group which was the Public Benefit and Privacy Panel. It was concluded this was not required if the researcher followed the steps she outlined above.

The Sub-Committee discussed the normal expectation for a potential participant to have a minimum of 24 hours to decide whether to participate in a research project and noted this study only allowed 30 minutes. Although it seems clear that the actual risk to potential participants is extremely low the Sub-Committee enquired whether any alternative approaches had been considered to allow a slightly longer period for participants to consider taking part in the study.
Miss Wright stated it may be possible for the nurse to alert the patient 24 hours in advance but sometimes the patients were booked in for drainage at very short notice and this would not be possible. She realised this was a short period of time but essentially she was only looking to take a sample which would otherwise be a waste product which would be discarded and there would be no risk to the patient as the fluid was being drained regardless of the study.
The Sub-Committee appreciated Miss Wright's explanation but advised that the normal procedure to ensure patients' were given time to consider participation would be to receive an information sheet in advance of their planned visit. However, it was clear this would not always be possible as more immediate measures may be necessary.
Miss Wright would make sure, where possible, that the nurse specialist provided patients with an information sheet and therefore advanced warning about the study, but should the procedure be organised at short notice, the approach may be more immediate. She would ensure patients were given as much time as possible to make a decision in both cases.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Covering letter on headed paper		15 April 2016
IRAS Checklist XML [Checklist_21042016]		21 April 2016
Participant consent form	5	20 April 2016
Participant information sheet (PIS)	4	12 April 2016
REC Application Form [REC_Form_21042016]		21 April 2016
Research protocol or project proposal	5	12 April 2016
Summary CV for Chief Investigator (CI) [Miss Emma Wright]		
Summary CV for supervisor (student research) [Miss Susan Moug]		

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

16/WA/0142	**Please quote this number on all correspondence**

Yours sincerely

pp. Mr Derek Lassetter
Joint Vice-Chair

Enclosures:	*List of names and professions of members who took part in the review*
	"After ethical review – guidance for researchers"
Copy to:	*Ms Emma Jane Gault* *Ms Elaine O'Neill, NHS GGC*

Wales REC 7

Attendance at PRS Sub-Committee of the REC meeting on 11 May 2016

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr John Buchan	Retired Medical Practitioner / Joint Vice-Chair	Yes	
Mr Owen Hughes	Psychologist	Yes	
Mr Derek Lassetter	Lay member / **Joint Vice-Chair**	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Ms Sue Byng	REC Manager

9.2 Appendix 2: NHS GGC Research & Development permission for the study – 'The effect of time on pH and lactate in ascitic fluid

Administrator: Mrs Elaine O'Neill
Telephone Number: 0141 232 1815
E-Mail: elaine.o'neill2@ggc.scot.nhs.uk
Website: www.nhsggc.org.uk/r&d

R&D Management Office
West Glasgow ACH
Dalnair Street
Glasgow G3 8SW

19 May 2016

Ms Susan Moug
Consultant Colorectal and General Surgeon
Royal Alexandra Hospital
Corsebar Road
Paisley PA2 9PN

NHS GG&C Board Approval

Dear Ms Moug,

Study Title:	**Effects of time on pH and lactate levels in ascitic fluid**
Principal Investigator:	**Ms Susan Moug**
GG&C HB site	**Royal Alexandra Hospital**
Sponsor	**NHS Greater Glasgow and Clyde**
R&D reference:	**GN16SG091**
REC reference:	**16/WA/0142**
Protocol no:	**V6 18/05/16**

I am pleased to confirm that Greater Glasgow & Clyde Health Board is now able to grant **Approval** for the above study.

Conditions of Approval

1. **For Clinical Trials** as defined by the Medicines for Human Use Clinical Trial Regulations, 2004

    a. During the life span of the study GGHB requires the following information relating to this site
        i. Notification of any potential serious breaches.
        ii. Notification of any regulatory inspections.

It is your responsibility to ensure that all staff involved in the study at this site have the appropriate GCP training according to the GGHB GCP policy (www.nhsggc.org.uk/content/default.asp?page=s1411), evidence of such training to be filed in the site file.

2. **For all studies** the following information is required during their lifespan.
   a. Recruitment Numbers on a monthly basis
   b. Any change of staff named on the original SSI form
   c. Any amendments – Substantial or Non Substantial
   d. Notification of Trial/study end including final recruitment figures
   e. Final Report & Copies of Publications/Abstracts

Please add this approval to your study file as this letter may be subject to audit and monitoring.

Your personal information will be held on a secure national web-based NHS database.

I wish you every success with this research study

Yours sincerely,

Mrs Elaine O'Neill
Senior Research Administrator

Cc: Ms Emma Wright

9.3 Appendix 3: Study protocol for the study – 'The effects of time on pH and lactate levels in ascitic fluid

Protocol Version 5

April 12th 2016

Title:

Effects of time on pH and lactate levels in ascitic fluid

Introduction

Anastomotic leak (AL) is the most dreaded complication in colorectal surgery. It leads to significant morbidity, mortality and is associated with a poorer oncological outcome [1]. Pre-, intra- and post-operative risk factors have been identified but as yet there is no reliable test to accurately detect or predict AL. Previous work has shown that measurement of clinical observations such as heart rate, blood pressure and temperature have poor specificity for detecting AL [2]. Blood parameters such as CRP and Procalcitonin have been shown to be useful markers of risk of anastomotic leak but again they lack specificity [3]. In cases of a suspected leak radiological studies, most often a computed tomographic (CT) scan, are performed in an attempt to confirm the diagnosis. Again, this modality is imperfect. It relies upon the experience and skill of the reporting radiologist. In addition, in the early stages a leak from an anastomosis maybe too subtle to be picked up on CT. Reports on the sensitivity and specificity of CT are variable but there is a consensus of opinion that CT alone is not a reliable test for AL [3,4].

Given the current limitations in the detection of AL there has been an attempt to identify new strategies. Much of this work has focused on so-called "biomarkers" for AL. Biomarkers are substances that can be measured locally at the anastomotic site and may give information about the health of the anastomosis [5]. To date, markers of inflammation (cytokines), wound repair (matrix metalloproteinases, MMPs),

ischaemia (lactate, pH) and detection of the presence of intra-abdominal bacteria have been studied [6,7,8,9]. These parameters have been analysed via microdialysis catheters placed close to the anastomosis and via peritoneal drain fluid. Elevated lactate, cytokines and reduced pH have emerged as factors which may help to predict/detect anastomotic leak several days prior to the onset of clinical symptoms.

These biomarkers reflect conditions in the milieu of the anastomosis and are therefore more specific than traditionally measured blood parameters which are not specific to the anastomosis.

Of these biomarkers, pH and lactate appear to show the most promise. They are easy and inexpensive to measure and results can be available rapidly. Different methods have been used to measure lactate and pH – intra-abdominal microdialysis, intra-luminal tonometry and analysis of peritoneal drain fluid. The simplest method is analysis of drain fluid. However, as drains are typically emptied once a day the fluid collected for analysis may have been out with the abdominal cavity sitting in the drainage bag for several minutes to hours before lactate and pH levels are measured. In 2009 Calatayud et al [10] took a sample of whole blood for lactate analysis and measured lactate levels at various time points over a two-hour period. They found that lactate levels increased over time. This could have important implications for the analysis of peritoneal drain fluid.

Aim

Part A – To determine the effect of time on pH and lactate levels in ascitic fluid.

Part B – To assess the validity of a handheld lactate sensor against a laboratory gold standard assay.

Patient risks/benefits

As the analysis will be carried out on surplus ascitic fluid that would otherwise be disposed of there is no risk/potential for harm to patients who agree to participate in the study. The benefit would be the knowledge that results obtained may ultimately help to reduce morbidity and mortality for patients undergoing colorectal surgery in the future.

Methods

All patients (approximately 20-30) attending the gastroenterology department at the Royal Alexandra Hospital (RAH) over an 8-week period for elective drainage of ascitic fluid will be eligible. Patients know to have blood borne viruses (HIV, hepatitis B, hepatitis C) will be excluded. The clinical team looking after the patient (liver nurse specialist and doctors from the gastroenterology department) will inform the researcher about potential patients for the study. The researcher will then screen their medical record. If the patient is eligible for the study they will be given an information sheet about the study when they attend for their ascitic drainage. They will have the opportunity to ask the researcher (or other suitably qualified member of the research team) questions about the study. If they are willing to participate they will sign a consent form.

The patients' community health index (CHI) number will be stored securely to allow patient demographics, underlying diagnosis and recent tests to be included in the analysis.

Part A – Effects of time on pH and lactate in ascitic fluid

At the time of insertion of an ascitic drain a 20ml sample of fluid will be collected into a white top container and stored at room temperature in a designated area in the high dependency unit (HDU). Lactate and pH will be measured at time zero, 30

minutes, 60 minutes, 120 minutes and 24 hours after collection. At the end of the 24 hour period the sample will be discarded. Lactate measurements will be made on a "Lactate Pro 2" handheld analyser (Arkray Global Business, Kyoto, Japan) and pH measurements on a PHH-7011 pocket tester (Omega products, UK).

Part B – Validation of handheld lactate sensor

10 – 15 of the fluid samples will have 10mls of fluid taken off and placed in a fluoride oxalate tube. The sample will then be taken to the Department of Biomedical Engineering at Strathclyde University within 6 hours where the lactate level will be analysed using a laboratory colorimetric assay (gold standard) on a clinical chemistry analyser.

Data handling/management

The patients' details/CHI number will be recorded. This information, along with the consent form, will be kept in a locked drawer in a locked office in the Royal Alexandra Hospital. Patients' will be assigned a unique study number. This will then be used on a database to store the study information and results, this will ensure that the results are anonymised. The database will be held on a hospital computer and encrypted memory stick.

Data analysis

Frequencies will be recorded and a students t test used to look for significant differences between samples, i.e. to test whether samples taken at different time points are significantly different from the baseline measurement taken at time zero.

Dissemination of results

The results will form part of the MD thesis for the researcher. It is also to be hoped that they will be accepted for publication in peer-reviewed scientific journals and also presented at relevant scientific/medical conferences.

Notifying participants of results

A section on the patient information sheet will invite participants to contact the researcher to find out the results of the study. It will outline that the study forms part of a larger piece of work towards a research degree and the results will be available from August 2017. Patients will be asked to use the contact details on the patient information sheet to contact the researcher after August 2017.

Ethics

The study will be submitted to an NHS Research Ethics Committee for review. No study activity will take place before a REC favourable opinion is obtained.

Finance

This work is being supported by grants awarded to the investigator from "The Royal College of Physicians and Surgeons of Glasgow" (Aileen Lynn Bequest Fund) and "The Ileostomy Association".

Indemnity

The principal investigator is an employee of NHS Greater Glasgow and Clyde and will therefore be covered by them for indemnity.

References

1. Boccola MA, Buettner PG, Rozen WM et al. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. World J Surg 2011;35:186–95.

2. Erb L, Hyman NH, Osler T. Abnormal vital signs are common after bowel resection and do not predict anastomotic leak. J Am Coll Surg 2014;218:1195-1200.

3. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. BJS 2015; 102:462-479.
4. Hirst N, Tiernan J, Millner P, Jayne D. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis* 2014; **16**: 95–109.

5. Komen N, De Bruin RWF, Kleinrensink GJ, Jeekel J, Lange JF. Anastomotic leakage, the search for a reliable biomarker. A review of the literature. Colorectal Dis 2008; 10: 109–15.

6. Cini C, Wolthuis A, D'Hoore A. Peritoneal fluid cytokines and matrix metalloprotinases as early markers of anstomotic leakage in colorectal anastomosis: a literature review and meta-analysis. Colorectal Dis 2013; 15:1070-1077.
7. Matthiessen P, Strand J, Jansson K et al. Is early detection of anastomotic leakage possible by intraperitoneal microdi-alysis and intraperitoneal cytokines after anterior resection of the rectum for cancer? Dis Colon Rectum 2007; 50: 1918–27.

8. Millan M, Garc_ıa-Granero E, Flor B, Garc_ıa-Botello S, Lledo S. Early prediction of anastomotic leak in colorectal cancer surgery by intramucosal pH. Dis Colon Rectum 2006; 49: 595–601.

9. Komen N, Morsink MC, Beiboer S et al. Detection of colon flora in peritoneal drain fluid after colorectal surgery: can RT-PCR play a role in diagnosing anastomotic leakage? J Microbiol Meth 2009; 79: 67–70.

10. Calatayud O, Tenlas JM. Effects of time, temperature and blood cell counts on levels of lactate in heparinized whole blood gas samples. Scand J Clin Lab Invest 2003; 63:311-314.

9.4 Appendix 4: Patient information sheet (PIS) for the study – 'The effect of time on pH and lactate levels in ascitic fluid (abdominal fluid)

Version 4
April 12th 2016

The effects of time on pH and lactate levels in ascitic fluid (abdominal fluid)

Information Sheet

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

Who is conducting the research?

The research is being carried out by Miss Emma Wright (surgical registrar) and Ms Susan Moug (Consultant Surgeon) from the Department of General Surgery. The research will contribute to the submission of a thesis for a higher degree (an MD) at the University of Glasgow by Miss Wright. This degree is being carried out over a 2 year period commencing November 2015.

What is the purpose of the study?

There is some research to suggest that measuring pH and lactate levels in abdominal fluid after bowel surgery may help to predict the development of a specific complication of bowel surgery - an anastomotic leak (a leak from a surgically created join between two cut ends of bowel). Leaks can be hard to predict and there is often a delay in diagnosing them. The incidence of anastomotic leaks is reported to vary from 5-20%. The consequences can be very serious for the patient. They become very unwell and often require further surgery possibly resulting in the need for a permanent stoma (colostomy or "bag") and admission to intensive care. Occasionally patients can die as a consequence of becoming very unwell after a leak.

Our overall aim is to develop a sensor or device that measures pH and lactate which can be placed inside the abdomen after bowel surgery. This would help us to detect anastomotic leaks more quickly and therefore reduce the complications experienced by the patient.

As part of further investigations in this area we need to find out whether a delay in analysing a fluid sample would affect the results, i.e. if a sample of abdominal fluid is taken but there is a delay of minutes to hours in analysing it will the pH and lactate levels be affected and therefore give a false result?

In this study we plan to obtain samples of abdominal fluid and measure the change in pH and lactate levels over time when the sample is stored at room temperature. This will ultimately help

us with the design of a sensor or device to detect anastomotic leaks early.

Why have I been invited?

You have been invited to take part in this study because you are here today to have excess abdominal fluid drained. Normally the majority of this fluid would be discarded. We hope to be able to take a small sample of this excess fluid to help with our study.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. You will be asked to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive or your future treatment.

What does taking part involve?

You do not have to actively do anything to take part. From the excess fluid that you are having drained we would simply take a 20ml sample which would otherwise be being discarded. This will not involve any additional time or discomfort to you.

The sample will be stored for 24 hours. During this time the lactate and pH levels will be checked a various time points – within 5 mins, at 30 mins, 60 mins, 2 hours and 24 hours after the sample was first taken. After this time the sample will be discarded. Approximately half of the samples taken will be transported to Strathclyde University for additional tests – these are to check the performance of the lactate sensor we use in the hospital against a “gold standard” lactate analyser kept at the university. Once this test has been performed the sample will be disposed of at the university. The university has special facilities in place to safely dispose of clinical/human waste.

What happens to the information?

Your identity and personal information will be completely confidential and known only to the researcher and the people overseeing the research, who may need to look at it to make sure the study is being conducted properly. We will assign each participant a number and anonymously record underlying medical problems and blood test results from your medical records so that we can compare the results from patients with different medical conditions. The information obtained will stored securely – identifiable information will be kept in the hospital in a secure office. The anonymised information with a unique participation number will be included in a database which will be stored on a hospital computer and an encrypted memory stick. This will ensure that when the data is being analysed it is anonymous and individual patients will not be able to be identified from the database.

What are the possible benefits of taking part?

By taking part in this research you may help to improve outcomes for patients undergoing bowel surgery in the future.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the 'Wales REC 7' Research Ethics Committee.

If you have any further questions?

We will give you a copy of the information sheet and signed consent form to keep. If you would like more information about the study and wish to speak to someone you can contact the following people:

Miss Emma Wright (Researcher)
Ward 26 Day Room
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN
Email: Emmawright8@nhs.net
Phone: 0141 887 9111

Ms Susan Moug (Consultant Colorectal & General Surgeon and Research Supervisor of Miss Wright)
Ward 26 Day Room
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN
Email: Susanmoug@nhs.net
Phone: 0141 887 9111

Or alternatively if you would like to speak to someone not closely linked to the study, please contact:

Mr Mark Vella (Consultant Colorectal & General Surgeon)
Ward 26 Day Room
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN
Email: Mark.Vella@ggc.scot.nhs.uk
Phone: 0141 887 9111

Another useful source of information about research activity within the NHS is the 'Patient Advice and Support Service'. This can be accessed via any Citizens Advice Bureau (CAB) in Scotland. Their contact details are as follows:

Version 4
April 12th 2016

Website: www.patientadvicescotland.org.uk

Phone: 0141 375 7328 (local CAB service for Renfrewshire)

If you would like to know the results of the study?

As previously outlined this study will form part of a larger research project which will be submitted as a thesis by the researcher to the University of Glasgow for a higher degree (an MD). It is anticipated that results will be available from August 2017. Should you wish to find out the results you are welcome to contact the researcher using the contact details provided above from August 2017 onwards.

If you have a complaint about any aspect of the study?

If you are unhappy about any aspect of the study and wish to make a complaint, please contact the researcher in the first instance but the normal NHS complaint mechanism is also available to you.

Thank-you for your time

Version 4 April 12th 2016

9.5 Appendix 5: Consent form for the study – 'The effect of time on pH and lactate levels in ascitic fluid (abdominal fluid)

Department of General Surgery,
Royal Alexandra Hospital,
Paisley

Version 5 April 20th 2016

The effects of time on pH and lactate levels in ascitic fluid (abdominal fluid)

Consent form

Please initial the boxes

I confirm that I have read and understand the information sheet dated 20/04/16 (version 5) for the above study and have had the opportunity to ask questions

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

I understand that sections of my medical notes may be looked at by the research team and the regulatory authorities where it is relevant to my taking part in the research. I give my permission for these people to have access to my records.

I agree to take part in the above study.

----------	----------	----------
Name of participant	Date	Signature
----------	----------	----------
Name of researcher	Date	Signature

1 copy to patient, 1 copy to researcher, 1 copy to notes

9.6 Appendix 6: Ethical approval for the study – 'Can serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?'

Reissue to correct document list

NHS
Health Research Authority

East Midlands - Leicester Central Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is an acknowledgement letter from the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

20 September 2016

Miss Emma C Wright
Senior Clinical Fellow (Morton Fellow)
NHS Greater Glasgow and Clyde
Ward 26 Day Room
Royal Alexandra Hospital
Paisley
PA2 9PN

Dear Miss Wright

Study title:	**Can the serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?**
REC reference:	**16/EM/0394**
IRAS project ID:	**210376**

Thank you for responding to the favourable opinion with conditions letter.

I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 02 September 2016

Documents received

The documents received were as follows:

Reissue to correct document list

Document	*Version*	*Date*
IRAS Checklist XML [Checklist_20092016]		20 September 2016
Participant consent form [Consent v3]	3	05 September 2016
Participant information sheet (PIS) [PISv4]	4	05 September 2016

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Covering letter on headed paper [Covering letter]		09 August 2016
IRAS Checklist XML [Checklist_22082016]		22 August 2016
IRAS Checklist XML [Checklist_20092016]		20 September 2016
Participant consent form [Consent v3]	3	05 September 2016
Participant information sheet (PIS) [PISv4]	4	05 September 2016
REC Application Form [REC_Form_22082016]		22 August 2016
Research protocol or project proposal [Protocol]	2	09 August 2016
Summary CV for Chief Investigator (CI)		
Summary CV for student		
Summary CV for supervisor (student research)		

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

16/EM/0394 **Please quote this number on all correspondence**

Yours sincerely

Tad Jones
REC Assistant

E-mail: nrescommittee.eastmidlands-leicestercentral@nhs.net

Copy to: *Ms Emma-Jane Gault*
Ms Kayleigh Pender, NHS GGC R&D Department

9.7 Appendix 7: NHS GGC Research & Development approval for the study - 'Can serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?'

Senior Research Administrator: Kayleigh Pender
Telephone Number: 0141 232 1826
E-Mail: Kayleigh.pender@ggc.scot.nhs.uk
website www.nhsggc.org.uk/r&d

Clinical Research & Development
West Glasgow ACH
Dalnair Street
Glasgow G3 8SJ
Scotland, UK

29/09/2016

Ms Emma Wright
NHS Greater Glasgow and Clyde
Ward 26 Day Room
Royal Alexandra Hospital
Paisley
PA2 9PN

NHS GG&C Board Approval

Dear Ms Wright,

Study Title:	Can the serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?
Principal Investigator:	Ms Emma Wright
GG&C HB site	Royal Alexandra Hospital
Sponsor	NHS Greater Glasgow & Clyde
R&D reference:	GN16GA471
REC reference:	16/EM/0394
Protocol no: (including version and date)	V2.0 09.08.16

I am pleased to confirm that Greater Glasgow & Clyde Health Board is now able to grant **Approval** for the above study.

Conditions of Approval

1. **For Clinical Trials** as defined by the Medicines for Human Use Clinical Trial Regulations, 2004
   a. During the life span of the study GGHB requires the following information relating to this site
      i. Notification of any potential serious breaches.
      ii. Notification of any regulatory inspections.

It is your responsibility to ensure that all staff involved in the study at this site have the appropriate GCP training according to the GGHB GCP policy (www.nhsggc.org.uk/content/default.asp?page=s1411), evidence of such training to be filed in the site file.

2. **For all studies** the following information is required during their lifespan.
   a. Recruitment Numbers on a quarterly basis
   b. Any change of staff named on the original SSI form
   c. Any amendments – Substantial or Non Substantial

d. Notification of Trial/study end including final recruitment figures
e. Final Report & Copies of Publications/Abstracts

Please add this approval to your study file as this letter may be subject to audit and monitoring.

Your personal information will be held on a secure national web-based NHS database.

I wish you every success with this research study

Yours sincerely,

Kayleigh Pender
Senior Research Administrator

9.8 Appendix 8: Protocol for the study - 'Can serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?'

Version 2

August 9th 2016

Title:

Can the serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?

Introduction

Anastomotic leak (AL) is the most dreaded complication in colorectal surgery. It leads to significant morbidity, mortality and is associated with a poorer oncological outcome (1). Pre-, intra- and post-operative risk factors have been identified but as yet there is no reliable test to accurately detect or predict AL. Previous work has shown that measurement of clinical observations such as heart rate, blood pressure and temperature have poor specificity for detecting AL Erb, Hyman (2). Blood parameters such as CRP and Procalcitonin have been shown to be useful markers of risk of anastomotic leak but again they lack specificity McDermott, Heeney (3). In cases of a suspected leak radiological studies, most often a computed tomographic (CT) scan, are performed in an attempt to confirm the diagnosis. Again, this modality is imperfect. It relies upon the experience and skill of the reporting radiologist. In addition, in the early stages a leak from an anastomosis may be too subtle to be picked up on CT. Reports on the sensitivity and specificity of CT are variable but there is a consensus of opinion that CT alone is not a reliable test for AL McDermott, Heeney (3) Hirst, Tiernan (4).

Given the current limitations in the detection of AL there has been an attempt to identify new strategies. Much of this work has focused on so-called "biomarkers" for AL. Biomarkers are substances that can be measured locally at the anastomotic site

and may give information about the health of the anastomosis Komen, de Bruin (5). To date, markers of inflammation (cytokines), wound repair (matrix metalloproteinases, MMPs), ischaemia (lactate, pH) and detection of the presence of intra-abdominal bacteria have been studied Cini, Wolthuis (6), Matthiessen, Strand (7), Millan, Garcia-Granero (8), Komen, Slieker (9). Elevated lactate, cytokines and reduced pH have emerged as factors which may help to predict/detect anastomotic leak several days prior to the onset of clinical symptoms.

These biomarkers reflect conditions in the milieu of the anastomosis and are therefore more specific than traditionally measured blood parameters which are not specific to the anastomosis.

Of these biomarkers, pH and lactate appear to show the most promise. In most of the existing research lactate has been measured via microdialysis (Matthiessen, Strand (7), Horer, Norgren (10), Daams, Wu (11), Pedersen, Qvist (12)). This involves inserting very small probes with dialysis membranes into the operative field at the end of a procedure. Fluid then flows through the circuit constantly allowing measurement of various analytes including lactate. It is expensive, technically challenging and technical failures are common (Daams, Wu (11)). One study has looked at measurement of lactate from peritoneal drains (Bini, Ferrari (13)). They studied lactate in relation to "major complications requiring reintervention" which included anastomotic leaks. They found that raised lactate levels were predictive of complications including AL. Of the two studies looking at pH (Millan, Garcia-Granero (8), Yang, Huang (14)) only one used peritoneal drain fluid (Yang, Huang (14)). They found that a low pH on post-operative day 3 was predictive of AL which occurred several days later. In the other study a catheter was sited intraluminally just proximal to the anastomosis and pH measured via tonometry (Millan, Garcia-Granero (8)). Measurement of lactate and pH via peritoneal drain fluid analysis is quick, easy and inexpensive, it is therefore an attractive alternative to microdialysis and intraluminal tonometry.

This study will measure peritoneal drain fluid pH and lactate levels following colorectal surgery. We aim to determine if the finding of Yang et al regarding the ability of day 3 pH to predict anastomotic leak can be replicated and we will be the first group to look at specifically at peritoneal drain fluid lactate in relation to prediction of anastomotic leak.

Aim

Part A - To determine if measurement of peritoneal drain fluid pH can predict colorectal anastomotic leak

Part B – To determine if measurement of peritoneal drain fluid lactate can predict colorectal anastomotic leak

Patient risks/benefits

As the analysis will be carried out on surplus ascitic fluid that would otherwise be disposed of there is no risk/potential for harm to patients who agree to participate in the study. The benefit would be the knowledge that results obtained may ultimately help to reduce morbidity and mortality for patients undergoing colorectal surgery in the future.

Methods

All patients attending the Royal Alexandra Hospital (RAH) over a 9-month period for elective colorectal resection will be eligible. The placement of a peritoneal drain and the timing of its removal following elective colorectal surgery is at the discretion of the operating surgeon, typically they are removed around post-operative day 5.

There are approximately 3-4 colorectal resections per week in the department but it is anticipated that not all will meet inclusion criteria/give consent to participate. The aim would be to have 30 patients in the study with samples taken daily for 3-5 days

following surgery. Little work has been done in this area so a power calculation is difficult.

Inclusion criteria:

- Age 18-100
- Attending for elective colorectal surgery
- Placement of a peritoneal drain during surgery

Exclusion criteria:

- Known to have blood bourne virus (HIV, hepatitis B, hepatitis C).

The patients' community health index (CHI) number will be stored securely to allow patient demographics, underlying diagnosis and recent tests to be included in the analysis. The following information will be obtained: age, sex, ASA grade, smoking status, alcohol intake, body mass index (BMI), neoadjuvant chemotherapy status, mechanical bowel preparation received, type of operation, duration of operation, blood loss, requirement for blood transfusion, the anastomosis technique used, presence/absence of a covering stoma and placement of peritoneal drain. The results of the patients routine daily blood tests taken during their admission including: albumin, white-cell count (WCC), C-reactive protein (CRP) and procalcitonin will be recorded. The patients' notes will be reviewed to determine the post-operative course – presence/absence of anastomotic leak or other complication during their admission. The case notes will be looked at again 30 days following discharge to determine if pre-admission for any complication including the late detection of AL has occurred.

Each morning a fresh (within 2 hours of emptying the drain) sample of fluid (10-20mls) from the peritoneal drain will be tested for lactate and pH levels. Lactate measurements will be made on a 'Lactate Pro 2" handheld analyser (Arkray Global Business, Kyoto, Japan) and pH measurements on a PHH-7011 pocket tester (Omega products, UK). This will be done daily until the drain is removed or the patient is

discharged. After measurement of lactate and pH levels the fluid will then be discarded via the standard hospital clinical waste stream.

In order to validate the use of the lactate and pH sensors 10-15 of the samples will have 10mls of fluid taken and placed in a universal container and transferred to the Department of Biomedical Engineering at Strathclyde University within 6 hours. At the university, lactate levels will be measured using a laboratory colorimetric assay (gold standard) and pH also measured using gold standard laboratory protocol. After testing the samples will be disposed of immediately via the universities clinical waste service.

Data handling/management

The patients' details/CHI number will be recorded. This information, along with the consent form, will be kept in a locked drawer in a locked office in the Royal Alexandra Hospital. Patients' will be assigned a unique study number. This will then be used on a database to store the study information and results, this will ensure that the results are anonymised. The database will be held on a hospital computer and encrypted memory stick.

Data analysis

The Mann-Whitney test will be used to look for significant differences between the samples for patients with AL versus those without AL. For lactate and pH the area under the curve (AUC) will be calculated for each post-operative day to assess whether lactate and pH can predict AL.

Dissemination of results

The results will form part of the MD thesis for the researcher. It is also to be hoped that they will be accepted for publication in peer-reviewed scientific journals and also presented at relevant scientific/medical conferences.

Notifying participants of results

A section on the patient information sheet will invite participants to contact the researcher to find out the results of the study. It will outline that the study forms part of a larger piece of work towards a research degree and the results will be available from November 2017. Patients will be asked to use the contact details on the patient information sheet to contact the researcher after November 2017.

Ethics

The study will be submitted to an NHS Research Ethics Committee for review. No study activity will take place before a REC favourable opinion is obtained.

Finance

This work is being supported by grants awarded to the investigator from "The Royal College of Physicians and Surgeons of Glasgow" (Aileen Lynn Bequest Fund) and "The Ileostomy Association".

Indemnity

The principal investigator is an employee of NHS Greater Glasgow and Clyde and will therefore be covered by them for indemnity.

References

1. Boccola MA, Buettner PG, Rozen WM, Siu SK, Stevenson AR, Stitz R, et al. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. World journal of surgery. 2011;35(1):186-95.
2. Erb L, Hyman NH, Osler T. Abnormal vital signs are common after bowel resection and do not predict anastomotic leak. J Am Coll Surg. 2014;218(6):1195-9.

3. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. Br J Surg. 2015;102(5):462-79.
4. Hirst NA, Tiernan JP, Millner PA, Jayne DG. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. Colorectal Dis. 2014;16(2):95-109.
5. Komen N, de Bruin RW, Kleinrensink GJ, Jeekel J, Lange JF. Anastomotic leakage, the search for a reliable biomarker. A review of the literature. Colorectal Dis. 2008;10(2):109-15; discussion 15-7.
6. Cini C, Wolthuis A, D'Hoore A. Peritoneal fluid cytokines and matrix metalloproteinases as early markers of anastomotic leakage in colorectal anastomosis: a literature review and meta-analysis. Colorectal Dis. 2013;15(9):1070-7.
7. Matthiessen P, Strandl J, Jansson K, Tornquist C, Andersson M, Rutegard J, et al. Is early detection of anastomotic leakage possible by intraperitoneal microdialysis and intraperitoneal cytokines after anterior resection of the rectum for cancer? Dis Colon Rectum. 2007;50(11):1918-27.
8. Millan M, Garcia-Granero E, Flor B, Garcia-Botello S, Lledo S. Early prediction of anastomotic leak in colorectal cancer surgery by intramucosal pH. Dis Colon Rectum. 2006;49(5):595-601.
9. Komen N, Slieker J, Willemsen P, Mannaerts G, Pattyn P, Karsten T, et al. Polymerase chain reaction for Enterococcus faecalis in drain fluid: the first screening test for symptomatic colorectal anastomotic leakage. The Appeal-study: analysis of parameters predictive for evident anastomotic leakage. Int J Colorectal Dis. 2014;29(1):15-21.
10. Horer TM, Norgren L, Jansson K. Intraperitoneal glycerol levels and lactate/pyruvate ratio: early markers of postoperative complications. Scand J Gastroenterol. 2011;46(7-8):913-9.
11. Daams F, Wu Z, Cakir H, Karsten TM, Lange JF. Identification of anastomotic leakage after colorectal surgery using microdialysis of the peritoneal cavity. Tech Coloproctol. 2014;18(1):65-71.
12. Pedersen ME, Qvist N, Bisgaard C, Kelly U, Pedersen S. Peritoneal microdialysis. Early diagnosis of anastomotic leakage after LAR for rectal cancer. Scandinavian Journal of Surgery. 2009.
13. Bini R, Ferrari G, Apra F, Viora T, Leli R, Cotogni P. Peritoneal lactate as a potential biomarker for predicting the need for reintervention after abdominal surgery. J Trauma Acute Care Surg. 2014;77(2):376-80.
14. Yang L, Huang X-E, Xu L, Zhou X, Zhou J-N, Yu D-S, et al. Acidic Pelvic Drainage as a Predictive Factor For Anastomotic Leakage after Surgery for Patients with Rectal Cancer. Asian Pacific Journal of Cancer Prevention. 2013;14(9):5441-7.

9.9 Appendix 9: Patient information sheet (PIS) for the study - 'Can serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?'

Version 3
August 22nd 2016

Can the serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?

Information Sheet

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

Who is conducting the research?

The research is being carried out by Miss Emma Wright (Surgical Registrar) and Ms Susan Moug (Consultant Surgeon) from the Department of General Surgery. The research will contribute to the submission of a thesis for a higher degree (an MD) at the University of Glasgow by Miss Wright. This degree is being carried out over a 2 year period commencing November 2015.

What is the purpose of the study?

There is some research to suggest that measuring pH and lactate levels in abdominal fluid after bowel surgery may help to predict the development

of a specific complication of bowel surgery - an anastomotic leak (a leak from a surgically created join between two cut ends of bowel). Leaks can be hard to predict and there is often a delay in diagnosing them. The incidence of anastomotic leaks is reported to vary from 5-20%. The consequences can be very serious for the patient. They can become very unwell and often require further surgery possibly resulting in the need for a permanent stoma (colostomy or "bag") and admission to intensive care. Occasionally patients can die as a consequence of becoming very unwell after a leak.

Following colorectal surgery the surgeon often (but not always) leaves a drain in the abdomen. This helps them to monitor the patients progress after the operation and may help to detect complications. In previous studies researchers have measured various substances from fluid in the drain to see if they can help to detect an anastomotic leak at an early stage. Lactate and pH have emerged as the most promising candidates.

Our overall aim is to develop a sensor or device which can be placed inside the abdomen after bowel surgery which will monitor the environment around the anastomosis and provide an early warning of any adverse changes which may indicate a leak. This would help us to detect anastomotic leaks more quickly and therefore reduce the complications experienced by the patient.

In trying to develop a sensor we have to decide which substances it should measure. In view of previous research in this area we think lactate and pH might be the best substances to measure. However, the studies that have previously been done are small. In order to help us to decide how to develop our sensor we need to carry out a further study to attempt to confirm or refute the previous finding that lactate and pH are helpful in detecting anastomotic leaks.

In this study we plan to measure the lactate and pH levels of fluid in the abdominal drain each day following colorectal surgery. The placement of a drain is at the discretion of the operating surgeon at the time of surgery. Those who have a drain placed will have the lactate and pH level of the drain fluid measured each day until the drain is removed, the timing of removal of the drain will be decided by the operating surgeon.

Version 3
August 22nd 2016

Why have I been invited?

You have been invited to take part in this study because you are having colorectal surgery that will involve the creation of an anastomosis and as part of the procedure your surgeon may choose to leave an abdominal drain in place at the end of your operation. If you consent to participate but your surgeon decides not to place a peritoneal drain then you will not enter the study.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. You will be asked to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive or your future treatment.

What does taking part involve?

You do not have to actively do anything to take part. When a drain is left in your abdomen the fluid collected in it is measured each day and then discarded. Prior to it being discarded we would measure the lactate and pH levels and then it would be disposed of via the normal procedure on the ward. Having the lactate and pH levels tested will not involve any additional time or discomfort to you. Approximately 10-15 of the samples taken in the study will be transported to Strathclyde University for additional tests – these are to check the performance of the lactate and pH sensors we use in the hospital against a "gold standard" analysers kept at the university. Once this test has been performed the samples will be disposed of at the university. The university has special facilities in place to safely dispose of clinical/human waste.

What happens to the information?

Your identity and personal information will be completely confidential and known only to the researcher and the people overseeing the research, who

may need to look at it to make sure the study is being conducted properly. We will assign each participant a number and anonymously record the lactate and pH levels of the drain fluid each day along with the following information: age, sex, ASA grade (fitness grading used by anesthetists), smoking status, alcohol intake, body mass index (BMI), neoadjuvant chemotherapy status, mechanical bowel preparation received, type of operation, duration of operation, blood loss, requirement for blood transfusion, the anastomosis technique used, presence/absence of covering stoma and placement of peritoneal drain and the results of routine post-operative daily blood tests. Your medical record will be checked again at 30 days after discharge to see if you have subsequently been re-admitted with a complication such as an anastomotic leak. The information obtained will stored securely – identifiable information will be kept in the hospital in a secure office. The anonymised information with a unique participation number will be included in a database which will be stored on a hospital computer and an encrypted memory stick. This will ensure that when the data is being analysed it is anonymous and individual patients will not be able to be identified from the database.

What are the possible benefits of taking part?

By taking part in this research you may help to improve outcomes for patients undergoing bowel surgery in the future.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the *BLANK* Research Ethics Committee.

If you have any further questions?

We will give you a copy of the information sheet and signed consent form

to keep. If you would like more information about the study and wish to speak to someone you can contact the following people:

Miss Emma Wright (Researcher)
Ward 26 Day Room
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN
Email: Emmawright8@nhs.net
Phone: 0141 887 9111

Ms Susan Moug (Consultant Colorectal & General Surgeon and Research Supervisor of Miss Wright)
Ward 26 Day Room
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN
Email: Susanmoug@nhs.net
Phone: 0141 887 9111

Or alternatively if you would like to speak to someone not closely linked to the study, please contact:

Mr Mark Vella (Consultant Colorectal & General Surgeon)
Ward 26 Day Room
Royal Alexandra Hospital
Corsebar Road
Paisley
PA2 9PN
Email: Mark.Vella@ggc.scot.nhs.uk
Phone: 0141 887 9111

Another useful source of information about research activity within the NHS is the 'Patient Advice and Support Service'. This can be accessed via any Citizens Advice Bureau (CAB) in Scotland. Their contact details are as

follows:

Website: www.patientadvicescotland.org.uk

Phone: 0141 875 7328 (local CAB service for Renfrewshire)

If you would like to know the results of the study?

As previously outlined this study will form part of a larger research project which will be submitted as a thesis by the researcher to the University of Glasgow for a higher degree (an MD). It is anticipated that results will be available from November 2017. Should you wish to find out the results you are welcome to contact the researcher using the contact details provided above from November 2017 onwards.

If you have a complaint about any aspect of the study?

If you are unhappy about any aspect of the study and wish to make a complaint, please contact the researcher in the first instance but the normal NHS complaint mechanism is also available to you.

Thank-you for your time

Version 3 August 22nd 2016

9.10 Appendix 10: Consent form for the study - 'Can serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?'

Department of General Surgery,
Royal Alexandra Hospital,
Paisley

Version 3 September 5th 2016

Can the serial measurement of pH and lactate in peritoneal drain fluid predict anastomotic leak after colorectal surgery?

Consent form

Please initial the boxes

I confirm that I have read and understand the information sheet dated 05/09/16 (version 4) for the above study and have had the opportunity to ask questions

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

I understand that sections of my medical notes may be looked at by the research team and the regulatory authorities where it is relevant to my taking part in the research. I give my permission for these people to have access to my records.

I agree to take part in the above study.

----------	----------	----------
Name of participant	Date	Signature
----------	----------	----------
Name of researcher	Date	Signature

9.11 Appendix 11: Laboratory protocol for measuring lactate

Lactate assay for Peritoneal Fluid Samples

Revision	Author	Description	Date
1.0	Joshua Paulinus (Strathclyde)	Original document	28/10/16
1.1	Emma Wright (Strathclyde)	Amended version	07/02/17

1. Introduction

This procedure details the method for determining lactate concentration (up to 20mM) in fluid samples using the Randox Lactate assay (LC2389) in a 96 well plate.

2. Equipment

1000µL pipette and tips

2.5µL pipette and tips

Computer controlled Labsystems Multiskan Ascent

Eppendorff tubes

50ml plastic tube

3. Materials

Randox Lactate Assay Kit (LC2389)

Sodium L-Lactate

Deionised water

Samples (peritoneal fluid)

PPE consisting of: blue nitrile gloves, face shield, lab coat

4. Methodology

4.1 Creating calibration solutions

a) Add 0.1221g of Sodium Lactate to 50mL of water in a 50mL plastic container to create a 20mM solution.

b) Serially dilute the 20mM of sodium lactate to make 10mM, 5mM, 2.5mM and 1.25mM concentrations in separate Eppendorf tubes using 1000µL pipette. (Table below to help)

Lactate solution concentration	
20mM	Add 2mL of 20mM to Eppendorf tube made from previous step
10mM	1mL 20mM lactate solution + 1mL dH_2O
5mM	1mL 10mM lactate solution + 1mL dH_2O
2.5mM	1mL 5mM lactate solution + 1mL dH_2O
1.25mM	1mL 2.5mM lactate solution + 1mL dH_2O

4.2 Equipment and Materials Preparation

a) Prior to setting up plate, make up the enzyme reagent found in the Randox lactate assay kit by adding 6mL of the buffer found in the kit. For this method, each bottle is enough for 24 samples so make up enough for your purposes (enzyme is stable for approximately 2 weeks in the fridge once buffer is added). Additionally, switch on the Multiskan plate reader and set up the experiment on the Skanit software found on the connected PC. Use the following settings:

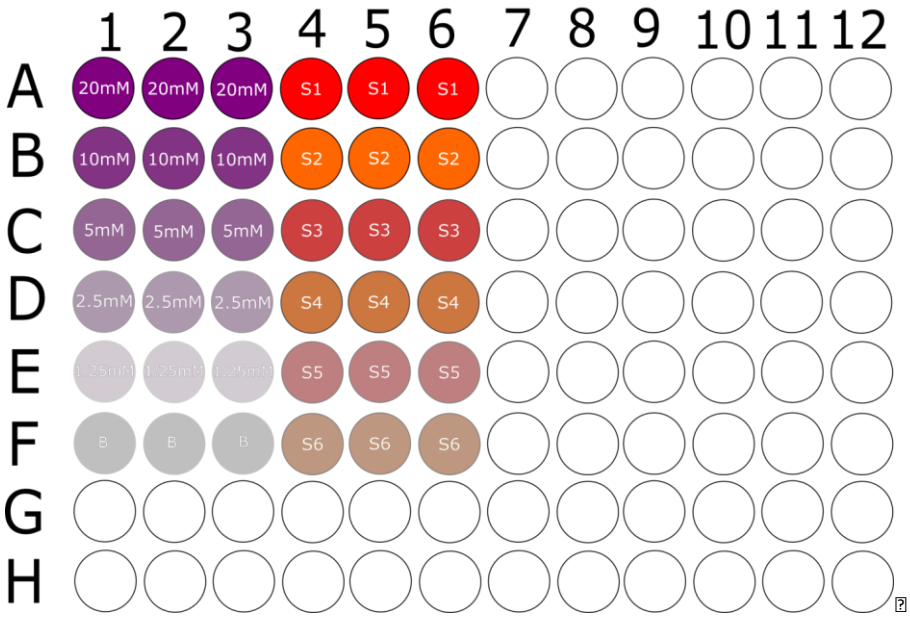
- Incubation temperature at 37°C

- Add shaking at low speed for 5 mins

- Add single absorbance measurement at 550nm (ensure shaking happens before this step)

Remember to add save results to an Excel file.

4.3 96 Well Plate set up



Key: B=Blank Sample, S=Sample Repeat (e.g. S1=Sample 1 repeat), Purple wells= calibration solutions

a) For each well, add 2.5µL of calibration solution first into the 96 well plate as shown above using a pipette. For the blank wells, add 2.5µL deionised water. More repeats can be added as required. Should you wish to check the accuracy of the calibration curve, you may add the standard solution found in the lactate assay kit for comparison. The concentration of the standard will be found in the box of the kit.

b) The sample of peritoneal fluid will be kept in a demarcated "biohazard" area of the lab. The 96 well plate should be brought to this area for the samples of peritoneal fluid to be added.

c) Once done, check the Multiskan Plate reader is at 37°C and then add 250µL of the enzyme reagent into all wells containing a sample/calibration solution using a pipette. Do this part quickly so all samples react at approximately the same time. It might be advisable to use a multi-pipette instead.

d) Place a cover over the 96 well plate and transfer it to the Multiskan and run the method programed earlier.

e) When the programme is complete the 96 well plate reader will be removed from the Multiskan and taken back to the demarcated "biohazard" area of the lab. The plate, and its contents, should be placed in Virkon solution. Thereafter samples and materials should be

disposed of as per the protocol outlined in the COOSH form for the handling of peritoneal fluid.

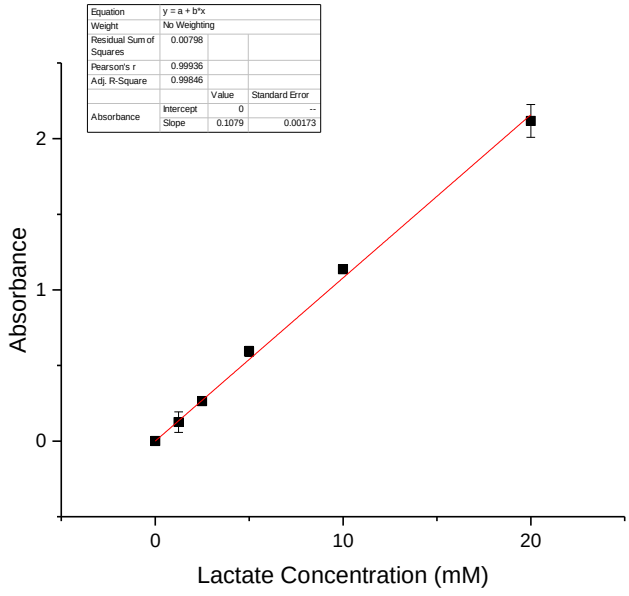
5. Analysis

Below is a sample calibration curve example (Table below). (0 is the blank reference)

Firstly, we will take an average (mean) of the absorbance values for each concentration (Column Mean (Y)). Standard deviation was also calculated to check how the range of data points for each reference (Column Standard Deviation (Y)). We then subtract the blank reference to get the correct absorbance value (seen in figure below in column F(Y)).

	A(X)	B(Y)	C(Y)	D(Y)	Mean(Y)	SD(yEr±)	E(Y)	F(Y)
Long Name	Lactate Concentration	Absorbance	Absorbance	Absorbance	Mean	Standard Deviation	Blank Reference	Absorbance
Units	mM							
Comments		1	2	3	Statistics On Rows of [Book1]Sheet1!Col("Absorbance")[1:6]	Statistics On Rows of [Book1]Sheet1!Col("Absorbance")[1:6]		Subtracted by "Blank Reference" on "Mean"
F(x)=								
1	20	2.055	2.26	2.219	2.178	0.10848	0.061	2.117
2	10	1.185	1.206	1.202	1.19767	0.01115	0.061	1.13667
3	5	0.626	0.645	0.691	0.654	0.03342	0.061	0.593
4	2.5	0.322	0.329	0.325	0.32533	0.00351	0.061	0.26433
5	1.25	0.187	0.119	0.254	0.18667	0.0675	0.061	0.12567
6	0	0.061	0.061	0.061	0.061	0	0.061	0
7								

Column A(X) and then column F(Y) are then plotted to get the calibration curve shown below.



Excel and origin (in this case) can be used to get a linear fit curve for which an equation can be developed. In this case, the y-intercept was set at 0 so the equation of the line is y=0.1079x. This relationship can be used to find the concentrations of lactate in samples.

9.12 Appendix 12: Laboratory protocol for measuring pH

pH testing of peritoneal drain fluid samples - Protocol

Revision	Author	Description	Date
1.0	Joshua Paulinus (Strathclyde)	Original document	17/11/16
1.1	Emma Wright	Revised & amended	13/04/17

1. Introduction

This procedure details the method for attaining pH measurements of peritoneal drain fluid samples using either Mettler Toledo InLab® Expert Pro-ISM or InLab® Micro with Mettler Toledo SevenCompact pH reader. It is advisable to use InLab® Expert Pro-ISM as it has temperature compensation.

2. Equipment

Description	**Qty**
pH probe (Either InLab® Expert Pro-ISM or InLab® Micro)	1
pH probe reader (SevenCompact pH reader)	1
Glass beakers	
Squeeze bottle containing deionised water	

3. Materials

Description	**Supplier**	**Qty**
Ethanol (required for biological testing)	Sigma 32221	
Deionised Water		
pH4 buffer solution tablets	Fisher Scientific B/4765/77	
pH7 buffer solution tablets	Fisher Scientific B/4760/77	
pH10 buffer solution	Acros Organics 258600010	
Virkon (For biological testing)		

4. Methodology

4.1 Preparation

4.1.1 Creating buffer solutions

pH4 buffer solution: Add one pH4 buffer solution tablet to 100mL of dH_2O

pH7 buffer solution: Add one pH7 buffer solution tablet to 100mL of dH_2O

4.2 Calibration of pH probe

Prior to experimentation, the pH probe requires calibration. Pour calibration solutions into separate glass beakers (enough to cover glass bulb) and enter calibration mode and calibrate according the manufacturer instructions. It is advisable to check calibration occasionally during experiments to check the probe is still reading correctly. Rinse probe with deionised water (use squeeze bottle over a beaker) prior to testing a different solution.

4.3 Preparation of the sample of peritoneal drain fluid

The sample will be handled as per the instructions in the COSHH form (Working with human peritoneal fluid (hazard group 2)) within the designated 'biohazard area'' of lab 4.09. It will be removed from the outer transport packaging and kept in the universal white top container that it was collected in.

4.4 Testing the peritoneal drain fluid sample

Prior to testing a sample rinse the probe with dH_2O (use squeeze bottle over a beaker) and then dip it in ethanol before thoroughly rinsing in dH_2O .To test a sample, simply place the pH probe into the solution. Use a pH probe stand to hold the pH probe in place and leave the probe until the reading given out stabilises and notes the value. The pH level will be measured 3 times to obtain an average reading. Rinse the pH probe with water (use squeeze bottle over a beaker) between taking each reading.

Once testing is complete, make up a 1% w/v Virkon solution and place the probe in this solution for 10-15mins before storing the pH probe in a solution of pH 4 buffer solution.

9.13 Appendix 13: COSHH form covering the handling of peritoneal fluid samples in the laboratory

University of Strathclyde Glasgow

CONTROL OF SUBSTANCES HAZARDOUS TO HEALTH
RISK ASSESSMENT FORM S21

Before completing this form, please read the University's COSHH Local Rules and Guidance Notes provided as separate documents in order to ensure the form is completed properly.

This form **MUST BE COMPLETED** prior to the commencement of any work involving hazardous substances, so that a suitable and sufficient assessment of any potential health risks can be made.

Individuals working under this risk assessment have a legal responsibility under the Health and Safety at Work Act, 1974 to ensure they are aware of the hazards associated with the work activities they are undertaking and that they follow the control measures stipulated to safeguard the health and safety of themselves and others.

SECTION 1

1.1 OPERATION / ACTIVITY		*Complete the relevant details of the activity being assessed.*	
Title:	Working with human peritoneal fluid (hazard group 2)		
Location(s) of work	Biomedical Engineering Lab, Wolfson Building	**Ref. No.**	

Brief description:
Permission has been obtained from the NHS Greater Glasgow and Clyde Research and Development (R&D) department to test the lactate and pH levels of peritoneal fluid obtained from patients undergoing ascitic drainage and those who have a peritoneal drain in-situ following colorectal surgery. Collection of samples from the first group has been approved by the Wales REC 7 NHS Ethics committee (reference no. 16/WA/0142) and for the post-operative patients by the Leicester Central NHS Ethics committee (reference no. 16/EM/0394). 10-15ml samples of peritoneal fluid will be obtained from patients at the Royal Alexandra Hospital in Paisley via normal clinical protocols. The samples will be collected into universal white-topped containers. These samples will be obtained and handled in the hospital as per the usual hospital standards.

When consulting the University guidelines on the transport of infectious substances and biological specimens', human ascitic fluid falls under the classification 'Category B biological substance (UN 3373)'. In accordance with the guidelines the specimen will be triple packed in a container which meets the requirements for transfer of a UN3373 substance. Briefly, the universal white top container will be placed in a secondary pack and a rigid outer pack. Between the specimen and secondary pack there will be absorbance pads. On the outside of the container a hazard label (UN 3373) will be displayed. Alongside this it will say 'Biological Substance, Category B'. The name/address of the person dispatching the specimen and the name/address of the person receiving the specimen will also be displayed. The researcher's name and contact number will be displayed as an emergency contact. The hospital has a contract with a taxi firm for the transport of clinical substances. They will be used to transport the sample from the hospital to the Wolfson Centre.

Transfer of the samples from the RAH to University of Strathclyde is covered by the generic material transfer agreement (MTA) that is in place between "Glasgow Biomedicine and The University of Strathclyde". This has been confirmed with Jane Hair from Biorepository and Paul Dearie from NHS GGC

R&D department. A copy of the generic MTA is attached to this form.

Samples will taken to lab 4.09. An area of the lab with be clearly demarcated with biohazard tape and signage. The samples will be placed in this area. Signs will be present on the door into the lab indicating the presence of potentially hazardous biological material. Experimental work will take place in the demarcated are of the lab. The lab is secure as access to the Wolfson building is restricted and requires a swipe card for entry. All persons with swipe card access are aware that biologically hazardous substances may be present and are alert to signs indicating where this is the case.

The samples will have their lactate and pH levels measured. These experiments will be carried out as per the attached protocols. Following use the samples will be disposed of through clinical waste in accordance with university policy and specific disposal instructions *(*see section 7*).*

1.2 PERSON RESPONSIBLE FOR THIS WORK			
Name:	Prof Patricia Connolly	**Position:**	
Department:		**Signature:**	

1.3 PERSON CONDUCTING THIS ASSESSMENT			
Name:	Emma Wright	**Position:**	Visiting Researcher
Date risk assessment undertaken:	19/01/17	**Signature:**	

1.4 ASSESSMENT REVIEW HISTORY

This assessment should be reviewed immediately if there is any reason to suppose that the original assessment is no longer valid. Otherwise, the assessment should be reviewed annually. The responsible person must ensure that this risk assessment remains valid during use.

REVIEW HISTORY				
	Review 1	**Review 2**	**Review 3**	**Review 4**
Due date				
Date conducted				
Conducted by				

A SIGNED COPY OF THIS ASSESSMENT MUST BE RETAINED BY THE DEPARTMENT

SECTION 2

2.1: SUMMARY CLASSIFICATION OF HAZARDOUS SUBSTANCES

Substances can be regarded as hazardous not just in the form in which they occur in the work activity but also in by-products and as intermediate substances in processes and waste residues.

CHEMICAL	V. Toxic ☐	Toxic ☐	Corrosive ☐	Harmful ☐	Mutagenic Category 1 ☐ 2 ☐ 3 ☐
Irritant ☐	Cytotoxic ☐	Flammable ☐	Oxidising ☐	Explosive ☐	Carcinogenic Category: 1 ☐ 2 ☐ 3 ☐
Toxic to reproductive system ☐		Environment ☐	Respiratory sensitiser ☐		Biological Hazard Group 1 ☐ 2 x 3 ☐

2.2: HAZARDS

List all the details of the hazardous substance used in the appropriate columns. Name of the substance including the chemical name where known. Quantity used in the process. Form of substance in use (e.g. liquid, powder, dust etc). The nature of the hazard should state whether the substance is very toxic, toxic, corrosive, harmful or irritant etc. For biological substances this should include the Hazard Grouping. If the substance has a Workplace Exposure Limit (WEL) it should be entered here. State whether a Material Safety Datasheet (MSDS) is available for the substance.

Substance	Quantity To be used	Form of Substance	Nature of Hazard or Biological Hazard Grouping	WEL (ppm or mg/m^3) 15 min or 8 hr	MSDS Available?
Human peritoneal (ascitic) fluid	10-15mls	Liquid	Biological hazard group 2	Yes ☐ No x	Yes ☐ No ☐
Sodium Hydroxide	Max. 100mL	Liquid (0.7%)	H319:Causes serious eye damage H315:Cause skin irritation	Yes ☐ No x	Yes ☐ No ☐
Virkon	500mL	Powder/Liquid (2%w/v)	H271:May cause fire or explosion; strong oxidiser H315: Cause skin irritation H318: Cause serious eye damage H335: May cause respiratory irritation	Yes ☐ No x	Yes ☐ No ☐
Ethanol	10ml	Liquid	H225: Highly flammable liquid and vapour H319: Causes serious eye damage	Yes ☐ No x	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐

				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐

2.2: HAZARDS

List all the details of the hazardous substance(s) used in the appropriate columns. Name of the substance including the chemical name where known. Quantity used in the process. Form of substance in use (e.g. liquid, powder, dust etc). The nature of the hazard should state whether the substance is very toxic, toxic, corrosive, harmful or irritant etc. For biological substances this should include the Hazard Grouping. If the substance has a Workplace Exposure Limit (WEL) it should be entered here. State whether a Material Safety Datasheet (MSDS) is available for the substance.

Substance	Quantity To be used	Form of Substance	Nature of Hazard or Biological Hazard Grouping	WEL (ppm or mg/m^3) 15 min or 8 hr	MSDS Available?
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐
				Yes ☐ No ☐	Yes ☐ No ☐

2.3: ROUTE(S) BY WHICH THE SUBSTANCES ARE HAZARDOUS *Complete all boxes that may apply*

Inhalation	Direct contact: skin, eyes	Skin absorption	Injection (via sharps)	Ingestion
X	x	☐	x	***x***

2.4: PROCESS FACTORS INFLUENCING THE RISK OF EXPOSURE

☐ Weighing x Pipetting ☐ Filtering X Shaking / Mixing ☐ Centrifugation

☐ Use of sharps ☐ Elevated temperatures ☐ High pressure ☐ Sonication

☐ Other (specify):

2.5: COMMENTS ON THE HAZARDS ASSOCIATED WITH THE SUBSTANCES

List any known or suspected health hazards associated with potential exposures to the substances used or generated in the operation or activity.

Normally within the peritoneal cavity there is up to 25ml of fluid present (peritoneal fluid). In a range of disease states, e.g. liver cirrhosis and heart failure, excess fluid can be created and accumulates in the peritoneal cavity. This excess peritoneal fluid generated by the body is often called 'ascites'. A sample of this fluid is often obtained sent for biochemical and microbiological analysis to help diagnose the underlying condition.

Following colorectal surgery there can also be increased levels of peritoneal fluid in the abdomen. This is due to tissue reaction to the surgery. In addition, the abdominal cavity is irrigated at the end of an operation with saline solution and the majority of this is removed via suction. However, inevitably some wash fluid remains so some of the additional fluid found in the peritoneal cavity may be residual saline wash. If a complication occurs, e.g. an anastomotic leak, then bowel content will leak into the peritoneal cavity and be present in the peritoneal fluid. The most common bacteria cultured from peritoneal fluid following colorectal surgery are e coli and e faecalis (Komen et al 2013). Transmission of these bacteria is by the faecal-oral route. These organisms are classified as hazard group 2 in the HSE approved list of biological agents.

Like any bodily fluid, peritoneal fluid has the potential to contain blood borne viruses (BBV) but infection is unlikely when diagnostic tests are being performed rather than cell/viral culture. In particular, the HSE guidelines state in paragraph 184 that work such as clinical chemistry on body fluid substances that contain or may contain BBVs can be carried out at containment level 2 (see attached HSE document). Taking this into consideration, ascites/peritoneal fluid, is considered to be a hazard group 2 substance. It is listed on the University of Strathclyde list of 'Chemicals and Biological Substances' as a hazard group 2 substance. In accordance with the university 'Guidance on the handling of blood and blood products', bodily fluids, where the screening status for BBVs is unknown, can be handled at category 2 containment. Use of a Microbiological Safety Cabinet is not specifically required.

The samples have the potential to cause laboratory acquired infection if they are ingested, inhaled or come into contact with open cuts/wounds or mucous membranes. Aerosol production is expected to be minimal. People working with peritoneal fluid samples should wear personal protective equipment (PPE) in the form of a laboratory coat, face shield and disposable nitrile gloves. Spillages should be cleared up using the disinfectant virkon. The laboratory standard operating procedures that will be used to ensure safe handling of samples are outlined below in section 11, experimental protocols are attached to the COSHH form.

Reference:

Komen N, Morsink MC, Beiboer S, Miggelbrink A, Willemsen P, van der Harst E, Lange JF and van Leeuwen WB (2009) Detection of colon flora in peritoneal drain fluid after colorectal surgery: can RT-PCR play a role in diagnosing anastomotic leakage? J Microbiol Methods 79 (1):67-70.

SECTION 3.0

3.0: IDENTIFICATION OF THOSE AT RISK OF EXPOSURE

Identify all categories of individuals who may be affected either directly or indirectly through the work activity.

x Dept. Staff	☐ Estates/Cleaning staff	x Pregnant Women or Women of reproductive age	
x Postgraduate Students	☐ Undergraduate students	☐ Young Workers	☐ Inexperienced Workers
☐ Immuno-compromised Individuals	☐ Contractors	☐ Visitors	☐ Other

SECTION 4.0

List the control measures taken to reduce risks. Can any substances be eliminated, or substituted with a less hazardous one? Are there any physical controls such as enclosure, local exhaust ventilation and PPE etc. Do not forget other controls including safe working procedures, information, instruction, training and supervision. Include details of maintenance and test schedules for physical controls.

4.1: CONTROL MEASURES – ELIMINATION / SUBSITUTION

Can any of the hazardous substances identified or the procedures required for this activity be eliminated or substituted with a less hazardous substance or procedure (e.g. by changing from a fine powder to a liquid form of the chemical)? ***If possible, please give details below:*** Yes ☐ No x

For research purposes, the fluid has in the same condition as it is taken from patients in order to properly determine lactate and pH levels.

4.2: CONTROL MEASURES – ENGINEERING & DESIGN

☐	Work can be carried out on the open bench without the use of control measures.			
☐	Local Exhaust Ventilation - Fume cupboard (FC)		FC No.	Location:
☐	Local Exhaust Ventilation (Other Partial Enclosure)		Specify:	
☐	Local Exhaust Ventilation (Full Enclosure)		Specify:	
☐	Microbiological Safety Cabinet	Class:	Cabinet No.	Location:
x	Biological Laboratory Containment Level:	2		
☐	Laminar Flow Cabinet		Specify:	
x	Other: please specify Work will be carried out in lab 4.09. It will take place in a clearly designated area of the lab. The bench top area will be marked off with tape and hazard signs will be displayed in the lab and on doors leading into the lab to alert staff/students to the hazard. Work will only be undertaken by those who have read, understood and signed this risk assessment.			

4.3: ADMINISTRATIVE CONTROLS *e.g. Risk assessments, SOPs, signage*

Entry to lab 4.09 where the work is undertaken is restricted through signage on door.

All staff and students conducting work on this project must have read and signed this risk assessment prior to commencing work.

Staff and students working with biological material must ensure that they have completed the BP2 biological safety

form on Pegasus. The BP2 form must be completed annually.

4.4: INSTRUCTION AND TRAINING

The following online University of Strathclyde biological safety modules must be completed by all staff and students who carry out work on peritoneal fluid:
- Module 1 Basic Biological Information
- Module 2 Controlling the Risk of Exposure to Infectious Agents
- Module 3 Safe working with Infectious Agents

These modules are available online under the University of Strathclyde Health and Safety webpage:
http://www.strath.ac.uk/wellbeing/safetyhealthandwellbeing/healthandsafetytraining/
Any problems accessing the training should be referred to the departmental safety convener.

Only the researcher, or other suitably qualified postgraduate students/members of staff who have read, signed and understood the relevant COSHH and risk assessments will work with peritoneal fluid samples. The researcher will demonstrate the protocols on a substitute (model) fluid for pipetting, experiment and disposal before the work starts.

4.5: SUPERVISION AND LONE WORKING

x The supervisor will approve straightforward routine work in progress.

☐ The supervisor will specifically approve the safe system of work.

☐ The supervisor will provide supervision personally to control the work.

Is lone working permitted for this activity? Yes **x**

A key aspect of our research is looking at lactate and pH levels in peritoneal drain fluid collected from patients on post-operative days 1, 2 and 3. The samples need to be tested shortly after collection from the drain. In the RAH there are 2 operating lists on a Thursday and 2 on a Friday. This means that post-operative days 1-3 fall over the weekend. For this reason lone working at the weekend is required so that these samples can be analysed in a timely manner following collection. The experiments being undertaken in the lab are low risk and therefore Prof Connolly feels that lone working is appropriate. However, when possible 2 people will work in the lab together testing the samples.

4.6: CONTROL MEASURES – PERSONAL PROTECTIVE EQUIPMENT

Eyes / Face	x Safety spectacles	☐ Goggles	X Face shield	***(specify)*** : General lab
Hand	x Gloves	***(specify)***: Disposable nitrile gloves EN374		
Respiratory	☐ Disposable respirator Type	☐ Full-face respirator Type	☐ Reusable half-face respirator Type	
	☐ Powered hood Type	☐ Breathing apparatus Type	☐ Other ***(specify)***	
Clothing	☐ Coverall Specify:	x Laboratory coat Specify: Blue side fastening Howie style	☐ Side –fastening coat Specify:	☐ Apron / Gown Specify:

	coat Other ☐ ***(specify)***

4.7: MONITORING AND HEALTH SURVIELLANCE

If monitoring or health surveillance is required for any of the hazardous substances relating to this assessment specify how this will be carried out.

Will monitoring for airborne contaminants be required? ***If "yes", give details below:***	Yes ☐ No x
Will health surveillance for workers be required? ***If "yes", give details below:***	Yes ☐ No x

SECTION 5.0: RISK EVALUATION RATING

Use the information above, and the guidance from the Hazard Identification and Risk Assessment section, taking into account the control measures in operation, decide the applicable SEVERITY and LIKELIHOOD, and ESTIMATE the risk rating

SEVERITY	LIKELIHOOD	RATING
1. Negligible injury or illness. *2. Minor injury or illness.* *3. Moderate injury or illness.* *4. Major injury or illness.* *5. Extreme loss, fatality, disaster.*	*1. Very Unlikely - Rarely happens* *2. Unlikely to occur.* *3. Possibly can occur.* *4. Likely to occur.* *5. Very Likely to occur.*	Rating = Severity x Likelihood
4	2	**8**

SECTION 6.0: STORAGE, TRANSPORT, HANDLING AND USE

Highlight any special circumstances relating to the safe method of storing, handling and using the substances. If there are any special requirements highlight them here.

Samples taken at RAH will be in universal white top containers. For transport, samples will be boxed and labelled as a 'Category B biological substance (UN 3373)' as outlined in section 1. Sample will be dispatched from the RAH to the Wolfson Centre in an authorised account taxi. On arrival at the Wolfson Centre samples will be taken to lab 4.09 and placed in the designated area. The samples will be handled and tested as per the protocol/SOP outlined in section 11.

During the work a container of Virkon solution will be in the designated area of the lab. All materials such as pipette tips and plate readers will be placed in the solution after being used. Used peritoneal fluid will also be poured into the Virkon solution. They will remain in the solution for 24 hours after which the liquid will be poured down the sink and plastic material will be autoclaved before disposal in the clinical waste stream.

Ethanol stock solution must be stored in the safety cabinet in W402. Lactate assay kit must be stored in fridge.

SECTION 7.0: WASTE DISPOSAL ROUTES

State how the substances or any excess or waste will be disposed of. If there are any special requirements due to the nature of the material, or if it should be treated as special waste, identify what procedures will be used for safe disposal.

☐	In-house to Council waste collection, after rendering safe.
☐	In-house to drain, after rendering safe.
☐	Solid chemical waste (disposed via Hazardous Waste Service)
☐	Liquid chemical waste (disposed via Hazardous Waste Service)
☐	Solvent waste (disposed via Hazardous Waste Service)
x	Biological Waste (disposed via Clinical Waste Service)
x	Other: please specify: During the work a container with Virkon solution will be in the designated area of the lab. All materials such as pipette tips and plate readers will be placed in the solution after being used. Used peritoneal fluid will also be poured into the Virkon solution. They will remain in the solution for 24 hours after which the liquid will be poured down the sink and plastic material will be autoclaved before disposal in the clinical waste stream.

SECTION 8.0: SPILLAGE / EMERGENCY PROCEDURES

Identify any specific instructions or requirements in the event of a spillage or emergency.

☐	Written emergency instructions will be provided to workers and others who might be affected. ***Specify:***
x	First Aid Provisions (for example, eye wash station, body shower, antidote, specialist hospital etc.) ***Specify:*** An eye wash and first aid kit is located within the laboratory. If fluid gets in a persons eye they should immediately was the eye out and then seek medical assistance.
x	Specific Spillage Procedures (such as neutralisation procedures, absorption granules disinfectants etc.) ***Specify:*** Virkon will be available at all times in the lab. In the event of a spillage the virkon should be poured over the fluid, left for 3 minutes then mopped up with a tissue. The contaminated tissues should be placed in a sterile bag for autoclaving. PPE should be worn when this is undertaken.
☐	Other ***Specify:***

SECTION 9.0: SUBSTANCES / ACTIVITY SUBJECT TO OTHER LEGISLATION

Refer to the Guidance Note to complete this section for any chemical substances / biological agents or activity which may be subject to other legislation. Where other legislation is applicable, please cross-reference with the appropriate details

☐ Anti-Terrorism, Crime and Security Act 2001 (ATCSA)

☐ Specified Animal Pathogens Order 1998 (SAPO)

☐ Home Office Drug Precursors or Controlled Drugs

☐ Dangerous Substances and Explosive Atmospheres Regulations (DSEAR)

☐ Risk Assessments

x Other (specify): Register as a biological worker with safety services using BP2 online form.

SECTION 10.0: SUMMARY OF ASSESSMENT RECOMMENDATIONS

Complete this section after the assessment. Include brief details of the assessment findings. Include details on all control measures e.g. monitoring, training, PPE, spillage procedures, health surveillance or any other relevant details.

1. Only fully trained staff will work with ascitic fluid
2. PPE consisting of labcoat, face shield, and gloves should be worn.
3. Any cuts/open wounds should be covered with a dressing.
4. All spillages should be disinfected in accordance with the specified spillage procedure.
5. Peritoneal fluid samples should be clearly labelled and kept in a sealed container in a designated area of lab 4.09. Samples should be on the bench for the duration of the experiment only. At the end of the experiment the samples must be disposed of as described in section 7.
6. Hands should be washed with soap and water upon entry and exit of the lab.
7. Only the Standard procedures (section 11) for handling peritoneal fluid and protocols for testing lactate and pH (see attachments) should be followed to reduced the likelihood of spillage or contamination and these are the only procedures authorised for these samples by this COSHH form
8. University ethical approval must be granted before any work can start.

SECTION 11.0: SAFE SYSTEM OF WORK (SOW) / STANDARD OPERATING PROCEDURE (SOP)

Specify if a SOW or SOP is required for the work activity and if so complete or attach the details of this.

Is a safe system of work or standard operating procedure deemed necessary for this work? Yes x No ☐

If yes, please give details:

11.1 Hazards and Risks

Ascitic Fluid
Ascitic fluid that is going be used in this work will be contaminated with blood and bacteria. Though it is possible to become infected by the fluid, it is unlikely to do so as long as good laboratory practice is followed. The route by which ascitic fluid is hazardous to your health is through ingestion, inhalation by direct contact with broken skin. The aerosol production is expected to be minimal.

Chemical Hazards and Risks
Ethanol is classified as harmful, flammable and an irritant. Use only in small amounts and store the stock solution in the fire cabinet in W4.02.

Virkon and Sodium Hydroxide are classified as Irritants.

11.2 Actions to be taken before commencing work

Training
Complete the online biological safety modules 1-3 provided by Safety Services. Additionally, if you are involved in the transport of biological fluid from the hospital to the university, also complete Biological Safety Module 6. More information can be found at:

http://www.strath.ac.uk/wellbeing/safetyhealthandwellbeing/healthandsafetytraining/

Complete lab safety induction with the departmental safety convener and training on machinery to be used for the experiment.

Equipment/Materials Required
Prepare 70% Ethanol Solution and keep blue roll nearby for spillages.

PPE Required
Face sheild and Nitrile gloves (EN374-2) to be used when handling chemicals and biological fluid. Use of a Howie style lab coat is also recommended.

11.3 Transport and Storage

Peritoneal fluid must be transported and stored in a triple packaging system in accordance with UN3373. The first layer being the bag containing the ascitic fluid. The secondary layer of an airtight bag large enough to hold layer the first layer. Being the first layer and second, there must be absorbent material that is capable of holding all the ascitic fluid should the first layer break. The third (outer) layer that allows transit of the fluid (airtight). This outer container must state “BIOLOGICAL SUBSTANCE, CATEGORY B”. Additionally, a diamond (2mm thick) with “UN3373” stated in the middle (at least 6mm high) must be put on this outer surface. Researcher details must be attached to the container including a phone number and from and to addresses. Hospital approved taxi service must be used and the driver must be told before transport occurs. Peritoneal fluid can be stored at room temperature, in the fridge or freezer as long as the triple packing system is used.

Ethanol must be stored in the fire cabinet in room W4.02. The lactate assay kit (containing sodium hydroxide) must be stored in the fridge.

11.4 Areas where work can be performed

Work will be performed in a designated area of lab 4.09. The area will be marked off with biohazard tape and signs nearby to indicate that a biological substance is being handled in this area. If machinery outside this area is required then as much of the experiment as possible will be prepared in the designated area prior to transfer to the relevant area of the lab e.g. plate reader. Before and after work is carried out the bench top should be wiped down with 70% ethanol. Any spillages will be dealt with as outlined below.

11.5 Step by Step Procedure

1) Wear lab coat, face shield and gloves when in labs

2) Avoid hand to mouth contact when in labs. Eating, drinking and use of mobile phones and headphone are not allowed.

3) Prepare 70% ethanol solution in a spray bottle and keep nearby working area. Additionally keep absorbent tissue and Virkon nearby. This is in preparation in case of spills. Additionally, if work is needs to be done in another lab, place plastic sheet (wipe down with Trigene prior to use) in appropriate area and mark area as a biohazard area. Prepare machinery needed to minimise the amount of time samples are outside the demarcated area.

4) Remove peritoneal fluid from triple packing only within the designated area of lab 4.09.

6) Prepare the sample for the experiment within the designated area. Carry out the experiment within the designated area if possible. If not then place a cover over the prepared sample for transfer to another area of the lab. Pre-prepare machines needed for the experiment and wipe them down with ethanol before and after use. Spillages in the plate reader are dealt with as per section 11.8.

7) At end of experiments, dispose of peritoneal fluid (see 11.8). Wipe down the bench with Trigene.

8) Wash hands thoroughly when leaving labs.

11.6 Spillages

Small spillages: Must be cleaned with 2%w/v Virkon or 70% ethanol solution and absorbent tissue.
Large spillages: Use of a spill kit (found in W4.05) or cover with Virkon powder and then clean with 2% w/v Virkon.
Spillages in the plate reader: if this occurs Brian Cartilage (lab technician) is to be contacted and he will ensure the machine is adequately cleaned and disinfected.

Ensure that spillage area has been fully disinfected.

11.7 First Aid Procedure

Peritoneal Fluid

Skin Contact: Wash hands immediately with soap and warm water. Remove and autoclave contaminated clothing.
Ingestion: Seek medical attention
Eyes: Rinse with water using eye washing station and seek medical attention.

Ethanol, Sodium Hydroxide and Virkon

Skin Contact: Wash off immediately with soap and warm water. Remove contaminated clothing
Inhalation: Move to well ventilated area and seek medical attention.
Ingestion: Seek medical attention immediately.
Eye: Rinse with water using eye washing station (for at least 15 mins) and seek medical attention.

First Aid can be received by calling extension 2222

11.8 Disposal Procedures

Peritoneal fluid can be poured into 2% w/v Virkon solution (volume of Virkon must be greater than the amount of biological fluid) for inactivation. All contaminated solid items (including gloves) must also be placed in the solution of 2% w/v Virkon solution as well. Leave for 24 hours and dispose of the fluid down the sink. All solid items should be placed in autoclave bags and be autoclaved (W4.05) at 126°C.

For equipment that cannot be autoclaved or reusable equipment: these items can be placed in 1% w/v Virkon solution for at least 15 mins before being stored away.

SECTION 12.0: RECEIPT AND AGREEMENT OF ASSESSMENT (**Reference No.**)

All individuals who are working to this risk assessment must sign and date to acknowledge that they have read and are aware of this risk assessment and the measures taken to safeguard the health of them and others. If this assessment is modified in any way, all current signatories must sign again to show they are aware of the modifications made.

	Version 1		Version 2	Version 3
NAME	SIGNATURE	DATE	Initials/Date	Initials/Date

Details of Changes Specify:	Sign/Dated Assessor	Signed/Dated Supervisor/PI	Date of Revision	Next review date
Version 2 - Changes				
Version 3 - Changes				

10 List of references

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(ACGBI), A. O. C. O. G. B. A. I. 2016. *Prevention, diagnosis and management of colorectal anastomotic leakage* [Online]. Available: http://www.acpgbi.org.uk/content/uploads/2016/03/management-of-colorectal-anastomtic-leakage.pdf [Accessed 31/08/2016 2016].

ABBAS, S. & HILL, A. 2008. Systematic review of the literature for the use of oesophageal Doppler monitor for fluid replacement in major abdominal surgery. *Anaesthesia,* 63**,** 44-51.

AGENTS, B. 2005. Managing the risks in laboratories and healthcare premises. *Advisory Committee on Dangerous Pathogens*.

AHMED, M. U., HOSSAIN, M. M., SAFAVIEH, M., WONG, Y. L., RAHMAN, I. A., ZOUROB, M. & TAMIYA, E. 2016. Toward the development of smart and low cost point-of-care biosensors based on screen printed electrodes. *Critical reviews in biotechnology,* 36**,** 495-505.

ALONSO, S., PASCUAL, M., SALVANS, S., MAYOL, X., MOJAL, S., GIL, M. J., GRANDE, L. & PERA, M. 2015. Postoperative intra-abdominal infection and colorectal cancer recurrence: a prospective matched cohort study of inflammatory and angiogenic responses as mechanisms involved in this association. *Eur J Surg Oncol,* 41**,** 208-14.

ALVES, A., PANIS, Y., POCARD, M., REGIMBEAU, J.-M. & VALLEUR, P. 1999. Management of anastomotic leakage after nondiverted large bowel resection. *Journal of the American College of Surgeons,* 189**,** 554-559.

ALVES, A., PANIS, Y., TRANCART, D., REGIMBEAU, J.-M., POCARD, M. & VALLEUR, P. 2002. Factors associated with clinically significant anastomotic leakage after large bowel resection: multivariate analysis of 707 patients. *World journal of surgery,* 26**,** 499-502.

AMBROSETTI, P., JENNY, A., BECKER, C., TERRIER, F. & MOREL, P. 2000. Acute left colonic diverticulitis—compared performance of computed tomography and water-soluble contrast enema. *Diseases of the colon & rectum,* 43**,** 1363-1367.

AMBROSETTI, P., ROBERT, J., MATHEY, P. & ROHNER, A. 1994. Left-sided colon and colorectal anastomoses: Doppler ultrasound as an aid to assess bowel vascularization. *International journal of colorectal disease,* 9**,** 211-214.

ANANTHAKRISHNAN, A. N. 2015. Epidemiology and risk factors for IBD. *Nature reviews Gastroenterology & hepatology,* 12**,** 205.

ANDREU, V., MENDOZA, G., ARRUEBO, M. & IRUSTA, S. 2015. Smart Dressings Based on Nanostructured Fibers Containing Natural Origin Antimicrobial, Anti-Inflammatory, and Regenerative Compounds. *Materials,* 8**,** 5154-5193.

ARCHAMPONG, T. & NKRUMAH, K. 2013. Inflammatory Bowel Disease in Accra: WhatNewTrends? *West African journal of medicine,* 32**,** 40-44.

ARENAL, J. J., BENITO, C., CONCEJO, M. P. & ORTEGA, E. 1999. Colorectal resection and primary anastomosis in patients aged 70 and older: prospective study. *European Journal of Surgery,* 165**,** 593-597.

ARMSTRONG, G., CROFT, J., CORRIGAN, N., BROWN, J., GOH, V., QUIRKE, P., HULME, C., TOLAN, D., KIRBY, A. & CAHILL, R. 2018. IntAct: Intraoperative Fluorescence Angiography (IFA) to Prevent Anastomotic Leak in Rectal Cancer Surgery: A Randomised Controlled Trial. *Colorectal Disease*.

ASHRAF, S., BURNS, E., JANI, A., ALTMAN, S., YOUNG, J., CUNNINGHAM, C., FAIZ, O. & MORTENSEN, N. 2013. The economic impact of anastomotic leakage after

anterior resections in English NHS hospitals: are we adequately remunerating them? *Colorectal Disease,* 15**,** e190-e198.
ASSICOT, M., BOHUON, C., GENDREL, D., RAYMOND, J., CARSIN, H. & GUILBAUD, J. 1993. High serum procalcitonin concentrations in patients with sepsis and infection. *The Lancet,* 341**,** 515-518.
ASTLES R, W. C., SEDOR F 1994. Stability of plasma lactate in vitro in the presence of anitglycolytic agents. *Clin Chem,* 40**,** 1327-1330.
AYDIN, H. N., REMZI, F. H., TEKKIS, P. P. & FAZIO, V. W. 2005. Hartmann's reversal is associated with high postoperative adverse events. *Diseases of the colon & rectum,* 48**,** 2117-2126.
BA, Z. F., YOKOYAMA, Y., TOTH, B., RUE, L. W., BLAND, K. I. & CHAUDRY, I. H. 2004. Gender differences in small intestinal endothelial function: inhibitory role of androgens. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 286**,** G452-G457.
BAKER, E. A. & LEAPER, D. J. 2003. Profiles of matrix metalloproteinases and their tissue inhibitors in intraperitoneal drainage fluid: relationship to wound healing. *Wound repair and regeneration,* 11**,** 268-274.
BAKKER, I., MORKS, A., TEN CATE HOEDEMAKER, H., BURGERHOF, J., LEUVENINK, H., VAN PRAAGH, J., PLOEG, R., HAVENGA, K., GROUP, C. C. S. S. & BAKKER, I. 2017. Randomized clinical trial of biodegradeable intraluminal sheath to prevent anastomotic leak after stapled colorectal anastomosis. *British Journal of Surgery,* 104**,** 1010-1019.
BALCI, C., SUNGURTEKIN, H., GÜRSES, E., SUNGURTEKIN, U. & KAPTANOĞLU, B. 2002. Usefulness of procalcitonin for diagnosis of sepsis in the intensive care unit. *Critical Care,* 7**,** 85.
BARRIER, A., FERRO, L., HOURY, S., LACAINE, F. & HUGUIER, M. 2003. Rectal cancer surgery in patients more than 80 years of age. *The American journal of surgery,* 185**,** 54-57.
BATTERSBY, N., BHANGU, A., CHAUDHRI, S., EL‐HUSSUNA, A., FRASSON, M., NEPOGODIEV, D., SINGH, B., VENNIX, S. & ZMORA, O. 2017. Relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo‐caecal resection: an international snapshot audit. *Colorectal Disease,* 19**,** e296-e311.
BEARD, J., NICHOLSON, M., SAYERS, R., LLOYD, D. & EVERSON, N. 1990. Intraoperative air testing of colorectal anastomoses: a prospective, randomized trial. *British Journal of Surgery,* 77**,** 1095-1097.
BELVERDE, B., FRATTAROLI, S., CARBONE, A. & VICECONTE, G. 2012. Anastomotic strictures in colorectal surgery: treatment with endoscopic balloon dilation. *G Chir,* 33**,** 243-245.
BEMELMAN, W. A., WARUSAVITARNE, J., SAMPIETRO, G. M., SERCLOVA, Z., ZMORA, O., LUGLIO, G., DE BUCK VAN OVERSTRAETEN, A., BURKE, J. P., BUSKENS, C. J. & COLOMBO, F. 2017. ECCO-ESCP consensus on surgery for Crohn's disease. *Journal of Crohn's and Colitis,* 12**,** 1-16.
BENOIST, S., PANIS, Y., ALVES, A. & VALLEUR, P. 2000. Impact of obesity on surgical outcomes after colorectal resection. *The American journal of surgery,* 179**,** 275-281.
BERGOFSKY, E. H. 1964. Determination of tissue O2 tensions by hollow visceral tonometers: effect of breathing enriched O2 mixtures. *journal of Clinical Investigation,* 43**,** 193.

BERTELSEN, C., ANDREASEN, A., JØRGENSEN, T. & HARLING, H. 2010. Anastomotic leakage after anterior resection for rectal cancer: risk factors. *Colorectal disease,* 12**,** 37-43.

BERTRAM, P., JUNGE, K., SCHACHTRUPP, A., GÖTZE, C., KUNZ, D. & SCHUMPELICK, V. 2009. Peritoneal Release of TNFα and IL-6 After Elective Colorectal Surgery and Anastomotic Leakage. *Journal of Investigative Surgery,* 16**,** 65-69.

BIANCHI, R. A., SILVA, N. A., NATAL, M. L. & ROMERO, M. A. C. 2004. Utility of base deficit, lactic acid, microalbuminuria, and C-reactive protein in the early detection of complications in the immediate postoperative evolution. *Clinical biochemistry,* 37**,** 404-407.

BINI, R., FERRARI, G., APRA, F., VIORA, T., LELI, R. & COTOGNI, P. 2014. Peritoneal lactate as a potential biomarker for predicting the need for reintervention after abdominal surgery. *J Trauma Acute Care Surg,* 77**,** 376-80.

BISGÅRD, A. S., NOACK, M. W., KLEIN, M., ROSENBERG, J. & GÖGENUR, I. 2013. Perioperative statin therapy is not associated with reduced risk of anastomotic leakage after colorectal resection. *Diseases of the Colon & Rectum,* 56**,** 980-986.

BOCCOLA, M. A., BUETTNER, P. G., ROZEN, W. M., SIU, S. K., STEVENSON, A. R., STITZ, R. & HO, Y.-H. 2011. Risk factors and outcomes for anastomotic leakage in colorectal surgery: a single-institution analysis of 1576 patients. *World journal of surgery,* 35**,** 186-195.

BOLAND, M. R., NOORANI, A., VARTY, K., COFFEY, J. C., AGHA, R. & WALSH, S. R. 2013. Perioperative fluid restriction in major abdominal surgery: systematic review and meta-analysis of randomized, clinical trials. *World journal of surgery,* 37**,** 1193-1202.

BONAVENTURA, J. M., SHARPE, K., KNIGHT, E., FULLER, K. L., TANNER, R. K. & GORE, C. J. 2015. Reliability and accuracy of six hand-held blood lactate analysers. *Journal of sports science & medicine,* 14**,** 203.

BOYLE, N., MANIFOLD, D., JORDAN, M. & MASON, R. 2000. Intraoperative assessment of colonic perfusion using scanning laser Doppler flowmetry during colonic resection. *Journal of the American College of Surgeons,* 191**,** 504-510.

BRANDSTRUP, B., TONNESEN, H., BEIER-HOLGERSEN, R., HJORTSO, E., ORDING, H., LINDORFF-LARSEN, K., RASMUSSEN, M., LANNG, C., WALLIN, L. & IVERSEN, L. 2003. Danish Study Group on Perioperative Fluid Therapy. Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens: a randomized assessor-blinded multicenter trial. *Ann Surg,* 238**,** 641-648.

BRETAGNOL, F., PANIS, Y., RULLIER, E., ROUANET, P., BERDAH, S., DOUSSET, B., PORTIER, G., BENOIST, S., CHIPPONI, J. & VICAUT, E. 2010. Rectal cancer surgery with or without bowel preparation: the French GRECCAR III multicenter single-blinded randomized trial. *Annals of surgery,* 252**,** 863-868.

BROWN, S. R., MATHEW, R., KEDING, A., MARSHALL, H. C., BROWN, J. M. & JAYNE, D. G. 2014. The impact of postoperative complications on long-term quality of life after curative colorectal cancer surgery. *Annals of surgery,* 259**,** 916-923.

BRUCE, J., KRUKOWSKI, Z., AL‐KHAIRY, G., RUSSELL, E. & PARK, K. 2001. Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. *British Journal of Surgery,* 88**,** 1157-1168.

BUCHS, N. C., GERVAZ, P., SECIC, M., BUCHER, P., MUGNIER-KONRAD, B. & MOREL, P. 2008. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. *International journal of colorectal disease,* 23**,** 265-270.

BÜLOW, S., BULUT, O., CHRISTENSEN, I. J. & HARLING, H. 2006. Transanal stent in anterior resection does not prevent anastomotic leakage. *Colorectal Disease,* 8**,** 494-496.

BURCH, J. M., FRANCIOSE, R. J., MOORE, E. E., BIFFL, W. L. & OFFNER, P. J. 2000. Single-layer continuous versus two-layer interrupted intestinal anastomosis: a prospective randomized trial. *Annals of surgery,* 231**,** 832-837.

BURTON, T. P., MITTAL, A. & SOOP, M. 2013. Nonsteroidal anti-inflammatory drugs and anastomotic dehiscence in bowel surgery: systematic review and meta-analysis of randomized, controlled trials. *Diseases of the Colon & Rectum,* 56**,** 126-134.

CAIN, S. M. 1965. Appearance of excess lactate in anesthetized dogs during anemic and hypoxic hypoxia. *American Journal of Physiology--Legacy Content,* 209**,** 604-610.

CALATAYUD, O. & TENIAS, J. 2003. Effects of time, temperature and blood cell counts on levels of lactate in heparinized whole blood gas samples. *Scandinavian journal of clinical and laboratory investigation,* 63**,** 311-314.

CANEDO, J., LEE, S. H., PINTO, R., MURAD‐REGADAS, S., ROSEN, L. & WEXNER, S. 2011. Surgical resection in Crohn′s disease: is immunosuppressive medication associated with higher postoperative infection rates? *Colorectal Disease,* 13**,** 1294-1298.

CAO, F., LI, J. & LI, F. 2012. Mechanical bowel preparation for elective colorectal surgery: updated systematic review and meta-analysis. *International journal of colorectal disease,* 27**,** 803-810.

CARLO, B. & VALERIO CORAZZA, A. 2012. The efficacy of intraoperative endoscopic control of the colorectal stapled anastomosis after anterior resection of the rectum for rectal cancer. *Colorectal Dis,* 14**,** 1-76.

CARLSTEDT, A., FASTH, S., HULTEN, L., NORDGREN, S. & PALSELIUS, I. 1987. Long-term ileostomy complications in patients with ulcerative colitis and Crohn's disease. *International journal of colorectal disease,* 2**,** 22-25.

CARTER, M. J., LOBO, A. J. & TRAVIS, S. P. 2004. Guidelines for the management of inflammatory bowel disease in adults. *Gut,* 53**,** v1-v16.

CASTELL, J. V., GÓMEZ-LECHÓN, M. J., DAVID, M., ANDUS, T., GEIGER, T., TRULLENQUE, R., FABRA, R. & HEINRICH, P. C. 1989. Interleukin‐6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS letters,* 242**,** 237-239.

CHALMERS, I., BRACKEN, M. B., DJULBEGOVIC, B., GARATTINI, S., GRANT, J., GÜLMEZOGLU, A. M., HOWELLS, D. W., IOANNIDIS, J. P. & OLIVER, S. 2014. How to increase value and reduce waste when research priorities are set. *The Lancet,* 383**,** 156-165.

CHAMBERS, J. A., CALLANDER, A. S., GRANGERET, R. & O'CARROLL, R. E. 2016. Attitudes towards the Faecal Occult Blood Test (FOBT) versus the Faecal Immunochemical Test (FIT) for colorectal cancer screening: perceived ease of completion and disgust. *BMC cancer,* 16**,** 96.

CHAN MKY, L. W. 2007. Use of chewing gum in reducing postoperative ileus after elective colorectal resection: A systematic review. *Dis Colon Rectum,* 50**,** 2149-2157.

CHANG, J. S., KEUM, K. C., KIM, N. K., BAIK, S. H., MIN, B. S., HUH, H., LEE, C. G. & KOOM, W. S. 2014. Preoperative chemoradiotherapy effects on anastomotic leakage after rectal cancer resection: a propensity score matching analysis. *Annals of surgery,* 259**,** 516-521.

CHAPMAN RW, C. J., HAYES PC 2006. Liver and biliary tract disease. *In:* BOON NA, C. N., WALKER BR (ed.) *Davidson's Principles and Practice of Medicine.* 20th ed. Edinburgh: Churchill Livingstone.
CHAPMAN, S., PERICLEOUS, A., DOWNEY, C. & JAYNE, D. 2018. Postoperative ileus following major colorectal surgery. *British Journal of Surgery,* 105**,** 797-810.
CHEN, J., ZHANG, Y., JIANG, C., YU, H., ZHANG, K., ZHANG, M., ZHANG, G.-Q. & ZHOU, S.-J. 2013. Temporary ileostomy versus colostomy for colorectal anastomosis: evidence from 12 studies. *Scandinavian journal of gastroenterology,* 48**,** 556-562.
CHOI, H.-K., LAW, W.-L. & HO, J. W. 2006. Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. *Diseases of the colon & rectum,* 49**,** 1719-1725.
CHOY, P. Y. G., BISSETT, I. P., DOCHERTY, J. G., PARRY, B. R., MERRIE, A. & FITZGERALD, A. 2011. Stapled versus handsewn methods for ileocolic anastomoses. *The Cochrane Library*.
CHUNG, R., HITCH, D. & ARMSTRONG, D. 1988. The role of tissue ischemia in the pathogenesis of anastomotic stricture. *Surgery,* 104**,** 824-829.
CINI, C., WOLTHUIS, A. & D'HOORE, A. 2013. Peritoneal fluid cytokines and matrix metalloproteinases as early markers of anastomotic leakage in colorectal anastomosis: a literature review and meta-analysis. *Colorectal Dis,* 15**,** 1070-7.
COFFEY, J. C., KIERNAN, M. G., SAHEBALLY, S. M., JARRAR, A., BURKE, J. P., KIELY, P. A., SHEN, B., WALDRON, D., PEIRCE, C. & MOLONEY, M. 2018. Inclusion of the mesentery in ileocolic resection for Crohn's disease is associated with reduced surgical recurrence. *Journal of Crohn's and Colitis*.
COFFEY, J. C., O'LEARY, D. P., KIERNAN, M. G. & FAUL, P. 2016. The mesentery in Crohn's disease: friend or foe? *Current opinion in gastroenterology,* 32**,** 267-273.
COLBURN, W., DEGRUTTOLA, V., DEMETS, D., DOWNING, G., HOTH, D., OATES, J., PECK, C., SCHOOLEY, R., SPILKER, B. & WOODCOCK, J. 2001. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Biomarkers Definitions Working Group. *Clinical Pharmacol & Therapeutics,* 69**,** 89-95.
CONSTANTINIDES, V. A., HERIOT, A., REMZI, F., DARZI, A., SENAPATI, A., FAZIO, V. W. & TEKKIS, P. P. 2007. Operative strategies for diverticular peritonitis: a decision analysis between primary resection and anastomosis versus Hartmann's procedures. *Annals of surgery,* 245**,** 94.
CORKE, C. & GLENISTER, K. 2001. Monitoring intestinal ischaemia.
CORRIGAN, N., MARSHALL, H., CROFT, J., COPELAND, J., JAYNE, D. & BROWN, J. 2018. Exploring and adjusting for potential learning effects in ROLARR: a randomised controlled trial comparing robotic-assisted vs. standard laparoscopic surgery for rectal cancer resection. *Trials,* 19**,** 339.
COSNES, J., GOWER–ROUSSEAU, C., SEKSIK, P. & CORTOT, A. 2011. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology,* 140**,** 1785-1794. e4.
DAAMS, F., WU, Z., CAKIR, H., KARSTEN, T. M. & LANGE, J. F. 2014. Identification of anastomotic leakage after colorectal surgery using microdialysis of the peritoneal cavity. *Tech Coloproctol,* 18**,** 65-71.
DAMHUIS, R., WERELDSMA, J. & WIGGERS, T. 1996. The influence of age on resection rates and postoperative mortality in 6457 patients with colorectal cancer. *International journal of colorectal disease,* 11**,** 45-48.
DANESE, S., VUITTON, L. & PEYRIN-BIROULET, L. 2015. Biologic agents for IBD: practical insights. *Nature reviews Gastroenterology & hepatology,* 12**,** 537.

DEARMOND, D. T., CARSWELL, A., LOUDEN, C. L., SIMMONS, J. D., BAYER, J., DAS, N. A. & JOHNSON, S. B. 2013. Diagnosis of anastomotic leak: electrolyte detection versus barium fluoroscopy. *J Surg Res,* 182**,** 192-7.
DEARMOND, D. T., CLINE, A. M. & JOHNSON, S. B. 2010. Anastomotic leak detection by electrolyte electrical resistance. *J Invest Surg,* 23**,** 197-203.
DEL GAUDIO, A., BOSCHI, L., PETRIN, C. & BERARDI, M. 1993. L'anastomosi colorettale bassa: sutura manuale e meccanica a confronto. *ANNALI ITALIANI DI CHIRURGIA,* 64**,** 545-545.
DELANEY, C. P., FAZIO, V. W., REMZI, F. H., HAMMEL, J., CHURCH, J. M., HULL, T. L., SENAGORE, A. J., STRONG, S. A. & LAVERY, I. C. 2003. Prospective, age-related analysis of surgical results, functional outcome, and quality of life after ileal pouch-anal anastomosis. *Annals of surgery,* 238**,** 221-228.
DINARELLO, C. A. 1984. Interleukin-1. *Review of infectious diseases,* 6**,** 51-95.
DINDO, D., DEMARTINES, N. & CLAVIEN, P.-A. 2004. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Annals of surgery,* 240**,** 205.
DIZEREGA, G. 1989. The peritoneum and its response to surgical injury. *Progress in clinical and biological research,* 358**,** 1-11.
DOSSA, F., CHESNEY, T. R., ACUNA, S. A. & BAXTER, N. N. 2017. A watch-and-wait approach for locally advanced rectal cancer after a clinical complete response following neoadjuvant chemoradiation: a systematic review and meta-analysis. *The lancet Gastroenterology & hepatology,* 2**,** 501-513.
DUBAY, D. A. & FRANZ, M. G. 2003. Acute wound healing: the biology of acute wound failure. *Surgical Clinics of North America,* 83**,** 463-481.
DUBROW, R. A., DAVID, C. & CURLEY, S. A. 1995. Anastomotic leaks after low anterior resection for rectal carcinoma: evaluation with CT and barium enema. *AJR. American journal of roentgenology,* 165**,** 567-571.
DUNLOP, M. 2007. The intestine and appendix. *In:* OJ GARDEN, A. B., JLR FORSYTHE, RW PARKS (ed.) *Principles and Practice of Surgery.* Edinburgh: Churchill Livingstone.
EBERL, T., JAGODITSCH, M., KLINGLER, A. & TSCHMELITSCH, J. 2008. Risk factors for anastomotic leakage after resection for rectal cancer. *The American journal of surgery,* 196**,** 592-598.
ECKMANN, C., KUJATH, P., SCHIEDECK, T., SHEKARRIZ, H. & BRUCH, H.-P. 2004. Anastomotic leakage following low anterior resection: results of a standardized diagnostic and therapeutic approach. *International journal of colorectal disease,* 19**,** 128-133.
EDNA, T.-H. & BJERKESET, T. 1997. Colorectal cancer in patients over 80 years of age. *Hepato-gastroenterology,* 45**,** 2142-2145.
EGOROV, V., SCHASTLIVTSEV, V., TURUSOV, R. & BARANOV, A. 2002. Participation of the intestinal layers in supplying of the mechanical strength of the intact and sutured gut. *European surgical research,* 34**,** 425-431.
ENGLAND, H. E. *Explore roles - general surgery* [Online]. Available: https://www.healthcareers.nhs.uk/explore-roles/surgery/general-surgery [Accessed 20/12/2016 2016].
ENGLAND, R. C. O. S. O. 2011. *The higher risk surgical patient: towards improved care for a forgotten group* [Online]. Available: https://www.rcseng.ac.uk/publications/docs/higher-risk-surgical-patient [Accessed 31/08/2016 2016].
ERB, L., HYMAN, N. H. & OSLER, T. 2014. Abnormal vital signs are common after bowel resection and do not predict anastomotic leak. *J Am Coll Surg,* 218**,** 1195-9.

FAZIO, V. W., MARCHETTI, F., CHURCH, M., GOLDBLUM, J. R., LAVERY, C., HULL, T. L., MILSOM, J. W., STRONG, S. A., OAKLEY, J. R. & SECIC, M. 1996. Effect of resection margins on the recurrence of Crohn's disease in the small bowel. A randomized controlled trial. *Annals of surgery,* 224**,** 563.

FAZIO, V. W., ZIV, Y., CHURCH, J. M., OAKLEY, J. R., LAVERY, I. C., MILSOM, J. W. & SCHROEDER, T. K. 1995. Ileal pouch-anal anastomoses complications and function in 1005 patients. *Annals of surgery,* 222**,** 120.

FINGERHUT, A., HAY, J.-M., ELHADAD, A., LACAINE, F., FLAMANT, Y. & RESEARCH, F. A. F. S. 1995. Supraperitoneal colorectal anastomosis: hand-sewn versus circular staples—a controlled clinical trial. *Surgery,* 118**,** 479-485.

FOUDA, E., EL NAKEEB, A., MAGDY, A., HAMMAD, E. A., OTHMAN, G. & FARID, M. 2011. Early detection of anastomotic leakage after elective low anterior resection. *J Gastrointest Surg,* 15**,** 137-44.

FRY RD, M. N., MARON DJ, BLEIER JIS 2012. Colon and rectum. *In:* TOWNSEND CM, B. R., EVERS BM, MATTOX KL, EDS. (ed.) *Sabiston Textbook of Surgery.* 19th ed. ed. Philadelphia, PA: Elsevier Saunders.

GAIESKI, D. F., DRUMHELLER, B. C., GOYAL, M., FUCHS, B. D., SHOFER, F. S. & ZOGBY, K. 2013. Accuracy of handheld point-of-care fingertip lactate measurement in the emergency department. *Western Journal of Emergency Medicine,* 14.

GARCIA-GRANERO, A., FRASSON, M., FLOR-LORENTE, B., BLANCO, F., PUGA, R., CARRATALÁ, A. & GARCIA-GRANERO, E. 2013. Procalcitonin and C-reactive protein as early predictors of anastomotic leak in colorectal surgery: a prospective observational study. *Diseases of the Colon & Rectum,* 56**,** 475-483.

GETZEN, L. 1966. Clinical use of everted intestinal anastomoses. *Surgery, gynecology & obstetrics,* 123**,** 1027-1036.

GHOSH, S., ARTHUR, B. & KLEIN, A. 2011. NICE guidance on CardioQTM oesophageal Doppler monitoring. *Anaesthesia,* 66**,** 1081-1083.

GLIMELIUS, B., TIRET, E., CERVANTES, A. & ARNOLD, D. 2013. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol,* 24.

GODLEE, F. 2018. Why aren't medical devices regulated like drugs? : British Medical Journal Publishing Group.

GORISSEN, K., BENNING, D., BERGHMANS, T., SNOEIJS, M., SOSEF, M., HULSEWE, K. & LUYER, M. 2012. Risk of anastomotic leakage with non‐steroidal anti‐inflammatory drugs in colorectal surgery. *British journal of surgery,* 99**,** 721-727.

GOSPODAROWICZ, M. K., BRIERLEY, J. D. & WITTEKIND, C. 2017. *TNM classification of malignant tumours*, John Wiley & Sons.

GOYAL, M., PINES, J. M., DRUMHELLER, B. C. & GAIESKI, D. F. 2010. Point-of-care testing at triage decreases time to lactate level in septic patients. *The Journal of emergency medicine,* 38**,** 578-581.

GRAFFNER, H., FREDLUND, P., OLSSON, S.-Å., OSCARSON, J. & PETERSSON, B.-G. 1983. Protective colostomy in low anterior resection of the rectum using the EEA stapling instrument. *Diseases of the Colon & Rectum,* 26**,** 87-90.

GROUP, C. C. L. O. O. R. S. 2005. Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. *The lancet oncology,* 6**,** 477-484.

GUENAGA, K. K., MATOS, D. & WILLE‐JØRGENSEN, P. 2009. Mechanical bowel preparation for elective colorectal surgery. *The Cochrane Library*.

GUILLOU, P. J., QUIRKE, P., THORPE, H., WALKER, J., JAYNE, D. G., SMITH, A. M., HEATH, R. M., BROWN, J. M. & GROUP, M. C. T. 2005. Short-term endpoints of

conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. *The lancet,* 365**,** 1718-1726.
GUTIERREZ, G., PALIZAS, F., DOGLIO, G., PUSAJO, J., WAINSZTEIN, N., KLEIN, F., GALLESIO, A., SAN ROMAN, E., PACIN, J. & DORFMAN, B. 1992. Gastric intramucosal pH as a therapeutic index of tissue oxygenation in critically ill patients. *The Lancet,* 339**,** 195-199.
H, Z. 1891. Ueber die Bildung von Milchsäure und Glykose in den Organen bei gestörter Circulation und bei der Blausäurevergiftung. . *Z Physiol Chem,* 15**,** 387–404.
HALL, N., FINAN, P., STEPHENSON, B., LOWNDES, R. & YOUNG, H. 1995. High tie of the inferior mesenteric artery in distal colorectal resections—a safe vascular procedure. *International journal of colorectal disease,* 10**,** 29-32.
HALLBÖÖK, O., JOHANSSON, K. & SJÖDAHL, R. 1996. Laser Doppler blood flow measurement in rectal resection for carcinoma–comparison between the straight and colonic J pouch reconstruction. *British journal of surgery,* 83**,** 389-392.
HALSTED, W. S. 1887. CIRCULAR SUTURE OF THE INTESTINE-AN EXPERIMENTAL STUDY. *The American Journal of the Medical Sciences,* 188**,** 436-460.
HAMMOND, J., LIM, S., WAN, Y., GAO, X. & PATKAR, A. 2014. The burden of gastrointestinal anastomotic leaks: an evaluation of clinical and economic outcomes. *Journal of Gastrointestinal Surgery,* 18**,** 1176-1185.
HERRLE, F., DIENER, M., FREUDENBERG, S., WILLEKE, F., KIENLE, P., BOENNINGHOFF, R., WEISS, C., PARTECKE, L., SCHULD, J. & POST, S. 2016. Single-Layer Continuous Versus Double-Layer Continuous Suture in Colonic Anastomoses—a Randomized Multicentre Trial (ANATECH Trial). *Journal of Gastrointestinal Surgery,* 20**,** 421-430.
HERWIG, R., GLODNY, B., KÜHLE, C., SCHLÜTER, B., BRINKMANN, O. A., STRASSER, H., SENNINGER, N. & WINDE, G. 2002. Early identification of peritonitis by peritoneal cytokine measurement. *Diseases of the colon & rectum,* 45**,** 514-521.
HESSE, D., TRACEY, K., FONG, Y., MANOGUE, K., PALLADINO JR, M., CERAMI, A., SHIRES, G. & LOWRY, S. 1988. Cytokine appearance in human endotoxemia and primate bacteremia. *Surgery, gynecology & obstetrics,* 166**,** 147-153.
HINCHEY, E., SCHAAL, P. & RICHARDS, G. 1978. Treatment of perforated diverticular disease of the colon. *Advances in surgery,* 12**,** 85-109.
HIRANO, Y., OMURA, K., TATSUZAWA, Y., SHIMIZU, J., KAWAURA, Y. & WATANABE, G. 2006. Tissue oxygen saturation during colorectal surgery measured by near-infrared spectroscopy: pilot study to predict anastomotic complications. *World J Surg,* 30**,** 457-61.
HIRST, N. A. 2014. *Development of biosensors for early detection of anastomotic leak and sepsis.* University of Leeds.
HIRST, N. A., TIERNAN, J. P., MILLNER, P. A. & JAYNE, D. G. 2014. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis,* 16**,** 95-109.
HOCHACHKA, P. W. & MOMMSEN, T. P. 1983. Protons and anaerobiosis. *Science,* 219**,** 1391-1397.
HOLLAND, I., MCCORMICK, C. & CONNOLLY, P. 2018. Towards non-invasive characterisation of coronary stent re-endothelialisation–An in-vitro, electrical impedance study. *PloS one,* 13**,** e0206758.
HOLTE K, K. D. 2000. Postoperative ileus: a preventable event. *Br J Surg,* 87**,** 1480-1493.

HORER, T. M., NORGREN, L. & JANSSON, K. 2011. Intraperitoneal glycerol levels and lactate/pyruvate ratio: early markers of postoperative complications. *Scand J Gastroenterol,* 46**,** 913-9.

HOURY, S., AMENABAR, J., REZVANI, A. & HUGUIER, M. 1994. Should patients over 80 years old be operated on for colorectal or gastric cancer? *Hepato-gastroenterology,* 41**,** 521-525.

HUANG, Y., TANG, S. R. & YOUNG, C. J. 2018. Nonsteroidal anti‐inflammatory drugs and anastomotic dehiscence after colorectal surgery: a meta‐analysis. *ANZ journal of surgery,* 88**,** 959-965.

HÜSER, N., MICHALSKI, C. W., ERKAN, M., SCHUSTER, T., ROSENBERG, R., KLEEFF, J. & FRIESS, H. 2008. Systematic review and meta-analysis of the role of defunctioning stoma in low rectal cancer surgery. *Annals of surgery,* 248**,** 52-60.

HYMAN, N. H., CATALDO, P. & OSLER, T. 2005. Urgent subtotal colectomy for severe inflammatory bowel disease. *Diseases of the colon & rectum,* 48**,** 70-73.

I, J. 1986. Blood lactate: Implications for training and sports performance. *Sports Medicine,* 3**,** 10-25.

INC, G. 2015. *Highlights of prescribing - Avastin (bevacizumab)* [Online]. Available: http://www.gene.com/download/pdf/avastin_prescribing.pdf [Accessed 30/08/2016 2016].

IVANOV, D., CVIJANOVIC, R. & GVOZDENOVIC, L. 2011. Intraoperative air testing of colorectal anastomoses. *Srp Arh Celok Lek,* 139**,** 333-338.

JAFARI, M. D., LEE, K. H., HALABI, W. J., MILLS, S. D., CARMICHAEL, J. C., STAMOS, M. J. & PIGAZZI, A. 2013. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. *Surgical endoscopy,* 27**,** 3003-3008.

JANSSON, K., REDLER, B., TRUEDSSON, L., MAGNUSON, A., MATTHIESSEN, P., ANDERSSON, M. & NORGREN, L. 2004. Intraperitoneal cytokine response after major surgery: higher postoperative intraperitoneal versus systemic cytokine levels suggest the gastrointestinal tract as the major source of the postoperative inflammatory reaction. *The American journal of surgery,* 187**,** 372-377.

JAYNE, D., PIGAZZI, A., MARSHALL, H., CROFT, J., CORRIGAN, N., COPELAND, J., QUIRKE, P., WEST, N., RAUTIO, T. & THOMASSEN, N. 2017. Effect of robotic-assisted vs conventional laparoscopic surgery on risk of conversion to open laparotomy among patients undergoing resection for rectal cancer: the ROLARR randomized clinical trial. *Jama,* 318**,** 1569-1580.

JHAVERI, K. S. & HOSSEINI-NIK, H. 2015. MRI of rectal cancer: an overview and update on recent advances. *American Journal of Roentgenology,* 205**,** W42-W55.

JUNG, S. H., YU, C. S., CHOI, P. W., KIM, D. D., PARK, I. J., KIM, H. C. & KIM, J. C. 2008. Risk factors and oncologic impact of anastomotic leakage after rectal cancer surgery. *Diseases of the Colon & Rectum,* 51**,** 902-908.

JUNGER, W., JUNGER, W., MILLER, K., BAHRAMI, S., REDL, H., SCHLAG, G. & MORITZ, E. 1995. Early detection of anastomotic leaks after colorectal surgery by measuring endotoxin in the drainage fluid. *Hepato-gastroenterology,* 43**,** 1523-1529.

KANELLOS, I., VASILIADIS, K., ANGELOPOULOS, S., TSACHALIS, T., PRAMATEFTAKIS, M., MANTZOROS, I. & BETSIS, D. 2004. Anastomotic leakage following anterior resection for rectal cancer. *Techniques in coloproctology,* 8**,** s79-s81.

KANG, C. Y., HALABI, W. J., CHAUDHRY, O. O., NGUYEN, V., PIGAZZI, A., CARMICHAEL, J. C., MILLS, S. & STAMOS, M. J. 2013. Risk factors for anastomotic leakage after anterior resection for rectal cancer. *JAMA surgery,* 148**,** 65-71.

KANTHAN, R., SHUAIB, A., GRIEBEL, R. & MIYASHITA, H. 1995. Intracerebral human microdialysis in vivo study of an acute focal ischemic model of the human brain. *Stroke,* 26**,** 870-873.

KARLICZEK, A., BENARON, D., BAAS, P., ZEEBREGTS, C., WIGGERS, T. & VAN DAM, G. 2010. Intraoperative assessment of microperfusion with visible light spectroscopy for prediction of anastomotic leakage in colorectal anastomoses. *Colorectal Disease,* 12**,** 1018-1025.

KARLICZEK, A., JESUS, E., MATOS, D., CASTRO, A., ATALLAH, A. & WIGGERS, T. 2006. Drainage or nondrainage in elective colorectal anastomosis: a systematic review and meta‐analysis. *Colorectal Disease,* 8**,** 259-265.

KARTHEUSER, A. H., LEONARD, D. F., PENNINCKX, F., PATERSON, H. M., BRANDT, D., REMUE, C., BUGLI, C., DOZOIS, E., MORTENSEN, N. & RIS, F. 2013. Waist circumference and waist/hip ratio are better predictive risk factors for mortality and morbidity after colorectal surgery than body mass index and body surface area. *Annals of surgery,* 258**,** 722-730.

KAUR, P., KARANDIKAR, S. & ROY-CHOUDHURY, S. 2014. Accuracy of multidetector CT in detecting anastomotic leaks following stapled left-sided colonic anastomosis. *Clinical radiology,* 69**,** 59-62.

KINGHAM, T. P. & PACHTER, H. L. 2009. Colonic anastomotic leak: risk factors, diagnosis, and treatment. *Journal of the American College of Surgeons,* 208**,** 269-278.

KJAER, M., KRISTJÁNSDÓTTIR, H., ANDERSEN, L., HEEGAARD, A.-M., ÅGREN, M. S. & JORGENSEN, L. N. 2018. The effect of gender on early colonic anastomotic wound healing. *International journal of colorectal disease,* 33**,** 1269-1276.

KLAUS, S., HERINGLAKE, M., GLIEMROTH, J., BRUCH, H.-P. & BAHLMANN, L. 2002. Intraperitoneal microdialysis for detection of splanchnic metabolic disorders. *Langenbeck's Archives of Surgery,* 387**,** 276-280.

KOLKERT, J. L., HAVENGA, K., HENK, O., ZUIDEMA, J. & PLOEG, R. J. 2011. Protection of stapled colorectal anastomoses with a biodegradable device: the C-seal feasibility study. *The American Journal of Surgery,* 201**,** 754-758.

KOMEN, N., DE BRUIN, R. W., KLEINRENSINK, G. J., JEEKEL, J. & LANGE, J. F. 2008. Anastomotic leakage, the search for a reliable biomarker. A review of the literature. *Colorectal Dis,* 10**,** 109-15; discussion 115-7.

KOMEN, N., MORSINK, M. C., BEIBOER, S., MIGGELBRINK, A., WILLEMSEN, P., VAN DER HARST, E., LANGE, J. F. & VAN LEEUWEN, W. B. 2009. Detection of colon flora in peritoneal drain fluid after colorectal surgery: can RT-PCR play a role in diagnosing anastomotic leakage? *J Microbiol Methods,* 79**,** 67-70.

KOMEN, N., SLIEKER, J., WILLEMSEN, P., MANNAERTS, G., PATTYN, P., KARSTEN, T., DE WILT, H., VAN DER HARST, E., DE RIJKE, Y. B., MURAWSKA, M., JEEKEL, J., LANGE, J. F. & GROUP, A. S. 2014a. Acute phase proteins in drain fluid: a new screening tool for colorectal anastomotic leakage? The APPEAL study: analysis of parameters predictive for evident anastomotic leakage. *Am J Surg,* 208**,** 317-23.

KOMEN, N., SLIEKER, J., WILLEMSEN, P., MANNAERTS, G., PATTYN, P., KARSTEN, T., DE WILT, H., VAN DER HARST, E., VAN LEEUWEN, W., DECAESTECKER, C., JEEKEL, H. & LANGE, J. F. 2014b. Polymerase chain reaction for Enterococcus faecalis in drain fluid: the first screening test for symptomatic colorectal anastomotic leakage. The Appeal-study: analysis of parameters predictive for evident anastomotic leakage. *Int J Colorectal Dis,* 29**,** 15-21.

KORNMANN, V. N., TRESKES, N., HOONHOUT, L. H., BOLLEN, T. L., VAN RAMSHORST, B. & BOERMA, D. 2013. Systematic review on the value of CT scanning in the diagnosis of anastomotic leakage after colorectal surgery. *International journal of colorectal disease,* 28**,** 437-445.

KOSTIC, Z., PANISIC, M., MILEV, B., MIJUSKOVIC, Z., SLAVKOVIC, D. & IGNJATOVIC, M. 2015. Diagnostic value of serial measurement of c-reactive protein in serum and matrix metalloproteinase-9 in drainage fluid in the detection of infectious complications and anastomotic leakage in patients with colorectal resection. *Vojnosanitetski pregled,* 72**,** 889-898.

KRANE, M. K., ALLAIX, M. E., ZOCCALI, M., UMANSKIY, K., RUBIN, M. A., VILLA, A., HURST, R. D. & FICHERA, A. 2013. Preoperative infliximab therapy does not increase morbidity and mortality after laparoscopic resection for inflammatory bowel disease. *Diseases of the Colon & Rectum,* 56**,** 449-457.

KRUSCHEWSKI, M., RIEGER, H., POHLEN, U., HOTZ, H. G. & BUHR, H. J. 2007. Risk factors for clinical anastomotic leakage and postoperative mortality in elective surgery for rectal cancer. *International journal of colorectal disease,* 22**,** 919-927.

KUDSZUS, S., ROESEL, C., SCHACHTRUPP, A. & HÖER, J. J. 2010. Intraoperative laser fluorescence angiography in colorectal surgery: a noninvasive analysis to reduce the rate of anastomotic leakage. *Langenbeck's Archives of Surgery,* 395**,** 1025-1030.

LACALLE, J. P., SOLE, J., PEY, G. C. & GARCIA, G. 1982. Rotated intestinal anastomoses. *Surgery, gynecology & obstetrics,* 154**,** 662-666.

LAW, W.-L., CHU, K.-W., HO, J. W. & CHAN, C.-W. 2000. Risk factors for anastomotic leakage after low anterior resection with total mesorectal excision. *The American journal of surgery,* 179**,** 92-96.

LAXAMANA, A., SOLOMON, M. J., COHEN, Z., FEINBERG, S. M., STERN, H. S. & MCLEOD, R. S. 1995. Long-term results of anterior resection using the double-stapling technique. *Diseases of the Colon & Rectum,* 38**,** 1246-1250.

LEICHTLE, S. W., MOUAWAD, N. J., WELCH, K. B., LAMPMAN, R. M. & CLEARY, R. K. 2012. Risk factors for anastomotic leakage after colectomy. *Diseases of the Colon & Rectum,* 55**,** 569-575.

LI, V. K. M., WEXNER, S. D., PULIDO, N., WANG, H., JIN, H. Y., WEISS, E. G., NOGEURAS, J. J. & SANDS, D. R. 2009. Use of routine intraoperative endoscopy in elective laparoscopic colorectal surgery: can it further avoid anastomotic failure? *Surgical endoscopy,* 23**,** 2459-2465.

LIPSKA, M. A., BISSETT, I. P., PARRY, B. R. & MERRIE, A. E. 2006. Anastomotic leakage after lower gastrointestinal anastomosis: men are at a higher risk. *ANZ journal of surgery,* 76**,** 579-585.

LOCKE, R., HAUSER, C. J. & SHOEMAKER, W. C. 1984. The use of surface oximetry to assess bowel viability. *Archives of Surgery,* 119**,** 1252.

LOGAN, R. F., PATNICK, J., NICKERSON, C., COLEMAN, L., RUTTER, M. D. & VON WAGNER, C. 2012. Outcomes of the Bowel Cancer Screening Programme (BCSP) in England after the first 1 million tests. *Gut,* 61**,** 1439-1446.

LOU, Z., ZHANG, W., YU, E., MENG, R. & FU, C. 2014. Colonoscopy is the first choice for early postoperative rectal anastomotic bleeding. *World journal of surgical oncology,* 12**,** 1.

LUONG, J. H., MALE, K. B. & GLENNON, J. D. 2008. Biosensor technology: technology push versus market pull. *Biotechnology advances,* 26**,** 492-500.

LYNCH, H. T. & DE LA CHAPELLE, A. 1999. Genetic susceptibility to non-polyposis colorectal cancer. *Journal of medical genetics,* 36**,** 801-818.

MACKAY, C., RAMSAY, G., RAFFERTY, A. & LOUDON, M. 2014. Impact of the Scottish Bowel Cancer Screening Programme on patient and tumour characteristics at a single centre. *Journal of evaluation in clinical practice,* 20**,** 7-11.

MÄKELÄ, J. T., KIVINIEMI, H. & LAITINEN, S. 2003. Risk factors for anastomotic leakage after left-sided colorectal resection with rectal anastomosis. *Diseases of the colon & rectum,* 46**,** 653-660.

MARIJNEN, C., KAPITEIJN, E., VAN DE VELDE, C., MARTIJN, H., STEUP, W., WIGGERS, T., KRANENBARG, E. K., LEER, J. & GROUP, C. I. O. T. D. C. C. 2002. Acute side effects and complications after short-term preoperative radiotherapy combined with total mesorectal excision in primary rectal cancer: report of a multicenter randomized trial. *Journal of Clinical Oncology,* 20**,** 817-825.

MARTENS, M. & HENDRIKS, T. 1991. Postoperative changes in collagen synthesis in intestinal anastomoses of the rat: differences between small and large bowel. *Gut,* 32**,** 1482-1487.

MARTENS, M., HUYBEN, C. & HENDRIKS, T. 1992. Collagen synthesis in fibroblasts from human colon: regulatory aspects and differences with skin fibroblasts. *Gut,* 33**,** 1664-1670.

MASKELL, N. & BUTLAND, R. 2003. BTS guidelines for the investigation of a unilateral pleural effusion in adults. *Thorax,* 58**,** ii8.

MAST, B. A. 1997. Healing in other tissues. *Surgical Clinics of North America,* 77**,** 529-547.

MATTHIESSEN, P., HALLBÖÖK, O., ANDERSSON, M., RUTEGÅRD, J. & SJÖDAHL, R. 2004. Risk factors for anastomotic leakage after anterior resection of the rectum. *Colorectal Disease,* 6**,** 462-469.

MATTHIESSEN, P., HALLBÖÖK, O., RUTEGÅRD, J., SIMERT, G. & SJÖDAHL, R. 2007a. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. *Annals of surgery,* 246**,** 207-214.

MATTHIESSEN, P., HENRIKSSON, M., HALLBÖÖK, O., GRUNDITZ, E., NORÉN, B. & ARBMAN, G. 2008. Increase of serum C‐reactive protein is an early indicator of subsequent symptomatic anastomotic leakage after anterior resection. *Colorectal Disease,* 10**,** 75-80.

MATTHIESSEN, P., STRAND, I., JANSSON, K., TORNQUIST, C., ANDERSSON, M., RUTEGARD, J. & NORGREN, L. 2007b. Is early detection of anastomotic leakage possible by intraperitoneal microdialysis and intraperitoneal cytokines after anterior resection of the rectum for cancer? *Dis Colon Rectum,* 50**,** 1918-27.

MCDERMOTT, F. D., HEENEY, A., KELLY, M. E., STEELE, R. J., CARLSON, G. L. & WINTER, D. C. 2015. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. *Br J Surg,* 102**,** 462-79.

MCNAIR, A., HEYWOOD, N., TIERNAN, J., VERJEE, A., BACH, S., FEARNHEAD, N., COLLABORATION, O., ARNOTT, R., ASHDOWN‐PHILLIPS, S. & BASON, N. 2017. A national patient and public colorectal research agenda: integration of consumer perspectives in bowel disease through early consultation. *Colorectal Disease,* 19**,** O75-O85.

MEAGHER, A., FAROUK, R., DOZOIS, R., KELLY, K. & PEMBERTON, J. 1998. J ileal pouch–anal anastomosis for chronic ulcerative colitis: complications and long‐term outcome in 1310 patients. *British Journal of Surgery,* 85**,** 800-803.

MEDICINE, J. H. 2016. *Sporadic (non-hereditary) Colorectal Cancer* [Online]. Available: https://gi.jhsps.org/GDL_Disease.aspx?CurrentUDV=31&GDL_Cat_ID=AF793A

59-B736-42CB-9E1F-E79D2B9FC358&GDL_Disease_ID=FB4F2BE3-FC13-44E4-BB69-2CCE936A6CD5 [Accessed 09/08/2016 2016].
MEISNER, M., LOHS, T., HUETTEMANN, E., SCHMIDT, J., HUELLER, M. & REINHART, K. 2001. The plasma elimination rate and urinary secretion of procalcitonin in patients with normal and impaired renal function. *European journal of anaesthesiology,* 18**,** 79-87.
MG, D. 2007. The intestine and appendix. *In:* GARDEN OJ, B. A., FORSYTHE JLR, PARKS RW (ed.) *Principles and practice of Surgery.* 5th ed. Edinbrugh: Churchill Livingstone.
MILLAN, M., GARCIA-GRANERO, E., FLOR, B., GARCIA-BOTELLO, S. & LLEDO, S. 2006. Early prediction of anastomotic leak in colorectal cancer surgery by intramucosal pH. *Dis Colon Rectum,* 49**,** 595-601.
MIRNEZAMI, A., MIRNEZAMI, R., CHANDRAKUMARAN, K., SASAPU, K., SAGAR, P. & FINAN, P. 2011. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. *Annals of surgery,* 253**,** 890-899.
MODASI, A., PACE, D., GODWIN, M., SMITH, C. & CURTIS, B. 2019. NSAID administration post colorectal surgery increases anastomotic leak rate: systematic review/meta-analysis. *Surgical endoscopy,* 33**,** 879-885.
MOHER, D., LIBERATI, A., TETZLAFF, J. & ALTMAN, D. G. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine,* 151**,** 264-269.
MOLD, C., GEWURZ, H. & DU CLOS, T. W. 1999. Regulation of complement activation by C-reactive protein. *Immunopharmacology,* 42**,** 23-30.
MONTEDORI, A., CIROCCHI, R., FARINELLA, E., SCIANNAMEO, F. & ABRAHA, I. 2010. Covering ileo‐or colostomy in anterior resection for rectal carcinoma. *The Cochrane Library*.
MORKS, A. N., HAVENGA, K. & PLOEG, R. J. 2011. Can intraluminal devices prevent or reduce colorectal anastomotic leakage: a review. *World J Gastroenterol,* 17**,** 4461-9.
MOYES, L., LEITCH, E., MCKEE, R., ANDERSON, J., HORGAN, P. & MCMILLAN, D. 2009. Preoperative systemic inflammation predicts postoperative infectious complications in patients undergoing curative resection for colorectal cancer. *British journal of cancer,* 100**,** 1236.
NACHIAPPAN, S., ASKARI, A., CURRIE, A., KENNEDY, R. H. & FAIZ, O. 2014. Intraoperative assessment of colorectal anastomotic integrity: a systematic review. *Surg Endosc,* 28**,** 2513-30.
NELSON, H., PETRELLI, N., CARLIN, A., COUTURE, J., FLESHMAN, J., GUILLEM, J., MIEDEMA, B., OTA, D. & SARGENT, D. 2001. Guidelines 2000 for colon and rectal cancer surgery. *Journal of the National Cancer Institute,* 93**,** 583-596.
NELSON, R. L., GLENNY, A. M. & SONG, F. 2009. Antimicrobial prophylaxis for colorectal surgery. *The Cochrane Library*.
NESBAKKEN, A., NYGAARD, K. & LUNDE, O. 2001. Outcome and late functional results after anastomotic leakage following mesorectal excision for rectal cancer. *British journal of surgery,* 88**,** 400-404.
NESBAKKEN, A., NYGAARD, K., LUNDE, O., BLÜCHER, J., GJERTSEN, Ø. & DULLERUD, R. 2005. Anastomotic leak following mesorectal excision for rectal cancer: true incidence and diagnostic challenges. *Colorectal Disease,* 7**,** 576-581.
NEUTZLING, C. B., LUSTOSA, S. A., PROENCA, I. M., DA SILVA, E. M. & MATOS, D. 2012. Stapled versus handsewn methods for colorectal anastomosis surgery. *Cochrane Database Syst Rev***,** Cd003144.

NG, S. C., TANG, W., CHING, J. Y., WONG, M., CHOW, C. M., HUI, A., WONG, T., LEUNG, V. K., TSANG, S. W. & YU, H. H. 2013. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology,* 145**,** 158-165. e2.

NICE. 2017. *Molecular testing stratgies for Lynch syndrome in people with colorectal cancer (Nice Guideline 57). Available at:* http://www.nice.org.uk/guidance/dg27/resources/molecular-testing-strategies-for-lynch-syndrome-in-people-with-colorectal-cancer-pdf-1053695294917 *(accessed 20 Aug 2018).* [Online]. Available: http://www.nice.org.uk/guidance/dg27/resources/molecular-testing-strategies-for-lynch-syndrome-in-people-with-colorectal-cancer-pdf-1053695294917 [Accessed].

NIETO, J. E., DECHANT, J. E., LE JEUNE, S. S. & SNYDER, J. R. 2015. Evaluation of 3 handheld portable analyzers for measurement of l - lactate concentrations in blood and peritoneal fluid of horses with colic. *Veterinary Surgery,* 44**,** 366-372.

NISANEVICH, V., FELSENSTEIN, I., ALMOGY, G., WEISSMAN, C., EINAV, S. & MATOT, I. 2005. Effect of intraoperative fluid management on outcome after intraabdominal surgery. *The Journal of the American Society of Anesthesiologists,* 103**,** 25-32.

NOH, S. M. 2003. Measurement of peritoneal fluid pH in patients with non-serosal invasive gastric cancer. *Yonsei Medical Journal,* 44**,** 45-48.

NOORDALLY, O. & VINCENT, J.-L. 1999. Evaluation of a new, rapid lactate analyzer in critical care. *Intensive care medicine,* 25**,** 508-513.

OBERKOFLER, C. E., RICKENBACHER, A., RAPTIS, D. A., LEHMANN, K., VILLIGER, P., BUCHLI, C., GRIEDER, F., GELPKE, H., DECURTINS, M. & TEMPIA-CALIERA, A. A. 2012. A multicenter randomized clinical trial of primary anastomosis or Hartmann's procedure for perforated left colonic diverticulitis with purulent or fecal peritonitis. *Annals of surgery,* 256**,** 819-827.

ORSAY, C., BASS, E., FIRFER, B., RAMAKRISHNAN, V. & ABCARIAN, H. 1995. Blood flow in colon anastomotic stricture formation. *Diseases of the colon & rectum,* 38**,** 202-206.

PÅHLMAN, L., GLIMELIUS, B. & FRYKHOLM, G. 1989. Ischaemic strictures in patients treated with a low anterior resection and perioperative radiotherapy for rectal carcinoma. *British journal of surgery,* 76**,** 605-606.

PAKKASTIE, T., OVASKA, J., PEKKALA, E., LUUKKONEN, P. & JÄRVINEN, H. 1997. A randomised study of colostomies in low colorectal anastomoses. *The European journal of surgery= Acta chirurgica,* 163**,** 929-933.

PALMER KR, P. I., PATERSON-BROWN S. 2006. Ailmentary tract and pancreatic disease. *In:* BOON NA, C. N., WALKER BR. (ed.) *Davidson's Principles and Practice of Medicine.* 20th ed. Edinburgh: Churchill Livingstone.

PARK, J. S., CHOI, G.-S., KIM, S. H., KIM, H. R., KIM, N. K., LEE, K. Y., KANG, S. B., KIM, J. Y., LEE, K. Y. & KIM, B. C. 2013. Multicenter analysis of risk factors for anastomotic leakage after laparoscopic rectal cancer excision: the Korean laparoscopic colorectal surgery study group. *Annals of surgery,* 257**,** 665-671.

PASTERNAK, B., MATTHIESSEN, P., JANSSON, K., ANDERSSON, M. & ASPENBERG, P. 2010. Elevated intraperitoneal matrix metalloproteinases-8 and -9 in patients who develop anastomotic leakage after rectal cancer surgery: a pilot study. *Colorectal Dis,* 12**,** e93-8.

PEDERSEN, M. E., QVIST, N., BISGAARD, C., KELLY, U. & PEDERSEN, S. 2009. Peritoneal microdialysis. Early diagnosis of anastomotic leakage after LAR for rectal cancer. *Scandinavian Journal of Surgery*.

PEEL, A. & TAYLOR, E. 1991. Proposed definitions for the audit of postoperative infection: a discussion paper. Surgical Infection Study Group. *Annals of the Royal College of Surgeons of England,* 73**,** 385.

PEPYS, M. B. & HIRSCHFIELD, G. M. 2003. C-reactive protein: a critical update. *The Journal of clinical investigation,* 111**,** 1805-1812.

PETRI, J. B., SCHURK, S., GEBAUER, S. & HAUSTEIN, U.-F. 1998. Cyclosporine A delays wound healing and apoptosis and suppresses activin beta-A expression in rats. *European journal of dermatology: EJD,* 8**,** 104-113.

PETROWSKY, H., DEMARTINES, N., ROUSSON, V. & CLAVIEN, P.-A. 2004. Evidence-based value of prophylactic drainage in gastrointestinal surgery: a systematic review and meta-analyses. *Annals of surgery,* 240**,** 1074-1085.

PIDDOCK, L., PLANAS, R., BERNARD, B. & INADOMI, J. 2000. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. *International ascites club. J Hepatol,* 32**,** 142-53.

PIMENTEL, J., DUARTE, A. & PATRICIO, J. 2003. The role of a protecting stoma in low anterior resection with TME and colonic J-pouch for rectal cancer; results of a prospective randomized trial. *Colorectal Disease Supplement,* 5**,** 21-22.

PLATELL, C., BARWOOD, N., DORFMANN, G. & MAKIN, G. 2007. The incidence of anastomotic leaks in patients undergoing colorectal surgery. *Colorectal Disease,* 9**,** 71-79.

PLATT, J. J., RAMANATHAN, M. L., CROSBIE, R. A., ANDERSON, J. H., MCKEE, R. F., HORGAN, P. G. & MCMILLAN, D. C. 2012. C-reactive protein as a predictor of postoperative infective complications after curative resection in patients with colorectal cancer. *Annals of surgical oncology,* 19**,** 4168-4177.

POSTON, G., TAIT, D., O'CONNELL, S., BENNETT, A. & BERENDSE, S. 2011. Diagnosis and management of colorectal cancer: summary of NICE guidance. *BMJ,* 343**,** d6751.

PRETE, F. P., PEZZOLLA, A., PRETE, F., TESTINI, M., MARZAIOLI, R., PATRITI, A., JIMENEZ-RODRIGUEZ, R. M., GURRADO, A. & STRIPPOLI, G. F. 2018. Robotic versus laparoscopic minimally invasive surgery for rectal cancer: a systematic review and meta-analysis of randomized controlled trials. *Annals of surgery,* 267**,** 1034-1046.

PYNE, D. B., BOSTON, T., MARTIN, D. T. & LOGAN, A. 2000. Evaluation of the Lactate Pro blood lactate analyser. *European journal of applied physiology,* 82**,** 112-116.

RAHBARI, N. N., WEITZ, J., HOHENBERGER, W., HEALD, R. J., MORAN, B., ULRICH, A., HOLM, T., WONG, W. D., TIRET, E. & MORIYA, Y. 2010. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery,* 147**,** 339-351.

RAHMAN, N. M., MISHRA, E. K., DAVIES, H. E., DAVIES, R. J. & LEE, Y. G. 2008. Clinically important factors influencing the diagnostic measurement of pleural fluid pH and glucose. *American journal of respiratory and critical care medicine,* 178**,** 483-490.

RAVO, B. 1997. Intracolonic bypass procedure. *Diseases of the colon & rectum,* 40**,** 628-629.

REED, G. F., LYNN, F. & MEADE, B. D. 2002. Use of coefficient of variation in assessing variability of quantitative assays. *Clin. Diagn. Lab. Immunol.,* 9**,** 1235-1239.

REISINGER, K. W., POEZE, M., HULSEWÉ, K. W., VAN ACKER, B. A., VAN BIJNEN, A. A., HOOFWIJK, A. G., STOOT, J. H. & DERIKX, J. P. 2014. Accurate prediction of anastomotic leakage after colorectal surgery using plasma markers for intestinal damage and inflammation. *Journal of the American College of Surgeons,* 219**,** 744-751.

RICCIARDI, R., ROBERTS, P. L., MARCELLO, P. W., HALL, J. F., READ, T. E. & SCHOETZ, D. J. 2009. Anastomotic leak testing after colorectal resection: what are the data? *Archives of Surgery,* 144**,** 407-411.

RICHARDS, C., CAMPBELL, V., HO, C., HAYES, J., ELLIOTT, T. & THOMPSON‐FAWCETT, M. 2012. Smoking is a major risk factor for anastomotic leak in patients undergoing low anterior resection. *Colorectal Disease,* 14**,** 628-633.

RICOS C, C. A., GARCIA-LARIO JV, JIMENEZ HCV, PERICH C, SIMON M 1999. Current databases on biological variation: pros, cons and progress. *Scandinavian Journal of Clinical and Laboratory Investigation,* 59**,** 491-500.

RISS, S., STIFT, A., MEIER, M., HAIDEN, E., GRÜNBERGER, T. & BERGMANN, M. 2010. Endo‐sponge assisted treatment of anastomotic leakage following colorectal surgery. *Colorectal Disease,* 12**,** e104-e108.

RONDELLI, F., BUGIANTELLA, W., VEDOVATI, M., BALZAROTTI, R., AVENIA, N., MARIANI, E., AGNELLI, G. & BECATTINI, C. 2014. To drain or not to drain extraperitoneal colorectal anastomosis? A systematic review and meta‐analysis. *Colorectal Disease,* 16**,** O35-O42.

RONDELLI, F., REBOLDI, P., RULLI, A., BARBERINI, F., GUERRISI, A., IZZO, L., BOLOGNESE, A., COVARELLI, P., BOSELLI, C. & BECATTINI, C. 2009. Loop ileostomy versus loop colostomy for fecal diversion after colorectal or coloanal anastomosis: a meta-analysis. *International journal of colorectal disease,* 24**,** 479-488.

ROOS, D., DIJKSMAN, L., TIJSSEN, J., GOUMA, D., GERHARDS, M. & OUDEMANS‐VAN STRAATEN, H. 2013. Systematic review of perioperative selective decontamination of the digestive tract in elective gastrointestinal surgery. *British Journal of Surgery,* 100**,** 1579-1588.

RULLIER, E., LAURENT, C., GARRELON, J., MICHEL, P., SARIC, J. & PARNEIX, M. 1998. Risk factors for anastomotic leakage after resection of rectal cancer. *British Journal of Surgery,* 85**,** 355-358.

SARTELLI, M., CATENA, F., ANSALONI, L., COCCOLINI, F., GRIFFITHS, E. A., ABU-ZIDAN, F. M., DI SAVERIO, S., ULRYCH, J., KLUGER, Y. & BEN-ISHAY, O. 2016. WSES Guidelines for the management of acute left sided colonic diverticulitis in the emergency setting. *World Journal of Emergency Surgery,* 11**,** 37.

SCARBOROUGH, J. E., MANTYH, C. R., SUN, Z. & MIGALY, J. 2015. Combined mechanical and oral antibiotic bowel preparation reduces incisional surgical site infection and anastomotic leak rates after elective colorectal resection. *Annals of surgery,* 262**,** 331-337.

SCHAEFER, J., WALKER, W. & THURLOW, W. 1993. Stenosis of stapled colorectal anastomosis. *Canadian journal of surgery. Journal canadien de chirurgie,* 36**,** 297.

SCHÄFFER, M. R., FUCHS, N., PROKSCH, B., BONGARTZ, M., BEITER, T. & BECKER, H.-D. 1998. TACROLIMUS IMPAIRS WOUND HEALING: A Possible Role of Decreased Nitric Oxide Synthesis1. *Transplantation,* 65**,** 813-818.

SCHOOLBAG.INFO. 2016. *Biology of the human: The digestive tract* [Online]. Available: http://schoolbag.info/biology/humans/20.html [Accessed 31/08/2016 2016].

SCHULTZ, M. & BUTT, A. G. 2012. Is the north to south gradient in inflammatory bowel disease a global phenomenon? *Expert review of gastroenterology & hepatology,* 6**,** 445-447.

SCOTLAND, N. H. 2017. *The new Scottish bowel screening test* [Online]. Available: http://www.healthscotland.scot/media/1619/bowel-screeening-inserts_nov17_english.pdf [Accessed 10/09/2019 2019].

SEBAG-MONTEFIORE, D., STEPHENS, R. J., STEELE, R., MONSON, J., GRIEVE, R., KHANNA, S., QUIRKE, P., COUTURE, J., DE METZ, C. & MYINT, A. S. 2009. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. *The Lancet,* 373**,** 811-820.

SEIKE, K., KODA, K., SAITO, N., ODA, K., KOSUGI, C., SHIMIZU, K. & MIYAZAKI, M. 2007. Laser Doppler assessment of the influence of division at the root of the inferior mesenteric artery on anastomotic blood flow in rectosigmoid cancer surgery. *International journal of colorectal disease,* 22**,** 689-697.

SENAGORE, A., MILSOM, J. W., WALSHAW, R. K., DUNSTAN, R., MAZIER, W. P. & CHAUDRY, I. H. 1990. Intramural pH: a quantitative measurement for predicting colorectal anastomotic healing. *Diseases of the Colon & Rectum,* 33**,** 175-179.

SENAGORE, A. J., DELANEY, C. P., MADBOULAY, K., BRADY, K. M. & FAZIO, C. V. W. 2003. Laparoscopic colectomy in obese and nonobese patients. *Journal of gastrointestinal surgery,* 7**,** 558-561.

SHAMIYEH, A., SZABO, K., WAYAND, W. U. & ZEHETNER, J. 2012. Intraoperative endoscopy for the assessment of circular-stapled anastomosis in laparoscopic colon surgery. *Surgical Laparoscopy Endoscopy & Percutaneous Techniques,* 22**,** 65-67.

SHANDALL, A., LOWNDES, R. & YOUNG, H. 1985. Colonic anastomotic healing and oxygen tension. *British journal of surgery,* 72**,** 606-609.

SHERIDAN, W. G., LOWNDES, R. H. & YOUNG, M. H. L. 1987. Tissue oxygen tension as a predictor of colonic anastomotic healing. *Diseases of the colon & rectum,* 30**,** 867-871.

SHERWINTER, D., GALLAGHER, J. & DONKAR, T. 2013. Intra‐operative transanal near infrared imaging of colorectal anastomotic perfusion: a feasibility study. *Colorectal Disease,* 15**,** 91-96.

SHERWINTER, D. A. 2012. Transanal near-infrared imaging of colorectal anastomotic perfusion. *Surgical Laparoscopy Endoscopy & Percutaneous Techniques,* 22**,** 433-436.

SHIKATA, S., YAMAGISHI, H., TAJI, Y., SHIMADA, T. & NOGUCHI, Y. 2006. Single-versus two-layer intestinal anastomosis: a meta-analysis of randomized controlled trials. *BMC surgery,* 6**,** 1.

SHIVANANDA, S., LENNARD-JONES, J., LOGAN, R., FEAR, N., PRICE, A., CARPENTER, L. & VAN BLANKENSTEIN, M. 1996. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut,* 39**,** 690-697.

SIMMEN, H.-P., BATTAGLIA, H., GIOVANOLI, P. & BLASER, J. 1994. Analysis of pH, pO 2 and pCO 2 in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. *Infection,* 22**,** 386-389.

SIMMEN, H.-P. & BLASER, J. 1993. Analysis of pH and pO 2 in abscesses, peritoneal fluid, and drainage fluid in the presence or absence of bacterial infection during and after abdominal surgery. *The American journal of surgery,* 166**,** 24-27.

SINGH, P., ZENG, I., SRINIVASA, S., LEMANU, D., CONNOLLY, A. & HILL, A. 2014. Systematic review and meta‐analysis of use of serum C‐reactive protein levels to predict anastomotic leak after colorectal surgery. *British Journal of Surgery,* 101**,** 339-346.

SLIEKER, J. C., DAAMS, F., MULDER, I. M., JEEKEL, J. & LANGE, J. F. 2013. Systematic review of the technique of colorectal anastomosis. *JAMA surgery,* 148**,** 190-201.

SLIEKER, J. C., KOMEN, N., MANNAERTS, G. H., KARSTEN, T. M., WILLEMSEN, P., MURAWSKA, M., JEEKEL, J. & LANGE, J. F. 2012. Long-term and perioperative corticosteroids in anastomotic leakage: a prospective study of 259 left-sided colorectal anastomoses. *Archives of surgery,* 147**,** 447-452.

SLIM, K., VICAUT, E., LAUNAY-SAVARY, M.-V., CONTANT, C. & CHIPPONI, J. 2009. Updated systematic review and meta-analysis of randomized clinical trials on the role of mechanical bowel preparation before colorectal surgery. *Annals of surgery,* 249**,** 203-209.

SMITH, R. L., BOHL, J. K., MCELEARNEY, S. T., FRIEL, C. M., BARCLAY, M. M., SAWYER, R. G. & FOLEY, E. F. 2004. Wound infection after elective colorectal resection. *Annals of surgery,* 239**,** 599.

SMITH, S., SESKI, J. C., COPELAND, L. J., GERSHENSON, D. M., EDWARDS, C. L. & HERSON, J. 1985. Surgical management of irradiation-induced small bowel damage. *Obstetrics & Gynecology,* 65**,** 563-567.

SMITH, S. A., ROBERTS, D. J., LIPSON, M. E., BUIE, W. D. & MACLEAN, A. R. 2016. Postoperative nonsteroidal anti-inflammatory drug use and intestinal anastomotic dehiscence: a systematic review and meta-analysis. *Diseases of the Colon & Rectum,* 59**,** 1087-1097.

SOMMER, T. & LARSEN, J. 2004. Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischaemia. *British journal of surgery,* 91**,** 855-861.

SØRENSEN, L., JØRGENSEN, T., KIRKEBY, L., SKOVDAL, J., VENNITS, B. & WILLE‐JØRGENSEN, P. 1999. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. *British Journal of Surgery,* 86**,** 927-931.

SPIEGEL, M. 1961. Chapter 4: The standard deviation and other measures of dispersion. *Theory and problems of statistics.* 1st ed. USA: McGraw-Hill Book Company.

STATE, P. 2002. *Energy III - Cellular Respiration* [Online]. Available: https://online.science.psu.edu/biol110_sandbox_8862/node/9003 [Accessed 31/08/2016 2016].

STEELE, R. 2014. Colonic cancer. *In:* RKS PHILLIPS, S. C. (ed.) *A companion to specialist surgical practice: Colorectal Surgery.* 5th ed. Edinburgh: Saunders Elsevier.

STERK, P., SCHUBERT, F., GUNTER, S. & KLEIN, P. 2001. [Anastomotic protection with a transanal tube after rectum resection and total mesorectal excision]. *Zentralbl Chir,* 126**,** 601-4.

T, A. 1891. Ueber die Bildung von Milchsäure und Glycose im Organismus bei Sauerstoffmangel. Zweite Mittheilung: Ueber die Wirkung von Morphium, Amylnitrit, Cocain. . *Z Physiol Chem,* 15**,** 546–561.

T, A. 1892a. Ueber Bildung von Glycose und Milchsäure bei Sauerstoffmangel. Entgegnung. . *Z Physiol Chem* 16**,** 201–204.

T, A. 1892b. Ueber die Bildung von Milchsäure und Glycose im Organismus bei Sauerstoffmangel. Dritte Mittheilung. . *Z Physiol Chem,* 16**,** 453–459.

T, A. (1891) Ueber die Bildung von Milchsäure und Glycose im Organismus bei Sauerstoffmangel. . *Z Physiol Chem* 15**,** 335–370.
TADROS, T., WOBBES, T. & HENDRIKS, T. 1992. Blood transfusion impairs the healing of experimental intestinal anastomoses. *Annals of surgery,* 215**,** 276.
TANNER, R. K., FULLER, K. L. & ROSS, M. L. 2010. Evaluation of three portable blood lactate analysers: Lactate Pro, Lactate Scout and Lactate Plus. *European journal of applied physiology,* 109**,** 551-559.
TARUI, T., MURATA, A., WATANABE, Y., KIM, S.-P., INOUE, M., SHIOZAKI, H., TAENAKA, N. & MONDEN, M. 1999. Earlier prediction of anastomotic insufficiency after thoracic esophagectomy by intramucosal pH. *Critical care medicine,* 27**,** 1824-1831.
TEAM, N. P. 2018. Fourth Patient Report of the National Emergency Laparotomy Audit. *RCoA London.*
TEEUWEN, P., DE GEUS-OEI, L., HENDRIKS, T., VAN GOOR, H., BREMERS, A., OYEN, W. & BLEICHRODT, R. 2012. Hybrid 18F-FDG PET/CT of colonic anastomosis. *Nuklearmedizin,* 51**,** 252-256.
TERA, H. & ABERG, C. 1976. Tissue holding power to a single suture in different parts of the alimentary tract. *Acta chirurgica Scandinavica,* 142**,** 343-348.
THOMPSON, S. K., CHANG, E. Y. & JOBE, B. A. 2006. Clinical review: Healing in gastrointestinal anastomoses, part I. *Microsurgery,* 26**,** 131-136.
THOMPSON-FAWCETT MW, M. N. 2014. Crohn's disease. *In:* PHILLIPS SK, C. S. (ed.) *A companion to specialist surgical practice: Colorectal surgery.* 5th ed. Edinburgh: Saunders Elsevier.
THORNTON, F. J. & BARBUL, A. 1997. Healing in the gastrointestinal tract. *Surgical Clinics of North America,* 77**,** 549-573.
UGRAS, B., GIRIS, M., ERBIL, Y., GOKPINAR, M., CITLAK, G., ISSEVER, H., BOZBORA, A. & OZTEZCAN, S. 2008. Early prediction of anastomotic leakage after colorectal surgery by measuring peritoneal cytokines: prospective study. *Int J Surg,* 6**,** 28-35.
UK, C. A. C. 2016a. *About inflammatory bowel disase* [Online]. Available: https://www.crohnsandcolitis.org.uk/about-inflammatory-bowel-disease [Accessed 11/08/2016 2016].
UK, C. R. 2016b. *About bowel cancer screening* [Online]. Available: http://www.cancerresearchuk.org/about-cancer/type/bowel-cancer/about/screening/about-bowel-cancer-screening [Accessed 17/08/2016 2016].
UK, C. R. 2016c. *Bowel cancer* [Online]. Available: http://www.cancerresearchuk.org/about-cancer/type/bowel-cancer/about/risks/high-risk-groups-for-bowel-cancer [Accessed 17/08/2016 2016].
UK, C. R. 2016d. *Survival statistics for bowel cancer* [Online]. Available: http://www.cancerresearchuk.org/about-cancer/type/bowel-cancer/treatment/statistics-and-outlook-for-bowel-cancer [Accessed 18/08/2016 2016].
VARADHAN, K. K., NEAL, K. R., DEJONG, C. H., FEARON, K. C., LJUNGQVIST, O. & LOBO, D. N. 2010. The enhanced recovery after surgery (ERAS) pathway for patients undergoing major elective open colorectal surgery: a meta-analysis of randomized controlled trials. *Clinical nutrition,* 29**,** 434-440.
VASEN, H. F., BLANCO, I., AKTAN-COLLAN, K., GOPIE, J. P., ALONSO, A., ARETZ, S., BERNSTEIN, I., BERTARIO, L., BURN, J. & CAPELLA, G. 2013. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. *Gut***,** gutjnl-2012-304356.

VERMA, R. P. & HANSCH, C. 2007. Matrix metalloproteinases (MMPs): chemical–biological functions and (Q) SARs. *Bioorganic & medicinal chemistry,* 15**,** 2223-2268.

VEYRIE, N., ATA, T., MUSCARI, F., COUCHARD, A.-C., MSIKA, S., HAY, J.-M., FINGERHUT, A., DZIRI, C. & RESEARCH, F. A. F. S. 2007. Anastomotic leakage after elective right versus left colectomy for cancer: prevalence and independent risk factors. *Journal of the American College of Surgeons,* 205**,** 785-793.

VIGNALI, A., GIANOTTI, L., BRAGA, M., RADAELLI, G., MALVEZZI, L. & DI CARLO, V. 2000. Altered microperfusion at the rectal stump is predictive for rectal anastomotic leak. *Diseases of the colon & rectum,* 43**,** 76-82.

WALSH, S., TANG, T., BASS, S. & GAUNT, M. 2008. Doppler‐guided intra‐operative fluid management during major abdominal surgery: systematic review and meta‐analysis. *International journal of clinical practice,* 62**,** 466-470.

WARSCHKOW, R., BEUTNER, U., STEFFEN, T., MÜLLER, S. A., SCHMIED, B. M., GÜLLER, U. & TARANTINO, I. 2012. Safe and early discharge after colorectal surgery due to C-reactive protein: a diagnostic meta-analysis of 1832 patients. *Annals of surgery,* 256**,** 245-250.

WARSCHKOW, R., STEFFEN, T., THIERBACH, J., BRUCKNER, T., LANGE, J. & TARANTINO, I. 2011. Risk factors for anastomotic leakage after rectal cancer resection and reconstruction with colorectostomy. A retrospective study with bootstrap analysis. *Annals of surgical oncology,* 18**,** 2772.

WIIK, H., KARTTUNEN, R., HAUKIPURO, K. & SYRJÄLÄ, H. 2001. Maximal local and minimal systemic cytokine response to colorectal surgery: the influence of perioperative filgrastim. *Cytokine,* 14**,** 188-192.

WINTER, D. 2014. Diverticular disease. *In:* RKS PHILLIPS, S. C. (ed.) *A companion to specialist surgical practice: Colorectal Surgery.* 5th ed. Edinburgh: Elsevier.

WONG, G. G. & CLARK, S. C. 1988. Multiple actions of interleukin 6 within a cytokine network. *Immunology today,* 9**,** 137-139.

WRIGHT, E. C., CONNOLLY, P., VELLA, M. & MOUG, S. 2017. Peritoneal fluid biomarkers in the detection of colorectal anastomotic leaks: a systematic review. *International journal of colorectal disease,* 32**,** 935-945.

YAMAMOTO, T., UMEGAE, S., MATSUMOTO, K. & SANIABADI, A. R. 2011. Peritoneal cytokines as early markers of peritonitis following surgery for colorectal carcinoma: a prospective study. *Cytokine,* 53**,** 239-42.

YANG, L., HUANG, X.-E., XU, L., ZHOU, X., ZHOU, J.-N., YU, D.-S., LI, D.-Z. & GUAN, X. 2013. Acidic Pelvic Drainage as a Predictive Factor For Anastomotic Leakage after Surgery for Patients with Rectal Cancer. *Asian Pacific Journal of Cancer Prevention,* 14**,** 5441-5447.

YOON, W.-H., SONG, I.-S. & CHANG, E.-S. 1994. Intraluminal bypass technique using a condom for protection of coloanal anastomosis. *Diseases of the colon & rectum,* 37**,** 1046-1047.

ZABANA, Y., VAN DOMSELAAR, M., GARCIA-PLANELLA, E., MAÑOSA, M., SAN ROMÁN, A. L., GORDILLO, J., CABRÉ, E. & DOMÈNECH, E. 2011. Perianal disease in patients with ulcerative colitis: a case-control study. *Journal of Crohn's and Colitis,* 5**,** 338-341.

ZAKRISON, T., NASCIMENTO JR, B. A., TREMBLAY, L. N., KISS, A. & RIZOLI, S. B. 2007. Perioperative vasopressors are associated with an increased risk of gastrointestinal anastomotic leakage. *World journal of surgery,* 31**,** 1627-1634.

ZEEH, J., INGLIN, R., BAUMANN, G., DIRSCH, O., RILEY, N. E., GERKEN, G., BÜCHLER, M. W. & EGGER, B. 2001. MYCOPHENOLATE MOFETIL IMPAIRS HEALING OF LEFT-SIDED COLON ANASTOMOSES 1. *Transplantation,* 71**,** 1429-1435.

ZHANG, H. & VINCENT, J. L. 1993. Oxygen extraction is altered by endotoxin during tamponade-induced stagnant hypoxia in the dog. *Circulatory shock,* 40**,** 168-176.
ZHOU, C., WU, X.-R., LIU, X.-H., CHEN, Y.-F., KE, J., HE, X.-W., HE, X.-S., HU, T., ZOU, Y.-F. & ZHENG, X.-B. 2018. Male gender is associated with an increased risk of anastomotic leak in rectal cancer patients after total mesorectal excision. *Gastroenterology report,* 6**,** 137-143.
ZULLO, A. 2018. Medical hypothesis: speculating on the pathogenesis of acute diverticulitis. *Annals of Gastroenterology,* 31**,** 1-3.