

**Improving patient assessment and outcomes in
contaminated abdominal wall reconstruction.**

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Declaration of Originality

I declare that the work described herein is the product of my own work and where data has been derived as a result of collaboration or with the assistance of others; this has been stated in full in each section. Quotations and work described by other researchers has been full referenced in accordance with standard practices.

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Abstract

This work aims to comprehensively evaluate patient assessment and explore avenues to improve outcomes for patients undergoing contaminated abdominal wall reconstruction (CAWR). A systematic review was performed to establish current outcomes from CAWR. Evaluation of national Hospital Episode Statistics data demonstrated contamination is a key risk factor for hernia recurrence (HR) justifying evaluation in isolation from clean defects.

Risk factors for the development of CAW defects were evaluated using national and local data. Factors including comorbidity, increasing BMI, emergency surgery and number of previous operations were found to be significant.

The use of two component separation techniques were evaluated using a systematic review and meta-analysis and found to be comparable across a range of outcomes.

Risk stratification was performed through development of logistic regression models evaluating surgical outcomes. Via international collaboration, a cohort of patients from 2004-2015 undergoing CAWR was used to develop the models. An independent cohort, from 2016-2017, was then evaluated to validate the models.

The use of CT-scanning in the evaluation of the CAW was investigated. After standardising measurement techniques, factors associated with surgical outcomes were assessed. Measurements related to hernia dimension are linked to long-term outcomes, such as HR.

Systematic review data demonstrated HR after using biologic mesh remained high, therefore a novel biologic mesh scaffold, using porcine AW tissue, was explored. A protocol for decellularisation was developed and tested for residual strength and pro-inflammatory proteins. The scaffold was then tested for *in vivo* biocompatibility demonstrating a significant increase in scaffold integration and a decrease in inflammatory reaction over 12-weeks, supporting the conclusion that the scaffold shows promise as a future biomaterial.

Overall, this work has offered an evaluation of risk prior to the development of contaminated defects, helped the surgeon in decision-making and counselling patients for CAWR and investigated novel avenues for future reconstruction.

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List of Abbreviations

APTS	3-aminopropyltiethoxysilane
AUMC	Amsterdam University Medical Centres
BMI	Body Mass Index
CAWR	Contaminated abdominal wall reconstruction
CD	Clavien Dindo
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
ECF	Enterocutaneous fistula
FCS	Foetal calf serum
GAG	Glycosaminoglycans
H&E	Haematoxylin and Eosin
IBD	Inflammatory bowel disease
ICC	Inter-class correlation coefficient
IDA	Industrial denatured alcohol
IH	Incisional hernia
IHD	Ischaemic heart disease
LSMI	Lumbar skeletal muscle index
NBF	Neutral buffered formalin
OACS	Open anterior component separation
PCSTAR	Posterior component separation with transversus abdominis release
PBS	Phosphate buffered saline
PSR-ME	Picro-Sirius red and Miller's Elastin
ROC	Receiver operating characteristic
SMH	St Mark's Hospital

SSI	Surgical site infection
SSO	Surgical site occurrence
VHWG	Ventral hernia working group
YI	Youden's Index

Chapter 1 – Introduction and literature review

1.1 History and background of abdominal wall reconstruction

Abdominal wall hernia is the most common anatomical abnormality in men and women and hernia repair remains a classic general surgical problem. Hernias have been described throughout history however it was not until the 19th century, with the advent of anatomical dissection, that functional hernia repair techniques were described. Following the anatomical appreciation of the inguinal and femoral canals, suture anatomical reconstruction was first described by Bassini in 1887 and further developed by Shouldice during the 1930s (1–3).

Bilroth first described early prosthetic repair in 1878 however effective prosthetic material was not developed until the 1960s. The tension-free mesh repair technique described by Lichtenstein in 1984 has become the mainstay of groin hernia surgery (4,5).

As open abdominal surgery grew in prevalence, the incidence of incisional hernia rose. Reported incidence of incisional hernia following midline laparotomy ranges from 2-30% (6–10). It is generally accepted that a figure of approximately 10% is likely. Non-midline incisions also carry a risk of hernia development, including transverse of 7.5% and para-median of 2.5%. Through the 1980s and 1990s the incidence of incisional hernia repair grew steadily in the USA by 3.6%/year (11) and is now the fifth most common operation performed in the USA.

The principles of hernia repair, developed in groin surgery, were applied to ventral/incisional hernias and initial techniques described accurate dissection of the

sac and anatomical restoration in the midline. If this was not possible or would place the wound under significant tension a bridging mesh was used. Stoppa first described the tension free mesh repair for incisional hernia in the 1980s (12–14). He described midline peritoneal closure with retro-muscular mesh placement followed anterior fascia re-approximation. This technique was developed and modified over time. Evidence for the use of mesh in incisional hernia repair has grown. Long-term follow-up studies and meta-analysis have demonstrated the superiority of mesh-reinforced repairs over suture reconstruction (15,16).

The question of how to repair hernias in the contaminated field, with concomitant bowel operations, pre-existing open wounds/infected mesh or enterocutaneous fistula has trouble surgeons for years. Early evidence pointed towards the risk of using mesh in the contaminated field, with a higher incidence of wound infection and mesh infection and further concerns arose with the increased occurrence of bowel adhesions and bowel fistula when meshes were used intra-peritoneally (17–19). The advent of biologic mesh in the early 1980s was thought to be the answer to this risk, as the biologic scaffold would react differently to the synthetic in the presence of contamination. The development of novel surgical techniques for local myofascial advancement to assist in obtaining fascial closure has also lead to improved outcomes within the field (20).

While the issue of how to repair contaminated hernias has been recognized and discussed in the literature, the condition remains a rare phenomenon and outcomes from contaminated cases tend to be grouped within cohorts of clean cases when discussed in the literature. Given contamination tends to be due to a concomitant

bowel operation, the hernia repair is often not the primary concern of the operating surgeon or the patient, however given improved survival of patients following major abdominal surgery, the risk of hernia recurrence and the risk associated with multiple/recurrent laparotomy, the importance of optimizing outcome in contaminated hernia surgery has grown. This thesis will exclusively examine contaminated abdominal hernia surgery. We will examine the natural history of the problem, including who gets contaminated defects and why. We will evaluate the techniques used in pre-operative decision making with the aim of improving patient risk stratification and surgical decision-making. We will also undertake the development of a novel biological mesh scaffold for use in contaminated abdominal wall reconstruction.

1.2 Reconstructive techniques and mesh choices

For smaller less complex defects direct suture closure still provides the mainstay of hernia repair. For larger more complex defects a number of techniques have been developed to assist in obtaining tension free fascial closure if not possible through simple dissection and to bridge large fascial gaps. These techniques can be used with or without mesh reinforcement.

In 1979, Huger *et al* defined three zones of the abdominal wall based on the regional blood supply (21). The superior and inferior deep epigastric arteries as well as the overlying skin principally supply Zone 1. Zone 2, in the inferior area, is supplied via the inferior epigastric and deep circumflex arteries as branches of the femoral arteries bilaterally. Zone 3, comprising the lateral portions of the abdominal wall, is supplied via the intercostal and lumbar arteries (Figure 1.1). This information can be useful in establishing the best repair technique for a particular patient and was built on by Mathes *et al* (22).

In 2000, they evaluated a cohort of 106 patients with a wide range of abdominal defects. They divide defect into two categories based on skin coverage and then go on to define the advised repair technique in different abdominal zones. They define Type 1 hernias as having intact or stable skin coverage and recommend prosthetic mesh reinforcement. Type 2 defects are defined as those without stable skin coverage or with absence of skin and they recommend closure using flap reconstruction. The abdominal wall zone and advised flap reconstruction for each area can be found in Figure 1.2.

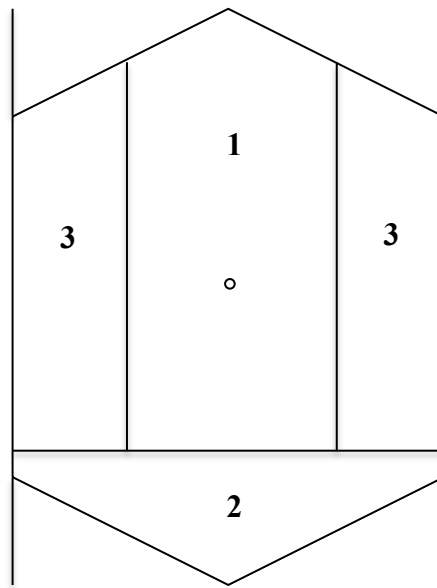


Figure 1.1: Abdominal wall zones as defined by Huger in 1979 (21).

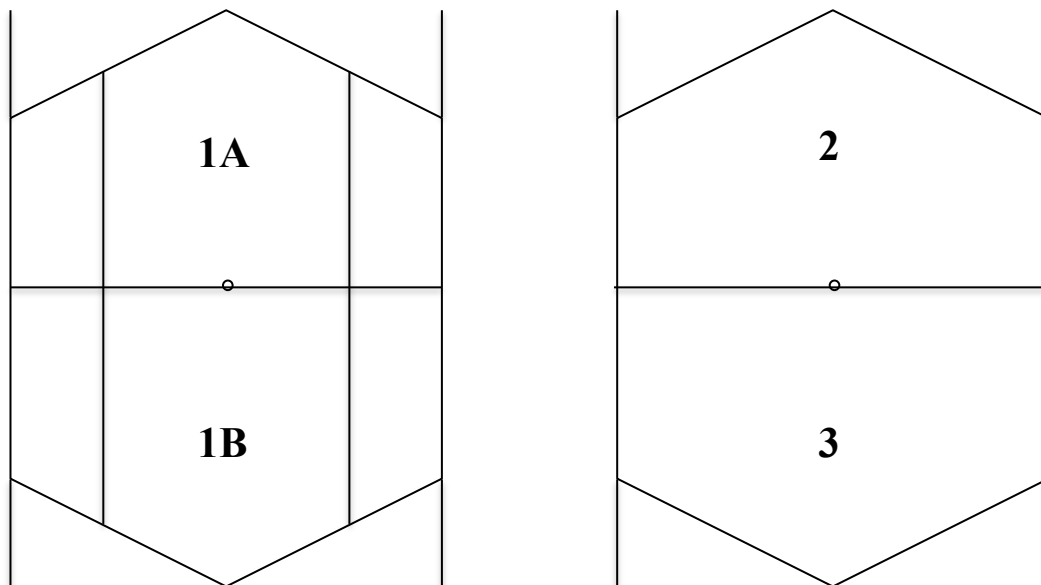


Figure 1.2: Mathes *et al* defined areas for flap reconstruction of abdominal wall defects. A rectus abdominis or tensor fascia lata flap can be used in any zone. Latissimus dorsi are recommended for Zone 2 and external oblique advancement for Zone 3. A rectus femoris flap could be used in Zones 1B or 3 (22).

The principle of obtaining tension free fascial closure is well established and, overtime, local myofascial advancement techniques were developed and have become preferred over distant flap coverage. This is due to the higher incidence of associated morbidity in distant flap reconstruction as well as donor site complications.

1.2.1 Reconstructive techniques

Ramirez *et al.* first described the technique of open anterior component separation, to help achieve fascial closure, in 1990 (20). The team performed dissection of the abdominal wall of 10 cadavers where the anterior subcutaneous fat is dissected from the rectus sheath to expose the linear semi-lunaris. The external oblique is then separated from the internal oblique to allow medialisation of the rectus sheath and restoration in the midline (Figure 1.3). The technique allows for an additional 5cm of lateral muscle mobilisation in the upper abdomen and 10cm in the lower abdomen. A case series of 11 patients was described where the technique was used to good effect.

Over the years it has been observed there are a number of problems arising as a result of open anterior component separation. The extensive subcutaneous dissection that is required lead to increased incidence of seroma, wound infection and abscess formation. Patients have developed a lateral bulge at the point of the created defect in the linear semi-lunaris necessitating further surgical repair. Recurrence rates have been reported as ranging from 9 to 18% and this raises the issue of what reconstruction techniques are subsequently possible if the midline defect recurs (23,24).

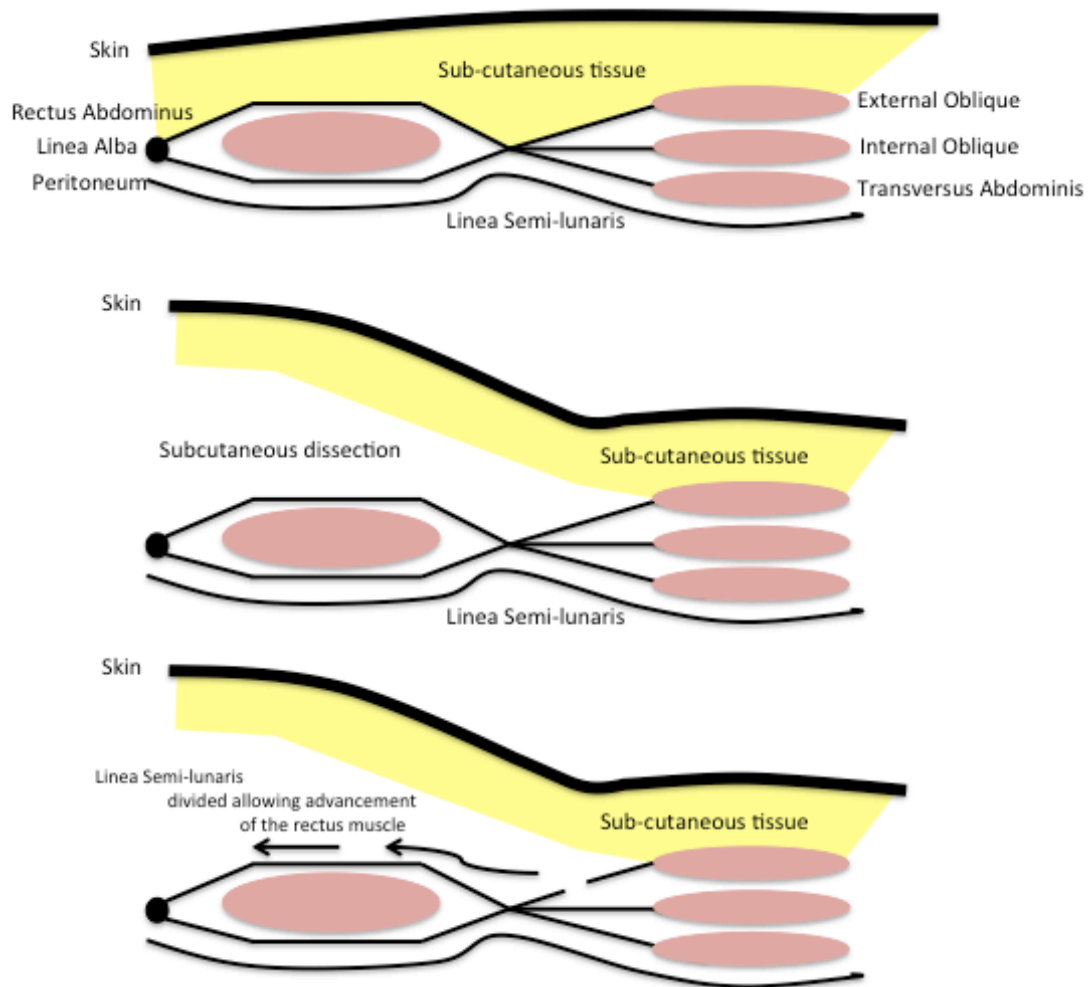


Figure 1.3: Open anterior component separation.

With the aim of reducing the associated wound morbidity, various modifications to the anterior component separation technique have been described. Following the principal of tissue and vascular preservation, “perforator sparing” modifications were initially trialed (25). This involves minimising the subcutaneous dissection by preserving the superficial arterial supply to the rectus complex and dividing the linear semi-lunaris around these pedicles. Endoscopic techniques have also been described involving two lateral incisions to identify the linear semi-lunaris (26). A dissecting balloon is then used to open the subcutaneous space before the linear semi-lunaris is divided using endoscissors or diathermy via an instrument port. These techniques

have the additional advantage of the facility to use with a laparoscopic approach to the main defect, if possible.

No studies have compared open anterior component separation with simple perforator sparing technique however basic surgical principles dictate there should be an advantage even if minimal and most surgeons would attempt to preserve perforating vessels if possible. A number of studies have been carried out to compare “minimally invasive” anterior component separation with standard open Ramirez techniques and meta-analysis of these studies have shown a likely advantage of minimally invasive techniques across a range of outcomes. A number of single centre studies have demonstrated lower rates of wound complications in patients treated with minimally invasive component separation compared to open (27–31). There was, however, no standardisation of wound complication outcome measures across the studies. The numbers of patients in these retrospective, non-randomised, case-control studies were small (<60) and while most describe comparable groups, in terms of pre-operative characteristics, Ghali *et al* reported larger defect size in the minimally invasive group ($405.4 \pm 193.6 \text{ cm}^2$ vs $273.8 \pm 186.8 \text{ cm}^2$; $p = 0.002$), while Azoury *et al* demonstrate the opposite ($162 \pm 145 \text{ cm}^2$ vs $195 \pm 253 \text{ cm}^2$). Increasing defect size is thought to be a risk factor for post-operative complications and minimally invasive techniques give less tissue medialisation therefore maybe less effective in larger defects. It was noted by Harth and Azoury that the minimally invasive group also had a lower operative time and length of stay, which would be as a result of an advantage of the minimally invasive technique or a reflection that those patients selected for these techniques had smaller less complex defects. Analysis of long-term hernia recurrence rates across all

the studies did not demonstrate a statistically significant difference between the two techniques.

Switzer *et al* undertook a systematic review and meta-analysis of open anterior and minimally invasive component separation techniques (32). They identified 63 studies including 3055 patients. The groups were comparable in terms of age and sex distribution while the long-term follow-up was shorter in the minimally invasive group (15.5 vs 25.8 months). Pooled data, systematically reviewed, demonstrated lower rates of superficial wound infection, skin dehiscence, necrosis/debridement, haematoma/seroma, fistula formation, fascial dehiscence and mortality in the minimally invasive group. The open group was found to have lower rates of intra-abdominal abscess formation and hernia recurrence. No statistical tests were undertaken to compare the groups. A smaller section of non-randomised controlled observational retrospective studies were identified with in the literature search including 387 patients. The rate of skin dehiscence was found to be significantly lower in the minimally invasive group (4.0% vs 14.6%; $p=0.02$; OR 3.18 (95%CI 1.16-8.69). No other significant difference was seen between the groups across a range of outcomes (superficial wound infection, abscess, skin necrosis/debridement, hematoma/seroma, fistula formation, fascial dehiscence, hernia recurrence and re-operation).

Some unresolved disadvantages of minimally invasive component separation techniques remain. Initial studies found the techniques allowed for a lower amount of tissue medialisation, likely as a result of the reduced overlying tissue dissection, and therefore they maybe less effective in larger defects. The issues with lateral

abdominal wall weakness and subsequent bulge remain as in the open technique. There are also a number of situations where minimally invasive techniques may not be usable, including the presence of a stoma or bilateral stomas or following previous abdominal wall infection and fibrosis. Overall, anterior component separation is a simple and effective technique for achieving medialisation of the rectus muscle to obtain midline fascial closure and its use remains widespread, both with and without mesh reinforcement, in abdominal wall reconstruction.

Given the ongoing issues with anterior component separation techniques further innovative techniques have recently been described. In 2012, Novitsky *et al* developed posterior component separation and transversus abdominis muscle release (33). This technique utilized the retromuscular space accessed by dissecting the posterior sheath back to the transversus abdominal muscle. The muscle can then be divided along the length of the medial border to access the space between the muscle and transversalis fascia (Figure 1.4). The dissection can then be continued laterally round to the psoas muscle to allow maximum mobilization, as required. They propose that, similar levels of fascial advancement can be achieved whilst preserving a large retromuscular space for mesh placement. They describe an initial cohort of 42 patients, 32 of whom had recurrent hernias. They report 10 patients developing wound complications and 2 recurrences at 2-year follow-up.

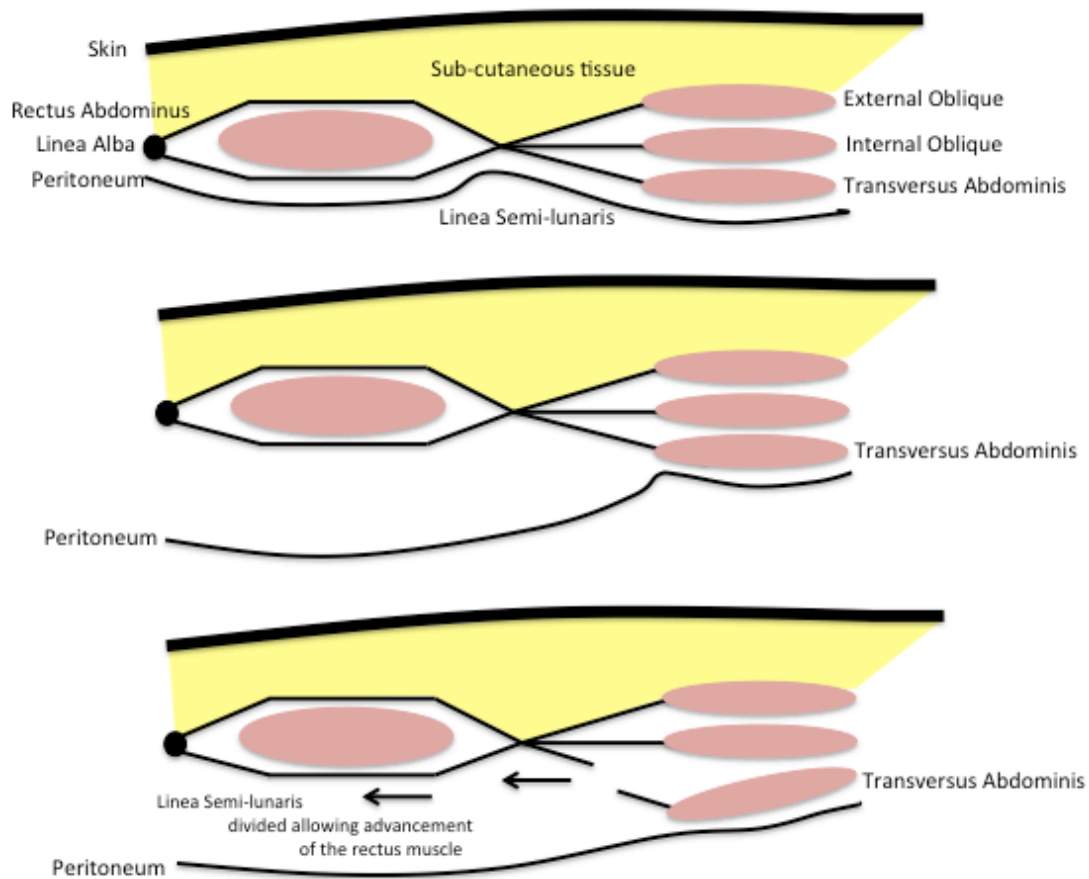


Figure 1.4: Posterior component separation with Transversus Abdominis release.

A further study by the same group published in 2012 compared outcomes from open anterior component separation and their posterior technique revealed an apparent advantage of their procedure. They found there were significantly more wound complications in the anterior component separation group (48.2% vs 25.5%; $p=0.01$) compared to posterior. There were also more long-term hernia recurrences in the anterior group however this was not statistically significant (14.3% vs 3.6%; $p=0.09$). It was noted that there was a significant difference in baseline demographics between the two groups in two key factors. The anterior component separation group was found to have significantly higher average BMI and significantly more patients with diabetes mellitus. These two factors are known to be associated with an increased

risk of developing wound complications and infections so this difference is a potential confounding factor. Overall, Novitsky's technique has been demonstrated to be a safe addition to the surgeon's armamentarium, however no independent comparison with anterior techniques has yet been performed.

A number of other plastic surgical techniques are available to bridge large defects in the anterior abdominal wall if local myofascial advancement is not an option. While the additional technical challenge, increased operative time and risk of donor site morbidity mean distant flaps should be restricted to certain situations where an innervated full thickness graft may be a valid alternative to a bridging mesh. A number of pedicled flaps are available including the tensor fascia lata, rectus femoris, gracilis and vastus lateralis in the lower abdomen. Of these, the tensor fascia lata is thought to be the most reliable with a large arc of rotation and the ability to cover defects in the lower two thirds of the abdomen. For the upper abdomen a pedicled latissimus dorsi flap is preferred. If, in extreme situations, a free flap is required a tensor fascia lata or innervated latissimus dorsi flap are preferred (34).

1.2.2 Mesh choices

The characteristics of the ideal scaffold for hernia repair as described by Cumberland and Scales, Hamer-Hodges and Scott, are as follows: non-carcinogenic; chemically inert; resistant to mechanical strain; sterile; able to limit the foreign-body reaction; modifiable to defect size; non-allergenic; resistant to infection; anti-adhesive; capacity to act like native tissue; associated with minimal surgical morbidity; easy to handle open and laparoscopically; and cost effective (35–37). There are a wide range of

commercially available products currently in use in ventral hernia repair. They can be broadly divided into synthetic and biological grafts.

Neither synthetic nor biologically derived materials can be considered novel. The first report of synthetic material used in the repair of hernias was in the 1800s and biologic grafts have been used since the late 1950s. However, the risk of graft infection in synthetic materials meant their use did not become widespread until relatively recently. It was initially found that problems with biocompatibility and cross-reaction between host and donor limited the use of biological grafts however advances in immunology, the preparation of bioprosthesis and decellularisation techniques made them a viable option.

The ultimate function of any prosthetic or bioprosthesis material is dependant on the initial tissue inflammatory response on implantation. A moderated inflammatory process at the time of implantation, mediated by early fibroblast and leucocyte interaction, will help support the material by promoting cell infiltration, re-vascularisation and colonisation. An uncontrolled exacerbated response however could lead to encapsulation and ultimately rejection of the material or if possible destruction (35).

1.2.2.1 Synthetic mesh

A wide range of commercially available synthetic meshes exists. They can be broadly divided into non-absorbable, absorbable and composite meshes. Non-absorbable meshes are advantageous as they are chemically inert and resistant to mechanical strain. They are easily modifiable to the size of the defect and are cost-

effective. However, they can promote a significant tissue reaction and lead to significant adhesion formation when used intra-peritoneally. Multiple studies have shown the risk of adhesion and enterocutaneous fistula formation associated with non-absorbable meshes intra-peritoneally (17–19). If mesh infection occurs with non-absorbable mesh it is extremely difficult to clear and is more likely to require explant, making their use in the contaminated field ill-advised (17).

Absorbable meshes are useful in many situations for similar reasons to non-absorbable meshes. However they can be used in the contaminated or complex setting judiciously, as if infection does occur this is unlikely to be chronic, as the mesh will eventually absorb, however because of this feature there is a high risk of long-term recurrence particularly when used to bridge large fascial defects. The most common use in the contaminated setting is as a bridge to a more definitive repair in a multi-staged procedure (38).

Composite meshes are designed to have mixed properties of absorbable and non-absorbable mesh. Some overcome the strength issues of absorbable mesh by being partially permanent. Some have dual surfaces with anti-adhesive properties and can therefore be safely used intra-peritoneally however none overcome all the disadvantages of other synthetic meshes whilst maintaining the strengths.

1.2.2.2 Biologic scaffolds

More recently development of biological prosthetics has heralded a new era of innovation. Biological mesh was first developed in the early 1980s for use in soft tissue reconstruction (39). Initial uses as soft tissue fillers and in the reconstruction of

burns demonstrated good graft integration and strength in contaminated fields. Because of this it was postulated that the materials would be ideal for use in abdominal wall reconstruction in the contaminated field overcoming the disadvantages of non-absorbable mesh, including tissue reaction, adhesion formation and infection risk with the added advantage of the ability to act like native tissue.

1.2.2.2.1 Biological scaffold creation

A biologic scaffold is created from donor tissue and can be autologous, allogeneic or xenogeneic. Autologous tissue scaffolds are derived from host tissue and have been discussed elsewhere as forms of tissue advancement (component separation) or flap reconstruction.

Allogeneic grafts from the reconstruction of abdominal wall defects have been described. Vascularised abdominal wall transplant surgery is in its infancy and has this far only been performed in conjunction with solid organ transplant (40). The need for life-long immunosuppression and high risks associated with the surgery have so far not been justified for hernia surgery alone. The difficulty in obtaining donor tissue is also an issue given the significantly higher incidence of ventral/incisional hernia, compared to solid organ transplant, as well as the timing of reconstructive surgery which cannot always be delayed to wait for a matched donor as the patient may require a concomitant bowel operation for a more urgent pathology, for example colon cancer or IBD.

Non-vascularised allogeneic scaffolds of rectus sheath have also been described and utilised for abdominal wall reconstruction at the same time as visceral transplant (41).

The muscle is harvested, fascia opened laterally and the muscle excised leaving only the rectus sheath and peritoneum. In a case series of sixteen, one graft excision and no long-term ventral hernias were seen. While this method has the advantage of being a like-for-like abdominal wall replacement, again, the paucity of donor tissue would make this ineffective on a large scale.

As a result of these difficulties, xenogeneic scaffolds of decellularised porcine and bovine dermis have been developed and utilised in abdominal wall reconstruction. This method provides an abundant supply of donor tissue whilst avoiding the need for immunosuppression. The process of decellularisation removes all immunogenic material from the scaffold and aims to maintain the tissues extracellular matrix. The process combines physical cellular disruption, such as freeze/thaw breakdown and mechanical agitation, with chemical and enzymatic processes to achieve a safe scaffold. Chemical processes, such as non-ionic detergents to disrupt lipid and protein interactions, ionic detergents to solubilise cellular membranes and hypo-/hypertonic solutions to assist in cell lysis, can be used. Enzymatic steps are used to digest DNA and RNA genetic materials (42).

While these methods are necessary and key in creating a non-immunogenic scaffold a balance must be achieved as overtreatment with any method can lead to disruption of the extra-cellular matrix, removal of group-specific antigen (GAG) and damage to collagen fibres that weaken the scaffold and therefore reduce its effectiveness in tissue reconstruction. Defining an appropriate decellularisation process is therefore important and assessment of the resultant scaffold is necessary before any biomaterial can be considered for further use.

The assessment of a decellularised scaffold has no clear guideline, however consensus in the literature suggests a resultant scaffold should be able to integrate into the host tissue without eliciting a significant immune response, whilst maintaining original structural integrity defined by histological appearance, residual collagen and GAG content and maintained mechanical strength and strain parameters. Proposed criteria for the assessment of biomaterials based on the features that should define non-immunogenicity are based on residual DNA, as DNA is directly linked to immune response in transplanted tissue (43). These features include less than 50ng of residual double stranded DNA/mg of scaffolds dry weight, less than 200bp DNA fragment length and lack of nuclear material on H&E staining.

Residual GAG, collagen and biomechanical strength can also be assessed by standard techniques and compared to native tissue to define an adequately decellularised scaffold. When this has been defined biocompatibility testing in a variety of *in vivo* models can be performed to determine host reaction to the graft, immune response, scaffold integration and effectiveness in tissue reconstruction. Following this, clinical studies must be carried out to determine to effectiveness of the scaffold in human hosts.

Current commercially available biologic scaffold utilise dermal or small intestinal tissue for host reconstruction. The effectiveness of these scaffolds in CAWR will be discussed in the next section however no study to date has yet combined the idea of xenogeneic reconstruction with the like-for-like replacement defined by Gondolesi *et al* (41).

1.2.2.2.2 The clinical evidence for biologic mesh

Saramh and Holl-Allen published the first study describing “porcine dermal collagen in the repair of incisional herniae” (44). They reported 11 cases of abdominal wall hernia treated with porcine dermal scaffold reinforcement with an average follow-up of 4.75 years (range 3-5.5). No hernia recurrences were reported after this time. Further investigation of the use of biologic grafts in the reconstruction of the abdominal wall following TRAM flap harvest were performed. In 2004, Buinewicz and Rosen reported the repair of 44 defects in patients with ventral hernia or following TRAM flap harvest (45). They report no hernia recurrences and low rates of wound complications (5 patients).

Baillie *et al* published a review of the available literature on the use of biological mesh in 2007 (46). To this point, 137 biologic mesh reconstructions had been described for the repair of defects ranging from post-TRAM flap creation (54 patients), recurrent ventral hernia with active infection or high risk for contamination (29 patients), management of open abdominal wounds (25 patients), following cancer resections (14 patients), abdominal wall reconstruction in the presence of enterocutaneous fistula (8 patients) and acute abdominal wall dehiscence (7 patients). Across this pooled cohort 4 hernia recurrences were reported and a single recurrent enterocutaneous fistula over a follow-up range of 6 to 18 months. Fourteen patients developed a wound infection and 18 a seroma. They concluded that bioprosthesis are safe and effective in the repair of abdominal wall defects given the issues with synthetic meshes. The needs for prospective and randomized studies are highlighted.

A range of commercially developed biologic meshes became available through the late 1990s and early 2000s. The majority of these consist of decellularised porcine dermis (Permacol, Strattice) however others, such as, Alloderm (human dermis) and Surgisis (small bowel intestinal mucosa) are available. A number of porcine dermal meshes can be broadly split into mesh that has undergone additional cross-linking and those that have not. Additional crosslinking is a process which aims to modify the collagen structure and strengthen the material and prevent rapid breakdown by host or bacterial collagenases (35). Because of this it was thought that this process would improve the mesh function in the contaminated surgical field.

Initial cohort studies (47,48) reported comparable outcomes, in terms of wound complications and hernia recurrences, to non-crosslinked studies. However another early study, by Connolly *et al*, reporting on abdominal wall reconstruction with cross-linked biologic mesh in the presence of enterocutaneous fistula, demonstrated a high rate of recurrent fistula (40%) by comparison to accepted rates of recurrent fistula (10%) (19,49,50). Because of this finding additional crosslinked mesh has not been taken on in abdominal wall reconstruction, with surgeons preferring non-crosslinked mesh instead.

To date the only prospective observational study of the use of non-crosslinked biologic mesh was published in 2012 by Itani *et al* (51). The Repair of Infected or Contaminated ventral incisional Hernias (RICH) study evaluated 80 prospectively recruited patients with potentially contaminated or actively contaminated defects with greater than 9cm² area with a standard follow-up of 2 years. They reported 66%

wound complications and 28% (22 patients) hernia recurrences. Of these recurrences, only 7 were clinically relevant and required further surgery.

More recent studies continue to demonstrate the safety of biologic mesh in ventral hernia surgery. Despite this reported hernia recurrence rates remain between 20-30% (52). A recent study found the non-crosslinked Strattice mesh delivered the lowest risk of infectious complications however it delivered similar rates of hernia recurrence compared to the crosslinked Permacol mesh (53).

Despite the widespread use of biological mesh most outcome data are derived from small, single-centre observational studies without robust comparisons between different meshes or repair techniques. This is likely as a result of the complexity and unique nature of the operation in this group of patients making it impossible to construct a trial protocol that is sufficiently reproducible. The data that are available suggest that biological meshes are associated with a 20% risk of hernia recurrence. Crosslinked meshes have been shown to have lower rates of wound morbidity, explant and enterocutaneous fistulae by comparison to non-crosslinked. Non-crosslinked meshes however have a higher rate of long-term recurrence (54). The variety of products available and lack of randomised trials adds to the confusion.

While it has been sufficiently demonstrated that biologic mesh is safe to use in abdominal wall reconstruction and likely has advantages compared to non-absorbable synthetic mesh the long-term hernia recurrence rates remain high. Non-crosslinked mesh appears safer than crosslinked when comparing fistula recurrence. Improving

decision-making regarding who would most benefit from biologic mesh requires further work, particularly given the initial cost of the mesh.

1.3 Current practice, guidelines and repair considerations

1.3.1 Current practice

While incisional hernia repair, and with increasing frequency abdominal wall reconstruction, is a common general surgical procedure, hernia repair in the contaminated field remains relatively rare. It is thought that a large number of centres perform a small number of operations each year as evidenced by the fact repairs with contamination tend to be reported in cohorts alongside clean defects.

What defines a complex hernia, or even the distinction between and ventral/incisional hernia repair and an abdominal wall reconstruction, is still up for debate. Campbell and Tufaro define complexity as any abdominal wall defect repair which includes any one of the following features: defect size $>40\text{cm}^2$; absence of stable skin coverage; recurrence; infected or exposed prosthetic mesh; compromised abdominal wall soft tissue due to radiotherapy or steroid use; concomitant visceral surgery; or a systemically compromised patient (for example immunosuppression) (55). While this list exhaustively describes a number of features which are known to affect hernia repair and increase the chance of adverse outcome, including wound complication and long-term hernia recurrence, these features have been shown to affect outcome to a variety of levels.

Surgery in the systemically compromised patient has shown to increase risk of general medical complications and those with diabetes, immunosuppression or the like are at increased risk of wound complications which in turn increase the risk of hernia recurrence (15,56). Surgery in the contaminated field or with unstable skin coverage also increases the risk of wound complications (57). Increasing defect size

has been demonstrated to be associated with a higher risk of hernia recurrence (58). No data exists that evaluates combinations of these factors as a result the difficulty with designing a trial and controlling for so many factors. Of all these factors contamination seems to be the single most significant factor in relation to risk of wound and hernia complications (59). However, even within this group there is a range of the level of contamination, from the presence of a stoma or controlled bowel resection to the presence of infected mesh or an enterocutaneous fistula.

Surgical wound contamination is best defined by the Centre for Disease Control Classification (60). Here wounds are classified from I to IV. Class II wounds are defined by the controlled presence of contamination such as entry into the GI tract without spillage or the presence of a stoma. Class III are defined by open wounds or accidental contamination. Class IV includes wounds with active infection, old trauma or perforated viscera. This method stratifies operative contamination and may prove to be helpful in defining contamination in abdominal wall reconstruction.

As well as differentiating between contamination and patients with comorbidity groups of patients exists who are very likely to be complicated by both these factors including those with intestinal failure (IF). IF is defined as the failure of the gastrointestinal tract to provide the body with adequate nutrition to survive without enteral or parenteral supplementation. This situation can be temporary or permanent and IF is sub-classified into three types; type 1 less than 28 days duration; type 2 greater than 28 days duration but still potentially reversible; and type 3 generally irreversible requiring long-term parenteral supplementation (61). Intestinal failure (IF) can commonly result from surgical complications, multiple laparotomies and

abdominal catastrophe, complex inflammatory bowel disease and ECF. All of these situations increase the risk of post-operative incisional hernia (IH) or laparostomy formation. Subsequent repair of these defects is therefore likely to be performed in the contaminated setting and a large proportion of IF patients require abdominal wall reconstructive operations making them an important sub-group of contaminated defects to be considered.

1.3.2 Current guidelines

Published in 2010, the Ventral Hernia Working Group (VHWG) consensus on the management of ventral/incisional hernia aimed to provide an overview of the best evidence on repair considerations, techniques and classify the defects by risk (62). Over a two-day consensus meeting they reviewed the available literature and discussed considerations and advice needed for all ventral hernia repairs.

Strong evidence identified at consensus defined the presence of infection and post-operative wound complications as a significant risk factor for short and long-term post-operative complications (63,64). Because of this they grade cases from 1 (clean defects in healthy patients) to 4 (contaminated defects) in order to help advise surgeons of the best repair technique and potential risk of surgery (Table 1.1).

Since this publication most groups report and stratify cohorts by VHWG grade. Given the higher prevalence of Grade 1 and 2 defects more case series reporting on these grades exist. Few studies exclusively evaluate Grades 3 and 4. Those papers that do consider Grade 3 and 4 tend to combine them together with clean repairs.

This reporting tendency means clear advice on how to deal with the more complex contaminated defects does not exist.

Grade 1	Grade 2	Grade 3	Grade 4
Low risk	Comorbid	Potentially contaminated	Infected
• Low risk for complications • No history of wound infection	• Smoker • Obese • Diabetic • Immunosuppressed • COPD	• Stoma present • Violation of GI tract • Previous wound infection	• Septic dehiscence • Infected mesh

Table 1.1: A table demonstrating the VHWG grading of abdominal wall defects reproduced from Breuing *et al* (62).

The utility of this grading system in the prediction of complications in more complex cases, Grade 3 and 4, remains contentious. There is no clear advice on long-term outcomes in these patients. In 2012, Kanters *et al*, attempted to validate the VHWG grades using a single centre cohort of 299 patients across all grades (65). They found that in the utility of predicting risk of surgical site infection the grading system worked better if Grade 3 and 4 were combined into a single “contaminated” grade (Table 1.2). They further recommended stratifying Grade 3 according to the CDC wound classification. Despite this association with wound complications they were not able to demonstrate a correlation with the grading system and long-term hernia recurrence (65).

Others have attempted to clarify the utility of the VHWG grading system. Diamond and Cryer describe a similar single centre cohort study of 125 ventral hernia repairs. They supported the use of separate grades in cases of severe contamination (66) as they found a significantly higher rate of wound related complications in VHWG

Grade 4 cases (>70%). They suggest a division between the “clean” VHWG Grade 1 and 2 cases and the “contaminated” VHWG Grade 3 and 4 cases particularly where mesh choice is concerned. They advocate synthetic mesh is safe to use in the clean grades. They also suggest that contaminated grades may be better treated in a staged approach as the use of component separation techniques, the use of biologic meshes and the use of bridging mesh were all associated with increased wound complications and subsequent instability of the hernia repair.

Two- or multi-staged repair of CAWR defects has been advocated by other groups as well. It is argued this technique should reduce operative time and reduce wound bioburden. This then leaves a clean field to reconstruct the abdominal wall and allow for safer mesh placement. Conversely this technique exposes the patient to a second major operation, with the associated morbidity (38,67)

Grade 1 Low risk	Grade 2 (modified) Comorbid	Grade 3 (modified) Contaminated
• Low risk for complications • No history of wound infection	• Smoker • Obese • Diabetic • Immunosuppression • COPD • Previous wound infection	• Stoma present • Violation of GI tract • Infected mesh • Septic dehiscence • (ECF/Intestinal failure)

Table 1.2: A table demonstrating a modification to the VHWG grading of abdominal wall defects described by Kanters et al (65). They found grading defects as above proved to better predict wound outcomes in each group within their dataset.

1.4 Repair considerations

1.4.1 Pre-operative assessment

Despite advances in repair technique and bioprosthetics, CAWR is still associated with high post-operative morbidity (62). Multiple pre-operative patient factors are thought to influence this including age, smoking and raised BMI. Systemic comorbidities including diabetes mellitus, chronic obstructive pulmonary disease, immunosuppression and long-term steroid use have also been implicated. Intra-operatively the abdomen often presents a unique challenge as a result of multiple previous laparotomies, previous peritonitis, enterocutaneous fistulae (ECF), stomas and, in some cases, prosthetic mesh from previous hernia repair.

The VHWG consensus recommend a number of intra-operative factors to control in order to maximise the effectiveness of the repair. They include ensuring optimal patient condition by improving nutritional status where necessary, controlling blood sugar levels prior to repair and long-term smoking cessation. The wound should be prepared appropriately by reducing levels of infection as much as possible prior to surgery. Intra-operatively all appropriate adhesions and fistulas should be taken down and restoration of the midline fascia achieved where possible, with the use of component separation techniques if required. The consensus also recommended underlay mesh placement and the use of biologic mesh in the contaminated surgical field. However a number of these recommendations are based on low-quality evidence or expert opinion and would require further investigation (Table 1.3).

Recommendation	Strength of recommendation	Level of evidence	Evidence
Reinforcement recommended for repair of all incisional ventral hernias	1	A/B	(15,16,68)
Centralise and re-approximate rectus muscles when feasible under physiological tension	1	C	(62,68–70)
Reduce bioburden prior to repair	1	B	(60,62)
Placement of repair material: underlay is the recommended technique for the placement of appropriate repair material for open and laparoscopic repairs; overlay placement of repair material should only be considered when complete fascia-to-fascia repair has been achieved	2	B	(62,68,71,72)
In the setting of gross, uncontrolled contamination, it is appropriate to consider delayed repair	1	C	(62)

Table 1.3: Reproduced from Breuing *et al* - VHWG recommendations in hernia repair techniques (62).

A number of studies have attempted to assess for risk factors for wound complications in this group of patients. Factors such as, active smoking, diabetes mellitus, COPD, number of previous hernia repairs, number of previous abdominal surgeries, and increasing operative time were found to be significant for wound complications (73–76). These studies however all used small cohorts (range 37-128) and are therefore at risk of type one error. No analysis has been performed in modified VHWG Grade 3 to look for risk factors associated with other post-operative co-morbidity, such as return to theatre.

One previous study has developed and validated a risk-stratification score for prediction of surgical site occurrence (SSO) and surgical site infection (SSI) in ventral hernia surgery (59). They successfully internally validate the score and then go on to externally validate it on a prospective cohort (77). The most significant predictor of SSO and SSI in their scoring system is wound contamination and therefore of limited use to discriminate risk in patients with modified-VHWG 3 defects. Previous studies, looking at enterocutaneous fistula repair alone, have shown underlying inflammatory

bowel disease and increasing comorbidity as factors associated with recurrent fistula (49).

The VHWG guidance is based around surgical technique and minimising the risk of infection (62). No evaluation of the patient's perspective is included. The Carolinas Equation for Determining Associated Risks (CeDAR) smart phone application, produced by the Carolinas Centre, also helps in the prediction of a particular patients risk when undergoing complex AWR (78). The application also provides patients with some information about what a hernia is and what to do if any complications of the hernia occur. No details of what to expect from the repair and possible complications are included.

1.4.2 Surgical goals

As previously stated the principal goal of hernia surgery is to complete an anatomically restored repair of the surrounding tissue and reinforce this with an appropriate mesh. Intra-operatively numerous techniques have evolved to aid this including component separation and bridging meshes. The evaluation of component separation in isolation as a risk factor for hernia recurrence is mainly related to its risk of wound morbidity. Open anterior techniques are accepted to increase wound problems and subsequently hernia recurrence. Component separation however is seen to be superior to bridging mesh, which is acknowledged to lead to a higher risk of long-term hernia recurrence.

Mesh placement techniques have been well described and studied. The placement of mesh can be performed in any layer of the muscular reconstruction (Figure 1.5). The

language used to describe the placement of mesh is varied and the choice of technique is often determined by specific patients circumstance. Underlay or intra-peritoneal mesh is defined as mesh placed within the peritoneal cavity without any tissue between the internal viscera and the mesh. This technique is often used when there has been significant abdominal wall tissue disruption, when it is not possible to close the underlying peritoneum or when the peritoneum has been excised. Retro-rectus or sublay mesh placement is defined as mesh placed between closed peritoneum and a closed muscle layer. Inlay or interposition mesh is an alternative description of a bridging mesh as the mesh can be described as laid in place of the muscle. Finally onlay mesh is placed above the muscle on top of close abdominal wall muscle.

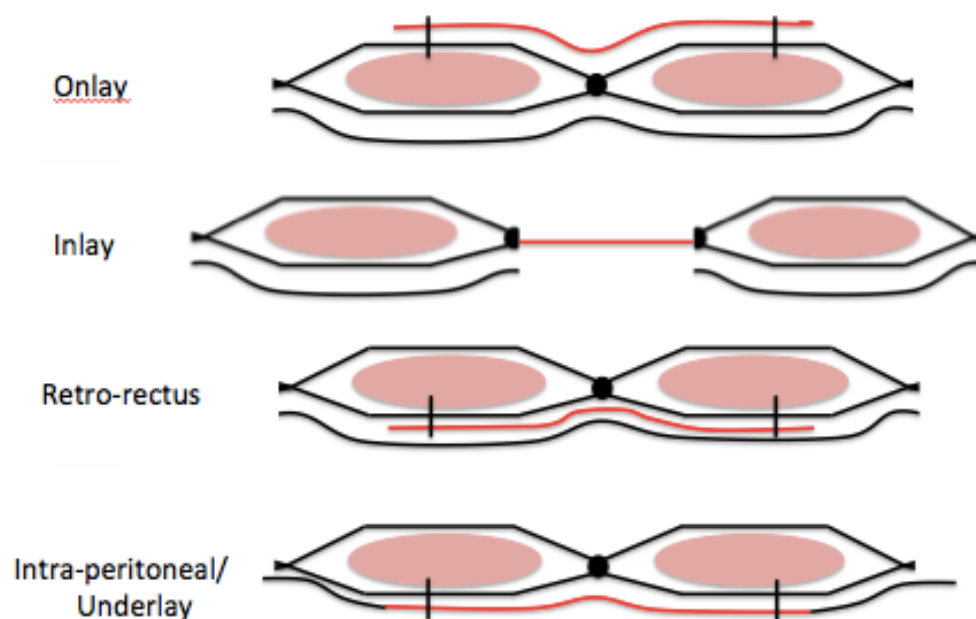


Figure 1.5: A description of mesh placement locations. Mesh = red.

Mesh placement choice varies significantly among studies and centres. There is no standardized way of choosing the best location and no studies have been performed to compare directly between locations in a controlled way.

Albino *et al* conducted a systematic review of studies evaluating the likelihood of post-operative complications depending on mesh placement (79). They compared the four mesh positions in a total of 5824-pooled patient. The majority of these patients 62% received an underlay mesh, 19% onlay mesh, 13% retrorectus and 6% interposition. Comparison of the baseline demographics revealed the only significant difference between the groups was that of the incidence of laparoscopic repair. All the patients who had a laparoscopic repair were in the underlay group.

They found, although there was no significant difference between overall complications based on mesh position ($p=0.23$), there was a significant advantage for retrorectus placement, when compared to the other three locations, for wound infection, seroma and hernia recurrence ($p\text{-value} = 0.001, 0.05$ and 0.01 respectively). When the mesh type was divided into synthetic and biologic this difference was still observed. Synthetic mesh in the retrorectus position had significantly lower overall complications and biologic in the same position lower recurrences. No direct comparison between synthetic and biologic meshes were possible however.

1.4.3 Mesh choices

While the evidence for mesh reinforcement in hernia surgery is well established, the best type of mesh to use in the case of abdominal wall reconstruction has not been identified.

Recommendations of mesh choice progresses from synthetic non-absorbable meshes in clean cases on to a consideration of biological mesh in contaminated cases (Table

1.4) (62). The use of synthetic non-absorbable mesh in the contaminated setting is not recommended in the VHWG guidelines and this is echoed in the European Society of Coloproctology guidelines on the management of IH in the context of IF (80). It is likely all defects within this cohort will be Grade 3 or 4 and the main reason cited for this recommendation is the risk of recurrent ECF.

Grade	Recommendation	Strength of recommendation	Level of evidence	Evidence
1	Choice of repair material by surgeon preference and patient factors	1	C	(62)
2	Increased risk for surgical site occurrence suggests additive risk of permanent synthetic repair material, and potential advantage for appropriate biologic reinforcement	1	B	(56,62,81–83)
3	Permanent synthetic repair material generally not recommended; potential advantage to biologic repair material	1	B	(84–87)
4	Permanent synthetic repair material not recommended; biologic repair material should be considered	1	A	(84,86–92)

Table 1.4: Reproduced from Breuing *et al* - the VHWG recommendations in mesh choice across their grades (62).

1.4.4 Radiology

CT scanning is a well-validated and accepted method of assessing the body prior to intervention. Early data have suggested that computer topography (CT) imaging could be useful in the diagnosis of IH (93), however further studies revealed that CT derived measurements of hernia dimensions underestimate the measurements made at operation (94). This is likely as a result of the associated dissection of surrounding tissues at the time of surgery and the use of muscle relaxant in anaesthesia, and it is thought that CT derived measurements could still be useful in predicting repair methods and outcomes.

In 2011, Sabbagh *et al*, reported that the peritoneal to hernia volume ratio could predict if a tension free fascial closure can be achieved. Tension free fascial closure is known to be a large factor in successful outcomes in hernia surgery. They state that a ratio of less than 20% allows for tension free fascial closure (95).

In 2013, Franklin *et al*, showed that measurements of the transverse size of the defect, the defect area and the percentage size of the abdominal wall defect in combination could predict whether fascial closure could be achieved after components separation (96). Blair *et al*, have also demonstrated that hernia defect size and abdominal wall thickness could predict the need for components separation in AWR and predict the likelihood of wound complications (97).

These studies are limited in their scope as the sizes of the cohorts are small. None of these studies specifically evaluated the use of imaging in contaminated abdominal wall defects (modified VHWG grade 3 (62,65)) and these cases were specifically excluded from the Blair study. Whilst these observations are useful, their use is limited when looking at exclusively contaminated cases. The scope for assessment of the abdominal wall prior to CAWR using CT imaging remains an area that requires further investigation.

While all the above studies describe methods for evaluating the abdominal wall and a variety of different measurements, no standardised techniques have been defined or assessment of evaluation between different observers. Sabbagh *et al* evaluate hernia volume using Myrian Expert Software and calculate the total using operator defined abdominal slices. They exclude retroperitoneal contents from the total volume.

Hernia defect dimensions were measured from the mid-point of the L3 vertebra however this may affect the size measured as not all defects are centred on this point.

Blair and Franklin using TeraRecon software and describe measurement of defect size from the point of maximal defect width and defect areas were calculated as the area of an ellipse. Further measurements of the rectus muscle and lateral abdominal wall were made in both papers however the techniques differed. Franklin *et al* proceeded to make these measurements on the same axial slice as the maximal hernia defect width however Blair chose a slice at the level of the umbilicus.

No consensus or investigation has been described on which of these is the better place to make these measurements however, logically to perform them at the same level of the maximal hernia defect width would make sense as any weakness at this point would contribute the most to affect the strength of any hernia repair.

1.5 Current outcomes in contaminated abdominal wall reconstruction – a systematic review of current practice

This work has been published in *Colorectal Disease* and has been reproduced with permission (*Appendix 8*). JD Hodgkinson, Y Maeda, CA Leo, J Warusavitarne, CJ Vaizey. *Colorectal Dis.* 2017Apr;19(4):319-330. doi: 10.1111/codi.13609.

1.5.1 Introduction

CAWR is still associated with high post-operative morbidity (4). Multiple pre-operative patient factors influence this including age, comorbidity, smoking and raised BMI. Intra-operatively the abdomen often presents a unique challenge as a result of multiple previous laparotomies, previous peritonitis, enterocutaneous fistula (ECF), stomas and, in some cases, prosthetic mesh from previous hernia repair.

While it is well established that the use of mesh reinforcement in hernia surgery greatly reduces the risk of recurrence (7), the role of mesh in complex AWR, particularly in the setting of contamination or ECF, is not clear (4). It is generally accepted that synthetic non-absorbable meshes should not be used due to risk of mesh infection, erosion and fistulisation, but synthetic absorbable and biological mesh maybe suitable in selected patients (4). The advent of biologic mesh was hoped to provide the solution to this and early results demonstrated satisfactory outcomes in contaminated settings (8, 9). Some issues have arisen around the use of crosslinked meshes and the risk of fistula recurrence (10) and the lack of data on long-term outcomes (6). The variety of products available and lack of randomized trials adds to the confusion.

There are very few studies exclusively investigating CAWR. This is likely a reflection of small patient numbers and lack of consensus in practice patterns amongst surgeons in this field. Data on outcomes of CAWR (VHWG grade 3/4) tends to be reported as part of larger cohorts of patients with all grades of abdominal wall hernia. There has therefore been minimal evaluation on this isolated group of complex patients. However, it is thought that rates of hernia recurrence are higher than in those with clean wounds. It is not known if there is a greater chance of developing a recurrent fistula after ECF repair in the presence of concurrent AWR.

The aim of this study is to evaluate the current literature on CAWR and establish the accepted rates of hernia and fistula recurrence.

1.5.2 Methods

Search Strategy

The study was conducted according to the PRISMA guidelines (11). A MEDLINE, EMBASE and pubmed search was carried out to identify all papers reporting on outcomes of CAWR. The reference lists of all related studies were reviewed and relevant papers were included.

Search terms

The following Medical Search Headings were used in combination with the functions ‘AND’ and ‘OR’: 1) ‘Enterocutaneous fistula’ AND ‘hernia’ OR ‘reconstruction’; 2) ‘Abdominal wall hernia’ AND ‘fistula’ OR ‘infection’ OR ‘contamination’; 3) ‘Abdominal wall defect’ AND ‘fistula’ OR ‘infection’ OR ‘contamination’; 4) ‘Abdominal wall reconstruction’ AND ‘fistula’ OR ‘infection’ OR ‘contamination’.

Inclusion criteria

Studies reporting exclusively on outcomes from single-staged CAWR (VHWG grade 3 or 4) from any time period were included. Outcomes of any reconstructive method including using prosthetic repairs, components separation with or without mesh placement, repair/resection of ECF and pedicled or free flaps. Only studies published in English were included.

Exclusion criteria

Studies that included outcomes from non-contaminated/clean cases (VHWG grade 1 or 2) or from non-complex (for example primary umbilical or inguinal) hernia repairs were excluded. Studies were excluded if they described management of acute

contaminated abdominal defects (for example trauma laparotomy) or if they dealt with the defect in a multi-staged approach. Outcomes of paediatric cohorts and case series reporting less than or equal to 5 cases were excluded. Review articles, conference abstracts, discussion and comment articles were excluded. Non-human, cadaveric and basic science studies were excluded.

Data collection

The search results were assessed and titles screened by the first reviewer (JDH). Papers were assessed by two independent reviewers (JDH and CAL) according to the inclusion and exclusion criteria. For papers published prior to the publication of the VHWG system, in 2010, the grade was retrospectively applied by the reviewers from the source data. Included papers were assessed using the Methodological Index for Non-randomized studies (MINORs) tool (12) and the Down and Blacks checklist for non-randomized study assessment (13). Data were extracted by the first reviewer and crosschecked independently by the second reviewer. Agreement on included papers and extracted data was reached following discussion between reviewers.

Data extraction

Data were extracted for; study demographics (population, study year, study design, sample size), patient demographics (sex, age, BMI, diabetes, smoking status), hernia details (contamination, presence of ECF, VHWG grade), operative details (suture repair, mesh repair/type of mesh, fascial closure achieved, use of component separation), post-operative complications (recurrence of hernia, recurrence of fistula, wound related morbidity, mortality), and average follow-up. Data from the included studies were stored in an Excel spreadsheet (Microsoft, Redmond, Washington,

USA). Fisher's exact test was used to test for statistical significance between groups.

1.5.3 Results

A database search identified 1631 studies. This was reduced to 1397 after removal of duplicates. One thousand, two hundred and twenty-three studies were excluded following title review and a further 160 were removed following assessment of abstracts and papers according to the inclusion criteria. This resulted in the inclusion of 14 studies (14-27). Review of reference lists of the screened studies identified a further 2 articles which met the inclusion criteria (10, 28). In total 16 studies were assessed for quality and included in the analysis (Figure 1.6 and Table 1.5). The MINORs tool revealed a range of scores from 8 to 11 out of a total of 16. Down and Blacks analysis demonstrated scores from 14 to 19 out of a possible total score of 32.

There was one prospective, multicenter, observational outcomes study (28) and one single-center study of a similar design (24). Five studies were on prospectively collected databases (10, 15, 18, 19, 25). The remaining studies were retrospective (14, 16, 17, 20-23, 26, 27). The time period covered in all studies ranged from 1987-2013. A total of 601 patients were included with an average follow-up of 26.7 months. Follow-up was performed by clinical assessment in 5 studies (17, 22-24, 28), a combination of clinical assessment and telephone interviews in 3 studies (15, 18, 20) and was not defined in 7 studies (10, 14, 16, 19, 21, 25-27). No study gave a clear definition of hernia recurrence and only one differentiated between hernia and bulge (17).

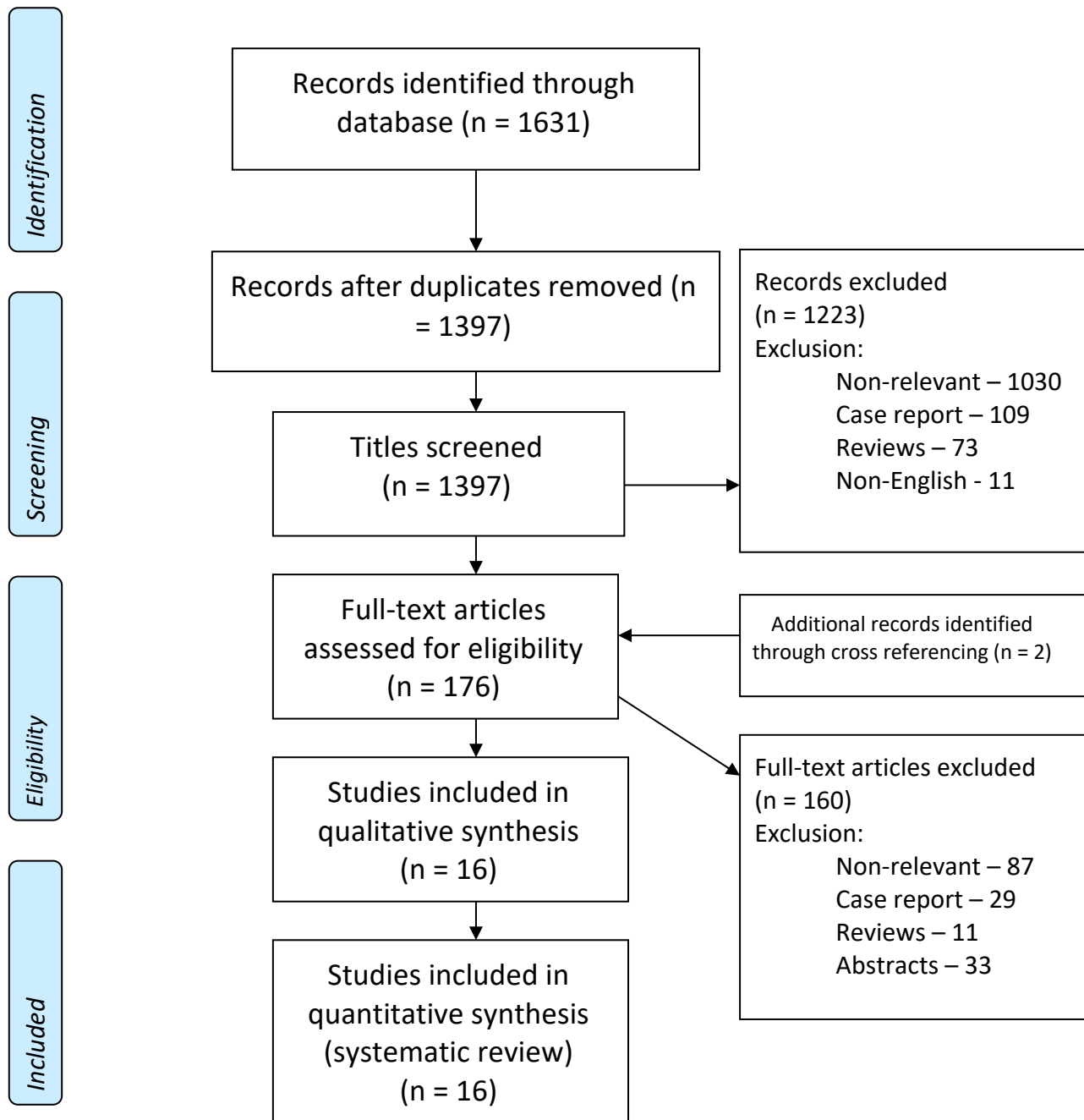


Figure 1.6: PRISMA flow diagram demonstrating study selection.

Paper	Title	Author	Publication year	Study type	Patient number	Year	MINORs	Down and Blacks
1	Outcomes in porcine acellular dermal matrix reinforcement of infected abdominal wall defects	Zerbib P <i>et al</i>	2012	Prospective enrolled outcomes study, single centre	14	2010-2012	10	15
2	Prospective study of single-stage repair of contaminated hernias using a biologic porcine tissue matrix: the RICH Study	Itani K <i>et al</i> , RICH study group	2012	Prospective enrolled outcomes study, multi-centre	80	2008-2012	11	18
3	Outcomes Analysis of Biologic Mesh Use for Abdominal Wall Reconstruction in Clean-Contaminated and Contaminated Ventral Hernia Repair	Sbitany H <i>et al</i>	2015	Prospective database, cohort, single centre	41	2009-2013	10	18
4	A retrospective review and observations over a 16-yr clinical experience on the surgical treatment of chronic mesh infection. What about replacing a synthetic mesh on the infected surgical field?	Birolini C <i>et al</i>	2015	Prospective database, cohort, single centre	41	1996-2012	11	15
5	A 5-year Clinical Experience With Single-Stage Repairs of Infected and Contaminated Abdominal Wall Defects Utilizing Biological Mesh	Rosen M <i>et al</i>	2013	Prospective database, cohort, single centre	128	2005-2012	11	18
6	Outcomes of simultaneous large complex abdominal wall reconstruction and enterocutaneous fistula takedown	Krpata DM <i>et al</i>	2013	Prospective database, cohort, single centre	37	2005-2012	9	17
7	Outcome of Reconstructive Surgery for Intestinal Fistula in the Open Abdomen	Connolly P <i>et al</i>	2008	Prospective database, cohort, single centre	63	1999-2006	10	16
8	Safety and Durability of One-Stage Repair of Abdominal Wall Defects with Enteric Fistulas	Slater NJ <i>et al</i>	2015	Retrospective, cohort, single centre	39	2000-2009	11	19
9	Abdominal wall reconstruction with components separation and mesh reinforcement in complex hernia repair	Nockolds C <i>et al</i>	2014	Retrospective, cohort, single centre	23	2009-2012	9	15
10	Surgical Treatment of Complex Enterocutaneous Fistulas in IBD Patients Using Human Acellular Dermal Matrix	Taner T <i>et al</i>	2008	Retrospective, cohort, single centre	11	2005-2007	8	17
11	Single-stage closure of enterocutaneous fistula and stomas in the presence of large abdominal wall defects using components separation technique	Wind J <i>et al</i>	2007	Retrospective, cohort, single centre	32	2000-2007	9	17
12	Laparoscopic component separation in the single-stage treatment of infected abdominal wall prosthetic removal	Rosen M <i>et al</i>	2007	Retrospective, cohort, single centre	7	2006-2007	9	15
13	The single-staged approach to the surgical management of abdominal wall hernias in contaminated fields	Aladeen D <i>et al</i>	2006	Retrospective, cohort, single centre	19	1999-2006	9	16
14	Surgical Treatment of Large Contaminated Abdominal Wall Defects	van Geffen H <i>et al</i>	2005	Retrospective, cohort, single centre	26	1996-2000	9	14
15	Definitive surgical treatment of infected or exposed ventral hernia mesh	Szczerba SR <i>et al</i>	2003	Retrospective, cohort, single centre	11	NR	10	14
16	Safety and outcome of use of nonabsorbable mesh for repair of fascial defects in the presence of open bowel	Geisler DJ <i>et al</i>	2003	Retrospective, cohort, single centre	29	1987-2001	9	15
				Total =	601			

Table 1.5: A table summarizing study details grouped by study design; including, year of publication, years of data collection, total patient numbers and study quality assessment; NR = not reported.

Patient demographics, grade of hernia and reason for contamination are reported in Table 1.6. Specific details of patient comorbidity and demographics were variably reported. Of the 16 papers included only 9 reported patients' BMI (14, 15, 17-20, 23-25), 7 included smoking status (15, 17-20, 25, 28), 6 had information on the size of hernia defect (15, 17, 18, 20, 22, 28) and 9 on whether the patient had diabetes (15, 17-20, 24, 25, 27, 28). Only 6 studies reported the number of previous laparotomies (10, 15, 17, 18, 20, 21) and 6 the number of previous hernia repairs (15, 17, 18, 20, 24, 28). As data reporting was not uniform we were unable to compare pre-operative demographics with operative factors and outcomes in most cases.

Paper	Pt no	Sex (Male) (%)	Age	Average BMI	Diabetes (%)	Smoker (%)	Average Follow-up (mths)	VHWG Grade 3	VHWG Grade 4	ECF	Bowel resection	Infected mesh	Stoma	Open/infected wound/sinus
1	14	4 (29)	60	34.1	4 (29)	NR	13	0	14	6	NR	8	NR	NR
2	80	47 (59)	57	NR	17 (21)	14 (18)	24	60	20	7	27	15	31	4
3	41	27 (66)	52.4	38.4	24 (59)	6 (15)	25	36	5	3	13	3	20	NR
4	41	18 (43.9)	53.4	31.2	10 (24.4)	16 (39)	74	0	41	0	NR	41	NR	NR
5	128	52 (41)	58.2	34.1	65 (51)	29 (23)	21.7	46	82	25	17	45	24	12
6	37	17 (46)	59	31.3	21 (57)	8 (22)	20	0	37	37	37	10	NR	NR
7	63	31 (49)	50	NR	NR	NR	29	0*	63*	63	63	NR	NR	NR
8	39	27 (69)	61.2	24.4	8 (21)	9 (23)	62.7	0	39	39	39	NR	NR	NR
9	23	15 (65)	57	NR	NR	NR	17	13	10	8	NR	NR	15	NR
10	11	5 (45)	46	NR	NR	NR	12	0*	11*	11	11	NR	NR	NR
11	32	22 (69)	43	21.7	NR	NR	20	0*	32*	15	9	0	21	24
12	7	4 (57)	54	37	3 (43)	1 (14)	4.5	0*	7*	0	NR	7	0	6
13	19	19 (100)	61	34.3	NR	NR	14	0*	19*	7	8	14	NR	3
14	26	15 (58)	49	NR	NR	NR	27	14*	12*	9	13	NR	12	4
15	11	5 (45)	54.5	NR	4 (36.4)	NR	24	0*	11*	3	4	11	NR	NR
16	29	11 (37.9)	67	NR	NR	NR	39	29*	0*	0	4	0	26	0
	601	319 (53.1)			156/398 (39.2)	83/373 (22.3)	26.7	198	403	233				

Table 1.6: A table demonstrating patient demographics including age, BMI, diabetes and smoking status. Further demographic details were sparsely reported. NR = not reported; ECF = enterocutaneous fistula; VHWG = Ventral Hernia Working Group; * = retrospectively applied grading as paper published prior to 2010.

Of the 601 CAWRs performed, 146 hernias recurred (24.3%) (Table 1.7) (10, 14-28).

Suture repair alone demonstrated the lowest level of recurrence with 14.2% (16/113).

When biological mesh was used to reinforce the hernia repair the recurrence rate was 25.8% (92/357). When dividing the biological mesh by manufacturing method it was found that when non-crosslinked mesh was used hernia recurrence was 25.1% (86/342). Very few crosslinked meshes were used in these studies and 6 of the 15 developed a recurrent hernia (40.0%). The use of synthetic mesh resulted in a recurrence rate of 53.1% (17/32) when using absorbable mesh and 21.2% (21/99) with non-absorbable mesh. When comparing the rate of recurrence based on fascial closure, independent of mesh use, hernias recurred significantly less frequently when fascia was closed compared to when a bridging mesh was used 16% (48/300) versus 40% (18/45) respectively ($p<0.001$)).

Paper	Suture Repair		Biological mesh		Synthetic mesh	
	Recurrence	Total	Recurrence	Total	Recurrence	Total
Zerbib P <i>et al</i>	0	0	6	14	0	0
Itani K <i>et al</i> , RICH study group	0	0	22	80	0	0
Sbitany H <i>et al</i>	0	0	5	41	0	0
Birolini C <i>et al</i>	0	0	0	0	3	41
Rosen M <i>et al</i>	0	0	40	128	0	0
Krpata DM <i>et al</i>	0	1	12	36	0	0
Connolly P <i>et al</i>	0	34	5	12	13	17
Slater NJ <i>et al</i>	10	26	0	0	2	13
Nockolds C <i>et al</i>	0	0	2	17	1	6
Taner T <i>et al</i>	0	0	0	11	0	0
Wind J <i>et al</i>	3	14	0	0	4	18
Rosen M <i>et al</i>	0	0	0	7	0	0
Aladeen D <i>et al</i>	0	5	0	11	2	3
van Geffen <i>et al</i>	2	22	0	0	0	4
Szczerba SR <i>et al</i>	1	11	0	0	0	0
Giesler DJ <i>et al</i>	0	0	0	0	13	29
Total =	16	113	92	357	38	131
Percentage =	14.2		25.8		29.0	

Table 1.7: A table demonstrating hernia recurrence rates stratified by repair method.

Two hundred and thirty-three of the operations included repair of an ECF of which 24 recurred (10.3%). Three studies reporting 33 ECF repairs, including 7 recurrences,

did not include enough detail on recurrence based on repair method and were excluded from this analysis (14, 22, 23). This resulted in 200 patients in the final pooled analysis (Table 1.8) (10, 15-21, 24, 27, 28). Recurrence was seen in 17 patients (8.5%). Suture repair alone was associated with the lowest rate of recurrence 1.6% (1/63). Repairs reinforced with non-biological mesh were found to have a recurrence rate of 10.3% (3/29) (absorbable mesh 11.8% (2/17); non-absorbable mesh 8.3% (1/12)). Biological mesh had the highest recurrence rate at 12.0% (13/108), however of the 15 crosslinked meshes used 5 developed a recurrent fistula (33.3%). Use of non-crosslinked biological mesh resulted in a recurrent fistula in 8.6% of cases (8/93).

Paper	Suture Repair		Biological mesh		Synthetic mesh	
	Recurrence	Total	Recurrence	Total	Recurrence	Total
Zerbib P <i>et al</i>	0	0	0	6	0	0
Itani K <i>et al</i> , RICH study group	0	0	1	7	0	0
Sbitany H <i>et al</i>	0	0	0	3	0	0
Birolini C <i>et al</i>	0	0	0	0	0	0
Rosen M <i>et al</i>	0	0	2	25	0	0
Krpata DM <i>et al</i>	0	0	5	36	0	0
Connolly P <i>et al</i>	0	34	5	12	2	17
Slater NJ <i>et al</i>	1	26	0	0	1	12
Nockolds C <i>et al</i>	0	0	0	8	0	0
Taner T <i>et al</i>	0	0	0	11	0	0
Wind J <i>et al</i>	NR	NR	NR	NR	NR	NR
Rosen M <i>et al</i>	0	0	0	0	0	0
Aladeen D <i>et al</i>	NR	NR	NR	NR	NR	NR
van Geffen H <i>et al</i>	NR	NR	NR	NR	NR	NR
Szczerba SR <i>et al</i>	0	3	0	0	0	0
Giesler DJ <i>et al</i>	0	0	0	0	0	0
Total =	1	63	13	108	3	29
Percentage =	1.6		12.0		10.3	

Table 1.8: A table demonstrating fistula recurrence rates stratified by repair method; NR = Not reported.

In some cases it was possible to compare a combination of closure techniques. Suture

repair alone is compared to component separation and suture and component separation and mesh repair to assess risk of hernia and fistula recurrence (Table 1.9).

Fistula recurrence with C/S	Number	Percent
Suture repair alone	0/32	0%
C/S and suture repair	3/32	9.4%
C/S and Biomesh	5/69	7.2%
C/S and synthetic mesh	2/16	12.5%
Hernia recurrence with C/S	Number	Percent
Suture repair alone	0/32	0%
C/S and suture repair	6/72	8.3%
C/S and Biomesh	28/145	19.3%
C/S and synthetic mesh	13/39	33.3%

Table 1.9: A table demonstrating the breakdown of hernia (10, 14, 16, 17, 21-24, 26-28) and fistula (10, 14, 19, 21, 22, 24, 27) recurrence according to the use of component separation techniques; C/S = component separation.

Table 1.10 demonstrates the total number of different mesh types used. 131 synthetic meshes were used; including 99 non-absorbable and 32 absorbable. The most prevalent biologic mesh used in these studies was Strattice™ (Lifecell Corporation, NJ, USA) (264). Of the remaining, 76 were non-crosslinked (Alloderm^R (Lifecell Corporation, NJ, USA), Biodesign^R (Cook Medical, USA), Xenmatrix™ (Bard Davol, USA)), 15 had additional crosslinking (Permacol™ (Covidien Medtronic, USA)) and 4 were a biosynthetic mesh (Bio A^R (Gore Medical, USA)).

Mesh Type	Mesh	Number used
Synthetic	Polypropylene	82
	Polyglactin	32
	Polyester	8
	Vypro	3
	Ultrapro	3
	Proceed	3
Biologic	Strattice	264
	Alloderm	53
	Biodesign	18
	Permacol	15
	XenMatrix	5
	BioA	4

Table 1.10: A table demonstrating total number of each type of mesh used.

Short-term post-operative complication rates in all studies were variably reported (Table 1.11). Two papers used the standardized Clavien Dindo scale of reporting complications (20, 24). Eight papers reported total numbers of patients who had complications (10, 14, 17, 20-23, 25). Only one paper (15) used a standardized method of reporting wound infection, therefore no further analysis was possible. Six papers reported data on medical complications post-operatively (17, 20-23). 46.1% (277/601) of patients were reported as having a wound-related complication. 15 deaths were reported (2.5%) (Table 1.12).

Author	All	Wound related	Standardised wound infection	Mesh related	Medical complications	Clavien - Dindo	Mortality
Zerbib P <i>et al</i>		•		•		•	•
Itani K <i>et al</i> , RICH study group		•		•			•
Sbitany H <i>et al</i>		•		•			•
Birolini C <i>et al</i>	•	•		•			•
Rosen M <i>et al</i>		•					•
Krpata DM <i>et al</i>		•	•		•		•
Connolly P <i>et al</i>	•	•		•	•		•
Slater NJ <i>et al</i>	•	•		•	•	•	•
Nockolds C <i>et al</i>		•		•			•
Taner T <i>et al</i>	•	•			•		•
Wind J <i>et al</i>	•	•		•	•		•
Rosen M <i>et al</i>	•	•			•		•
Aladeen D <i>et al</i>	•	•		•	•		•
van Geffen H <i>et al</i>	•	•			•		•
Szczerba SR <i>et al</i>		•					•
Giesler DJ <i>et al</i>		•					•
Total =	8	16	1	9	8	2	16

Table 1.11: A table revealing consistency of reporting complication data across all papers. Clavien Dindo first validated in 2004.

Author	All	Wound related	Medical complications	Mortality
Zerbib P <i>et al</i>	NR	8	NR	0
Itani K <i>et al</i> , RICH study group	NR	53	NR	5
Sbitany H <i>et al</i>	NR	22	NR	0
Rosen M <i>et al</i>	NR	61	NR	3
Krpata DM <i>et al</i>	NR	24	NR	1
Connolly P <i>et al</i>	52	23	NR	3
Slater NJ <i>et al</i>	28	18	23	1
Birolini C <i>et al</i>	14	16	NR	0
Nockolds C <i>et al</i>	NR	13	NR	0
Taner T <i>et al</i>	5	5	0	0
Wind J <i>et al</i>	22	9	10	0
Rosen M <i>et al</i>	3	2	1	0
Aladeen D <i>et al</i>	9	7	5	1
van Geffen H <i>et al</i>	17	8	5	1
Szczerba SR <i>et al</i>	NR	2	NR	0
Giesler DJ <i>et al</i>	NR	6	NR	0
Total =		277		15

Table 1.12: A table demonstrating short-term postoperative complication rates. NR = Not reported.

1.5.4 Discussion

This review aimed to evaluate the rates of hernia and fistula recurrence following CAWR. No previous systematic review has focused on outcomes of CAWR. Across all VHWG grades systematic review data reveals a hernia recurrence rate of 15.2% with an average follow-up of 13.6 months (29). A separate review focusing on biological mesh repair found a hernia recurrence rate of 20% over a similar follow-up period (30). The current literature review suggests that in the contaminated setting biological mesh repairs have a similar recurrence rate over an average follow-up of 2 years. No long-term follow-up data for patients undergoing CAWR with suture repair alone exists. There are no controlled trials of suture repair versus mesh repair or trials comparing different meshes therefore these groups are not directly comparable. The heterogeneity inherent in this patient group is demonstrated by the lack of consistent reporting of patient comorbidity and previous laparotomy/hernia repair. The lower rate of hernia recurrence rate observed in the suture repair group is not consistent with existing literature that mesh repair is superior to suture alone. It is difficult to ascertain the reason for this however it is possible the smaller less complex hernias were repaired without a mesh and therefore a lower recurrence rate would be expected. Any suggestion that mesh repair increases the recurrence rate in the contaminated field would not be supported by other data in the literature. It is likely that biological meshes were used in the more complex cases however these details are not reported and therefore it is impossible to directly compare mesh versus suture reconstruction.

Opinion is divided on how best to manage large ventral hernias with coexisting ECF. This review focuses on single-staged repair however some surgeons advocate multi-

staged surgical management consisting of initial surgical closure/treatment of the fistula and wound closure with a bridging mesh or simple skin grafting followed by definitive AWR at a later date (31-34). The advantages of this technique are thought to include a reduction in operative time and a reduction in medical and wound morbidity. Conversely this approach exposes the patient to a second, or even third, operation increasing the hospitalization cost and anaesthetic risk. Additionally there is a risk of recurrent or de novo fistula formation (33, 34).

This study demonstrates the rates of recurrent ECF following single-staged repair with AWR and cohorts of isolated ECF repair are comparable, approximately 10% (35-37). Mortality in this cohort is also found to be low (2.5%) and in keeping with reports of ECF repair in isolation (35, 36). Wound morbidity is expected to be high in a population of patients undergoing surgery in a contaminated or dirty field and a rate of 46.1% is in keeping with data for surgical site infection in dirty wounds (38). It can therefore be concluded that concurrent ECF repair and single-staged AWR is a safe method of dealing with both complex surgical problems in a single operation thus reducing the number of operations, cost of hospital stay and associated morbidity.

Breaking down the data by use of component separation some studies did not give sufficient detail of the recurrent cases to establish what repair technique had been used. When this was possible, numbers in each category were significantly lower and therefore not possible to draw robust conclusions, however these data seem to imply that the use of component separation techniques in CAWR does not affect the rates of hernia or fistula recurrence. The low hernia recurrence rate (0%) for suture repair implies these cases had less complex defects than those requiring component

separation and/or mesh repair and therefore it is not prudent to draw any significant conclusions from this figure.

This study may demonstrate the advantage of biological over synthetic mesh in this field, with lower rates of both hernia and fistula recurrence, however the heterogeneity of the groups makes it difficult to make this comparison. The use of biological mesh in the contaminated field remains contentious. All meshes provoke an inflammatory response when implanted and it is a balance between this reaction and the integration of the mesh that dictates its long-term function (39). The presence of inflammatory cells in the infected wound may provoke a larger than normal response to the mesh resulting in mesh degradation. Some meshes undergo a process of additional cross-linking in order to strengthen the prosthetic against enzymatic degradation. The only additionally crosslinked mesh used in the included studies was Permacol. Early data suggested a high rate of recurrent fistula when using crosslinked meshes (10) and this could explain why so few were used in these studies. Some studies have suggested that the additional crosslinking can modulate the inflammatory response and improve mesh integration, tissue quality over time and reduce recurrence rates when compared to non-crosslinked mesh (39). However, further data comparing different biological mesh found additional crosslinked meshes resulted in higher rates of infection and explanting (40). The recently published guidelines from the European Society of Coloproctology (41) on surgery in intestinal failure patients recommend the use of non-crosslinked mesh in AWR in patients with concurrent ECF repair. These data suggest non-crosslinked mesh is comparatively safe to use in the presence of ECF however hernia recurrence is still high.

The drawbacks of this review are clear and are a recurring problem with data in this field. There are no prospective controlled trials in the repair of ECF and AWR. No comparative studies have thus far been conducted and the bulk of data presented here comes from small retrospective cohorts. This is a reflection of the paucity of cases in this specialist field and that patients have unique considerations that make designing robust trials impossible. More concerning than this is the lack of consistent data reporting. Assessment of quality of the studies included in the analysis revealed there were no ideal studies however scores were consistent across the studies. Researchers should aim to maximize the effectiveness of the data available by keeping prospective databases, performing collaborative studies and reporting key outcomes measures. These should include patient demographic data of factors that are known to affect hernia recurrence and wound infection rates, such as smoking status and BMI. Likewise few studies used a standardized measure for reporting complications. One study used the CDC wound infection scale, however other definitions and methods of assessing wound complications exist. The validated Clavien and Dindo classification of surgical complications was only used in two studies. These data highlight the lack of consensus amongst surgical researchers as to how to report and grade complications that contributes to the difficulty in comparing studies.

This study demonstrates the feasibility of single-staged ECF repair and complex AWR. Fistula recurrence rates are comparable with reported series of fistula repair alone. Non-crosslinked biological mesh would appear to be safer to use than crosslinked mesh with regards to fistula recurrence.

Hernia recurrence rates remain high in the contaminated setting even when biological

mesh is used to reinforce the repair. While it is not possible to directly compare suture and mesh repair in these studies, due to significant heterogeneity between the groups, this analysis would suggest a controlled trial of suture versus mesh repair in this group is warranted. There should be standardisation of reporting of patient demographics, wound and patient complications in future studies in this field.

1.6 Gaps in the literature and aims of thesis

Null hypothesis – Contaminated abdominal wall defects are a rare problem, of uncertain origin and evaluating patients with these defects and exploring novel treatment pathways cannot improve patient outcome or experience.

This thesis aims to comprehensively evaluate and improve outcomes in complex contaminated abdominal wall reconstruction. As a surgical problem CAWR is niche and currently not quantified. Factors leading to the development of contaminated abdominal wall defects have never been studied and from our overview of the literature it would seem that the presence of contamination is a key factor in outcome from repair of these defects by comparison to clean cases, even in the presence of patient co-morbidity. The first aim of this thesis is therefore to quantify the problem and determine risk factors for its development.

Aim 1 – Determine the prevalence of contaminated abdominal wall defects and identify risk factors for the development of contaminated defects.

Minimal data exists on outcomes from CAWR and cohorts currently tend to be published along with clean cases. Cohorts reporting exclusively on CAWR are limited to small numbers and no analysis of factors associated with poor outcome has been undertaken. We hypothesise that novel factors, other than wound contamination, and including the use of CT-scanning can be identified and used to aid in pre-operative decision making, pre-operative patient counseling, choice of repair technique and risk stratification.

Aim 2 – Evaluate factors influencing patient outcome to better determine the risk of a patient undergoing repair of a contaminated abdominal wall defect, including comparison of repair techniques, risk stratification use pre-operative factors and evaluation of CT-scanning in the assessment of patients.

The question of whether we have found the ideal mesh for use in CAWR remains unanswered. Synthetic meshes lead to high recurrence rates and have the risk of infection and fistulisation. Biologic meshes remain expensive and the question of whether they reduce long-term morbidity remains unanswered. We hypothesize there is scope for exploring novel mesh strategies for use in CAWR.

Aim 3 – Develop and evaluate *in vivo* biocompatibility of a novel biological mesh.

Chapter 2 – Prevalence and risk factors for the development of contaminated abdominal wall defects

In this chapter we will discuss who develops contaminated abdominal wall defects, how big a problem they are and evaluate the modified VHWG grading system as a method of stratifying patients undergoing ventral hernia surgery.

2.1 Incisional/ventral hernia surgery in England: a population study of prevalence and prognosis using Hospital Episode Statistics

2.1.1 Introduction

Hernia surgery encompasses a wide range of the general surgical workload. The incidence of incisional hernia following laparotomy is thought to be approximately 10% (6–10) and 10% of these hernias recur and will require further surgical repair (11). Population level studies of incisional hernia surgery are rare. This is likely as a result of the large variation the scope and scale of hernia surgery.

Defining the complexity of incisional/ventral hernia surgery has proved difficult with multiple factors thought to play a role in complicating a hernia operation, such as patient comorbidity, the presence of contamination or the defect size. A number of attempts have been made to classify ventral hernia defect in order to define complexity and aid comparison between studies (72,98,99). Although many of these were described a number of years ago, none have been taken up across the surgical community or successfully validated across all cases.

In 2010, the Ventral Hernia Working Group defined a simple grading system for ventral hernia surgery with the aim of grouping patients by increasing risk (62). They graded defects from 1, clean defects in healthy patients, to 4, contaminated defects. The overall validity of this system has never been proven.

A single centre study by Kanters *et al*, aimed to validate the system for Surgical Site Occurrence (SSO). They found the risk of a SSO was better predicted by combining

the contaminated Grade 3 and 4 into a single group and stratifying these patients according to the Centre for Disease Control wound classification (65). No study has demonstrated the validity of the VHWG grades for long-term outcomes, such as, hernia recurrence, however, systematic review data has suggested that hernia recurrence is more likely in contaminated cases (100) (*Chapter 1.5*).

No study has attempted to evaluate the population of patients undergoing ventral hernia surgery to establish risk factors for the development of contaminated defects as opposed to clean defects.

This study aims to assess the scale of ventral/incisional hernia surgery in England using the Hospital Episode Statistics database. Data for all operations occurring prior to the ventral hernia repair episode, including risk factors, will be evaluated for factors associated with more complex (contaminated) hernia development. These data will then be used to evaluate the ability of the VHWG grading system to predict long-term outcomes.

2.1.2 Methods

Defining the dataset

The Hospital Episodes Statistics (HES) database was setup in 1987 aiming to capture every hospital episode, out-patient appointment, emergency attendance and elective admission, across England for all patients. All operations carried out during the admission are coded using OPCS codes. Other diagnoses and comorbidities relevant to the admission are identified using the ICD-10 classification system.

The HES database from 1st April 1997 to 31st March 2012 was evaluated. Ethical approval for use of the HES dataset was given by the London board (HRA NRES 2013: REC reference 13/LO/1235). All patients who underwent an operation for incisional and ventral hernia were identified using the OPCS codes in Table 2.1. All hospital admissions for these patients were then identified and isolated from the full HES database using the patients' unique HES identification code. Cases were excluded if they were under the age of 18 at the time of their index hernia operation and if there were missing data within the index hernia admission episode.

Hernia operations were retrospectively graded using the modified-VHWG classification system (65). Grade 3 cases were identified using OPCS code for operations that involve violation of the GI tract at the same operation as the hernia repair (Table 2.1). These cases were then removed from the dataset resulting in the presence of only 'clean' cases. These were then divided into Grade 2 by identifying relevant hernia comorbidity defined by the grading system (diabetes mellitus, chronic obstructive pulmonary disease (COPD), immunosuppression) using ICD-10 codes both at the time of hernia surgery and listed at a previous admission for the same case.

The remaining cases, without comorbidity or concomitant GI tract violation, were classified as Grade 1.

Further relevant comorbidity coded for at the index hernia operation or admissions prior to the hernia repair, such as ischaemic heart disease (IHD), inflammatory bowel disease and Ehlers-Danlos Syndrome, were also highlighted using ICD-10 codes (Table 2.1). Other demographics captured by HES, including Index of Multiple Deprivation (IMD), emergency admission and planned daycase admission, were identified.

Hernia Procedure OPCS Code	Comorbidity ICD-10 Code	Concomitant GI tract violation OPCS Code
T25 – Primary repair of incisional hernia	E10-14 – Diabetes Mellitus	G01-11, 26-29, 30-36, 40-41, 49, 50-53, 60-61, 63, 68-69, 70-79 – Small bowel violation
T26 – Repair of recurrent incisional hernia	J44 – Chronic Obstructive Pulmonary Disease	H01-19, 29, 32-33 – Colon violation
T27 - Primary repair of ventral hernia	D80-84 – Immunodeficiency	J19, 27.1-3, 29.1-2, 30.1-3, 34-36, 54.1, 56.1-3, 56.8-9 – Other GI tract violation
T28 – Abdominal wall reconstruction	Y434 – Immunosuppressive medication	
Y71.2 – Modifier for recurrent hernia	K50-51 – Inflammatory Bowel Disease	
	I25 – Chronic Ischaemic Heart Disease	
	I50 – Heart Failure	
	Q796 – Hypermobility/Ehlers Danlos Syndrome	

Table 2.1: OPCS codes used to identify incisional/ventral hernia surgery in the HES database and ICD-10 and OPCS codes used to group patients according to the VHWG classification.

Previous operations...how did we get here?

In order to evaluate risk factors for developing contaminated defects all hospital admission prior to the index hernia operation were identified for each patient. Within these admissions, all abdominal operations were identified using the OPCS codes described in Table 2.2, including patients who had multiple abdominal procedures in the same admission. Data were identified for the total number of abdominal operations prior to the index hernia operation, as well as the time from the last operation to the index hernia operation and whether the last operation was performed as an emergency. Patients with prior operative data were then compared with the index hernia operation data for these patients only. This is due to the fact that comparing them to patients who had not had previous surgery or who potentially had surgery prior to the first HES date may lead to bias and would not be a valid comparison.

OPCS codes for abdominal surgery/incision
Small bowel surgery – G01-11, 23-29, 30-36, 40-41, 49, 50-53, 60-61, 63, 68-69, 70-79
Colon surgery – H01-19, 29, 32-33
Hepatobiliary surgery –J01-36, 50-59, 60, 62-65, 69-72
Gynaecological surgery – R17-18, 252; Q07, 09.2, 2-4, 50-54
Vascular surgery – L18.3-9, 19.3-9, 41, 42, 45, 46, 48-54
Urology – M35-37, 39, 41, 61, 62, 641
Miscellaneous – Y502, Y751, Y752

Table 2.2: OPCS procedure codes for abdominal surgery/incision.

Looking forward...outcomes from hernia surgery and validation of the VHWG classification

Outcomes from the index hernia operation identified from the HES database, including post-operative length of stay. Future hospital admissions were evaluated for further hernia operations, identified using the same OPCS codes. Time to further hernia surgery and number of further hernia operations were evaluated. The HES dataset was also combined with Office of National Statistics (ONS) data to define dates of death where relevant. In this way, 30 and 90-day post-operative mortality were identified. Patients were censored either by the earliest applicable date; date of death (if relevant); date of first further hernia surgery; or the last HES date (31st March 2012).

Statistical analysis

Statistical analysis was performed using SPSS (V20, IBM, New York, USA). Categorical demographic and outcome data are presented as numbers and percentages. Continuous data are expressed as a mean and standard deviation, if parametric, and a median, inter-quartile range and range, if non-parametric. Point prevalence was determined by calculating rates per 100,000 of the population based on the total population of England (ONS) for all hernia surgery and per 100 hernia operations for repairs in the presence of contamination.

Risk factors for the development of contaminated defects were identified using binary logistic regression. Uni-variate analysis revealed variables, considered to be significant if p-value was less than 0.01, that were carried forward to multi-variate analysis. Results are presented as odds ratios with a 95% confidence interval.

Evaluation of the modified-VHWG grading system, as a stratification tool for hernia recurrence, was performed using cox regression analysis of time to censor. Variables were assessed for significance using uni-variate cox regression. Proportional hazards were assessed using log-log and Schoenfeld plots and those variables that did not meet the assumptions were re-evaluated with time dependent co-variables. Factors found to be significant on uni-variate analysis ($p \leq 0.01$) were placed into a multi-variate cox regression model with backward-stepwise analysis based on likelihood ratio to determine the optimal factors for inclusion in the final grading system. Factors deemed significant for inclusion in the final model (inclusion $p=0.05$; exclusion $p=0.10$) were collated into a modified-grading system based on the original VHWG grade. Results are presented as unadjusted and adjusted hazard ratios. A survival curve, demonstrating time to further hernia surgery, for the final model for further hernia surgery was evaluated using receiver-operating characteristics.

2.1.3 Results

Baseline demographics at index hernia operation

The database search identified 221,537 hernia operations with 2,816,939 hospital admissions over the time period. Cases with missing data were identified and excluded resulting in 214,082 cases included in the final analysis (7,455 excluded (3.37%)).

The majority of included patients were female (113,539, 52.6%) with a mean age of 56.59 (SD 15.9) and the majority of operations were performed in the 60-69 age category (21.9%). The hernia operation was planned as a daycase procedure in 21.9% (46,798 patients) and performed as an emergency in 16.1% (34,456 patients) (Table 2.3).

When the cohort was divided and classified according to the modified-VHWG grades, 80.2% (171,783) were Grade 1, 9.97% (21,436) were Grade 2 and 9.8% (20,953) were Grade 3. Hernia comorbidity was present overall in 11.9% (24,481 patients) (Table 2.3).

When evaluating the number of hernia operations performed in each complete year for the HES database (1998-2011) the point prevalence has increase from 22.7/100,000 in 1998 to 34.3/100,000 in 2011. Of these, the point prevalence of contaminated repairs has also increase over time, from 8.9/100 in 1998 to 12.4/100 in 2011 (Figure 2.1).

	Modified VHWG Grade N (%)			Whole Cohort N (%)
	1	2	3	Total
Sex (Male)	81,271 (47.3)	9,905 (46.4)	10,257 (49.0)	101,433 (47.4)
Age (Mean +/- SD)	54.63 (15.8)	64.05 (12.2)	64.98 (14.7)	56.59 (15.9)
• 18-29	9,759 (5.7)	120 (0.6)	402 (1.9)	10,281 (4.8)
• 30-39	23,731 (13.8)	592 (2.8)	957 (4.6)	25,280 (11.8)
• 40-49	33,729 (19.6)	2,095 (9.8)	4,982 (9.5)	37,806 (17.7)
• 50-59	35,122 (20.4)	4,063 (19.0)	3,226 (15.4)	42,411 (19.8)
• 60-69	34,786 (20.2)	6,791 (31.8)	5,221 (24.9)	46,798 (21.9)
• 70-79	26,000 (15.1)	5,865 (27.5)	5,945 (28.4)	37,810 (17.7)
• >80	8,656 (5.0)	1,820 (8.5)	3,220 (15.4)	13,696 (6.4)
Hernia Comorbidity	N/A	21,346 (100)	4,102 (19.6)	25,448 (11.9)
• Diabetes	N/A	14,779 (69.2)	2,604 (12.4)	17,383 (8.1)
• COPD	N/A	7,676 (36.0)	1,747 (8.3)	9,423 (4.4)
• Immuno- deficiency	N/A	88 (0.4)	18 (0.0)	106 (0.0)
IHD	5,553 (3.2)	2,658 (12.5)	1,741 (8.3)	9,952 (4.6)
IBD	1,674 (1.0)	247 (1.2)	1,484 (7.1)	3,405 (1.6)
• Crohn's	1,152 (0.7)	137 (0.6)	870 (4.2)	2,159 (1.0)
• UC	522 (0.3)	110 (0.5)	614 (2.9)	1,246 (0.6)
Ehlers-Danlos	20 (0.0)	1 (0.0)	3 (0.0)	24 (0.0)
Contaminated hernia repair	N/A	N/A	20,953 (100)	20,953 (9.8)
Emergency operation	21,305 (12.4)	4,337 (20.3)	8,814 (42.1)	34,456 (16.1)
Planned as day case	53,695 (31.3)	3,053 (14.3)	463 (2.2)	57,211 (26.7)

Table 2.3: Baseline demographics of the whole cohort split by modified VHWG grade.

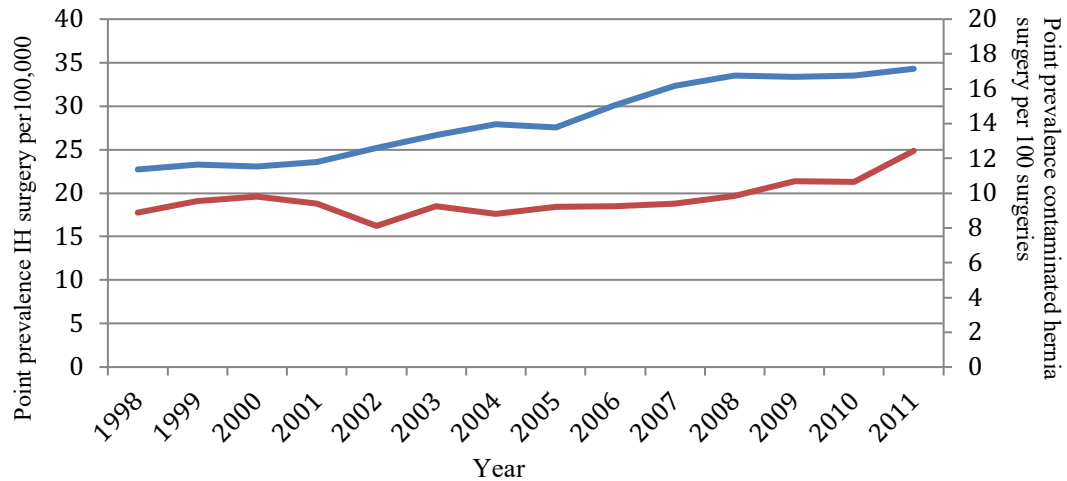


Figure 2.1: Point prevalence of incisional hernia (IH) surgery (blue) per 100,000 of the population and of contaminated hernia surgery per 100 hernia surgeries (red).

Risk factors for the development of contaminated hernia defects

Of the whole cohort, 37.4% (80,765 patients) had open abdominal surgery prior to their index hernia operation. Of these, 39.5% (31,706 patients) were male and the mean age was 54.77 (SD 15.6). Again the majority of operations took place in the 6th decade of life (23.4%, 60-69). The median number of operations prior to the index hernia operation was 1 (IQR 1-2; range 1-27). The median time from last operation to index hernia operation was 20 months (IQR 11-40; range 0-148) (Table 2.4).

	Total N = 80,347 (%)
Male	31,706 (39.5)
Age (Mean +/- SD)	54.77 (15.6)
• <18	207 (0.3)
• 18-29	4,488 (5.6)
• 30-39	11,798 (14.6)
• 40-49	13,503 (16.7)
• 50-59	15,857 (19.6)
• 60-69	18,861 (23.4)
• 70-79	13,361 (16.5)
• >80	2,735 (3.4)
Hernia Comorbidity	11,755 (14.6)
• Diabetes	8,063 (10.0)
• COPD	4,295 (5.3)
• Immunodeficiency	58 (0.1)
IHD	6,064 (7.5)
• IBD	4,441 (5.5)
• Crohn's	2,332 (2.9)
• UC	2,109 (2.6)
Ehlers-Danlos	12 (0.0)
Emergency operation	21,608 (26.9)
No previous operations (Median; IQR; Range)	1 (1-2; 1-20)
No previous operations	78,483 (97.7)
• 1-3	1,750 (2.2)
• 4-6	114 (0.1)
• >6	
Multiple previous op	23,385 (29.1)
Time from last operation to hernia repair (Months) (Median; IQR; Range)	20 (11-40; 0-178)

Table 2.4: Demographics of patients who had an abdominal operation prior to the index hernia operation.

Binary logistic regression revealed multiple factors were associated with the development of a contaminated hernia defect. Increasing age (OR 1.03; 95% CI 1.029-1.032; $p < 0.0001$) and male sex (OR 1.27; 95% CI 1.22-1.32; $p < 0.0001$) were both associated with future hernia surgery in the presence of contamination.

Pre-existing hernia comorbidity, such as diabetes (OR 1.23; 95% CI 1.15-1.31; $p<0.0001$) and COPD (OR 1.29; 95% CI 1.19-1.39; $p<0.0001$), as well as increasing number of previous operations and last operation performed as an emergency (OR 1.72; 95% CI 1.64-1.80; $p<0.0001$) were all associated with future hernia surgery in the presence of contamination. Most significantly, inflammatory bowel disease present at the prior operation (Crohn's Disease (OR 3.87; 95% CI 3.51-4.26; $p<0.0001$) and UC (OR 4.13; 95% CI 3.75-4.54; $p<0.0001$) was associated with the highest risk of developing a contaminated defect (Table 2.5).

	Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95% C.I.)	p-value	Odds ratio (95% C.I.)	p-value
Age	1.03 (1.028-1.031)	<0.0001	1.03 (1.029-1.032)	<0.0001
Sex (Male)	1.66 (1.59-1.73)	<0.0001	1.27 (1.22-1.32)	<0.0001
Diabetes	1.40 (1.32-1.49)	<0.0001	1.23 (1.15-1.31)	<0.0001
COPD	1.78 (1.65-1.93)	<0.0001	1.29 (1.19-1.39)	<0.0001
Immunodeficiency	0.90 (0.41-1.97)	0.78		
IHD	1.67 (1.56-1.78)	<0.0001	1.05 (0.98-1.13)	0.16
IBD				
• Crohn's	3.00 (2.74-3.29)	<0.0001	3.90 (3.52-4.29)	<0.0001
• UC	3.94 (3.59-4.31)	<0.0001	4.14 (3.76-4.56)	<0.0001
Hypermobility	1.45 (0.31-6.71)	0.64		
Emergency Surgery	1.88 (1.80-1.96)	<0.0001	1.72 (1.64-1.80)	<0.0001
Previous operations				
• 1-3	1			
• 4-6	1.34 (1.18-1.53)	<0.0001	1.50 (1.32-1.72)	<0.0001
• >6	2.04 (1.33-3.15)	0.001	2.25 (1.43-3.54)	<0.0001

Table 2.5: Binary logistic regression of risk factors for future hernia surgery in the presence of contamination.

Hernia surgery outcomes

Median length-of-stay post-index hernia operation was 2 days (IQR 1-6; range 0-792). Thirty-day post-operative mortality was 1.4% (2,941 patients) and 90-day mortality was 2.1% (4,550 patients) for all cases. The short-term mortality rates

increased across the modified-VHWG grades. Thirty-day mortality was 0.6% in Grade 1, 1.8% in Grade 2 and 7.3% in Grade 3. The same pattern was seen at 90-day mortality; 1.0% in Grade 1; 2.9% in Grade 2; and 10.6% in Grade 3 (Table 2.6).

	VHWG Grade			Total (N, %)
	1 (N, %)	2 (N, %)	3 (N, %)	
LOS (days) (Median; IQR; Range)	2 (0-4; 0-564)	3 (1-7; 0-378)	11 (6-20; 0-792)	2 (1-6; 0-792)
30-day mortality	1,012 (0.6)	391 (1.8)	1,538 (7.3)	2,941 (1.4)
90-day mortality	1,703 (1.0)	625 (2.9)	2,222 (10.6)	4,550 (2.1)
Recurrent hernia surgery	13,889 (8.1)	1,566 (7.3)	2,259 (10.7)	17,714 (8.3)
Multiple hernia recurrences	2,412 (17.37)	260 (16.6)	468 (20.7)	3,140 (17.7)

Table 2.6: Outcomes from index hernia operation. Multiple hernia recurrences are presented as a proportion of the total hernia recurrences.

An admission for further hernia surgery was found in 8.3% of the cases (17,714 patients). The highest numbers of further hernia operations were seen in Grade 3 cases (10.7%, 2,259 patients). The median number of further hernia operations was 1, with a range of 0-11. Of those who had a further hernia operation approximately 80% did not need a second further hernia surgery. In Grade 1, 17.4% of patients needed multiple further operations, 16.6% in Grade 2 and 20.7% in Grade 3 (Table 2.6).

Evaluation of the modified-VHWG classification for stratification of patients by likelihood of further hernia surgery

The modified-VHWG grading system was evaluated as a stratification tool using cox regression. Factors identified within HES thought to be associated with an increased risk of recurrence were evaluated with uni-variate analysis. Only the presence of

hypermobility pre-operatively was found to be not significant (HR 2.53; 95% CI 0.95-6.74; $p=0.06$). Proportion hazards assumptions were violated by sex, hernia comorbidity, IHD pre-operatively, IBD pre-operatively, contaminated hernia repair and emergency hernia repair. No violation was found with patient age.

Uni-variate cox regression was then repeated with time-dependent co-variables for the relevant factors. Patient sex and emergency hernia surgery were then found to not be significantly associated with further hernia surgery ($p=0.14$ and $p=0.29$ respectively). The remaining factors were entered into the multi-variate model to determine the variables with the most influence on the likelihood of further hernia surgery (Table 2.7).

	Unadjusted analysis		Adjusted multi-variate analysis	
	Hazard ratio (95% C.I.)	p-value	Hazard ratio (95% C.I.)	p-value
Age	1.002 (1.001-1.003)	<0.0001	0.997 (0.994-1.001)	0.45
Sex (Male)	1.03 (0.99-1.08)	0.14		
Hernia Comorbidity	1.09 (1.02-1.17)	0.01	1.05 (1.00-1.10)	0.05
IHD	1.17 (1.06-1.30)	0.003	1.05 (0.98-1.10)	0.18
IBD	1.76 (1.55-2.01)	<0.0001	1.52 (1.38-1.67)	<0.0001
Hypermobility	2.53 (0.95-6.74)	0.06		
Contamination	1.78 (1.67-1.89)	<0.0001	1.69 (1.62-1.77)	<0.0001
Emergency Surgery	1.03 (0.97-1.09)	0.29		

Table 2.7: Unadjusted and adjusted proportionate hazard cox regression model evaluating factors linked to further hernia surgery.

The multi-variate model retained contaminated hernia repair, IBD at the time of hernia surgery and hernia comorbidity as significant factors for the risk of further hernia surgery (Table 2.7). Given IBD at the time of further hernia surgery was found to be a significant factor in the likelihood of further hernia surgery, it was included in

the modified-VHWG classification as a comorbidity that defines a Grade 2 patient. Survival analysis including this additional modification to the VHWG grading system revealed a significant difference between the three grades in the prediction of further hernia surgery (Table 2.8). Compared to Grade 1, the hazard ratio (HR) of further hernia surgery increased in Grade 2 (HR 1.19; 95% CI 1.13-1.25; $p<0.0001$) and further increased in Grade 3 (HR 1.79; 95% CI 1.72-1.88; $p<0.0001$).

St Mark's Modified VHWG Grade	Patient number (N, %)	Further hernia surgery (N, %)	Hazard ratio (95% CI)	p-value
1	170,109 (79.5)	13,661 (8.0)	1	
2	23,020 (10.8)	1,794 (7.8)	1.19 (1.13-1.25)	<0.0001
3	20,953 (9.8)	2259 (10.8)	1.79 (1.72-1.88)	<0.0001

Table 2.8: Cox regression analysis of the St Mark's modified VHWG grading system for its ability to discriminate between patients risk of further hernia surgery.

Survival curves for each Grade demonstrated a likelihood of further hernia surgery of 11% in Grade 1, 14% in Grade 2 and 20% in Grade 3 at 15-year follow-up (Figure 2.2). Interestingly, the majority of further hernia surgery took place in the first 3-years from index operation (6% in Grade 1, 8% in Grade 2 and 12% in Grade 3). Analysis with ROC revealed the area under the curve to be 0.73 (95% CI 0.73-0.74) demonstrating a moderate ability to discriminate between patients (Figure 2.3).

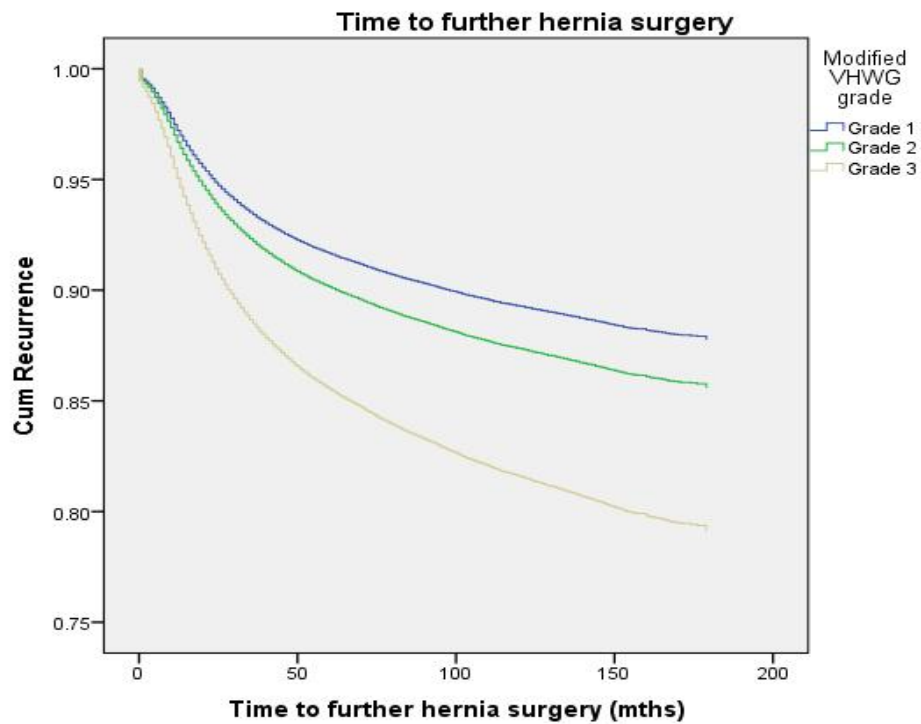


Figure 2.2: Survival curve demonstrating the ability of the St Mark's modified VHWG grading system to discriminate between the risk for further hernia surgery.

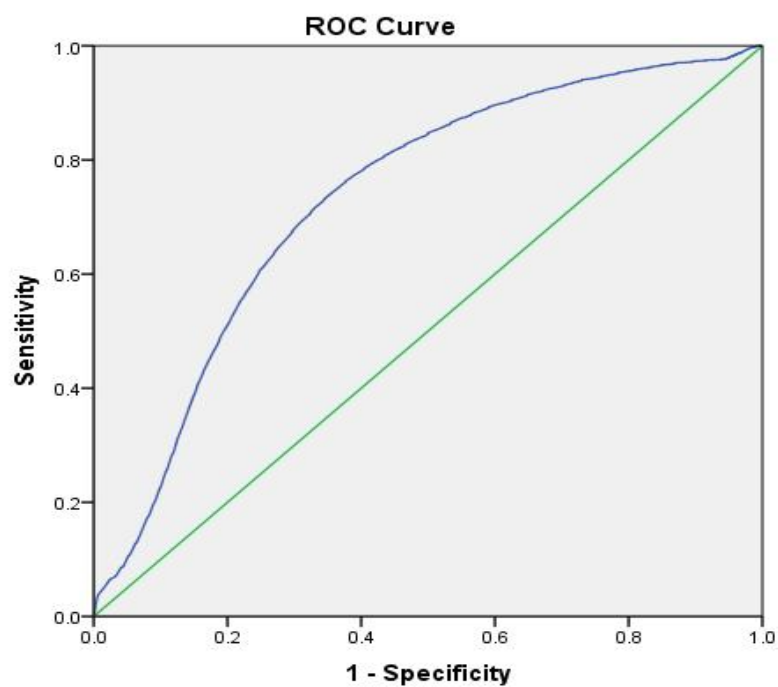


Figure 2.3: Receiver operating characteristic curve assessing the validity of the St Mark's modified VHWG grading system (AUC 0.73; 95% CI 0.728-0.736).

2.1.4 Discussion

This study demonstrates the point prevalence of ventral/incisional hernia surgery has increased over time, as has the point prevalence of contaminated cases. The incidence of further hernia surgery is approximately 10%, which is consistent with previously published rates (58,101). Of those patients who had further hernia surgery, 20% went on to have multiple further hernia operations (102). This is double the rate seen in previously published data and suggests hernia recurrence following previous recurrence surgery is a bigger problem than previously realised. It is the first study to demonstrate the modified-VHWG classification system is a valid tool to stratify patients by risk of their likelihood of requiring further hernia surgery and clearly shows contamination at the time of hernia surgery as the largest discriminating factor.

Contaminated defects make up approximately 10% of the overall burden of ventral hernia surgery. When looking at patients who go on to develop the need for contaminated hernia surgery multiple factor are implicated, including underlying diabetes, COPD and inflammatory bowel disease, as well as an increasing number of previous operations and the last surgery being performed as an emergency. Most of these have been implicated as risk factors in previous studies however the association of inflammatory bowel disease is novel (6). This would be consistent with the increasing need for abdominal surgery over time in this cohort of patients and the likelihood of concomitant bowel surgery in inflammatory bowel disease.

Overall, these data suggest that with increasing complexity of patient, comorbidity and higher number of previous operations, the risk of future contaminated hernia surgery increases. This section of the analysis is limited by the fact this HES database

only has a maximum retrospective view of a patients previous admissions up to 14 years. There is no record of admissions or operations prior to April 1997 and this could be significant for some patients. However it is reassuring to note the median time from prior operation to index hernia operation is less than 2 years (20 months). This implies that the majority of people who have had surgery prior to the index hernia operation will have been captured by this dataset.

When evaluating the risk of further hernia surgery some risk factors implicated in further hernia surgery are consistent with those previously described in the literature including grouped hernia comorbidity (diabetes, COPD and immunosuppression) and contaminated hernia surgery. Inflammatory bowel disease is shown here as a novel risk factor for further hernia surgery ($p < 0.0001$). This could be consistent with the likelihood that patients with inflammatory bowel disease are more likely to undergo more abdominal procedures (103–105) however, further analysis of IBD as a factor in the recurrence of incisional hernia would be interesting.

While the modified-VHWG classification has been shown to adequately risk stratify patients on the basis of wound outcome (65), no study to date has demonstrated its use in the stratification of long-term hernia recurrence outcomes. These data suggest the addition of inflammatory bowel disease as a relevant hernia comorbidity related to recurrence would improve the accuracy of the model as a tool for stratifying patients on the basis of likelihood of long-term further hernia surgery with moderate accuracy based on ROC analysis.

The principle limitation of this analysis is the use of further hernia surgery as an outcome measure for recurrence. As discussed by Muysoms *et al* (99), the hierarchy of quality of follow-up in hernia surgery places further surgery as the lowest quality of evidence based on the likelihood of underestimating recurrence rates. This would be consistent with the presented data as reported rates of incisional hernia recurrence are estimated to be approximately 10% (range 5-45%) (58,101,106) where as our observed rate of further surgery is only 8.3%. While this is likely an under estimate of the overall rate of recurrence, collecting prospective data on this scale with diagnosis of hernia recurrence based on imaging finding (as per Muysoms *et al*), is unrealistic. It can be argued therefore that this analysis is likely to be as accurate in its validation given the cohort size.

The other limitations of this study are consistent with any large retrospective administrative database analysis. There is a possibility of coding error, particularly in relation to the accuracy of hernia operation OPCS codes. The index hernia operation was taken to be the first operation within the dataset for an incisional or ventral hernia. It was shown that although some of these were coded as primary ventral hernias the patient had undergone (sometimes multiple) previous abdominal operations. Given that the incidence of incisional hernia is 10% and the rate of recurrent hernia is around 20% following repair the chance of there being a large enough number of patients to affect the analysis in a cohort of this size is low. As well as this there is no way of distinguishing between midline hernias and lateral abdominal wall hernias in the OPCS codes and different incisions have been shown to have different rates of incisional and recurrence hernia (11). Again the number of

patients affected and the differences in overall incisional and hernia recurrence rates are likely to be small and therefore unlikely to affect the final results.

Finally there are a number of co-factors implicated in the development of incisional hernia and the rate of hernia recurrence that are not coded for within HES. While size of defect is thought to be important the largest two missing factors are smoking status and body mass index (BMI). These two factors would increase the number of patients classified as Grade 2 cases and probably explains the limited size of this cohort as a proportion of the overall case mix by comparison to previous studies (59,65). Given that the difference in long-term further hernia surgery rates between Grade 1 and 2 is significantly different and it is likely the inclusion of smoking and BMI would only widen this gap, it is relatively safe to assume the validation of the modified-VHWG classification is accurate. The question of whether the inclusion of these factors could aid in further sub-classifying the contaminated Grade 3 patients remains to be answered.

2.2 Incisional hernia and enterocutaneous fistula in patients with chronic intestinal failure: prevalence and risk factors in a cohort of patients referred to a tertiary centre.

This section has been published in *Colorectal Disease* and has been reproduced with permission (*Appendix 8*). Hodgkinson JD, Oke SM, Warusavitarne J, Hanna GB, Gabe SM, Vaizey CJ. *Colorectal Dis.* 2019 Nov;21(11):1288-1295. doi: 10.1111/codi.14735. Epub 2019 Jul 15.

2.2.1 Introduction

Intestinal failure (IF) is defined as “the reduction of gut function below the minimum necessary for the absorption of macronutrients and/or water and electrolytes, such that intravenous supplementation is required to maintain health and/or growth” (107) (*Introduction 1.3.1*).

A number of these patients will go on to have further surgery to restore intestinal continuity or maximise available bowel length for nutritional purposes. The complexity of the surgery in these patients can be due to underlying enterocutaneous fistula or the presence of large incisional hernias. All patients undergoing surgery in this cohort with abdominal wall defects are classified as Grade 3 patients (65) by virtue of concomitant bowel surgery, the presence of stoma or enterocutaneous fistula. The scale of this problem has not been defined previously.

While certain underlying disease states, such as Crohn’s disease, may drive the development of IF, many patients develop the condition as a result of post-operative complications or ECF formation both of which may be preventable (80). Although

overall incidence of ECF following abdominal surgery is poorly studied following emergency laparotomy for trauma, it is reported to be around 1-2% (108,109). Following treatment with an open abdomen the incidence is recognised to be higher, around 20% (110). Other factors such as, failure to recognise iatrogenic bowel injury, multiple reoperations within 3 to 6 months of index surgery and anastomotic failure have been associated with ECF formation (110–112).

The incidence of incisional hernia is globally recognised to be around 10% following laparotomy (6–10). Multiple factors have been implicated in the development of incisional hernia post-laparotomy. These include operative factors such as emergency or unplanned surgery, post-operative surgical site infection and anastomotic leak (113). Patient related factors such as advancing age, obesity, smoking, ischaemic heart disease, chronic obstructive pulmonary disease (COPD), benign prostatic hypertrophy and underlying liver disease have also been associated with the occurrence of incisional hernia (113).

Patients with IF often require complex abdominal surgery necessitating specialist surgical management. The complexity of surgery maybe further compounded by the presence of either an IH or ECF. This study aims to determine the prevalence of incisional hernia and ECF within the context of IF and identify factors associated with their development within this cohort.

2.2.2 Methods

Patient identification and data collection

The local hospital ethics committee approved the study (LNWH R+D Office, granted 11th January 2017, Ref: SE16/027). The prospectively maintained St Mark's database of patients on home parenteral nutrition was evaluated between the 1st January 2011 and the 31st December 2016. All patients started on home parenteral nutrition during this time were identified and a retrospective case note review was undertaken to extract underlying demographic data, abdominal wall status on referral to our institution and details of previous surgical intervention. Patients were included in the study if they had undergone an abdominal surgical procedure prior to referral to our centre for nutritional assessment. Cases were excluded if the patient had not had a surgical procedure prior to developing IF, or if IF developed prior to the age of 18.

Data were collected by two researchers (JDH and SO). Where disagreement arose or data were not clear, agreement was reached by discussion between the assessors and clinical team or the factor was highlighted as unknown or unclear. Data were collected for abdominal wall status on referral and was evaluated by clinical examination, noting in particular the presence of an incisional hernia or ECF or both. The underlying aetiology of IF, as well as the ESPEN criteria for diagnosis, were identified (107). A surgical complication as the aetiology of IF was defined as a significant abdominal catastrophe leading to re-laparotomy and IF, including formation of high jejunostomy and enterocutaneous fistula. Patient demographic data including age, sex, BMI at referral, IHD, diabetes, inflammatory bowel disease, COPD, smoking status, immunosuppression, use of biologics and hypermobility were collected. Data from previous surgical procedures such as previous emergency

surgery, operations through the same incision, number of previous operation, treatment with an open abdomen (defined as laparostomy for intra-abdominal sepsis or septic dehiscence) and surgical site infection (SSI) at last surgery, intra-abdominal septic complication (IASC) (defined as intra-abdominal collection or anastomotic leak) and anastomotic leak, were separately identified. Data on types of previously used mesh and use of VAC therapy were not well documented in the referral paperwork and were not collected. Given the retrospective nature of these data, patients were categorised as having, or not having an operation or a complication from surgery, only when it was explicitly stated in previous letters or the referral paperwork to our institution. If the history was unclear or unknown this was clearly marked.

Data analysis

Data were stored on an Excel Spreadsheet (Microsoft, Redmond, USA). Statistical analysis was undertaken on SPSS (V22, IBM, New York, USA). Patient demographics, previous operative factors and abdominal wall status on referral are presented as numbers and percentages. Chi-squared test was used to test for significant differences between groups. Risk factors for the occurrence of incisional hernia and enterocutaneous fistulae were identified using uni-variate logistic regression with a p-value of ≤ 0.05 and entered into a backward stepwise multi-variate binary logistic regression model to determine dependent factors. Factors were removed from the model if $p \geq 0.10$ and considered significant if $p \leq 0.05$. Risk factors for the occurrence of incisional hernia alone, ECF alone and both together were evaluated using multinomial logistic regression. Outcomes were reported as odds ratios and a p-value of < 0.05 was taken to be significant.

2.2.3 Results

Patient demographics

Four hundred and forty-seven patients were identified from the database search. Of these, 349 (78.1%) had abdominal surgery prior to the development of IF and were included in the study. Of the included patients, mean age was 55.1 (SD 16.1) with 174 males (49.9%). The mean value of BMI was 23.8 (SD 5.3) at the time of referral. Further patient demographics and underlying co-morbidity are shown in Table 2.9.

In this series, 125 (35.8%) patients had developed IF as a result of a surgical complication, 101 (22.6%) due to underlying inflammatory bowel disease, 73 (16.3%) following resection for mesenteric infarction and the remaining 148 (33.1%) as a result of underlying malignancy, pseudo-obstruction, radiation enteritis, volvulus or other causes (Figure 2.4).

Details of previous surgery, post-operative surgical site infections and anastomotic leak following the last operation are shown in Table 2.10.

<u>Demographics</u>	<u>N (%)</u> n = 349
Mean age (SD, range)	55.1 (16.1, 16-87)
Sex (Male)	174 (49.9)
Mean BMI (SD, range)	23.8 (5.3, 13.9-43.7)
IF Aetiology • Surgical complication • Inflammatory bowel disease • Malignancy • Mesenteric Infarct • Pseudo-obstruction • Radiation enteritis • Volvulus • Congenital • Other	125 (35.8) 90 (25.8) 28 (8.0) 73 (20.9) 3 (0.9) 11 (3.2) 5 (1.4) 1 (0.3) 13 (3.7)
ESPEN Criteria • Short Bowel • Intestinal fistula • Intestinal dysmotility • Mechanical obstruction • Mucosal disease • Unclassifiable	190 (54.4) 68 (19.5) 3 (0.9) 62 (17.8) 24 (6.9) 2 (0.6)
Ischaemic heart disease	56 (16.0)
Diabetes	33 (9.5)
Inflammatory bowel disease • Crohn's Disease • Ulcerative Colitis	81 (23.2) 22 (6.3)
Chronic obstructive pulmonary disease	18 (5.2)
Smoking status • No • Active smoker • Ex-smoker • Unknown	73 (20.9) 54 (15.5) 6 (1.7) 212 (60.7)
Immunosuppression	45 (12.9)
Confirmed diagnosis of hypermobility	3 (0.9)
Anti-TNF alpha therapy pre-operatively	11 (3.2)
Anti-TNF alpha therapy post-operatively	11 (3.2)

Table 2.9: Baseline demographics of intestinal failure patients with prior abdominal surgery.

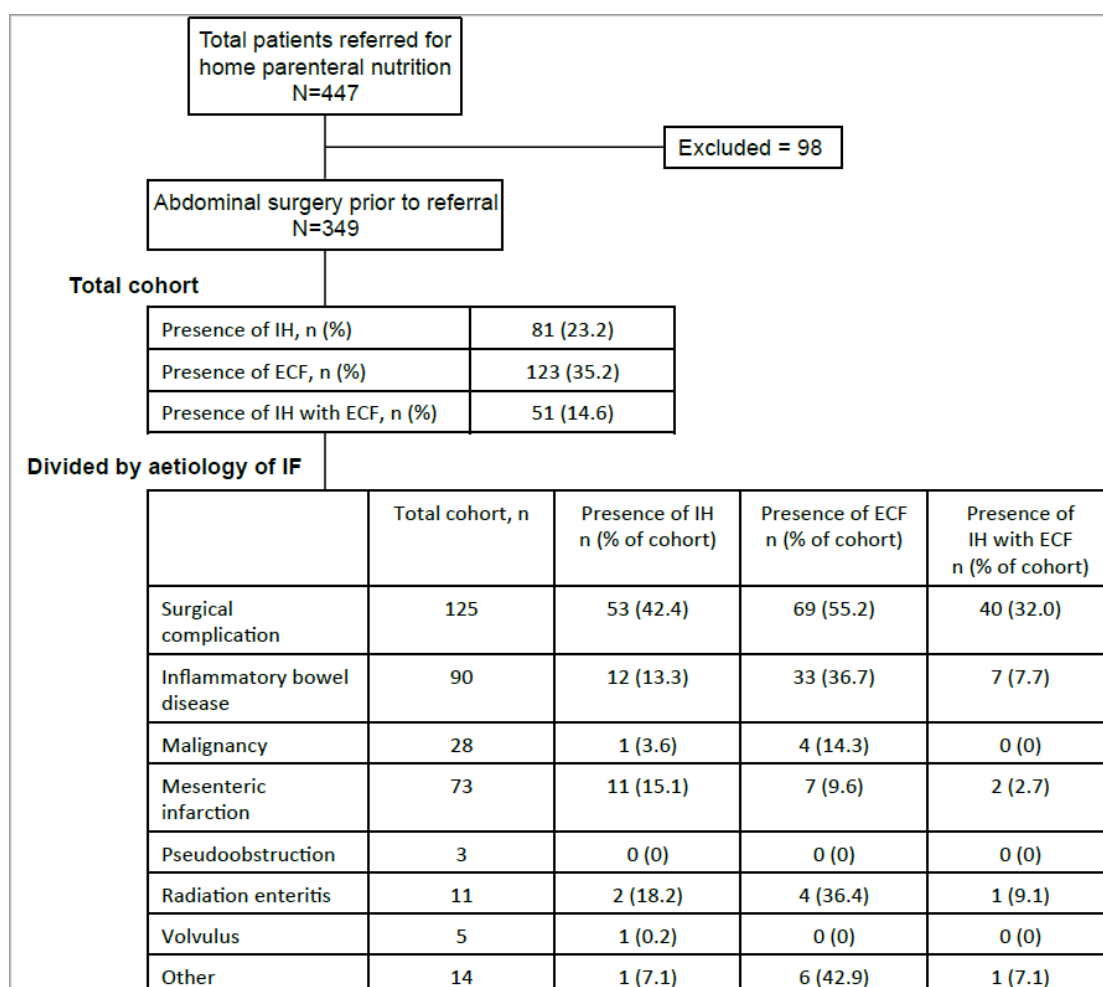


Figure 2.4: Flow chart. The numbers of patients with IH on referral, ECF on referral and IH/ECF combined is demonstrated followed by the numbers categorised by aetiology of IF.

Prevalence of incisional hernia and enterocutaneous fistula

Eighty-one (23.2%) patients had an incisional hernia and 123 (35.2%) had an ECF at the time of referral to our unit. Of these, 51 (14.6%) had both incisional hernia and ECF present (Figure 2.4).

<u>Previous surgery</u>	<u>N (%)</u> n = 349
Last operation performed as emergency	207 (59.3)
Multiple operation within 6 months of last surgery through same incision	127 (36.4)
Multiple operations through same incision but more than 6 months apart	212 (60.7)
Number of previous abdominal operations (median, range)	3 (1-13)
Grouped number of previous operations • 1-3 • 4-6 • Greater than 6 • Unknown	244 (69.9) 75 (21.5) 24 (6.9) 6 (1.7)
<u>Wound status following previous surgery</u>	<u>N (%)</u> n = 349
Treatment with laparostomy or septic dehiscence • Yes • No • Unknown	29 317 3
SSI following last surgery • Yes • No • Unknown	141 (40.4) 139 (39.8) 69 (19.7)
IASCs at last operation • Yes • No • Unknown	131 (37.5) 168 (48.1) 50 (14.3)
Anastomotic leak at last operation • Yes • No • Unknown	67 (19.2) 254 (72.8) 28 (8.0)

Table 2.10: Details of previous surgery and the wound status following last operation.

When reviewing the prevalence of incisional hernia and stratifying the patients by the aetiology of IF, it was found that only the group with a surgical complication leading to IF had a rate (42.4%) higher than expected in the literature (approx. 10%). This was a statistically significant difference when compared to all other aetiology groups ($p < 0.001$). When the patients treated with a long-term open abdomen ($n=32$) were

excluded from this analysis, the prevalence of incisional hernia within the group with a surgical complication resulting in IF (27/99 (27.3% (excluded = 26)), remained significantly higher ($p < 0.001$) when compared to all other aetiology groups combined (22/218 (10.1% (excluded = 6)). The prevalence of ECF was found to be highest in the group with a surgical complication leading to IF (69/125, 55.2%), followed by inflammatory bowel disease (33/90, 36.7%). Patient numbers in other groups were much smaller and therefore difficult to compare (Figure 2.4).

Risk factors for incisional hernia and enterocutaneous fistula

Uni-variate analysis for incisional hernia formation revealed the following significant risk factors; increasing BMI ($p < 0.001$), significant surgical complication resulting in IF ($p < 0.001$), previous emergency surgery ($p < 0.02$), multiple operations within 6 months of IF ($p < 0.001$), increasing number of previous surgeries ($p < 0.001$), underlying IHD ($p < 0.02$) and SSI ($p < 0.001$), IASC ($p < 0.001$) or anastomotic leak at last operation ($p < 0.001$) (Table 2.11). Previous treatment with an open abdomen was excluded from this analysis as all patients who were treated with an open abdomen went on to develop an incisional hernia. Following backward stepwise multi-variate regression only the following factors remained significantly associated with the development of incisional hernias; high BMI ($p < 0.05$), significant surgical complication resulting in IF ($p < 0.01$), emergency surgery as last operation ($p < 0.04$), increasing number of previous operations ($p < 0.02$) and SSI ($p < 0.01$) (Table 2.12).

	<u>Incisional hernia</u>		<u>Enterocutaneous fistula</u>	
<u>Associated factors</u>	<u>Odds Ratio</u>	<u>p-value</u>	<u>Odds Ratio</u>	<u>p-value</u>
Age	1.01 (0.99-1.02)	0.30	1.00 (0.99-1.02)	0.53
Sex (Female)	1.64 (0.99-2.71)	0.06	0.75 (0.48-1.17)	0.20
BMI	<u>1.09 (1.04-1.15)</u>	<u>0.001</u>	1.04 (1.00-1.10)	0.08
IF Aetiology				
• Surgical	<u>5.43 (2.89-10.21)</u>	<u><0.001</u>	<u>6.63 (3.70-11.89)</u>	<u><0.001</u>
• Complications	1.14 (0.51-2.53)	0.76	<u>3.12 (1.65-5.87)</u>	<u><0.001</u>
• IBD	1	NA	1	NA
• Other				
Emergency Surgery at last operation	<u>1.88 (1.10-3.24)</u>	<u>0.02</u>	1.05 (0.67-1.65)	0.82
Multiple operations within last 6 mths	<u>3.75 (2.23-6.32)</u>	<u><0.001</u>	<u>1.85 (1.18-2.91)</u>	<u>0.01</u>
Multiple operations greater than 6 mths	0.91 (0.54-1.51)	0.70	1.39 (0.87-2.20)	0.17
Number of operations prior to IF	<u>1.35 (1.18-1.55)</u>	<u><0.001</u>	<u>1.21 (1.07-1.37)</u>	<u>0.002</u>
Categorical number of operations	1	NA	1	NA
• 1-3 operations	<u>2.00 (1.10-3.63)</u>	<u><0.001</u>	<u>2.05 (1.21-3.48)</u>	<u>0.01</u>
• 4-6 operations	<u>9.62 (3.87-23.93)</u>	<u><0.001</u>	<u>3.90 (1.64-9.33)</u>	<u>0.002</u>
• >6 operations				
Ischaemic heart disease	<u>2.11 (1.14-3.91)</u>	<u>0.02</u>	1.33 (0.74-2.40)	0.34
Diabetes	1.27 (0.57-2.86)	0.56	0.55 (0.24-1.26)	0.16
Inflammatory bowel disease				
• Crohn's disease	0.72 (0.39-1.34)	0.30	<u>1.68 (1.01-2.80)</u>	<u>0.05</u>
• Ulcerative colitis	0.29 (0.07-1.29)	0.11	<u>0.09 (0.01-0.69)</u>	<u>0.02</u>
COPD	1.29 (0.45-3.74)	0.64	<u>3.02 (1.14-8.00)</u>	<u>0.03</u>
Active smoker	2.49 (0.99-6.28)	0.05	1.86 (0.89-3.86)	0.10
Immunosuppression	0.64 (0.29-1.44)	0.28	0.83 (0.43-1.59)	0.57
Hypermobility	6.74 (0.60-75.36)	0.12	3.68 (0.33-41.04)	0.29
Biologics 3 months pre-operatively	0.73 (0.15-3.45)	0.69	1.53 (0.46-5.10)	0.49
Biologics 3 months post-operatively	0.80 (0.21-3.09)	0.75	2.23 (0.67-7.45)	0.19
Treatment with open abdomen at last operation	NA	NA	<u>10.89 (4.03-29.38)</u>	<u><0.001</u>
SSI at last operation	<u>7.04 (3.57-13.91)</u>	<u><0.001</u>	<u>13.95 (7.65-25.44)</u>	<u><0.001</u>
IASC at last operation	<u>2.90 (1.67-5.03)</u>	<u><0.001</u>	<u>12.93 (7.38-22.66)</u>	<u><0.001</u>
Anastomotic leak at last operation	<u>2.59 (1.45-4.62)</u>	<u>0.001</u>	<u>5.23 (2.92-9.36)</u>	<u><0.001</u>

Table 2.11: Uni-variate analysis demonstrating significant factors associated with incisional hernia and enterocutaneous fistula.

Significant risk factors associated with ECF were previous surgical complications resulting in IF ($p<0.001$), inflammatory bowel disease ($p<0.001$), multiple operations within the last 6 months ($p<0.01$), increasing number of operations prior to IF ($p<0.002$), underlying Crohn's disease ($p<0.05$), underlying COPD ($p<0.03$), previous treatment with open abdomen ($p<0.001$) and SSI and anastomotic leak ($p<0.02$) at last operation as significant (Table 2.11). Multi-variate analysis confirmed surgical complication resulting in IF ($p<0.001$), previous treatment with open abdomen ($p<0.03$), SSI ($p<0.001$), intra-abdominal septic complication at last operation ($p<0.001$) and anastomotic leak at last operation ($p<0.02$) as predisposing factors to ECF (Table 2.13).

<u>Incisional hernia</u>	<u>Odds Ratio</u>	<u>p-value</u>
BMI	1.06 (1.00-1.13)	0.05
IF Aetiology		
• Surgical Complications	3.06 (1.33-7.04)	0.01
• Inflammatory bowel disease	0.81 (0.30-2.23)	0.69
• Other	1	NA
Emergency Surgery at last operation	2.26 (1.04-4.92)	0.04
Number of operations prior to IF	1.25 (1.03-1.50)	0.02
SSI at last operation	5.78 (2.41-13.89)	<0.001

Table 2.12: Multi-variate analysis demonstrating significant factors associated with incisional hernia (ROC area under curve = 0.827 (95% CI 0.769-0.885)).

<u>Enterocutaneous fistula</u>	<u>Odds Ratio</u>	<u>p-value</u>
IF Aetiology		
• Surgical Complications	5.88 (2.65-13.02)	<0.001
• Inflammatory bowel disease	2.56 (0.57-12.00)	0.23
• Other	1	NA
Treatment with open abdomen	4.23 (1.24-14.47)	0.02
SSI at last operation	4.70 (1.95-11.31)	0.001
IASC at last operation	6.29 (2.81-14.12)	<0.001
Anastomotic leak at last operation	2.60 (1.15-6.14)	0.02

Table 2.13: Multi-variate analysis demonstrating significant factors associated with enterocutaneous fistula (ROC area under curve = 0.909 (95% CI 0.876-0.941)).

Multinomial logistic regression was used to identify factors associated with both incisional hernia and ECF (Table 2.14). Significant associations were found with BMI ($p<0.001$), male sex ($p<0.04$), active smoking at operation ($p<0.03$), multiple operations within 6 months ($p<0.001$) and increasing number of operations prior to IF ($p<0.001$) were found to be significant. Surgical site infection ($p<0.001$), IASC ($p<0.001$) or anastomotic leak following earlier surgery were also significant ($p<0.001$).

<u>Risk factors for combined</u>	<u>Odds Ratio</u>	<u>p-value</u>
Reference = No IH/No ECF Combined IH and ECF	1	
Age	1.01 (0.99-1.03)	0.34
<u>Sex (Male)</u>	<u>1.94 (1.03-3.66)</u>	<u>0.04</u>
<u>BMI</u>	<u>1.12 (1.05-1.19)</u>	<u>0.001</u>
Emergency Surgery at last operation	1.89 (0.96-3.72)	0.07
<u>Multiple operations within last 6 mths</u>	<u>4.65 (2.42-8.95)</u>	<u><0.001</u>
Multiple operations greater than 6 mths	0.95 (0.51-1.77)	0.87
Ischaemic heart disease	2.07 (0.96-4.47)	0.07
Diabetes	0.74 (0.24-2.26)	0.59
Inflammatory bowel disease	0.73 (0.36-1.49)	0.38
COPD	2.65 (0.72-9.78)	0.14
<u>Active smoker</u>	<u>3.62 (1.12-11.71)</u>	<u>0.03</u>
Immunosuppression	0.50 (0.17-1.50)	0.22
Biologics 3 months pre-operatively	0.75 (0.09-6.58)	0.80
Biologics 3 months post-operatively	1.27 (0.13-12.44)	0.84
<u>Number of operations prior to IF</u>	<u>1.45 (1.23-1.72)</u>	<u><0.001</u>
<u>SSI at last operation</u>	<u>31.50 (11.54-85.97)</u>	<u><0.001</u>
<u>IASC at last operation</u>	<u>13.05 (6.09-28.00)</u>	<u><0.001</u>
<u>Anastomotic leak at last operation</u>	<u>7.07 (3.26-15.34)</u>	<u><0.001</u>

Table 2.14: Multinomial logistic regression demonstrating factors associated with developing an incisional hernia and enterocutaneous fistula together.

2.2.4 Discussion

As far as we are aware this is the first study to evaluate the prevalence of incisional hernia and enterocutaneous fistula in a large cohort of patients with Type 2 or 3 IF. Our results show that patients who develop IF following surgery are more likely, than other post-operative patients, to develop features associated with the need more complex future abdominal surgery that may require specialist management. In this group of patients the prevalence of incisional hernia was significantly higher (23.3%) than was expected for patients undergoing laparotomy (approximately 10%) (8–10). When an incisional hernia was present it was more likely to be found with an ECF. This association makes further surgery on these patients potentially more complex increasing the likelihood of further complications when compared to patients undergoing repair of clean ventral hernias (62,114,115) (*Chapter 1.5*).

Only 8.3% of patients in our series had been managed with an open abdomen (laparostomy). It was more commonly seen in patients who had suffered previous septic complications. The use of laparostomy in the management of intra-abdominal sepsis remains controversial. Its' use has been advocated in selected patients and shown to reduced mortality (116–118), however the risk of developing an ECF in these patients is thought to be high and our data supports this, with 24 of the 29 patients managed with a long-term open abdomen presenting with an ECF. Current ESCP guidelines on the prevention of acute IF recommend achieving early fascial closure to help prevent the formation of ECF and incisional hernia and the results of the study support this recommendation (80).

This study also demonstrates important patient, procedure and surgical site related factors associated with the development of complex surgical factors in this cohort of patients. Regression analysis revealed patient factors such as increasing BMI, active smokers and Crohn's disease as significantly associated with the development features needing more complex future abdominal surgery. These data demonstrate patients with these three factors are more likely to develop ECF alone or incisional hernia and ECF together than those without them.

Significant factors associated with both incisional hernia and enterocutaneous fistula included emergency surgery, multiple operations within the previous 6 months and increasing number of operations. Current guidance on the prevention of IF, albeit from studies in trauma patients, emphasise the importance of minimising the number of returns to theatre to reduce the incidence of ECF formation (110). Similar data exist for patients with Crohn's disease (119). Our results lend support to this and reinforces the view that definitive reconstructive surgery should be delayed until at least 6 months following initial complication (108,120,121).

The findings that surgical site complications, including wound infections, intra-abdominal septic complications and anastomotic leaks, are associated with incisional hernia and ECF are not surprising and emphasise the importance of surgical technique in minimising risk to the patient (113). Our results support recommendations in ESCP guidelines in which it is suggested that the formation of anastomoses in the setting of infection and an unwell patient should be avoided even if the alternative is to form a high jejunostomy and accept a period of type 2 IF as this is more likely to facilitate subsequent reconstructive surgery (80).

We recognise certain limitations to this study; firstly, patients may not be a representative group. Our cohort comprised patients that have survived an initial septic insult to get to a point of stability where they can be transferred to a specialist centre. The number of patients who did not survive to the point of referral cannot be known and therefore these associations are limited in their predictive ability. Secondly, there are also a significant number of patients who have complex surgical needs and contaminated abdominal wall defects that do not go on to develop IF. Given the strength of the association of some factors in this cohort it is reasonable to assume they do play a role in the development of the need for more complex abdominal surgery and are therefore useful in the decision making process when managing the complex acute abdomen. Thirdly, retrospective data are limited in their interpretation and data collection may be incomplete particularly in respect to factors such as smoking and wound complications. Finally, there may also be incomplete data as most cases were referred from other institutions and therefore access to old operation notes and presenting factors was not always available. Nonetheless, it must be emphasised that every effort was made to ensure the robustness of the data collected and where there was doubt any in the accuracy cases were excluded.

In conclusion, this group of patients have complex medical, surgical and wound care needs. Current guidance states that they should be managed in a multidisciplinary team approach in a dedicated specialist centre. The current study demonstrates that more than 40% of patients with Type 2 or 3 IF will have a 'complex' surgical abdomen. In a cohort of patients with IF the prevalence of incisional hernia is more than double that expected following laparotomy and the prevalence of ECF is 1 in 3.

Certain patient and surgical factors appear to be associated with the development of a more 'complex' surgical abdomen in patients with IF.

2.3 Conclusions

Why contamination is key?

This chapter has established the prevalence of contaminated incisional/ventral hernia surgery within the context of abdominal wall hernia surgery and shown that it is increasing over time. We have shown the broad validity of the modified VHWG grading system in stratifying patients by risk of further hernia surgery. Additionally we have demonstrated underlying IBD to be a significant risk factor for long-term further hernia surgery and would recommend it should be included in the definition of Grade 2 patients.

Kanters' evaluation of the validity of the VHWG grading system was a relatively small, retrospective, single centre, cohort study and as such was able to assess more specific details regarding surgical technique and short-term outcomes (65). They include 299 patients in the study, 90% of the total number performed in the institution, due to missing data in the remaining 10%. Within their cohort, 36 patients were defined as Grade 1, 100 as Grade 2, 110 as Grade 3 and 53 as Grade 4. With their modification to the grading system (combining Grades 3 and 4), 163 (54%) of the cases were included in the contaminated category. This distribution of cases is reflective of the specialised practice of this institution and of the single surgeon whose data was analysed. As demonstrated in this and other large database analysis (122) the actual distribution of patients in the general population places the vast majority of ventral hernia surgery in Grade 1 (70-80%) and only approximately 10% within a contaminated group. This therefore potentially could lead to underlying population and selection bias in the Kanters study. As well as this the small numbers of patients in each group mean that any multi-variate analysis of risk factors is not possible.

Although there are also limitations in the use of large databases to study outcomes from hernia surgery due to the lack of some of the detailed technical and procedural details, such as defect size, mesh choice, suture technique or CDC wound classification (as used by Kanters), a broad stratification system for all ventral hernias, such as the VHWG classification, is important in day-to-day surgical practice. Simply stratifying patients based on the Kanters VHWG system with the St Mark's modification, would allow for better patient counselling on short- and long-term outcome from surgery, influence repair decisions for more complex cases and allow for better design of future research studies and trials if universally accepted.

Contamination at the time of hernia repair remains the key underlying discriminatory risk factor for poor outcome, as previously shown by Berger *et al* and Liang *et al* for wound complication (59,77) and here for long-term further hernia surgery (2.1.3). Given the strength of contamination as a risk factor for short- and long-term adverse outcomes evaluating Grade 3 patients in isolation from clean cases therefore should be recommended. With this knowledge studies can be designed to better risk stratify patients within Grade 3 based on more detailed patient, technical and procedural factors without the contamination factor potentially skewing the analysis given its strong influence on outcome. In chapter 3 we will evaluate exclusively contaminated hernia defects and attempt to better risk stratify this complex group of patients who have been shown to have higher risk of poor short- and long-term outcome from hernia repair.

Who is at risk of developing a contaminated hernia defect?

Both parts of this chapter have sought to determine risk factors for patients who go on to develop contaminated hernia defects by evaluating national and local data. Both studies showed emergency surgery at the last operation, increasing number of previous surgeries, male sex and comorbidity, such as, diabetes, COPD and inflammatory bowel disease to be associated with contaminated future hernia surgery. Given the more detailed nature of the local cohort, further factors, such as surgical site occurrence at previous surgery and anastomotic leak to be associated with a chronic contaminated abdominal wall defect.

In day-to-day surgical practice these factors are important considerations for all patients undergoing abdominal surgery. We know approximately 10% will go on to develop an incisional hernia and the prevalence of incisional hernia surgery is increasing. By minimising the incidence of surgical site occurrence and anastomotic leak and minimising the number of returns to theatre in the deteriorating surgical patient, particularly in high risk groups, such as patients with diabetes, COPD and IBD, we would hypothesise a reduction in the development of incisional hernia and contaminated defects. These data recommend the routine collection of these factors in studies evaluating the incidence of long-term incisional hernia. Comparative studies, controlling for these factors, could also be designed to evaluate novel abdominal wall repair techniques given their influence on hernia formation.

However a number of key limitations remain. The retrospective nature of the data presented here, as well as, the use of further hernia surgery rates as a surrogate for recurrence rate, limit the predictive ability of risk factors determined here for

contaminated hernia surgery. A number of likely confounding factors were missing from both studies including smoking status, BMI at the time of prior surgery and defect size. The next chapter aims to reduce these limitations by looking at local outcome data, evaluation of defect size and prospective clinical evaluation of hernia recurrence.

Chapter 3 – Improving peri-operative care in patients undergoing contaminated abdominal wall reconstruction

In this chapter we will evaluate component separation techniques used in contaminated abdominal wall reconstruction, develop a novel method for risk stratifying patients and evaluate the use of CT imaging in patients undergoing contaminated abdominal wall reconstruction.

3.1 A meta-analysis comparing open anterior component separation with posterior component separation and transversus abdominis release in the repair of midline ventral hernias

This section has been published in *Hernia* and is reproduced with permission (*Appendix 8*). Hodgkinson JD, Leo CA, Maeda Y, Bassett P, Oke S, Vaizey CJ, Warusavitarne J. *Hernia*. 2018 Aug;22(4):617-626. doi: 10.1007/s10029-018-1757-5.

The student declares that the statistical meta-analysis carried out in this section was performed by the medical statistician Mr Paul Bassett, as acknowledged in the published work.

3.1.1 Introduction

In order to help achieve anatomical restoration Ramirez *et al* first described the technique of open anterior component separation (OACS) in 1990 as described in the introduction (20). It was observed that there were a number of problems arising as a result of the OACS technique including seromas, wound infections and abscess formation that could be exacerbated in the contaminated setting. These complications have been shown to be reduced by the use of minimally invasive anterior component separation, such as perforator sparing or endoscopic techniques, but these techniques are often not possible in contaminated cases.

Novitsky *et al* first described the posterior component separation and transversus abdominis muscle release (PCSTAR) in 2012 as described in the introduction (33). This technique utilized the retromuscular space, accessed by incising the posterior rectus sheath and dissecting the posterior sheath back to the transversus abdominal muscle and therefore is hypothesized to be useful in the contaminated setting. This

study aims to compare the outcomes of PCSTAR with OACS and establish if one technique is better across a range of reported outcomes including short-term complications and hernia recurrence.

3.1.2 Methods

Search Strategy and Terms

The study was conducted according to the PRISMA guidelines. MEDLINE, EMBASE and Pubmed database searches were carried out to identify all papers reporting on outcomes of midline ventral hernia repair using “component separation” techniques. The search term “component separation” was used. The reference lists of all related studies were reviewed and relevant papers were included.

Inclusion criteria

Studies reporting exclusively on outcomes from midline ventral hernia repairs utilizing component separation techniques from 1990 onwards were included. Outcomes of studies describing PCSTAR in conjunction with retromuscular mesh placement were identified. Studies describing OACS using only retromuscular mesh placement were identified and matched to the included PCSTAR studies post-hoc by comparing demographics. Only studies published in English and investigating human subjects were included.

Exclusion criteria

Studies that included outcomes from non-midline hernia repairs (for example lateral abdominal wall and parastomal) were excluded. Studies were excluded if they described cohorts that included some patient who did not have component separation techniques used as part of their hernia repair. Studies describing minimally invasive anterior component separation and/or non-retromuscular mesh placement (eg onlay mesh placement) were excluded. Outcomes of paediatric cohorts and case series reporting less than or equal to 5 cases were excluded. Review articles, conference

abstracts, discussion articles and commentaries were excluded. Non-human, cadaveric and basic science studies were also excluded.

Data collection

The search results were assessed and titles screened by the first reviewer (JH). Papers were assessed by two independent reviewers (JH and CL) according to the inclusion and exclusion criteria. Included papers were assessed using the Methodological Index for Non-randomized studies (MINORs) tool and the Down and Blacks checklist for non-randomized study assessment (123,124). Data were extracted by the first reviewer and cross-checked independently by the second reviewer. Agreement on included papers and extracted data was reached following discussion between reviewers. It was noted that the majority of the papers describing PCSTAR were published from the same institution. To ensure there was no significant overlap in patients included in the cohorts, we contacted the corresponding author who informed us there was minimal overlap (<10 patients) between two of the studies (14, 15) and studies (15) and (16). There was no overlap between studies (14) and (16). Based on this additional information the decision was made to include all the identified papers in the analysis.

Data extraction

Data were extracted for; study demographics (population, study year, study design, sample size), patient demographics (sex, age, BMI, diabetes, smoking status), hernia details (defect size, number of previous hernia repairs, presence of contamination), operative details (type of repair, use of mesh, use of bridging mesh, use of component separation), post-operative complications (length of stay, recurrence of hernia,

wound-related morbidity, mortality), and average follow-up. Data from the included studies were stored in an Excel spreadsheet (Microsoft, Redmond, Washington, USA).

Statistical analysis

Statistical analysis was performed using SPSS (IBM Analytics, New York, USA).

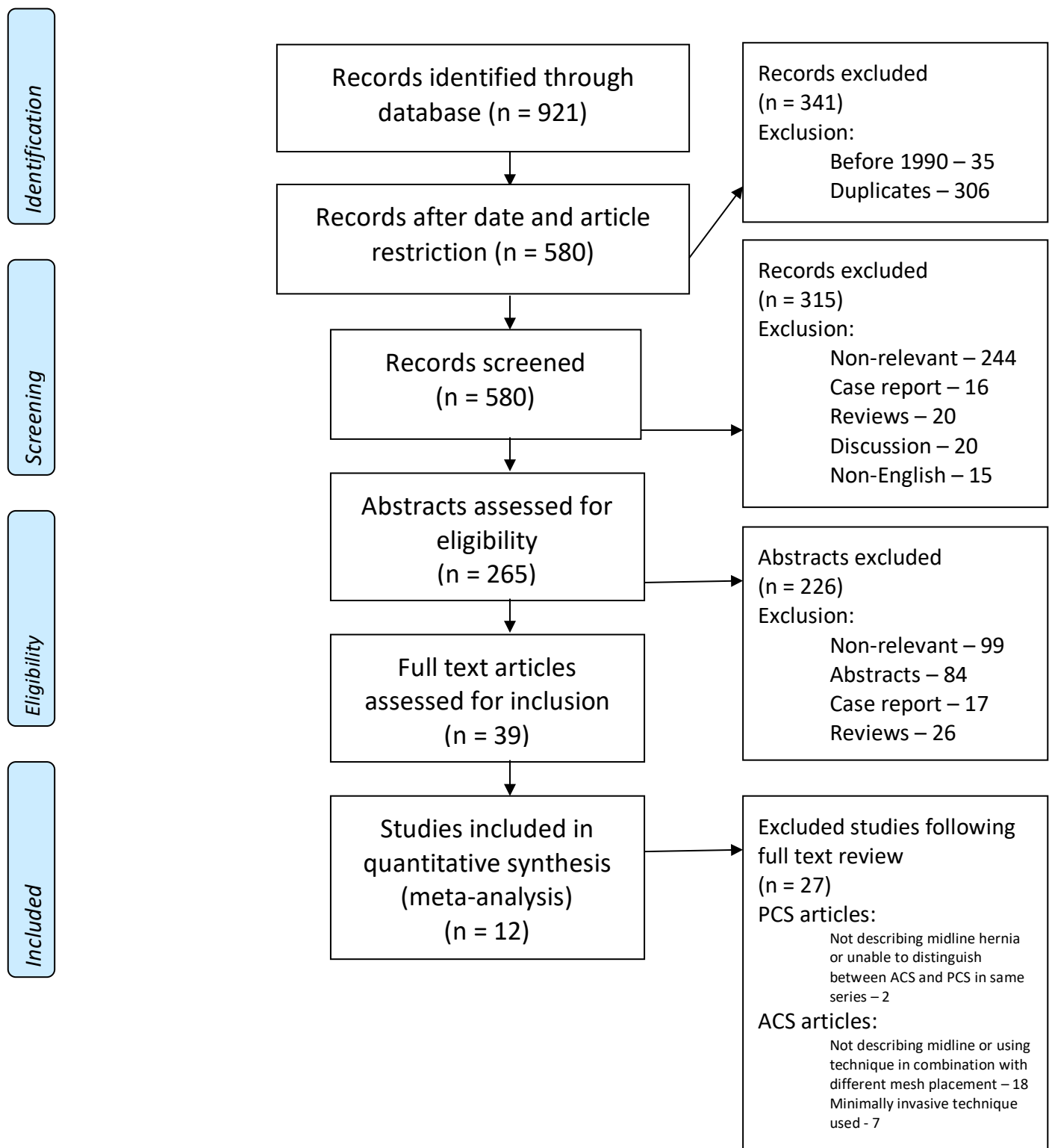
Pooled data were analysed for anterior and posterior component separation for outcome including hernia recurrence, use of bridging mesh, wound complications (all related, superficial, deep), re-operation and length of stay. I^2 values were calculated to determine heterogeneity. Student t-test, chi-squared and Mann-Whitney U tests were used to test for statistically significant differences between groups.

3.1.3 Results

A database search identified 921 studies. Papers published before 1990 (the year “component separation” was first described) were excluded and duplicates were removed leaving 580 studies. Three hundred and fifteen studies were excluded following title review, and a further 226 were removed following assessment of abstracts. The resulting 39 articles were reviewed in full and a further 27 were excluded according to the criteria. This resulted in the inclusion of 12 studies; one study comparing outcomes of posterior and anterior component separation (125); six discussing posterior component separation only (33,126–130); and 5 discussing anterior component separation only (131–135). In total 12 studies were assessed for quality and included in the meta-analysis (Figure 3.1 and Table 3.1). Assessment of the quality of included studies revealed there were no ideal studies. Scores ranging from 8 to 11 on MINORs and 12 to 16 on Down and Black’s were seen. Studies lost marks on both scores, primarily as a result of the lack of comparative data, lack of prospective data collection or sample size calculation and comparative statistics.

There were no randomized trials or prospective controlled studies of the two techniques. Only one paper, utilizing a prospectively maintained database, compared the two techniques directly (125). Of the papers describing PCSTAR, four also utilized prospectively maintained databases to identify the cohort (126–129) and the remaining two describe retrospective cohorts (130,136). Of the papers describing OACS, one uses a cohort from a prospectively maintained database (132) and four describe retrospective cohorts (131,133–135).

Figure 3.1: A PRISMA flow chart demonstrating study selection.



Title	Author	Publication year	Study type	Patient number	Mesh used		Year	MINORs	D&B's
					Biologic	Synthetic			
Posterior and open anterior components separations: a comparative analysis (13)	Krpata <i>et al</i>	2012	Prospective database comparative cohort, single centre	111	61	50	2006-2011	9	12
Initial UK experience with transversus abdominis muscle release for posterior components separation in abdominal wall reconstruction of large or complex ventral hernias: a combined approach by general and plastic surgeons (18)	Appleton <i>et al</i>	2016	Retrospective, cohort, single centre	12	9	3	2013-2014	11	14
Outcomes of Retromuscular porcine biologic mesh repairs using transversus abdominis release reconstruction (17)	Fayezizadeh <i>et al</i>	2016	Prospective database, cohort, single centre	77	77	0	2007-2014	11	16
Repair of massive ventral hernias with "quilted mesh" (16)	Posielski <i>et al</i>	2015	Prospective database, cohort, single centre	32	0	32	NR	9	15
Posterior component separation and transversus abdominis muscle release for complex incisional hernia repair in patients with a history of an open abdomen (15)	Petro <i>et al</i>	2015	Prospective database, cohort, single centre	29	15	19	2011-2013	9	16
Posterior component separation with transversus abdominis release successfully addresses recurrent ventral hernias following anterior component separation (14)	Pauli <i>et al</i>	2015	Prospective database, cohort, single centre	55	5	24	2006-2011	9	15
Transversus abdominis muscle release: a novel approach to posterior component separation during complex abdominal wall reconstruction (10)	Novitsky <i>et al</i>	2012	Retrospective, cohort, single centre	42	0	42	2006-2009	10	13
Total abdominal wall reconstruction with component separation, reinforcement, and ventral abdominoplasty in patients with complex ventral hernias (19)	Espinosa <i>et al</i>	2016	Retrospective, cohort, single centre	58	NR	NR	2010-2014	8	13
Outcome in porcine acellular dermal matrix reinforcement of infected abdominal wall defects: a prospective study (20)	Zerbib <i>et al</i>	2015	Prospective database, cohort, single centre	18	18	0	2010-2012	10	15
Comparison of synthetic and biologic mesh in ventral hernia repair using components separation technique (21)	Sandvall <i>et al</i>	2014	Retrospective, cohort, single centre	72	45	27	2006-2010	9	14
Component separation with porcine acellular dermal reinforcement is superior to traditional bridged mesh repairs in the open repair of significant midline ventral hernia defects (22)	Richmond <i>et al</i>	2014	Retrospective, cohort, single centre	40	40	0	2006-2012	10	15
Indications and outcomes following complex abdominal reconstruction with component separation combined with porcine acellular dermal mesh reinforcement (23)	Patel <i>et al</i>	2011	Retrospective, cohort, single centre	41	41	0	2007-2011	9	15

Table 3.1: A table demonstrating included papers and study quality divided by study type (top – comparative study; middle – PCSTAR only; bottom – OACS only). NR – not reported.

Pooled data for 281 patients who received PCSTAR are demonstrated in Table 3.2. Heterogeneity across these papers for all outcomes, except for length of stay was not significant. The heterogeneity seen for length of stay is likely due to the fact only 3 papers reported on this finding. Pooled hernia recurrence rate was found to be 5.7% (CI 3.0-8.5).

Pooled data for 285 patients who received OACS are demonstrated in Table 3.3. Heterogeneity across these papers was significant for all outcomes. This is likely the result of the widespread use of this technique for abdominal wall reconstruction resulting in varying hernia grades and patient comorbidity. The pooled hernia recurrence rate was found to be 9.5% (CI 4.0-14.9).

Outcome	Number of studies	Heterogeneity		Pooled % (95% CI) N = 281
		p-value	I ²	
Hernia recurrence	7	0.49	0%	5.7% (3.0%, 8.5%)
Wound complications (superficial)	7	0.63	0%	10.9% (7.3%, 14.5%)
Wound complications (deep)	7	0.39	5%	9.5% (6.0%, 13.0%)
Re-operation	5	0.81	0%	3.8% (0.4%, 7.3%)
Bridging Mesh	6	0.28	20%	3.1% (0.0%, 7.2%)
Length of stay (days)	4	<0.001	92%	7.7 (5.2, 10.2)

Table 3.2: A table demonstrating pooled data for PCSTAR for all outcomes.

Outcome	Number of studies	Heterogeneity		Pooled % (95% CI) N = 285
		p-value	I ²	
Hernia recurrence	6	0.05	56%	9.5% (4.0%, 14.9%)
Wound complications (superficial)	5	<0.001	89%	21.6% (7.4%, 35.8%)
Wound complications (deep)	3	0.17	43%	12.7% (3.4%, 22.1%)
Re-operation	4	0.56	0%	5.9% (2.0%, 9.8%)
Bridging Mesh	6	0.02	63%	7.5% (1.7%, 13.4%)
Length of stay (days)	3	0.10	56%	9.0 (5.3, 12.8)

Table 3.3: A table demonstrating pooled data for OACS for all outcomes.

Pooled data for patient demographics were analyzed and compared between the PCSTAR and OACS groups (Table 3.4). There was no statistically significant difference between the patient mean age, mean BMI, mean defect size, diabetes, the presence of pre-operative contamination, mortality and average follow-up. The PCSTAR group had significantly more male patients and more who had previous hernia repairs. The OACS group had significantly more patients with wound contamination at the time of surgery.

Patient demographics	PCSTAR N = 281	OACS N = 285	p-value
Sex male (%)	49.8	39.3	0.03
Age (years) (mean, SD)	56.14 (3.2)	57.23 (2.95)	0.54
BMI (mean, SD)	34.8 (3.05)	35.75 (1.61)	0.58
Defect size (cm ²) (mean, SD)	457.42 (158.74)	355.17 (185.11)	0.61
Number previous hernia repair (%)	90.1	63.4	<0.001
Diabetes (%)	30.1	38.0	0.06
Smoker (%)	16.7	20.7	0.26
Pre-operative contamination (VHWG grade 3/4) (%)	34.1	47.4	0.01
Mortality (%)	0.0	0.01	0.87
Median follow-up (mths, IQR)	18 (9-26)	14 (12-20)	1.00

Table 3.4: Pooled data comparing patient demographics of studies of PCSTAR with OACS. It can be seen that there are significantly more contaminated wounds in the OACS group and more patients who have had a previous hernia repair in the PCSTAR group. No other difference in the two groups was statistically significant.

Results from the meta-analysis of the outcome data can be found in Table 3.5 and forest plots comparing the outcomes in Figure 3.2. No statistically significant difference between the two techniques was seen across any of the observed outcomes.

Outcome	Difference % (95% CI)	P-value
Hernia recurrence	-3.7% (-9.8%, 2.4%)	0.23
Wound complications (superficial)	-10.7% (-25.3%, 3.9%)	0.15
Wound complications (deep infection)	-3.2% (-13.2%, 6.8%)	0.53
Re-operation	-2.1% (-7.3%, 3.2%)	0.44
Bridging Mesh	-4.4% (-11.5%, 2.7%)	0.22
Length of stay	-1.3 (-5.8, 3.2)	0.57

Table 3.5: A table demonstrating the meta-analysis of outcomes comparing PCSTAR with OACS. Difference was calculated by subtracting OACS from PCSTAR. A negative value implies favouring PCSTAR however no significant difference was seen for any outcome.

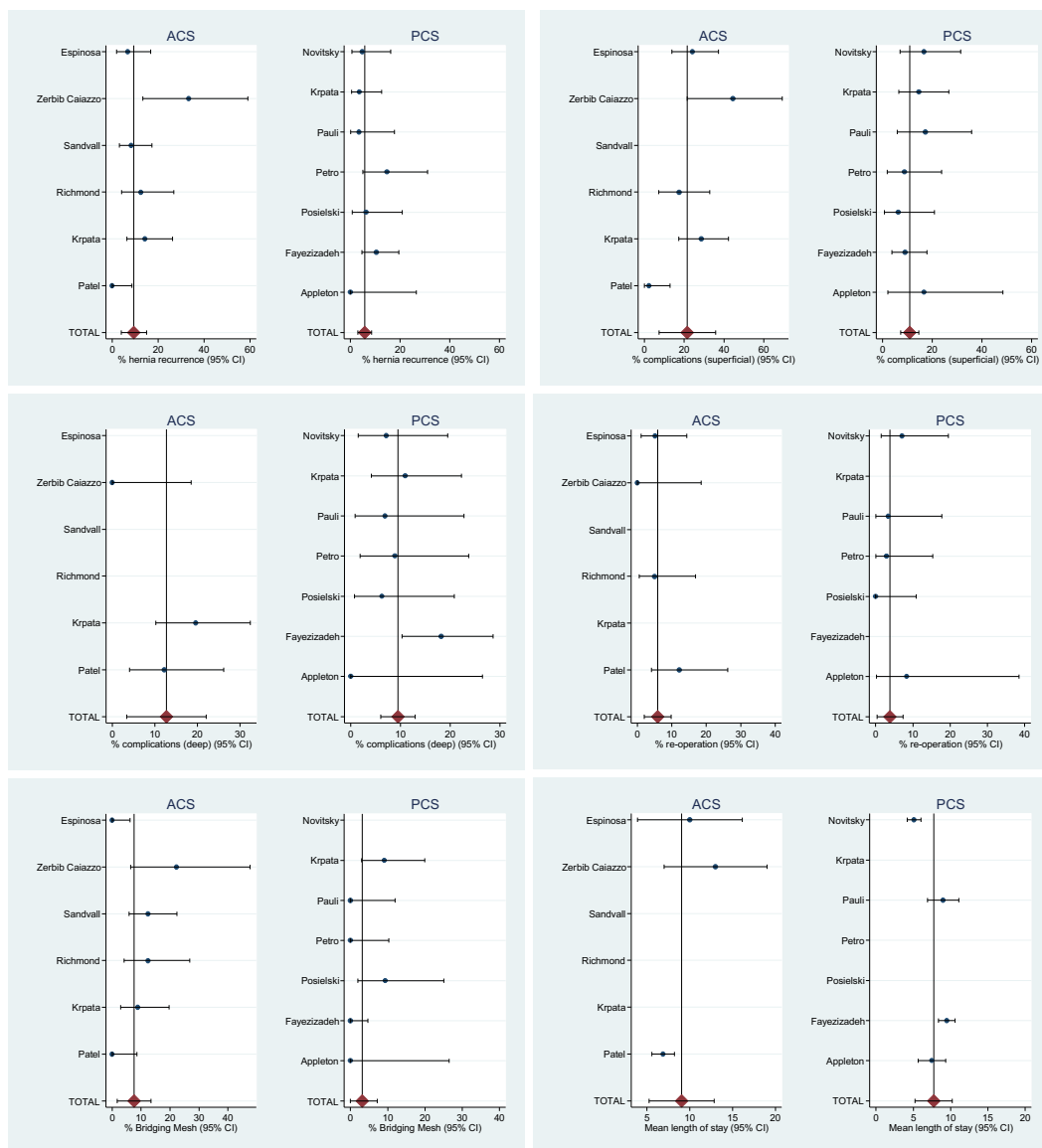


Figure 3.2: Forest plots demonstrating a comparison of the observed outcomes and pooled results.

3.1.4 Discussion

These data demonstrate that PCSTAR has comparable outcomes with OACS across a number of key outcomes relevant to hernia surgery. No statistical difference in outcomes between the two groups was observed, to favour one technique over the other. The use of bridging mesh was also comparable; implying a similar amount of fascial medialisation can be achieved with the two techniques.

Comparing the difference between the two groups, demonstrates that there is no statistically significant difference between the two, in most of the measured patients' demographic markers. The only differences observed were in number of previous hernia repairs (more in the PCSTAR group) and pre-existing wound contamination (more in the OACS group). Although this method of comparing the two groups is not ideal the fact they are comparable across a number of pre-operative factors that are known risk factors for hernia recurrence aids the meta-analysis.

The lack of comparative studies and randomized trials makes direct comparison between the two groups difficult. In addition to this the inherent heterogeneity in the OACS group makes it hard to draw a robust conclusion from these data. Given PCSTAR is a relatively new technique this is the first attempt in the literature that has been made to compare this procedure with the more established OACS. The heterogeneity observed in the PCSTAR group is minimal. It could be argued that this is as a result of some overlap of patients between studies. Given that this overlap was confirmed as only of a few patients in two studies, it is felt this would unlikely lead to a statistically significant result. This, therefore, aids the comparison and is reassuring that the outcomes in this group are likely to be consistent. Every effort to minimize

heterogeneity in the OACS group has been made by trying to standardize the remaining elements of the procedure including midline incision and site of mesh placement. Nevertheless given this technique is widely used it is likely a comparison of outcomes across 285 patients will give a good estimate of the potential outcomes of this technique as a whole. We believe this is because it is used in across a patient group with significant inherent heterogeneity, and the observed rates in our study for all measured outcomes were comparable with other published studies for OACS outcomes (137).

At the very least these data suggests PCSTAR is as safe as OACS with comparable levels of hernia recurrence. Superficial wound complications trended towards favouring PCSTAR. OACS is acknowledged to have issues relating to wound complications and various minimally invasive and “perforator sparing” methods have been described and have been found to reduce the incidence of wound morbidity in direct comparison of the two techniques (138–140). Given PCSTAR was only performed via midline laparotomy in the included studies, studies investigating outcomes from minimally invasive OACS were excluded from the analysis. Despite this a significant amount of heterogeneity in wound outcomes from OACS was seen.

The only paper included in the analysis that directly compare the two techniques comes from the group who developed PCSTAR (125). They retrospectively compared the outcomes from these two procedures, with 56 OACS and 55 PCSTAR patients. They attempt to make the groups comparable by measuring the difference between a range of pre-operative factors. Defect size, smoking, contamination, number of previous hernia repairs and abdominal surgeries were comparable. Other

factors such as age, sex, BMI and diabetes were significantly different between the two groups. It was found that recurrence rates were comparable, possibly trending to favour PCSTAR. It reported significantly better rates of wound complications in the PCSTAR group however they also noted that the OACS group had significantly more patients with pre-existing wound infection and diabetes, two key risk factors for this complication (141). This method of retrospectively comparison is not ideal and a prospective controlled cohort comparison would be the best way of ensuring the comparison was valid.

A randomized controlled trial or a comparative controlled trial to create comparable groups in order to assess these techniques would be recommended. The inherent difficulty in finding comparable patients in the field of abdominal wall reconstruction include the range of ventral hernia working group grades, range of other operative factors, such as mesh type, and the range of potential comorbidities in this patient group. A simple sample size calculation to compare just these two factors would require 438 patients, in each group, to establish statistical significance for hernia recurrence (assuming a 95% CI; 80% power; rates of 10% for OACS and 5% for PCSTAR) and 197 patient for superficial wound complications (assuming a 95% CI; 80% power; rates of 20% for OACS and 10% for PCSTAR). These numbers would only be achievable by an international multi-centre collaboration. Alternatively, analysis of a large hernia-specific outcome registry, such as the Americas Hernia Society Quality Collaborative or the European Registry of Abdominal Wall Hernias, may give some insight into 'real life' outcomes. The main drawbacks in the use of large-scale registry data include selective reporting and the reliability of long-term follow-up.

Posterior component separation with transversus abdominis release and anterior component separation have comparable outcomes for complex abdominal wall reconstruction of midline ventral/incisional hernias. The lack of directly comparable data limits scope of any significant conclusions but no previous attempt has been made to establish the relative outcomes of these two procedures. Future controlled comparative trials are recommended to explore the conclusions more accurately as well as standardization of outcome measures, particularly in reporting wound morbidity. PCSTAR is a useful addition to the surgeon's armamentarium.

3.2 Development of risk stratification models for peri-operative assessment of patients undergoing contaminated abdominal wall reconstruction

The student declares all data from the AUMC were collected by Dr F de Vries and Mr J Claessen. Statistical analysis was performed primarily by myself with the assistance, in parts, and guidance of the medical statistician Mr Paul Bassett. I would like to thank and acknowledge the British Hernia Society for the kind research award that has funded the online risk calculator.

3.2.1 Introduction

Short and long-term outcomes for patients undergoing reconstruction of contaminated (modified-VHWG 3) defects have not been studied in sufficiently high numbers. Our systematic review demonstrated high overall wound complication rates (46% (range 18-85)), which may be expected in a group of patients with pre-operative active wound infection/contamination (*Chapter 1.5*). Mortality rate was acceptably low at 2.3%.

A number of studies have attempted to assess for risk factors for wound complications in this group of patients. Factors such as, active smoking, diabetes mellitus, COPD, number of previous hernia repairs, number of previous abdominal surgeries, and increasing operative time were found to be significant for wound complications (73–76). These studies however all used small cohorts (range 37-128) and are therefore at risk of type one error. No analysis has been performed in modified VHWG grade 3 to look for risk factors associated with other post-operative co-morbidity, such as return to theatre or long-term hernia recurrence.

One previous study has developed and validated a risk-stratification score for prediction of surgical site occurrence (SSO) and surgical site infection (SSI) following ventral hernia repair (59). They successfully validate the score on an external prospective cohort (77). The most significant predictor of SSO and SSI in their scoring system is wound contamination and therefore of limited use to discriminate risk in patients with modified-VHWG 3 defects. It can be hypothesized that evaluating this cohort in isolation would lead to a more accurate model of risk stratification therefore this study aims to develop and validate risk stratification models for CD grade ≥ 3 postoperative complications for patients with modified-VHWG grade 3 abdominal wall defects.

3.2.2 Methods

A collaboration was set up between St Mark's Hospital, London, UK (SMH) and the Academic University Medical Centres, location AMC, Amsterdam, Netherlands (AUMC), two national referral centres for complex gastrointestinal surgery and intestinal failure. Ethical approval was granted by the local institutional review board and national ethics committee in both centres (UK: REC ref 16/EE/0348; IRAS 210325). The initial cohort used to develop the model was established from consecutive patients undergoing elective repair of a midline modified VHWG grade 3 abdominal wall defect (2), between January 2004 and December 2015, through local operative databases. The presence of contamination, to define Grade 3 cases, was taken as the presence of a stoma, concomitant gastrointestinal tract violation, the presence of an infected mesh, septic dehiscence or enterocutaneous fistula. In order to validate the models, an independent cohort of consecutive patients was identified from January 2016 to December 2017. At both centres, data were collected via as retrospective review of prospectively collected clinical databases.

Pre-operative patient characteristics, operative details and short-term post-operative outcomes were collected by retrospective case note review. Factors were selected for inclusion based on likely risk of adverse outcome following hernia surgery based on previous risk factors identified in previous CAWR cohorts or other hernia surgery. If patient records were missing, unclear or incomplete then the patient was excluded from the final analysis. All patients who had survived to the present day were contacted by post and invited to attend a one-off out-patient appointment to determine long-term hernia outcomes.

In order to validate the models an independent cohort of patients was identified from January 2016 to December 2017 from the same centres. At SMH patients with intestinal failure were identified and operative details collected prospectively using a local clinical database. Factors not included in the database and patients without intestinal failure were collected by retrospective case note review. At AUMC patients were identified using a local operative database and patient details were collected by retrospective case note review. As the length of follow-up was not sufficient to identify all possible hernia recurrences validation was restricted to short-term outcomes only therefore prospective follow-up was not undertaken.

Patient demographics and outcomes

Data were collected for baseline patient demographics including sex, age and BMI at time of surgery, co-morbidity, intestinal failure, number of previous abdominal operations and previous hernia repairs. Previous open abdomen was defined as long-term management with laparostomy. Operative details including length of operation, technique of abdominal wall reconstruction and concurrent procedures were recorded.

The validated Clavien Dindo grade of surgical complications was used as the primary short-term outcome measure (142). Wound morbidity was divided into any wound complication, and the wound intervention required to treat the complication (conservative/bedside measures vs radiological or surgical intervention) was recorded. Similarly, intra-abdominal septic complications (IASC) (anastomotic leak, intra-abdominal collection and recurrent fistula) were separately defined by their occurrence and required intervention to treat the complication (conservative/bedside

measures vs radiological or surgical intervention). This is consistent with recent recommendations in outcome reporting in this cohort (12).

Short-term outcomes identified and modelled for included post-operative Clavien-Dindo complication of grade 3 or greater, wound intervention (defined as radiological or surgical intervention excluding wounds opened at the bedside) and interventions for intra-abdominal septic complications (IASCs, defined as radiological intervention or further surgery for an intra-abdominal collection or anastomotic leak). Long-term outcomes including hernia recurrence, further hernia surgery and time to further surgery were also collected and modelled.

Statistical analysis

Baseline characteristics are presented as proportions and compared between cohorts using the Mann-Whitney test. Given the paucity of analysis of risk factors for these outcomes in this group of patients this analysis has been performed using uni-variate binary logistic regression analysis was used to identify factors of significance within the development cohort. For each outcome a p-value of <0.05 was seen as significant. Multi-variate binary logistic regression, using a backward selection process, was then undertaken on these factors of significance to determine a risk stratification model. The logit-p equation was used to determine predicted probabilities for each outcome using factors retained in multi-variate analysis ($\log(p/1-p) = y = \text{constant} + \text{risk factor A (regression coefficient)} + \text{risk factor B (regression coefficient)} + \dots$) where the probability of complication = $P = (e^y / 1 + e^y)$. Receiver operating characteristics (ROC) curves were analysed to assess the predictive ability of the models.

Using the validation cohort, models were then tested for discrimination and calibration using the validation cohort. Discrimination, a measure of the model's ability to distinguish between high and low risk patients, was tested by examining the difference in predictive probability of a complication when it occurred compared to when it did not. The predicted probabilities were found to be non-parametric and therefore were compared using the Mann-Whitney test. Discrimination was then further evaluated using the c-statistic with a 95% confidence interval.

Calibration of the model determines whether the predicted probabilities of an outcome occurring are similar to those in the validation cohort. This was evaluated using the Hosmer-Lemeshow test to compare the predicted and observed number of adverse events. Differences between these groups were then assessed using a Chi-squared test with one degree of freedom.

Both discrimination and calibration were evaluated together by dividing patients into three categories based on the predicted risk of an outcome occurring. Categorical cut-offs were decided based in an even distribution of patients into each category and the observed percentage of an outcome was compared with the predicted risk.

3.2.3 Results

Development cohort - baseline patient demographics and outcomes

The development cohort identified from 1st January 2004 to the 31st December 2015 consisted of 269 consecutive patients who underwent 287 procedures. Fifteen patients, who had one procedure each had incomplete or missing records, were therefore excluded from the analysis. This resulted in 272 procedures in 254 patients included in the final analysis. The mean age was 58.0 (SD 13.6 years) and 58.8% were male. The mean ASA classification across the cohort was 2.43 (SD 0.5). The majority of patients (58.1%) had an enterocutaneous fistula and 47.1% had underlying intestinal failure (Table 3.6).

The median number of abdominal operations performed previously in each patient was 4 (IQR 2-5) and 43.8% had undergone a previous hernia repair. Component separation and/or mesh were used in two thirds of the reconstructions (67.3% and 66.9% respectively) and fascial closure was achieved in 72.5% of patients (Table 3.6).

Patient characteristics			
	Development cohort	Validation cohort	
	272 procedures in 254 patients N (%)	114 procedures in 112 patients N (%)	p-value
Age, mean (SD)	58.0 (SD 13.6)	58.1 (12.6)	0.94
Sex male	160 (58.8)	62 (54.4)	0.42
BMI, median (IQR)	26.0 (22.6-29.6)	26.0 (22.9-30.5)	0.59
ASA classification, mean (SD)	2.43 (0.5)	2.45 (0.6)	0.70
1	0 (0)	3 (2.6)	
2	162 (59.6)	61 (53.5)	
3	103 (37.9)	46 (40.4)	
4	7 (2.6)	4 (3.5)	
Active smoker	62 (22.8)	16 (14.0)	0.06
Diabetes	50 (18.4)	16 (14.0)	0.43
Immunosuppression	21 (7.7)	9 (7.9)	0.96
Cardiac comorbidity	63 (23.2)	28 (24.6)	0.77
Pulmonary comorbidity	55 (20.2)	34 (29.8)	0.04
COPD	29 (10.7)	11 (9.6)	0.75
Hypertension	83 (30.5)	34 (29.8)	0.89
Abdominal malignancy	50 (18.4)	31 (27.2)	0.06
IBD	39 (14.3)	24 (21.1)	0.06
Intestinal failure	129 (47.1)	48 (42.1)	0.47
History of open abdomen	128 (47.1)	38 (33.3)	0.01
Presence of intestinal fistula	159 (58.1)	60 (52.6)	0.42
Operative characteristics			
Number of previous abdominal surgeries, median (IQR)	4 (2-5)	3 (2-5)	0.18
Undergone previous hernia repair/s	119 (43.8)	49 (43.0)	0.90
Anastomosis constructed	200 (73.5)	84 (73.7)	
Mesh removal	59 (21.7)	23 (20.2)	0.74
Component separation technique performed	183 (67.3)	85 (74.6)	0.20
Mesh used	182 (66.9)	77 (67.5)	0.90
Fascial closure achieved	197 (72.5)	99 (86.8)	0.01

Table 3.6: Baseline demographics of the development and validation cohorts. Differences between the cohorts have been evaluated using the Mann-Whitney test.

The median post-operative length of stay was 18 days (IQR 10-31). Eighty-four percent of patients had a post-operative complication on the Clavien Dindo scale, of which 43.8% were of grade 3 or higher. A wound complication was recorded in 58.5% and an IASC in 18.8% (Table 3.7).

<u>Outcome</u>	<u>Development</u> <u>N=272 (%)</u>	<u>Validation</u> <u>N=114 (%)</u>
Length of stay (median days, IQR)	18 (10-31)	19 (10-37)
Clavien Dindo complication		
0	41 (15.1)	22 (19.3)
1	47 (17.3)	14 (12.3)
2	65 (23.9)	33 (28.9)
3a	38 (14.0)	15 (13.2)
3b	19 (7.0)	8 (7.0)
4a	44 (16.2)	9 (7.9)
4b	11 (4.0)	11 (9.6)
5	6 (2.6)	3 (2.6)
Post-operative wound complication	159 (58.5)	58 (50.9)
Wound intervention required (interventional radiology or surgery)	71 (26.1)	14 (12.3)
Intra-abdominal septic complication	51 (18.8)	27 (23.7)
Intra-abdominal septic complication intervention	42 (15.4)	22 (19.3)
Return to theatre	33 (12.1)	12 (10.5)
Re-admission within 30 days of discharge	35 (12.9)	6 (5.3)
Recurrent enterocutaneous fistula	20 (7.4)	4 (3.5)

Table 3.7: Short-term post-operative outcomes from the development and validation cohorts.

Validation cohort – baseline demographics and outcomes

The validation cohort identified from 1st January 2016 to the 31st December 2017 consisted of 112 consecutive patients who underwent 114 procedures. All patient records were complete therefore all cases were included in the final analysis. The mean age was 58.1 (SD 12.6 years) and 54.4% were male. The mean ASA classification across the cohort was 2.45 (SD 0.6). Again the majority of the patients had an enterocutaneous fistula (52.6) (Table 3.6). No significant difference was seen between the pre-operative demographics between the validation and development

cohorts with the exception of the number of patients with a history of treatment with an open abdomen ($p=0.01$).

The median number of abdominal operations performed previously in each patient was 3 (IQR 2-5) and 43.0% had undergone a previous hernia repair. Significantly more patients in the validation cohort achieved fascial closure at surgery ($p=0.01$), which could be reflected in the fact that more received components separation, however this difference was not statistically significant.

The average length of stay was 19 days (IQR 10-37), which was comparable to the development cohort. Any complication on the Clavien Dindo scale was seen in 80.7% of patients, with 40.2% having a complication of grade 3 or higher. A wound complication was recorded in 50.9% and an IASC in 23.7% (Table 3.7).

Model 1 – Clavien Dindo complication grade 3 or more

Uni-variate analysis identified a number of factors associated with a Clavien Dindo complication of grade 3 or higher. Underlying patient factors such as cardiac comorbidity, previous treatment with an open abdomen, presence of intestinal failure and presence of an enterocutaneous fistula were associated with higher grade of complication. Interestingly pre-operative diagnosis of inflammatory bowel disease was protective against significant complication. Operative factors, such as, total operative time, number of bowel anastomoses, the need for component separation techniques and the inability to obtain fascial closure were associated with increasing likelihood of significant complication (Table 3.8).

Risk factor	Clavien Dindo Grade ≥ 3 (OR (95%CI))	Wound intervention (OR (95%CI))	Intra-abdominal septic complication (IASC) intervention (OR (95%CI))
Age	1.01 (0.99-1.03)	0.99 (0.97-1.01)	1.00 (0.97-1.02)
Sex (Female)	1.10 (0.67-1.79)	1.24 (0.72-2.14)	0.97 (0.50-1.89)
Obese	0.92 (0.51-1.65)	0.93 (0.48-1.78)	0.77 (0.33-1.77)
Smoker	0.61 (0.33-1.11)	0.61 (0.30-1.23)	0.52 (0.21-1.30)
Diabetes	1.02 (0.55-1.91)	0.76 (0.37-1.58)	1.48 (0.68-3.26)
Immunosuppression	1.31 (0.54-3.19)	2.29 (0.92-5.68)	3.09 (1.16-8.18)
Cardiac comorbidity	2.29 (1.29-4.05)	0.76 (0.39-1.49)	1.22 (0.57-2.58)
Pulmonary comorbidity	1.61 (0.89-2.92)	0.85 (0.43-1.69)	1.09 (0.49-2.44)
COPD	1.59 (0.73-3.43)	0.89 (0.36-2.18)	1.16 (0.42-3.23)
IBD	0.44 (0.20-0.93)	0.83 (0.37-1.84)	0.78 (0.29-2.12)
Intestinal Failure	2.59 (1.58-4.25)	2.05 (1.18-3.57)	2.90 (1.43-5.87)
Pre-op resistant bacteria	1.43 (0.67-3.06)	0.83 (0.34-2.02)	1.09 (0.39-3.02)
N. previous abdominal surgeries ≥ 3	2.31 (1.27-4.19)	3.00 (1.39-6.42)	5.27 (1.57-17.67)
N. previous hernia surgeries			
• 1	1.41 (0.81-2.47)	0.86 (0.47-1.61)	0.97 (0.47-2.01)
• ≥ 2	0.61 (0.30-1.26)	0.38 (0.15-0.95)	0.22 (0.05-0.97)
Recurrent hernia	0.95 (0.58-1.55)	0.57 (0.32-1.00)	0.64 (0.32-1.28)
Previous component separation	1.48 (0.59-3.69)	0.95 (0.33-2.72)	0.60 (0.13-2.69)
Previous open abdomen	1.71 (1.05-2.79)	1.94 (1.12-3.36)	2.30 (1.16-4.55)
Wound classification			
• Contaminated	1.85 (1.04-3.30)	2.18 (1.13-4.24)	3.57 (1.49-8.57)
• Dirty	1.41 (0.72-2.76)	0.92 (0.40-2.10)	0.69 (0.19-2.47)
ECF	1.76 (1.06-2.90)	1.69 (0.96-2.99)	2.60 (1.22-5.53)
Stoma	1.14 (0.70-1.90)	0.84 (0.48-1.46)	1.33 (0.66-2.70)
Time from last operation	1.78(0.75-4.23)	2.16 (0.72-6.49)	2.44 (0.56-10.71)
Operation time			
• 3.5-5.5 hours	0.93 (0.43-2.03)	0.91 (0.37-2.26)	0.66 (0.17-2.59)
• 5.5-7.5 hours	2.02 (0.95-4.26)	2.13 (0.93-4.92)	3.40 (1.16-9.97)
• ≥ 7.5 hours	9.15 (3.62-23.1)	4.48 (1.73-10.1)	4.85 (1.59-14.81)
Mesh removal	0.96 (0.53-1.72)	0.58 (0.28-1.20)	0.69 (0.29-1.63)
Number of anastomosis			
• 1	1.58 (0.78-3.21)	3.22 (1.07-9.65)	1.77 (0.57-5.50)
• ≥ 2	3.46 (1.55-7.69)	10.0 (3.21-31.2)	4.40 (1.38-14.04)
Stoma takedown/relocation	1.42 (0.87-2.31)	1.40 (0.82-2.42)	1.46 (0.75-2.82)
Anterior component separation	1.85 (1.08-3.18)	2.79 (1.41-5.54)	2.15 (0.95-4.87)
Drain use	1.71 (0.86-3.39)	1.29 (0.60-2.75)	4.60 (1.07-19.77)
S/C drain	1.90 (1.00-3.62)	1.48 (0.72-3.07)	2.00 (0.75-5.37)
IA drain	1.20 (0.74-1.94)	1.26 (0.74-2.17)	1.56 (0.80-3.03)
Fascial closure	1.83 (1.06-3.17)	1.58 (0.87-2.87)	1.72 (0.85-3.48)

Table 3.8: Uni-variate analysis demonstrating significant factors associated with three key outcomes. Highlighted factors demonstrate a p-value of ≤ 0.05 and were used in multi-variate analysis.

The factors found to have a significant effect on the likelihood of a Clavien Dindo complication were entered into a backward stepwise multi-variate logistic regression model with removal and inclusion significance levels set at a p-value of 0.05. The multi-variate model retained the presence of intestinal failure ($p < 0.01$) and the total operative time ($p < 0.01$) as significant and were included in the final risk model (Table 3.9). Evaluation of the model using receiver operating characteristics revealed a c-statistic of 0.72 (95% CI 0.66-0.79) suggesting a reasonable ability of the model to discriminate between high and low risk patients.

Assessment of the model's discriminative ability was performed on the validation cohort. A comparison of the predictive percentage of the model on the validation cohort revealed a p-value of < 0.0001 suggesting the model has some ability to discriminate between patients (Figure 3.3). Receiver operating characteristics assessment of the model on the validation cohort demonstrated a c-statistic of 0.73 (95% CI 0.63-0.82) again suggesting the model can discriminate between patients for this outcome. Analysis of the model calibration was performed by splitting the cohort into three groups based on the predicted and observed probability of the outcome. This revealed evidence of good calibration as the predicted and observed complication rates were comparable in the three groups. Examination of this using the Hosmer-Lemeshow test showed a p-value of 0.12 consistent with good calibration of the model for this outcome (Table 3.10).

<u>Outcome</u>	<u>Variable</u>	<u>Category</u>	<u>Odds ratio (95% CI)</u>	<u>Regression coefficient</u>	<u>p- value</u>	<u>c-statistic</u>
Clavien Dindo ≥3	Constant	-	-	-1.80	-	0.72 (0.66-0.79)
Significance 0.05	Intestinal Failure	No Yes	1 3.08 (1.56-6.06)	1.12	<0.01	
Removal 0.05 Inclusion 0.05	Operation time	≤ 3.5 hours 3.5 - 5.5 hours 5.5 - 7.5 hours >7.5 hours	1 1.34 (0.53-3.35) 3.53 (1.46-8.52) 11.39 (3.92-33.16)	0.29 1.26 2.43	<0.01	
Wound Intervention	Constant	-	-	-3.02	-	0.76 (0.68-0.83)
Significance 0.05	Immuno- suppression	No Yes	1 3.02 (0.98-9.29)	1.11	0.05	
Removal 0.05 Inclusion 0.05	Intestinal Failure	No Yes	1 2.20 (1.11-4.37)	0.79	0.02	
	Operation time	≤ 3.5 hours 3.5 - 5.5 hours 5.5 - 7.5 hours >7.5 hours	1 0.88 (0.29-2.72) 2.20 (0.78-6.24) 3.17 (1.06-9.43)	-0.12 0.79 1.15	0.04	
	Number of anastomosis	0 1 2+	1 2.41 (0.63-9.21) 6.04 (1.46-25.0)	0.88 1.80	0.01	
IASC Intervention	Constant	-	-	-3.60	-	0.72 (0.64-0.80)
Significance 0.05	Immuno- suppression	No Yes	1 2.86 (1.03-7.90)	1.05	0.04	
Removal 0.05 Inclusion 0.10	N. previous abdominal surgeries	≤ 2 3+	1 4.36 (1.28-14.83)	1.47	0.02	
	Intestinal Failure	No Yes	1 2.61 (1.26-5.41)	0.96	0.01	

Table 3.9: Risk stratification models for Clavien Dindo complications ≥3, wound intervention, IASC intervention and further hernia surgery developed using multi-variate backward selection logistic regression. C-statistics derived using receiver-operating characteristic demonstrate values all greater than 0.70 suggesting a moderate discriminatory ability of all models.

Model 2 – Wound intervention

Uni-variate analysis demonstrated patient factors including the presence of intestinal failure, previous treatment with an open abdomen and the number of previous abdominal operations being greater than or equal to three as associated with the

requirement for wound intervention. Operative factors were also significantly associated with wound intervention, such as increasing operative time, the number of anastomoses and the use of component separation techniques (Table 3.8).

Multi-variate analysis retained pre-operative immunosuppression ($p=0.05$), intestinal failure ($p=0.02$), increasing operative time ($p=0.04$) and increasing number of anastomosis ($p=0.01$) in the final model. Assessment of the c-statistic demonstrated a reasonable ability of the model to discriminate between high and low risk patients ($c=0.76$; 95%CI 0.68-0.83) (Table 3.9).

On assessment of the model using the validation cohort, poor discrimination of the model was demonstrated. The predicted percentage of those who had a wound intervention versus those who did not were not significantly different on Mann-Whitney testing ($p=0.61$) and evaluation of this data with the c-statistic revealed a value of 0.54 (95% CI 0.36-0.73) (Figure 3.3). Calibration testing also did not demonstrate validation of the model with poor correlation between predicted and observed interventions and a significant difference between the observed number and the predicted values ($p<0.001$) (Table 3.10).

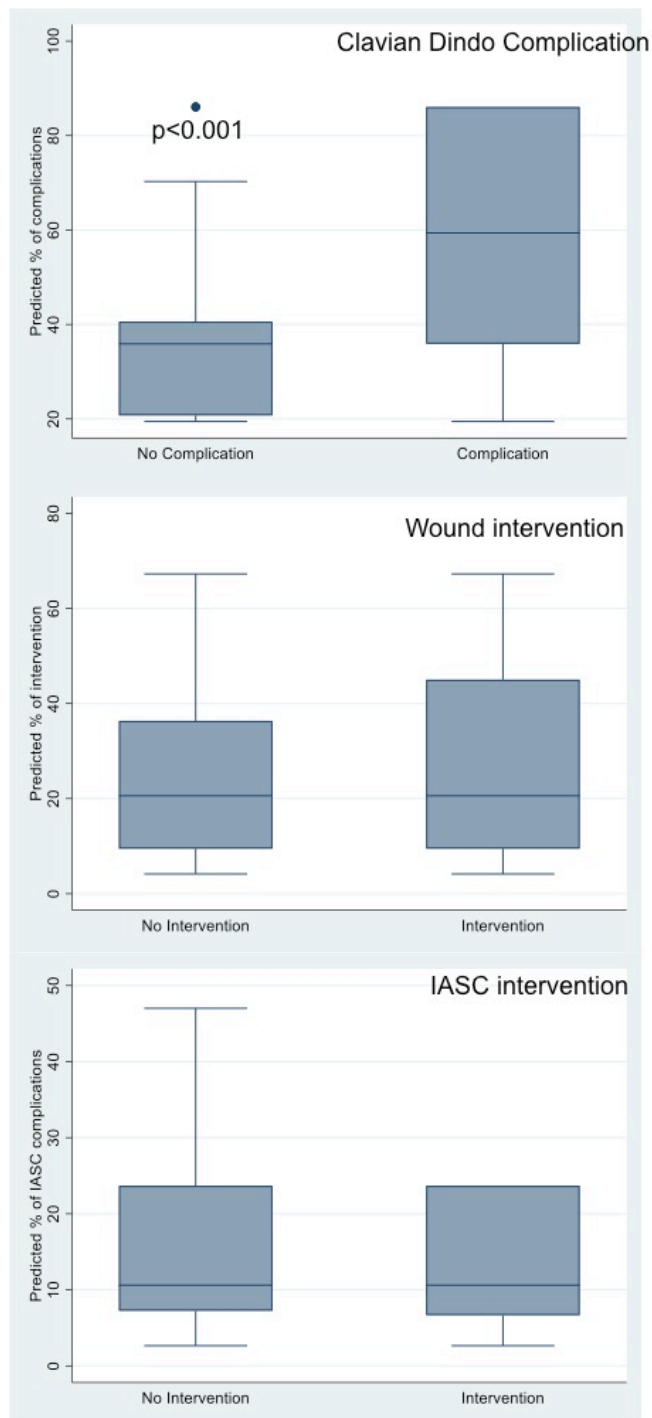


Figure 3.3: Evaluation of the discriminative ability of the models using predictive probability of a given outcome. A significant difference was seen between predicted groups when using the Clavian Dindo model suggesting the model has a moderate ability to discriminate between patients. The other two models did not demonstrate a significant difference.

Model 3 – Intra-abdominal septic complications

Analysis with uni-variate regression revealed only five factors to be associated with an IASC; pre-operative immunosuppression; pre-operative intestinal failure; previous treatment with an open abdomen; presence of an enterocutaneous fistula and a number of previous abdominal operations of three or more (Table 3.8). These factors were therefore entered into multi-variate analysis where pre-operative immunosuppression ($p=0.04$), pre-operative intestinal failure ($p=0.02$) and the number of previous abdominal operations ($p=0.02$) were retained in the final model. Again, evaluation with the ROC revealed a reasonable ability of the model to predict this outcome ($c=0.72$; 95% CI 0.64-0.80) (Table 3.9).

Assessment of the model using the validation cohort revealed no significant difference in the predicted percentage of intervention versus no intervention ($p=0.62$). Evaluation of this result using the c-statistic revealed a value of 0.47 (95% CI 0.32-0.61) suggesting poor ability of the model to discriminate between low and high-risk patients (Figure 3.3). On assessment of the calibration of the model there was no observed consistent pattern between the observed and predicted rate of intervention in each group and revealed the model significantly under-estimated the rate of intervention in the low risk group. Hosmer-Lemeshow testing showed a significant difference between the observed and predicted number of intervention ($p<0.001$) suggesting poor calibration of the model to predict this outcome (Table 3.10).

Model/Risk group	Number of patients	Observed number of complications (%)	Predicted number of complications (%)	Chi-squared statistic (p-value)
Clavien Dindo ≥ 3				
$\leq 25\%$	42	10 (23.8)	8.5 (20.4)	2.5 (0.12)
26-50%	35	12 (34.3)	13.5 (38.5)	
$\geq 50\%$	36	23 (63.9)	26.6 (74.0)	
Wound intervention				
$\leq 15\%$	43	5 (11.6)	3.4 (8.0)	19.7 (<0.001)
16-30%	39	5 (12.8)	8.4 (21.4)	
$\geq 30\%$	31	4 (12.9)	15.5 (50.1)	
IASC intervention				
$\leq 10\%$	34	9 (26.5)	1.6 (4.6)	37.2 (<0.001)
11-20%	41	4 (9.8)	4.2 (10.8)	
$\geq 20\%$	39	9 (23.1)	9.6 (24.5)	

Table 3.10: A table demonstrating calibration of the models. Comparison of the predicted vs observed percentage complications within each risk group reveals similarity in for the Clavien Dindo model but differences for the remaining two models. Hosmer-Lemeshow test demonstrates no significant difference in the Clavien Dindo model suggesting good calibration. The significant difference observed for the remaining two models suggests poor calibration.

Model 4 – Further hernia surgery

The long-term risk of further hernia surgery was evaluated and a model designed using the development cohort. Uni-variate analysis found patients with a BMI of more than 30, active smoking at the time of surgery and the inability to obtain fascial closure to be significantly associated with an increased risk of further hernia surgery (Table 3.11). Multi-variate analysis retained a BMI of more than 30 ($p=0.07$) and the inability to obtain fascial closure ($p=<0.01$) in the final model. Discriminatory ability of the model was assessed using the c-statistic, which demonstrated a moderate ability of the model ($c=0.72$; 95% CI 0.63-0.82) (Table 3.12).

Model 5 – Hernia recurrence

The final model designed evaluated overall long-term hernia recurrence. Uni-variate analysis only demonstrated the ability to obtain fascial closure as a significant factor associated with the development of recurrent hernia ($p < 0.01$) (Table 3.11). As a single factor evaluation with the c-statistic revealed a value of 0.70 (95% CI 0.63-0.77) suggesting some ability to discriminate between high and low risk patients (Table 3.12).

	Hernia recurrence	Further hernia surgery
Risk factor	OR (95%CI)	OR (95%CI)
Age	1.00 (0.98-1.02)	0.98 (0.96-1.01)
Sex (Female)	1.11 (0.66-1.86)	1.60 (0.78-3.27)
Obese	1.55 (0.85-2.85)	2.29 (1.06-4.93)
Smoker	1.53 (0.85-2.76)	2.17 (1.02-4.65)
Diabetes	0.40 (0.18-0.86)	0.69 (0.25-1.88)
Immunosuppression	0.89 (0.33-2.40)	1.73 (0.54-5.52)
Cardiac comorbidity	0.65 (0.34-1.25)	0.41 (0.14-1.22)
Pulmonary comorbidity	0.60 (0.30-1.20)	0.79 (0.31-2.01)
COPD	0.64 (0.26-1.55)	0.46 (0.10-2.02)
IBD	0.49 (0.22-1.12)	0.51 (0.15-1.75)
Intestinal Failure	0.75 (0.45-1.26)	0.73 (0.36-1.51)
Pre-op resistant bacteria	0.75 (0.32-1.75)	0.69 (0.20-2.42)
N. previous abdo surgeries ≥ 3	0.92 (0.51-1.65)	0.83 (0.37-1.84)
N. previous hernia surgeries		
• 1	0.74 (0.39-1.38)	1.02 (0.44-2.40)
• ≥ 2	1.67 (0.83-3.33)	1.66 (0.67-4.14)
Recurrent hernia	1.27 (0.80-2.14)	1.33 (0.65-2.71)
Previous component separation	1.06 (0.39-2.92)	0.74 (0.16-3.35)
Previous open abdomen	0.68 (0.41-1.15)	0.63 (0.30-1.31)
Wound classification		
• Contaminated	0.88 (0.49-1.60)	0.67 (0.30-1.52)
• Dirty	0.77 (0.38-1.55)	0.72 (0.28-1.87)
ECF	1.06 (0.63-1.79)	0.64 (0.31-1.20)
Stoma	0.62 (0.36-1.04)	0.49 (0.24-1.00)
Time from last operation	1.76 (0.68, 4.54)	0.23(0.03-1.77)
Operation time		
• 3.5-5.5 hours	1.58 (0.69-3.64)	1.32 (0.47-3.74)
• 5.5-7.5 hours	1.67 (0.73-3.81)	0.86 (0.28-2.63)
• ≥ 7.5 hours	1.74 (0.70-4.35)	0.99 (0.29-3.78)
Mesh removal	1.29 (0.70-2.39)	1.32 (0.58-3.00)
Number of anastomosis		
• 1	1.20 (0.59-2.47)	0.64 (0.24-1.70)
• ≥ 2	1.28 (0.56-2.88)	1.15 (0.40-3.28)
Stoma takedown/relocation	1.06 (0.63-1.78)	0.81 (0.40-1.68)
Component separation	1.01 (0.58, 1.76)	1.11 (0.50-2.46)
Fascial closure	4.50 (2.51-8.06)	3.99 (1.88-8.47)

Table 3.11: Uni-variate analysis demonstrating significant factors associated with long-term outcomes. Highlighted factors demonstrate a p-value of ≤ 0.05 and were used in multi-variate analysis.

<u>Outcome</u>	<u>Variable</u>	<u>Category</u>	<u>Odds ratio</u> (95% CI)	<u>Regression</u> coefficient	<u>p-</u> value	<u>c-statistic</u>
Hernia Recurrence Significance 0.05 Inclusion 0.05 Removal 0.10	Constant	-	-	-0.68	-	0.70 (0.63-0.77)
	Fascia Closed	Yes No	1 4.25 (2.21-8.18)	1.45	<0.01	
Further hernia surgery Significance 0.05 Removal 0.05 Inclusion 0.10	Constant	-	-	-2.77	-	0.72 (0.63-0.82)
	BMI >30	No Yes	1 2.20 (0.95-5.11)	0.79	0.07	
	Fascial closure achieved	Yes No	1 5.28 (2.35-11.87)	1.66	<0.01	

Table 3.12: Risk stratification models for hernia recurrence and further hernia surgery developed using multi-variate backward selection logistic regression. C-statistics derived using receiver-operating characteristic demonstrate values all greater than 0.70 suggesting a moderate discriminatory ability of all models.

3.2.4 Discussion

This study aims to evaluate short-term outcomes and risk-stratify patients undergoing contaminated (VHWG grade 3) complex abdominal wall reconstruction. This is an inherently heterogeneous and complex group of patients as evidenced by the extensive number of co-morbidities, high mean ASA grade, high number of previous abdominal operations and inherent surgical site contamination. Allowing for this, we demonstrate good short-term outcomes with acceptable rates of wound complication and post-operative enterocutaneous fistula recurrence. While the total number of patients who experienced any complication (84.9%) and the median length of stay are high, most complications were of low grade (CD1/2) this would be expected from such a complex type of surgery (144,145).

Here, we have demonstrated the development and validation of a risk stratification model for predicting Clavien Dindo complications in CAWR. The model appears to have good predictive ability both in discrimination and calibration tests. This is the first risk stratification tool designed and validated for use in contaminated ventral hernia surgery and the first that predicts the risk of a validated outcome measure (CD grade).

Models predicting other short-term outcomes (wound intervention and IASC intervention) have not demonstrated good predictive ability on validation. Previous studies in smaller cohorts have demonstrated some of the factors identified in these models to also be significant. The choice of wound and abdominal intervention as outcome measures has been selected based on giving the maximal clinical ability to counsel patients pre-operatively. For example, the ability to predict and inform a

patient they have high risk for a wound complication in a cohort of patients was a 50% chance of having a wound complication is of limited use (*Chapter 1.5*). The aim of these models is to aid in discrimination by defining a risk for a given patient of requiring surgical or radiological intervention for wound complications compared to simple measures, such as oral antibiotics. It is unclear why these models proved less effective on validation however it is likely due to the smaller size of the validation cohort. This was necessitated due to the available length of follow-up available. Work was undertaken to establish new collaborations for validating the model however no centre with adequate volume and a similar case mix, which were willing to collaborate, was identified. As the prevalence of these cases at these two institutions has increased in recent years a further 2-3 years of data should provide the evidence to validate or redevelop the current models.

The Ventral Hernia Risk Score (VHRS) is the best attempt, currently, to risk stratify patients undergoing ventral hernia surgery (59). This is a positive integer scoring system that groups patients into 3 categories for risk of SSO and 5 for risk of SSI. A variety of factors are included in both scores including concomitant hernia repair and skin flaps for both, use of mesh for SSO and ASA score greater than or equal to 3 and BMI greater than or equal to 40 for SSI. Both scores also include contaminated wound class as an independent predictor however if a patient has a contaminated wound this immediately places them into the highest risk bracket defined by the score despite the presence or absence of any other risk factor. This therefore limits its ability to discriminate in our cohort of patients. Our models aim to fill this gap.

Models for the prediction of long-term outcomes have also been developed and assessed. The ultimate aim of all hernia surgery prediction tools is to evaluate the highest risk patients for long-term hernia recurrence. This aim has proved elusive previously and continues to prove difficult to evaluate. On using hernia recurrence as the outcome the only factor found to be significant in stratifying patients was the ability to obtain fascial closure. This has been demonstrated previous as significant and as a single factor does not provide reasonable discrimination between patients. The need for further hernia surgery has always been regarded as a poor outcome measure for hernia surgery as, by definition, a number of patients who have, potentially debilitating, recurrences are excluded from this statistic. However in the context of significant contamination we have observed anecdotal evidence that patients with a small, asymptomatic, non-contaminated recurrence are happy with this outcome as they no longer have the source of contamination, for example the infected mesh or enterocutaneous fistula. Therefore we also evaluated further hernia surgery as an outcome as these are most likely to be the patients with a large symptomatic recurrence or on going or recurrent infection.

The model evaluating the risk of further hernia surgery proved to be a better tool for discriminating patients with an AUC of 0.72 on ROC analysis. The factors in this model have been shown to be related to hernia recurrence previously (75) and therefore this analysis is consistent with published literature. Further work would need to be performed to validate this model. Analysis performed by our collaborator at AMC on the time to recurrence and further hernia surgery demonstrated that both outcomes were most likely to occur within the first 3 years of the index operation. The average follow-up possible to achieve in our validation cohort was only 1 year

therefore a further analysis and follow-up of this cohort would need to be performed in 2-3 years time to aim to validate this model. Additionally collaboration with other centres could help in this validation however as previously discussed this was not possible as part of this work.

The principle limitation of this study is the retrospective nature of the majority of the data, particularly in the development cohort. Every effort has been made to ensure the robustness of the data collection including cross-checking case notes and clinic letters, review by independent data collectors and discussion with patients when they attended for prospective follow-up. Cases with co-existent intestinal failure were prospectively collected in the validation cohort thanks to a new clinical operative database, however this was not possible at the AMC. The age of some of this data also limits effectiveness. Given the changes in practice patterns, introduction of biologic mesh and novel wound therapies such as negative pressure have all occurred over the course of the included time period these are a number of possible confounders in operative decision making. Conversely the rarity of contaminated complex abdominal wall defects necessitated the inclusion of such a long time period to ensure sufficient patient numbers were included for regression analysis. When looking at the overall short- and long-term outcomes, while it does not definitively appear some factors, such as mesh choices, have made a large difference to the outcomes analysed, more recent developments, such as closed incision NPWT, may have effected wound outcomes particularly in the validation cohort and therefore may have effected the validation for this outcome.

Another limitation lies within the use of regression analysis and is affected by the paucity of previous analysis in to risk factors in this cohort. The ideal methodology for regression analysis would use previously identified risk factors and perform multi-variate analysis to identify the overall effect of each factor as well as which are should be included in the risk model. In order to avoid over-fitting the model a factor can only be included in the multi-variate analysis for every 10 events for that outcome occurring in the dataset. Due to the lack of previous analysis of risk factors in this cohort we have used uni-variate analysis to identify significant variables to include in the multi-variate analysis that puts the model at risk of over-fitting. However, the use of subsequent backward selection in multi-variate and good area under the curve minimise this and improve the chances of model validation. This is demonstrated in the successful validation of the model for Clavien Dindo outcome.

The unsuccessful validation of the other short-term models could be related to over-fitting but could also be due to the lower number of events in the validation cohort, which could weaken the model and amplify the effect of small differences in the cohorts. The other factor that could have affected the result for the wound complication model is the implementation of NPWT, which was used much more frequently in the validation cohort and could have reduced the number of wounds requiring radiological or surgical intervention. These issues could be dealt with by increasing the patient number within the validation cohort or redesigning the model for these outcomes combining the development and validation cohorts a using a further prospective validation cohort with more up-to-date practice patterns.

The acceptable AUC seen for the further hernia surgery model implies a moderate likelihood of future validation of this model. This could be achieved by further evaluation of the current validation cohort in 2-3 years given the required follow-up time period and likely number of recurrence surgeries within the cohort (112 patients with a likelihood of 20% requiring further hernia surgery) allowing for maintaining the 10 to 1 rule for regression analysis.

Despite these limitations this remains the largest study to evaluate risk factors and risk models for patients undergoing CAWR. The ability to accurately predict the likelihood of Clavien Dindo outcome in these patients will vastly improve the ability of the surgeon to counsel patients pre-operatively as well as resource plan for the surgery, including ensuring the availability of high dependence post-operative care for high-risk patients. This model is available online at smbari.co.uk/smjconv2?v=3.01/ for use by clinicians.

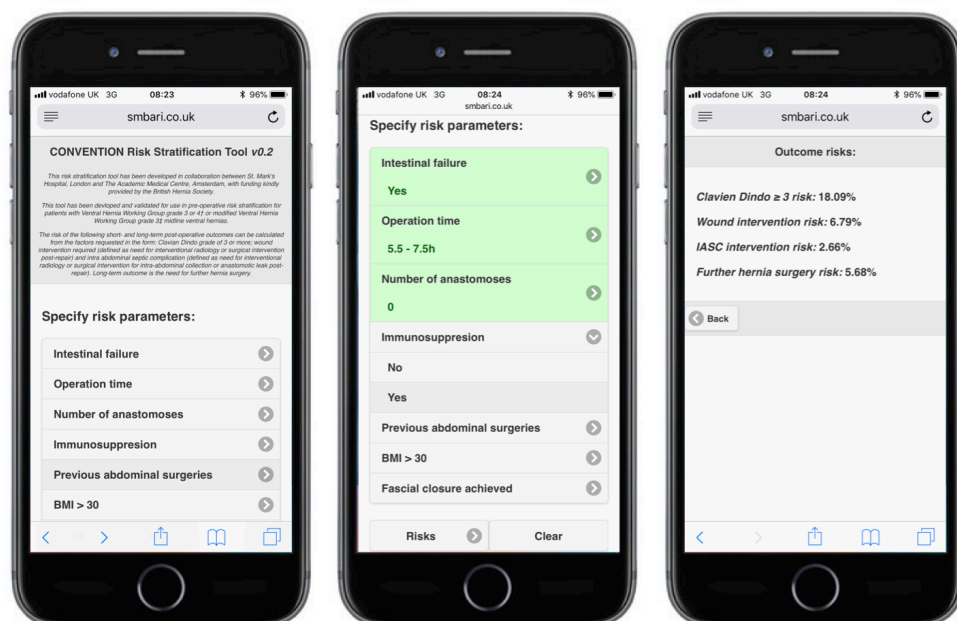


Figure 3.4: Online webtool calculator available at smbari.co.uk/smjconv2?v=3.01/.

While the other models developed in this study require further work to evaluate, this study marks an initial step in the targeted evaluation of these complex patients pre-operatively. Given the rarity of the condition the only way to prospectively evaluate short- and long-term outcomes is through collaboration and national/international database development. The identification of significant risk factors in this study could provide the basis of what factors should be stored in such a database given the routine collection of the number of factors initially analysed in this cohort would unlikely be feasible in the long term.

3.3 Evaluation of the use of CT imaging in the assessment of patients undergoing contaminated complex abdominal wall reconstruction

The statistical analysis performed in this chapter was carried out by myself with the assistance of Professor S Pokrel of Brunel University.

3.3.1 Introduction

We have demonstrated the ability of pre-operative factors in assisting the assessment of patients undergoing CAWR. As discussed in the introduction (*Chapter 1.4.4*) previous data has suggested CT imaging may provide additional information as to this assessment, however these data have only been evaluated in clean cases and small cohorts. This aim of this study is to evaluate CT imaging in contaminated ventral hernia cases.

The first step of this process was to define the measurements to be taken and evaluate their assessment. Previous studies have described a wide range of possible measurements, however no standardisation of these measurements has ever been described. Secondly, we aim to evaluate these measurements and establish which are related to the outcomes evaluated in 3.2 and therefore would be beneficial to measure. Ideally CT measurements would be ultimately included in our risk stratification models however lack of previous evaluation of CT and the fact CT has not been a standard technique for evaluation of the abdominal wall in the past means at present the goal is not possible. This chapter therefore aims to establish which measurements could be focussed for inclusion in our risk models by evaluating currently available CT data.

3.3.2 Methods

3.3.2.1 Patient population, CT validity and definitions

Patients were drawn from both the development and validation cohorts described in *Chapter 3.2*. Ethical approval was obtained as part of the overall study design both locally and nationally at both centres. Briefly consecutive patients undergoing contaminated abdominal wall reconstruction at St Mark's Hospital and the Academic University Medical Centres in Amsterdam, from 2004 to 2017, were evaluated. Those patients who had a CT scan performed for operative planning were included in this study. CT scans were included if they had been performed within 1 year prior to the CAWR operation date. Scans were excluded if the scan was performed outside of this timeframe, within 1 month of a prior operation or if there was a co-existent, potentially confounding, acute pathology, for example small bowel obstruction.

Patient demographics and short-term outcomes, including use of bridging mesh and wound complications, were identified by retrospective case note review. Patients with more than 2-year follow-up were invited to attend a single out-patient appointment to evaluate long-term hernia recurrence. Data were anonymised and stored in an Excel spreadsheet (Microsoft, Redmond, USA). Data analysis was performed using SPSS V24 (IBM Analytics, New York, USA).

3.3.2.2 Determination and standardisation of measurement techniques

Measurements were performed as described by previous studies (95,97,146–148) and can be seen in Table 3.13. Two independent consultant radiologists evaluated the first 50 complete scans identified at St Mark's Hospital. Assessors were blinded to patient demographics, operative details and follow-up outcomes.

<u>Radiology measurement consensus statement</u>
<u>Defect size</u> - Defect width – measured at widest point of defect (A) - Defect length – Longest point of defect in the midline (B) - Defect area – calculated from length and width as area of an ellipse ($\pi(A/2)(B/2)$) Additional points: - If pre-existing mesh include as part of defect as it would be excised during surgery. - If multiple midline defects chose largest for width and use length incorporating all defects and they would most likely be jointed into one during laparotomy. - If significant rectus divarication only include length measurement up to point of previous scarring or evidence of infection.
<u>Rectus muscle dimensions</u> - Rectus width – measurement at point of greatest defect width; measure up to rectus fascia (measured right C and left D) - Average width calculated as mean of left and right measurement ($(C+D)/2 = E$) - Rectus thickness – measurement at point of greatest defect width; measure up to rectus fascia (measured right F and left G) - Average width calculated as mean of left and right measurement ($(F+G)/2 = H$) - Rectus muscle area – calculated from average width and thickness ($\pi(E/2)(H/2)$)
<u>Lateral abdominal wall thickness</u> - Muscle – measurement at point of greatest defect width; measure at 3 and 9 o'clock (I) - Pannus – measurement at point of greatest defect width; measure at 3 and 9 o'clock (J) - Total – calculated from above two measurements (I+J)
<u>Circumference</u> - Abdominal circumference – measurement at point of greatest defect width; measure total circumference of skin including hernia sac (K) - Muscle circumference – measurement at point of greatest defect width; measure circumference of muscle with straight line drawn across hernia defect between rectus muscles (L) - Circumference difference – calculated from above two measurements (K-L) - Defect percentage – calculated from two measurements ($(A/K) \times 100$)
<u>Volumes</u> - Intra-abdominal volume – total intra-abdominal volume including all intra-peritoneal organs (excluding retroperitoneum) (M) - Hernia volume – volume of contents of hernia sac (N) - Total volume – calculated from above two measurements (M+N)=O - HV:IAV ratio – (N/O)

Table 3.13: Details of CT measurements taken, consensus between assessors and how derived measurements were calculated.

Differences between the assessors were evaluated using inter-class correlation coefficient, values of greater than 0.6 were considered good correlation and greater than 0.75 were considered excellent. On initial evaluation some values, such as defect length and rectus measurements, were found to have unacceptable inter-rater reliability. Subsequent discussion between raters demonstrated slight differences in measuring style. Consensus was reached and further independent assessment of these variables improved the reliability to acceptable levels and the agree measurements are described in Table 3.13. Further composite variables were calculated from measured values, such as defect area, to give a more complete assessment of the hernia and patient.

3.3.2.3 Evaluation of CT measurements for whole cohort outcomes

CT measurements across the whole cohort were evaluated to assess for factors associated with adverse outcome. The selected outcomes of interest were the same as in *Chapter 3.2* to allow an understanding of what CT measurements maybe included in each model. The primary outcome was Clavien Dindo grade of 3 or more. Secondary outcomes were wound complication, wound intervention, IASC, IASC intervention, hernia recurrence and further hernia surgery, as previously described (*Chapter 3.2*).

3.3.2.4 Statistical analysis

Given the retrospective nature of this study and the fact some of the CT scans were not performed for explicit evaluation of the abdominal wall, some measured factors were found to have a level of missing data. These data was determined to be missing at random and therefore multiple imputation with predictive mean matching (imputed

datasets n=5; SPSS V24, IBM, New York, USA) was used to derive missing values for continuous CT derived measurements (149). Reports for number of missing values and distribution of imputed data can be found in *Appendix 1*.

Uni-variate binary logistic regression analysis was used to determine CT measurements associated with selected outcomes. Given no previous study has evaluated CT measurements in this cohort a lower threshold for inclusion in multi-variate analysis was chosen. A p-value of <0.10 was taken to be significant and significant factors were assessed using multi-variate regression to determine factors predictive of worse outcome.

Further analysis was performed to define “cut-offs” for where continuous CT measured values were likely to be significant for variables identified on multi-variate analysis. Receiver operating curve analysis was performed to calculate sensitivity and specificity values across the range of significant CT variables. Youden’s Index was calculated to determine cut-off values where the CT derived measurement was most significant.

3.3.3 Results

3.3.3.1 Patient demographics

Across the two centres, 366 patients were identified with a total of 386 procedures in the date range. Of these, 139 had a CT that met the inclusion criteria. One of these procedures was complicated by intra-operative haemorrhage that required packing and 24 hour management with a laparostomy. As this could have influenced the short and long-term outcome in this case, it was excluded from the final analysis leaving 138 patients remaining.

The mean age of the cohort was 58.81 (SD 13.1), with 76 (55.1%) males. Seventy-five patients (54.3%) had pre-existing intestinal failure and 57 (41.3%) had a history of treatment with an open abdomen. All defects were defined as modified-VHVG grade 3, including 85 (61.6%) with an enterocutaneous fistula.

The median length of stay was 21 days (IQR 11-35). Sixty-four patients (46.4%) had a complication of grade 3 or higher on the Clavien Dindo classification. Seventy-eight patients (56.5%) had a surgical site occurrence, of which, 32 (23.2%) required radiological or surgical intervention. The median long-term follow-up was 24 months (IQR 8.5-41) and 27 patients, of 105 with long term-follow-up, (25.7%) had a recurrent hernia. Further demographic data can be found in Table 3.14.

	N = 138 (%)
Age (Mean/SD)	58.81 (13.1)
Sex (Male)	76 (55.1)
BMI (Mean/SD)	27.45 (6.22)
ASA classification	
1	1 (0.7)
2	73 (53.6)
3	62 (44.9)
4	2 (1.4)
Active smoker	27 (19.6)
Diabetes	29 (21.0)
IBD	25 (18.1)
Immunosuppression	12 (8.7)
Cardiac Comorbidity	34 (24.6)
Pulmonary Comorbidity	43 (31.2)
Hypertension	44 (31.9)
Intestinal Failure	75 (54.3)
No previous abdominal operations (median, IQR)	3 (2-5)
No previous hernia repairs (median, IQR)	0 (0-1)
History of open abdomen	57 (41.3)
CDC wound classification	
C-C	26 (18.8)
Contaminated	60 (43.5)
Dirty	52 (37.7)
Presence of enterocutaneous fistula	85 (61.6)
Previous hernia repair	65 (47.1)
Presence of stoma	83 (60.1)
Operative details	
Use of bridging mesh	24 (17.6)
Component separation used	89 (64.5)
Mesh type used	
No mesh	50 (36.2)
Biologic	69 (50.0)
Synthetic non-absorbable	10 (7.2)
Synthetic absorbable	9 (6.5)
Post-operative outcomes	
Length of stay (days) (median (IQR))	21 (11-35)
Average follow-up (months) (median, IQR)	24 (8.5-41)
Clavien Dindo complication >3	64 (46.4)
Surgical site occurrence	78 (56.5)
Wound intervention required	32 (23.2)
Intra-abdominal septic complication	28 (20.3)
IASC intervention	23 (16.7)
Recurrent hernia (n=105)	27 (25.7)
Recurrent fistula	7 (5.1)

Table 3.14: Baseline demographics of all included patients (n=138).

3.3.3.2 Inter-rater variability assessment

All CT measurements were performed on the first 50 identified scans that met the inclusion criteria. As there has been no previous standardisation of measurement techniques, initial measurements were taken and assessed for inter-rater variability. A consensus meeting was then performed between the assessor and primary investigator. Where disagreement was discovered in measured values, between the assessors, discussion and consensus, as to how the measurements were taken, was reached. A consensus statement detailing these findings can be found in Table 3.13.

Measured variable	Inter-class correlation coefficient Single measures (95%CI)	Inter-class correlation coefficient Average measures (95%CI)
Defect Width	0.930 (0.876-0.960)	0.963 (0.934-0.980)
Defect Length	0.946 (0.905-0.970)	0.972 (0.950-0.985)
Defect Area	0.955 (0.920-0.975)	0.977 (0.959-0.987)
Rectus width average	0.697 (0.509-0.821)	0.821 (0.675-0.902)
Rectus thickness average	0.753 (0.592-0.856)	0.859 (0.743-0.922)
Rectus area average	0.790 (0.648-0.879)	0.882 (0.786-0.935)
Lateral wall muscle average	0.735 (0.531-0.858)	0.847 (0.694-0.924)
Lateral wall pannus average	0.922 (0.850-0.960)	0.959 (0.919-0.980)
Lateral wall total average	0.934 (0.869-0.967)	0.966 (0.930-0.983)
Abdominal circumference	0.819 (0.693-0.896)	0.900 (0.819-0.945)
Muscle circumference	0.736 (0.546-0.846)	0.848 (0.723-0.916)
Circumference difference	0.909 (0.840-0.949)	0.952 (0.913-0.974)
Defect percentage	0.893 (0.814-0.940)	0.944 (0.897-0.969)
Intra-abdominal volume	0.881 (0.793-0.933)	0.937 (0.884-0.966)
Hernia volume	0.928 (0.872-0.960)	0.963 (0.932-0.980)
Hernia:peritoneal volume ratio	0.918 (0.855-0.954)	0.957 (0.922-0.977)

Table 3.15: Inter-class correlation co-efficient for CT derived measurements comparing the assessment of two independent consultant radiologists.

Final analysis was performed on measured variables derived following the consensus meeting and all inter-class correlation coefficients were found to be acceptable to excellent (Table 3.15).

3.3.3.3 Whole cohort analysis of measurements

Mean values for whole cohort CT measurements can be found in Table 3.16. The average defect width is 11.80cm (SD 5.68) with an average defect area of 18.98cm² (SD 5.89). Composite measurements including the ratio of lateral abdominal wall muscle to pannus thickness and the intra-abdominal to hernia volumes ratio are also calculated. It can be seen that the average defects across the cohort are large with a significant loss of domain.

The percentage missing values for each measurement can also be found in Table 3.16. Following multiple imputation with predicted mean matching the derived mean values are presented and compared to real values and no significant difference is seen (Table 3.16).

3.3.3.4 Determining CT measured risk factors for modelled outcomes

3.3.3.4.1 Model 1 – Clavien Dindo grade 3 or more

Uni-variate analysis of the original data revealed larger lateral pannus thickness ($p=0.04$), higher lateral pannus to muscle ratio ($p=0.06$) and great difference between muscle and abdominal circumference ($p=0.03$). On multi-variate analysis none of these factors retained significance for developing a Clavien Dindo complication of grade 3 or more (Table 3.17).

When evaluating the imputed dataset in the same way the same three factors were found to be significant ($p=0.01$, $p=0.01$ and $p=0.02$ respectively). Additionally higher total lateral abdominal wall thickness ($p=0.03$) and lower LSMI ($p=0.05$) were also statistically significant. On multi-variate analysis higher lateral pannus to muscle ratio ($p=0.01$) and lower LSMI ($p=0.04$) were found to be related to Clavien Dindo outcome (Table 3.17).

	Original data Mean (SD)	Imputed data Mean (SD)	Percentage missing in original set
Defect Width (cm)	11.80 (5.68)	11.80 (5.68)	0
Defect Length (cm)	18.98 (5.89)	18.98 (5.89)	0
Defect Area (cm ²)	18.81 (12.19)	18.81 (12.19)	0
Rectus muscle width average (mm)	49.55 (17.56)	49.55 (17.56)	0
Rectus muscle thickness average (mm)	11.95 (5.05)	11.95 (5.05)	0
Rectus area average (mm ²)	49.09 (26.87)	49.09 (26.87)	0
Lateral abdominal wall muscle thickness average (mm)	21.10 (6.60)	21.22 (6.25)	16.7
Lateral abdominal wall pannus thickness average (mm)	19.44 (12.47)	20.30 (12.33)	21.7
Lateral abdominal wall thickness total average (mm)	40.69 (15.43)	41.52 (15.08)	21.7
Average ratio lateral muscle:pannus	0.96 (0.63)	1.01 (0.63)	21.7
Defect percentage	11.1 (4.77)	11.31 (10.02)	9.4
Abdominal circumference	102.29 (14.77)	103.14 (14.26)	9.4
HV:IAV ratio	5.92 (6.07)	6.33 (5.47)	37.0
Lumbar skeletal muscle index	42.18 (12.13)	41.08 (10.75)	37.7

Table 3.16: A table demonstrating the mean CT based measurements derived from included patients. The real observed values are compared to the imputed values for all variables without significant difference.

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	1.02 (0.96-1.08)	0.55			1.03 (0.97-1.09)	0.42		
Defect Length (mm)	0.99 (0.93-1.05)	0.67			1.00 (0.95-1.06)	0.90		
Defect Area (cm ²)	1.00 (0.97-1.03)	0.94			1.01 (0.98-1.04)	0.58		
Rectus muscle average width	1.00 (0.98-1.02)	0.89			1.01 (0.99-1.03)	0.34		
Rectus muscle average thickness	1.02 (0.95-1.09)	0.64			1.03 (0.96-1.10)	0.45		
Rectus muscle average area	1.00 (0.99-1.02)	0.52			1.01 (0.99-1.02)	0.17		
Average lateral muscle thickness	1.00 (0.95-1.06)	0.90			1.00 (0.95-1.06)	0.89		
Average lateral pannus thickness	1.04 (1.00-1.07)	0.04			1.04 (1.01-1.07)	0.01		
Average lateral total thickness	1.02 (0.99-1.05)	0.11			1.03 (1.00-1.05)	0.03		
Average muscle:pannus ratio	1.96 (0.98-3.90)	0.06			2.11 (1.19-3.74)	0.01	2.16 (1.20-3.87)	0.01
Circumference difference	1.05 (1.00-1.09)	0.03			1.04 (1.01-1.09)	0.02		
Defect percentage	1.00 (0.93-1.08)	0.95			1.00 (0.94-1.08)	0.91		
HV:IAV ratio	1.05 (0.98-1.13)	0.19			1.03 (0.97-1.09)	0.47		
LSMI	0.99 (0.95-1.02)	0.46			0.97 (0.94-1.00)	0.05	0.97 (0.94-0.99)	0.04
Sarcopenia muscle	0.65 (0.27-1.55)	0.33			1.46 (0.74-2.87)	0.28		

Table 3.17: Uni- and multi-variate analysis evaluating Clavien Dindo outcome for original and imputed data.

3.3.3.4.2 Model 2 – Wound complication

The analysis of the original CT measurements in patients who had a surgical site occurrence demonstrated the average lateral abdominal wall pannus ($p=0.01$), total lateral abdominal wall thickness ($p=0.02$) and the average ratio of lateral abdominal wall muscle to pannus ($p=0.02$) were found to be significant on uni-variate analysis. Multi-variate analysis retained only higher muscle to pannus ratio (OR 2.08; $p=0.04$) as significant risk factor for SSI (Table 3.18).

When considering the analysis of the imputed data the same three factors were found to be significant on uni-variate analysis ($p=0.01$, $p=0.02$ and $p=0.02$ respectively). Additionally circumference difference was found to be significant ($p=0.03$). On multi-variate analysis only higher muscle to pannus ratio (OR 1.98; $p=0.03$) was retained as a significant risk factor for SSO, which is consistent with the original data (Table 3.18).

3.3.3.4.3 Model 3 – Wound intervention

On analysis of the CT data to predict radiological or surgical intervention for a wound complication no factors were found to be significant on uni-variate analysis. When evaluating the imputed data only a higher pannus to muscle ratio (OR 2.09; $p=0.02$) was found be associated with wound intervention (Table 3.19).

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	1.01 (0.95-1.08)	0.66			1.01 (0.96-1.08)	0.66		
Defect Length (mm)	1.01 (0.95-1.07)	0.73			1.01 (0.95-1.07)	0.73		
Defect Area (cm ²)	1.00 (0.98-1.03)	0.77			1.00 (0.98-1.03)	0.77		
Rectus muscle average width	1.01 (0.99-1.03)	0.39			1.01 (0.99-1.03)	0.39		
Rectus muscle average thickness	0.99 (0.93-1.06)	0.76			0.99 (0.93-1.06)	0.76		
Rectus muscle average area	1.01 (0.99-1.02)	0.32			1.01 (0.99-1.02)	0.32		
Average lateral muscle thickness	1.01 (0.96-1.07)	0.66			1.02 (0.96-1.07)	0.55		
Average lateral pannus thickness	1.04 (1.00-1.08)	0.03			1.04 (1.01-1.07)	0.01		
Average lateral total thickness	1.03 (1.00-1.06)	0.05			1.03 (1.00-1.05)	0.02		
Average muscle:pannus ratio	2.08 (1.05-4.10)	0.04	2.08 (1.05-4.10)	0.04	1.98 (1.10-3.55)	0.02	1.98 (1.10-3.55)	0.02
Circumference Difference	1.03 (0.99-1.07)	0.20			1.04 (1.01-1.08)	0.03		
Defect percentage	0.99 (0.92-1.07)	0.79			1.00 (0.93-1.05)	0.93		
HV:IAV ratio	0.96 (0.92-1.07)	0.24			0.99 (0.93-1.05)	0.66		
LSMI	0.99 (0.95-1.02)	0.52			0.99 (0.96-1.02)	0.61		
Sarcopenia muscle	0.72 (0.30-1.70)	0.45			1.45 (0.73-2.88)	0.29		

Table 3.18: Binary logistic regression analysis comparing patients who had a surgical site occurrence versus those who did not. The higher the average ratio of lateral abdominal wall muscle to pannus was found to be associated with worse outcome suggesting patients with a higher BMI are more likely to develop a SSO.

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	0.98 (0.92-1.06)	0.65			0.98 (0.92-1.06)	0.65		
Defect Length (mm)	1.00 (0.93-1.07)	0.89			0.99 (0.93-1.06)	0.89		
Defect Area (cm ²)	0.99 (0.96-1.02)	0.55			0.99 (0.96-1.02)	0.55		
Rectus muscle average width	1.00 (0.98-1.02)	0.99			1.00 (0.98-1.02)	0.99		
Rectus muscle average thickness	0.98 (0.90-1.06)	0.59			0.98 (0.90-1.06)	0.59		
Rectus muscle average area	1.00 (0.99-1.02)	0.84			1.00 (0.99-1.02)	0.84		
Average lateral muscle thickness	0.96 (0.86-1.03)	0.27			0.95 (0.89-1.02)	0.17		
Average lateral pannus thickness	1.01 (0.97-1.05)	0.62			1.02 (0.99-1.05)	0.17		
Average lateral total thickness	1.00 (0.97-1.03)	0.89			1.01 (0.98-1.03)	0.57		
Average muscle:pannus ratio	1.68 (0.84-3.36)	0.14			2.09 (1.13-3.88)	0.02		
Circumference Difference	1.01 (0.97-1.06)	0.67			1.03 (0.99-1.07)	0.13		
Defect percentage	0.98 (0.89-1.07)	0.60			0.98 (0.90-1.06)	0.60		
HV:IAV ratio	0.91 (0.75-1.10)	0.33			0.99 (0.94-1.02)	0.78		
LSMI	0.99 (0.93-1.06)	0.74			0.98 (0.94-1.02)	0.25		
Sarcopenia muscle	0.49 (0.09-2.71)	0.42			0.86 (0.39-1.92)	0.71		

Table 3.19: Binary logistic regression analysis comparing patients who had wound intervention versus those who did not. Only muscle to pannus ratio was found to be significant in the imputed data.

3.3.3.4.4 Model 4 – Intra-abdominal septic complication

When looking at IASCs lower rectus muscle thickness ($p=0.09$), higher lateral pannus ($p=0.07$) and total ($p=0.09$) lateral abdominal thickness, and lower hernia to intra-abdominal volume ratio ($p=0.04$) were found to be significant on uni-variate analysis. No factors retained significance on multi-variate analysis (Table 3.20).

When evaluating the imputed dataset, lower rectus muscle thickness ($p=0.09$) and lower hernia to intra-abdominal volume ratio ($p=0.04$) were again found to be significant. Higher pannus to muscle ratio ($p=0.09$) and higher circumference difference ($p=0.06$) were also found to be significant. The multi-variate model retained higher circumference difference (OR 1.05; $p=0.03$) and lower hernia to intra-abdominal volume ratio (OR 0.89; $p=0.02$) were found to be associated with IASC (Table 3.20).

3.3.3.4.5 Model 5 – IASC intervention

When looking at the original data for IASC intervention only lower hernia to intra-abdominal volume ratio ($p=0.09$) was found to be significant. When looking at the imputed data a higher lateral abdominal pannus to muscle ratio ($p=0.05$) and circumference difference ($p=0.07$), as well as lower LSMI ($p=0.09$), were found to be significant on uni-variate analysis. No factors retained significance on multi-variate analysis in either the original or the imputed data (Table 3.21).

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	0.96 (0.89-1.04)	0.34			0.96 (0.89-1.04)	0.34		
Defect Length (mm)	0.95 (0.89-1.02)	0.16			0.95 (0.89-1.02)	0.16		
Defect Area (cm ²)	0.97 (0.94-1.01)	0.16			0.97 (0.94-1.01)	0.16		
Rectus muscle average width	0.99 (0.97-1.02)	0.53			0.99 (0.97-1.02)	0.53		
Rectus muscle average thickness	0.92 (0.84-1.01)	0.09			0.92 (0.84-1.01)	0.09		
Rectus muscle average area	0.99 (0.97-1.01)	0.25			0.99 (0.97-1.01)	0.25		
Average lateral muscle thickness	1.01 (0.95-1.08)	0.73			1.01 (0.94-1.08)	0.83		
Average lateral pannus thickness	1.03 (0.99-1.07)	0.07			1.03 (0.99-1.06)	0.11		
Average lateral total thickness	1.03 (0.99-1.06)	0.09			1.02 (0.99-1.05)	0.16		
Average muscle:pannus ratio	1.77 (0.88-3.53)	0.11			1.73 (0.92-3.24)	0.09		
Circumference Difference	1.02 (0.98-1.07)	0.30			1.04 (1.00-1.08)	0.06	1.05 (1.01-1.09)	0.03
Defect percentage	0.92 (0.84-1.02)	0.11			0.95 (0.87-1.04)	0.25		
HV:IAV ratio	0.82 (0.68-0.99)	0.04			0.90 (0.82-0.99)	0.04	0.89 (0.80-0.98)	0.02
LSMI	1.01 (0.96-1.06)	0.77			0.99 (0.95-1.03)	0.50		
Sarcopenia muscle	0.63 (0.19-2.10)	0.45			0.75 (0.32-1.73)	0.50		

Table 3.20: Binary logistic regression analysis comparing patients who had an IASC versus those who did not.

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	1.00 (0.92-1.08)	0.92			1.00 (0.92-1.08)	0.92		
Defect Length (mm)	1.00 (0.93-1.08)	0.95			1.00 (0.93-1.08)	0.95		
Defect Area (cm ²)	0.99 (0.96-1.03)	0.77			0.99 (0.96-1.03)	0.77		
Rectus muscle average width	1.00 (0.98-1.03)	0.89			1.00 (0.98-1.03)	0.89		
Rectus muscle average thickness	0.97 (0.88-1.06)	0.52			0.97 (0.88-1.06)	0.52		
Rectus muscle average area	1.00 (0.98-1.02)	0.99			1.00 (0.98-1.02)	0.99		
Average lateral muscle thickness	0.98 (0.91-1.06)	0.68			0.99 (0.92-1.06)	0.73		
Average lateral pannus thickness	1.03 (0.99-1.07)	0.15			1.03 (0.99-1.06)	0.12		
Average lateral total thickness	1.02 (0.99-1.05)	0.30			1.02 (0.99-1.05)	0.26		
Average muscle:pannus ratio	1.86 (0.88-3.92)	0.10			2.00 (1.01-3.95)	0.05		
Circumference Difference	1.02 (0.97-1.07)	0.42			1.04 (0.99-1.08)	0.07		
Defect percentage	0.95 (0.85-1.05)	0.32			0.98 (0.89-1.07)	0.62		
HV:IAV ratio	0.77 (0.75-1.04)	0.09			0.93 (0.84-1.02)	0.13		
LSMI	0.97 (0.90-1.04)	0.33			0.96 (0.92-1.01)	0.09		
Sarcopenia muscle	0.40 (0.07-2.19)	0.29			1.00 (0.41-2.47)	1.00		

Table 3.21: Binary logistic regression analysis comparing patients who had intervention for an IASC versus those who did not.

3.3.3.4.6 Model 6 – Hernia recurrence

Uni-variate analysis of the original data revealed higher defect width ($p=0.03$) and defect area ($p=0.04$) and lower lateral abdominal muscle thickness ($p=0.02$) to be significant. Multi-variate analysis retained larger defect width ($p=0.07$) and lower lateral abdominal muscle thickness ($p=0.02$) as significantly associated with hernia recurrence.

Analysis of risk factors for long-term hernia recurrence compared to imputed CT measurements found patients who had a recurrence were significantly more likely to have larger defects (defect width ($p=0.03$), defect area ($p=0.04$) and defect percentage (0.04)). Average lateral muscle thickness ($p=0.01$) was found to be lower in those whose hernias recurred. Higher LSMI ($p=0.01$) and the absence of sarcopenia ($p=0.02$) were also found to be associated with hernia recurrence. Multi-variate analysis retained defect width (OR 1.13; $p=0.007$) and lower average lateral abdominal wall muscle thickness (OR 0.85; $p=0.005$) and higher LSMI (OR 1.11; $p=0.002$) as significant risk factors for hernia recurrence (Table 3.22).

3.3.3.4.7 Model 7 – Further hernia surgery

Uni-variate analysis revealed lower circumference difference ($p=0.08$), higher LSMI ($p=0.06$) and the absence of sarcopenia ($p=0.04$) were significantly associated with further hernia surgery. However, none of these factors were retained in the multi-variate model as significant.

When evaluating the imputed data lower lateral abdominal muscle thickness ($p=0.08$) and higher LSMI ($p=0.04$) and the absence of sarcopenia ($p=0.04$) were significantly

associated with further hernia surgery. The multi-variate model retained lower lateral abdominal muscle thickness ($p=0.03$) and the absence of sarcopenia ($p=0.01$) as factors significantly associated with further hernia surgery (Table 3.23).

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	1.09 (1.01-1.17)	0.03	1.08 (0.99-1.18)	0.07	1.09 (1.01-1.17)	0.03	1.13 (1.03-1.24)	0.007
Defect Length (mm)	1.08 (1.00-1.17)	0.05			1.08 (0.99-1.17)	0.05		
Defect Area (cm ²)	1.04 (1.00-1.07)	0.04			1.04 (1.00-1.07)	0.04		
Rectus muscle average width	0.98 (0.96-1.01)	0.13			0.98 (0.96-1.01)	0.13		
Rectus muscle average thickness	0.94 (0.85-1.03)	0.20			0.94 (0.85-1.03)	0.20		
Rectus muscle average area	0.99 (0.98-1.01)	0.54			0.99 (0.98-1.01)	0.54		
Average lateral muscle thickness	0.89 (0.80-0.98)	0.02	0.88 (0.80-0.98)	0.02	0.90 (0.82-0.98)	0.02	0.85 (0.75-0.95)	0.005
Average lateral pannus thickness	1.01 (0.97-1.05)	0.71			1.00 (0.97-1.04)	0.81		
Average lateral total thickness	0.98 (0.95-1.02)	0.38			0.99 (0.96-1.02)	0.44		
Average muscle:pannus ratio	2.11 (0.99-4.50)	0.06			1.60 (0.82-3.15)	0.17		
Circumference Difference	0.97 (0.91-1.03)				1.02 (0.98-1.06)	0.43		
Defect percentage	1.11 (0.99-1.23)	0.06			1.10 (1.01-1.21)	0.04		
HV:IAV ratio	1.00 (0.89-1.12)	1.00			1.02 (0.94-1.10)	0.68		
LSMI	1.01 (0.99-1.13)	0.10			1.06 (1.01-1.11)	0.01	1.11 (1.04-1.18)	0.002
Sarcopenia muscle	0.30 (0.07-1.28)	0.10			0.32 (0.12-0.83)	0.02		

Table 3.22: Binary logistic regression analysing long-term hernia recurrence.

	Original data				Imputed data			
	Uni-variate analysis		Multi-variate analysis		Uni-variate analysis		Multi-variate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Defect Width (mm)	1.06 (0.97-1.16)	0.19			1.06 (0.97-1.16)	0.19		
Defect Length (mm)	1.09 (0.98-1.20)	0.10			1.09 (0.98-1.20)	0.10		
Defect Area (cm ²)	1.03 (0.99-1.07)	0.17			1.03 (0.99-1.07)	0.17		
Rectus muscle average width	1.00 (0.97-1.03)	0.85			1.00 (0.97-1.03)	0.85		
Rectus muscle average thickness	0.99 (0.87-1.11)	0.80			0.99 (0.87-1.11)	0.80		
Rectus muscle average area	1.01 (0.98-1.03)	0.60			1.01 (0.98-1.03)	0.60		
Average lateral muscle thickness	0.92 (0.82-1.02)	0.11			0.91 (0.81-1.01)	0.08	0.86 (0.75-0.99)	0.03
Average lateral pannus thickness	0.98 (0.95-1.02)	0.98			1.00 (0.96-1.05)	0.92		
Average lateral total thickness	0.98 (0.94-1.03)	0.42			0.99 (0.95-1.03)	0.53		
Average muscle:pannus ratio	1.86 (0.80-4.36)	0.15			1.62 (0.96-1.07)	0.25		
Circumference Difference	0.92 (0.83-1.01)	0.08			1.01 (0.96-1.07)	0.66		
Defect percentage	1.05 (0.93-1.20)	0.44			1.07 (0.95-1.20)	0.26		
HV:IAV ratio	1.03 (0.91-1.16)	0.62			1.01 (0.91-1.11)	0.90		
LSMI	1.07 (1.00-1.15)	0.06			1.06 (1.00-1.12)	0.04		
Sarcopenia muscle	0.11 (0.01-0.95)	0.04			0.25 (0.07-0.95)	0.04	0.17 (0.04-0.70)	0.01

Table 3.23: Binary logistic regression analysing long-term further hernia surgery.

3.3.3.5 Determination of cut-offs for significance

Cut-off values for all significant risk factors were calculated using receiving operator characteristics and analysis of Youden's Index (YI) and can be found in Table 3.24 and Table 3.25. When looking at the original data, SSI was found to be associated with an average lateral muscle to pannus ratio of greater than 1.00 (YI 1.28). A defect width of greater than 12.75cm (YI 1.35) and an average lateral abdominal wall muscle thickness of less than 16.75mm were associated with hernia recurrence (YI 1.27).

Agreement was seen in these factors when considering the imputed data. For SSI the same ratio of 1.01 was seen with a similar YI of 1.28. When comparing risk for hernia recurrence, a defect width of greater than 12.75cm had the highest YI (1.35). The optimal lateral muscle thickness was slightly higher in the imputed data, greater than 22.75mm (YI 1.21). The multi-variate model in the imputed data also retained higher LSMI. Using YI an LSMI of greater than 39.57 was most likely to be associated with hernia recurrence.

Three outcomes (Clavien Dindo, IASC and further hernia surgery) did not have any factors that retained significance in the multi-variate model when evaluating the original data, however did have significant factors in the imputed data. For the Clavien Dindo outcome, an average pannus to muscle ratio of greater than 1.18 (YI 1.22) and an LSMI of less than 42.75 (YI 1.20), were associated with poor outcome. IASCs were most likely to be associated with a circumference difference of more than 10cm (YI 1.19) and a hernia to intra-abdominal volume ratio of less than 8.00 (YI 1.23). Further hernia surgery was most likely to be associated with a lateral average muscle thickness of less than 20.75 (YI 1.01).

Outcome	Cut-off	Sensitivity	Specificity	Youden's Index
<u>Surgical site infection</u>				
Average muscle to pannus ratio	1.01	0.49	0.79	1.28
<u>Hernia recurrence</u>				
Defect width (cm)	12.75	0.67	0.68	1.35
Lateral muscle thickness (mm)	16.75	0.48	0.79	1.27

Table 3.24: Determination of Youden's Index using the original data.

Outcome	Cut-off	Sensitivity	Specificity	Youden's Index
<u>Clavien Dindo</u>				
Average muscle to pannus ratio	1.18	0.45	0.77	1.22
LSMI	42.75	0.69	0.51	1.20
<u>Surgical site infection</u>				
Average muscle to pannus ratio	1.00	0.54	0.73	1.27
<u>IASC</u>				
Circumference difference	10.00	0.93	0.26	1.19
HV:IAV ratio	8.00	0.86	0.37	1.23
<u>Hernia recurrence</u>				
Defect width (cm)	12.75	0.67	0.68	1.35
Lateral muscle thickness (mm)	22.75	0.85	0.36	1.21
LSMI	39.73	0.74	0.59	1.33
<u>Further hernia surgery</u>				
Lateral muscle thickness (mm)	20.75	0.47	0.54	1.01

Table 3.25: Determination of Youden's Index using the imputed data.

3.3.4 Discussion

This is the first study to describe and evaluate the effectiveness of CT derived measurements in the assessment of patients undergoing CAWR. It shows the successful validation of CT measurements with all ICC values demonstrating adequate inter-rater reliability with most showing excellent reliability. Post-operative outcomes from the cohort are found to be comparable to those of previous studies in this group (144) (*Chapter 1.4.4*), including a high incidence of SSI (56.5%) and acceptable rate of hernia recurrence (25.7%) with an adequate follow-up period (median 24 months).

On evaluation of short-term outcomes it appears factors related to CT measurements concerned with body composition. Higher average pannus to muscle ratio is implicated in Clavien Dindo complication of grade 3 or more and the incidence of surgical site infection. Along with this a higher circumference difference between the muscle and total abdominal circumference was associated with IASCs. These findings would imply that more obese patients are more likely to have worse short-term outcomes from CAWR, which is consistent with previous literature (75). However, given the fact increasing BMI was not a factor in these models, discussed in *Chapter 3.2*, these findings suggest there are other ways of evaluating body composition that could prove helpful in the evaluation of patients undergoing CAWR.

LSMI of less than 42.75 has also been found to be related to Clavien Dindo outcome suggesting patients with sarcopenia are more likely to have significant short-term post-operative complications consistent with the current literature (150,151). Interestingly a lower hernia to intra-abdominal volume was associated with the

occurrence of IASCs. Previous studies have suggested higher volume ratio is associated with bridging and recurrence (148), however these studies were performed in clean cases. The volume ratio has not been found to be associated with recurrence in contaminated cases based on these data.

When looking at long-term outcomes a defect width of more than 12.75cm was most likely to predict hernia recurrence. This is in keeping with current knowledge that larger defects are more likely to recur (73–75,152), however most publications use an arbitrary measurement of 10cm as the cut-off between low and high-risk patients. These data suggest for contaminated defects a more accurate cut-off to analysis is 12.75cm and may prove useful in including this cut-off in the risk stratification model.

Lower lateral abdominal wall muscle thickness (<21mm) was associated with both hernia recurrence and further hernia surgery implying an assessment of abdominal wall muscle quality is important in predicting long-term outcome. These are the first data to suggest this measurement of the muscle quality would help in the evaluation of the abdominal wall and aid in predicting hernia recurrence.

Interestingly a higher LSMI was found to be associated with hernia recurrence. It is not clear why this is the case as overall sarcopenia was not associated with hernia recurrence. Separately the absence of sarcopenia was associated with further hernia surgery. This is most likely as a result of sarcopenic patients not coming to further surgery as they would not be fit for the second operation.

Although this is the largest cohort of CAWR yet evaluated using CT measurements the numbers remain small. This is partly due to the rarity of the condition and that abdominal CT has not previously been a standard investigation in the pre-operative work-up of these patients. This study is also limited by the retrospective nature of the data and missing data for some measurements. Predictive mean matching methods were chosen to account for the non-normal distribution of some of the variables in order to minimise the risk of altering the imputed dataset from the real data and an $n=5$ of iterations was used to minimise the risk of bias and allow adequate sampling variation. Nevertheless this the methodology could contribute to bias in the study if missing data was not missing at random, as has been assumed, or because of the relatively small sample size which could lead to significant differences between real and imputed values. Comparing the real versus imputed means this does not seem to be the case however a larger complete dataset would be preferable.

The aim of this analysis is to highlight potentially important CT measurements in the evaluation of contaminated abdominal wall defects. We have demonstrated the methodology of measuring CT dimensions in these cases to be valid and reproducible. Measuring all possible CT dimensions is time consuming and potentially not productive therefore focusing on the factors identified in this work for future evaluation would be recommended.

3.4 Conclusion

This chapter aims to assess and improve the evaluation and risk stratification of patients undergoing CAWR. The development of novel techniques, such as component separation, is key in the continued improvement of outcomes and on going marginal gains from these developments continue to demonstrate this finding. The novel technique of posterior component separation with transversus abdominis release has been shown to demonstrate reasonable outcomes in cohort studies however no attempt has been made to compare this technique with more established component separation techniques. Our analysis demonstrates outcomes are at least comparable however further directly comparable data are necessary to draw robust conclusions. The required number of included patients necessary to make this comparison could be prohibitively large however such a comparison could be performed using large-scale registry data.

Risk stratification modelling

Development of risk stratification models for complex surgery is a valid method of improving outcomes and patient counselling pre-operatively. The ability to accurately predict the likelihood of a $CD \geq 3$ outcome in these patients will improve the ability of the surgeon to counsel patients pre-operatively as well as resource plan for the surgery, including ensuring the availability of high dependence post-operative care for high-risk patients. Further work is necessary to improve the accuracy of other models and validate long-term outcomes. The principle that the inclusion of CT measurements within these models would aid in the improved stratification of patients' risk has been demonstrated in a subset of these cases. On going work is required to do this with the collection of large-scale prospective registry data

focussing on the factors identified in these studies. The inclusion of CT scanning in the assessment of patients undergoing CAWR would be recommended as a result of this work.

Further work to assess the impact of the models on patient care would also be recommended. The evaluation of user interface with the model would be required in the first instance. Then how patients react to the model outcomes would also need to be evaluated. These models could therefore aid in the facilitation of personal healthcare decisions and evaluation of the patients' decision making by surveying their thought process pre- and post-model deployment.

Chapter 4 - Development and characterisation of a novel

decellularised porcine rectus sheath scaffold for use in abdominal wall reconstruction

The work in this chapter was funded by the St Mark's Hospital Foundation. All experiments described in this chapter were carried out by the student with the exception of animal implants and terminations, which were performed by Dr Tahera Ansari, and collagen and GAG quantification, which were performed by Miss Lauren Jones.

4.1 Introduction

The use of decellularised biologic grafts as tissue reinforcement in the repair of complex ventral hernias has become commonplace. Commercially available biological meshes are derived from human, porcine or bovine dermis, porcine intestinal submucosa or bovine pericardium (52). Their use was first trialled in contaminated surgical fields given the increased risk of mesh infection, erosion and fistulisation when using synthetic grafts in this setting (17–19).

All materials are decellularised to leave a collagen matrix that acts as a scaffold for host tissue integration. Current data suggests that, over two year follow-up, biological meshes are associated with a 25% risk of hernia recurrence and 10% risk of fistulisation in the contaminated setting suggesting that the ideal mesh has not yet been found (*Chapter 1.5*).

Some meshes undergo a process of additional crosslinking to modify the collagen structure with the aim of strengthening the material and preventing rapid breakdown by collagenases thereby reducing hernia recurrence rates (35). Crosslinking can be performed in a number of ways in current commercially available meshes, such as chemical treatment with glutaraldehyde (Peri-guard) or hexamethylene diisocyanate (Permacol), with the aim of increasing the number of covalent bonds between collagen molecules (52). However crosslinked meshes may be associated with higher rates of wound infection, the need for removal and fistula recurrence and therefore unsuitable for use in the contaminated setting (19,153). The most effective way of maintaining graft strength whilst minimising infection and fistulisation rates has yet to be established.

Non-vascularised meshes of human rectus sheath have been described and utilised for abdominal wall reconstruction at the same time as visceral transplant (41). The muscle is harvested, fascia opened laterally and the muscle excised leaving only the rectus sheath and peritoneum. In a case series of sixteen, one graft excision and no long-term ventral hernias were seen. However the paucity of donor tissue would make this ineffective on a large scale.

The use of porcine derived rectus sheath may offer the benefits of human derived rectus sheath whilst overcoming the supply issue. It is “like-for-like” repair and the residual extra-cellular peritoneal matrix may prevent severe adhesions of bowel to the fascia itself. Herein is described the development of a standard protocol for porcine rectus sheath decellularisation, biomechanical strength and strain assessment of the resultant graft and in vivo biocompatibility testing using a rat model.

4.2 Methods

4.2.1 Tissue harvest

Abdominal wall tissue was harvested from post-mortem specimens from Large-White/Landrace crossbreed pigs following euthanasia. All animals were previously utilised for unrelated studies and had undergone midline laparotomy or laparoscopy. Full thickness specimens were harvested in one piece (if laparoscopic abdominal surgery had been undertaken) or as two rectus complexes (if prior midline laparotomy had been performed).

The abdominal wall was dissected into four different specimens in preparation for decellularisation. Section of: i) posterior sheath and peritoneum; ii) anterior and posterior sheath with peritoneum; iii) full thickness; and iv) anterior sheath and muscle were created (Figure 4.1). Dissection was performed using careful sharp dissection with scalpel or scissors, as a single piece.

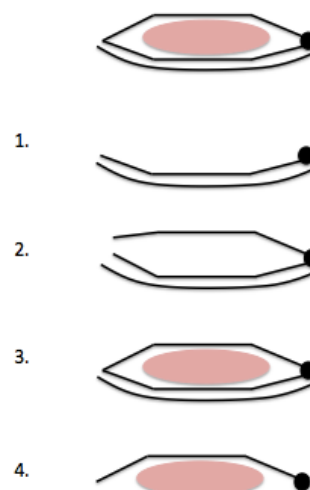


Figure 4.1: Complete rectus complex with peritoneum and dissected sections for decellularisation. 1) posterior sheath and peritoneum; 2) anterior and posterior sheath with peritoneum; 3) full thickness; and 4) anterior sheath.

Tissue samples could be as large as 30x20cm depending on the size of the donor animal (Figure 4.2). The dissected specimen was divided into smaller 1-2cm square sections for further experimental testing. Dissection of a full size abdominal wall graft can be performed in less than 30 minutes.

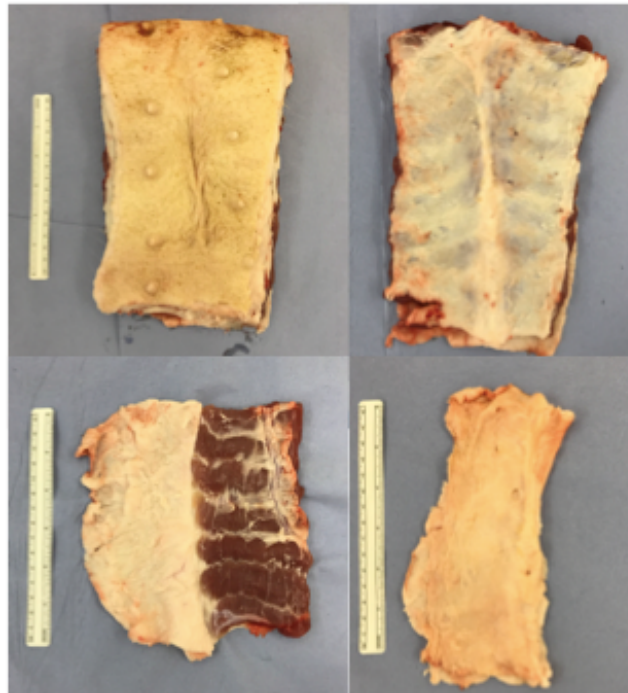


Figure 4.2: A demonstration of the dissection required to identify the required tissue for decellularisation.

Samples of all sections were snap frozen in liquid nitrogen and stored at -80°C as control tissue ($n=3$). Samples for decellularisation were stored at -20°C in preparation for experimentation.

4.2.2 Decellularisation protocol

The decellularisation protocol was developed and adapted from existing in-house decellularisation protocols (Table 4.1). Specimens from 15 different animals were

use to develop and optimise the protocol. It quickly became apparent that sufficient treatment time with detergent, in order to penetrate to the centre of the tissue, lead to the decision not to continue with samples iii) and iv). Progressive protocol modifications were evaluated histologically and residual molecular structure (DNA, GAG, collagen) until an adequate scaffold was produced. Finally, due to time and monetary constraints, sample i) was chosen for *in vivo* testing. A further n=3 specimens were used for final decellularisation for molecular analysis, *in vivo* biocompatibility and strength and strain testing.

The whole decellularisation protocol was carried out using a medium desiccator (Sigma-Aldrich, UK) and under vacuum. The vacuum was created with a Telstar Vacuum Pump 2F-10 fitted with a digital vacuum gauge (Pendle Refrigeration Wholesale Ltd, UK) to a level of 2000 microns. Following an initial freeze-thaw step, Latrunculin-B (50nM) (Sigma-Aldrich, UK) in high-glucose Dulbecco's modified Eagle's medium (Sigma-Aldrich, UK) treatment was used to assist in residual muscle breakdown by preventing polymerisation of actin fibres. The sample was then washed with detergent, Triton-X (non-ionic) and sodium deoxycholate (SOC) (ionic), to remove cell residue and disrupt and dissociate protein interactions. DNase and RNase nuclease enzymes were used to cleave nucleic acid sequences. A combination of deionised water, PBS buffer and PBS/Tween 20 washer buffer were used to clean the scaffold between the treatment steps. All steps, apart from enzymatic digestion, were conducted at room temperature on a rocker at 75rpm. Enzymatic digestion was performed at 37°C on a rocker at 100rpm.

Step	Treatment	Time	Agitation	Volume	Pressure	Temp
1	Thaw	1hr	Nil	N/A	Atm	RT
2	Latrunculin B	1hr	75rpm	250mls	-2000	RT
3	Deionised H2O	1hr	75rpm	250mls	-2000	RT
4	Deionised H2O	1hr	75rpm	250mls	-2000	RT
5	Detergent - Triton 0.25% + SOC 0.25%	2hr	75rpm	250mls	-2000	RT
6	PBS	1hr	75rpm	250mls	-2000	RT
7	Detergent - Triton 0.25% + SOC 0.25%	2hr	75rpm	250mls	-2000	RT
8	PBS + 0.2% Tween	1hr	75rpm	250mls	-2000	RT
9	Deionised H2O	1hr	75rpm	250mls	-2000	RT
10	PBS	12hr	75rpm	250mls	-2000	RT
11	Latrunculin B	1hr	75rpm	250mls	-2000	RT
12	Deionised H2O	1hr	75rpm	250mls	-2000	RT
13	Detergent - Triton 0.25% + SOC 0.25%	1hr	75rpm	250mls	-2000	RT
14	Deionised H2O	1hr	75rpm	250mls	-2000	RT
15	Enzymes	2hr	100rpm	250mls	-2000	37
16	Deionised H2O	1hr	75rpm	250mls	-2000	RT
17	Enzymes	2hr	100rpm	250mls	-2000	37
18	Deionised H2O	1hr	75rpm	250mls	-2000	RT
19	PBS	12hr	75rpm	250mls	-2000	RT
20	Deionised H2O	1hr	75rpm	250mls	-2000	RT
21	Enzymes	1hr	100rpm	250mls	-2000	37
22	PBS + 0.2% Tween	30min	75rpm	250mls	-2000	RT
22	PBS + 0.2% Tween	30min	75rpm	250mls	-2000	RT
23	PBS + 0.2% Tween	1hr	75rpm	250mls	-2000	RT
24	PBS + 0.2% Tween	1hr	75rpm	250mls	-2000	RT
25	Deionised H2O	1hr	75rpm	250mls	-2000	RT
26	PBS	4hr	75rpm	250mls	-2000	RT

Table 4.1: Final decellularisation protocol for porcine abdominal wall Sample i).

Following decellularisation, representative samples were fixed in 10% NFB solution and routinely embedded in paraffin. Further samples were snap frozen using liquid nitrogen and stored at -80°C for molecular analysis. Samples for mechanical strength and strain and *in vivo* biocompatibility testing were sterilised using progressive concentrations of ethanol (*Appendix 2*) and stored in PBS solution with 1% antibiotic and antimycotic solution at 4°C.

4.2.3 *In vivo biocompatibility testing*

Animal handling and surgical procedures were performed in accordance with The Animals (Scientific Procedures) Act 1986 and Home Office Code of Practice. All surgical procedures were performed by Dr Tahera Ansari who holds both a personal and project licence.

Six male Sprague-Dawley rats (Harlan Ltd, UK) were anaesthetised with 0.25ml IM Hyponorm and 1mg IP diazepam and their fur was cleaned and shaved over the abdomen. Under sterile conditions, a midline incision was made and bilateral subcutaneous pockets were created. A single 1x1cm decellularised scaffold was placed into each pocket. The skin was closed using interrupted 3-0 Mersilk sutures and a spray adhesive dressing was applied (Figure 4.3). Animal number 1 was found with an open wound on Day 1 post-operatively and required re-suturing. Unfortunately the animal was seen to be distressed and the wound reopened therefore it was terminated on Day 3.

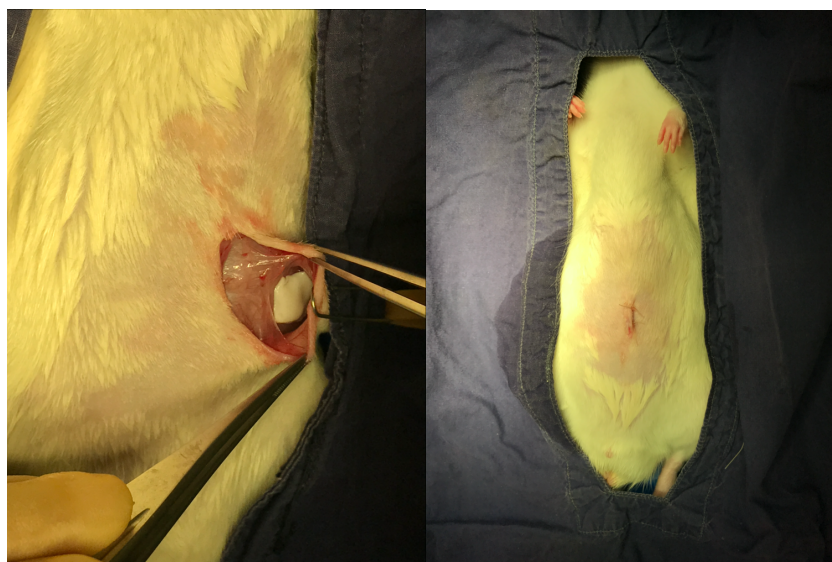


Figure 4.3: Implant of decellularised scaffold in to animal model.

Animals were terminated at 4 (n=1), 8 (n=2) and 12 (n=2) weeks and the residual scaffold was excised with underlying muscle tissue. Explanted tissue was placed in 10% NBF and fixed routinely in paraffin. Five micrometre sections were cut and processed for H&E and immunohistochemistry to assess for scaffold degradation and cellular infiltration. Histological analysis was undertaken by three independent assessors according to criteria defined in previously published papers related to mesh evaluation (Table 4.2) (154–156). Briefly, 5 randomly selected representative areas on each of the explanted sample (4 weeks n=2, 8 weeks n=4, 12 weeks n=) were selected and evaluated according to the criteria in Table 4.2.

Score	0	1	2	3
Cellular infiltration	Zero cells in contact with scaffold	Cells contact periphery – no penetration into scaffold	Cells infiltrate scaffold – non reach centre	Cells penetrate into centre of scaffold
Inflammatory cells	Inflammatory cells present – no fibroblasts	Primarily inflammatory cells – few fibroblasts	Primarily fibroblasts – few inflammatory cells	Fibroblasts only – no inflammatory cells
Fibrous Capsule	Extensive encapsulation (50-100% of periphery)	Moderate encapsulation (25-50% of periphery)	Mild encapsulation (<25% of periphery)	No fibrous encapsulation
Vascularisation	Zero bloods vessels present	Vessels present at scaffold periphery – no penetration into scaffold	Vessels infiltrate scaffold but none reach centre of scaffold	Vessels penetrate into centre of scaffold
Degradation	Original scaffold intact, borders clearly demarcated	Scaffold partially degraded, layers separated by cells, blood vessels, host tissue, etc	Scaffold extremely degraded, difficult to distinguish scaffold from host tissue	Scaffold completely degraded, no evidence of original scaffold

Table 4.2: Histology scoring.

4.2.4 Histological assessment

All tissue samples for histology were stored in 10% NFB solution and routinely embedded in paraffin (TP1020, Lecia, Germany). Standard 5 micrometre sections were cut using a microtome. Samples for haematoxylin and eosin (H&E) and picrosirius red and Miller's elastin (PSR-ME) were mounted on standard slides. Samples for immunohistochemistry were mounted on slides coated with 3-aminopropyl triethoxysilane (APTS) (Sigma-Aldrich, UK). All slides were warmed to 40°C for 1 hour before storage at room temperature.

Representative samples from n=3 runs of the standardised decellularisation protocol were fixed in 10% NFB solution and routinely embedded in paraffin. Sections were stained with H&E to assess for the presence of residual cellular and nuclear tissue. PSR-ME were used to assess underlying structural integrity of the remaining collagen and elastin and compared to control tissue.

H&E and PSR-ME staining were performed according to standard in house protocols (*Appendix 3 and 4*). H&E staining is used to observe intra-cellular structure, nucleus and differentiate between different cell types. Haematoxylin binds to basophilic structures such as DNA and RNA resulting in a violet or dark blue colour. Eosin binds to acidic and cytoplasmic structures, such as amino acids, resulting in a red or pink stain.

PSR-ME staining evaluates tissue collagen content. Under bright light elastin fibres are observed to be blue and collagen red and under polarised light demonstrates larger intact collagen as bright yellow or orange and damaged collagen as green or black.

4.2.5 Immunohistochemistry

Following tissue processing and mounting on APTS coated slides, immunohistochemistry was performed to evaluate total macrophage activation at all *in vivo* test time points using CD68 antibodies (BioRad, UK) (Table 4.3). Slides were dewaxed and rehydrated prior to antigen retrieval using proteinase K (20ug/ml, Sigma Aldrich, UK) at 37°C for 5 minutes. Endogenous blocking was carried out using 10% hydrogen peroxide with methanol for 30 minutes and non-specific binding sites were blocked with 2.5% horse serum (Vector Laboratories, UK) for 1 hour at room temperature. The CD68 primary antibody (mouse-anti-rat CD68 monoclonal antibody, MCA341R, BioRad, UK) was added to the samples at a concentration of 1:100, diluted in PBS, for 2 hours followed by anti-mouse secondary antibody (Vector Laboratories Ltd, UK) for 1 hour. Washing between all steps was performed using 1% foetal calf serum diluted with PBS solution for 3x10 minutes. The samples were stained using the chromogenic substrate Diaminobenzidine (Impact, Vector Laboratories, UK) for 20 seconds. The reaction was quenched with PBS and the samples were counterstained with haematoxylin for 1 minute before differentiating. The samples were then fixed, dehydrated and mounted with coverslips (Fisher scientific, UK).

Step	Protocol	Incubation time
1	Deparaffinise sections in Xylene 1	10 min
2	Xylene 2	5 min
3	Rehydrate sections in 100% IDA	2 min
4	95% IDA	2 min
5	70% IDA	2 min
6	Running water	2 min
7	Carry out antigen retrieval – Proteinase K 37 degrees	5 min
8	Rinse sections in PBS +FCS	3 x 10 min
9	Endogenous block. Incubate sections in 3% hydrogen peroxide made in methanol at room temperature.	30 min
10	Rinse sections in PBS +FCS	3 x 10 min
11	Non-specific block. Incubate sections with Vector's ready to use 2.5% normal horse serum at room temperature (remove from fridge ~30 before use)	30 min
12	Primary antibody. Prepare the primary antibody to the suggested dilution with TBS and incubate sections (at room temperature/4°C)	2 hr
13	Rinse sections in PBS +FCS	3 x 10 min
14	Polymer detection system. Incubate sections with the appropriate Vector ImmPRESS kit at room temperature (remove from fridge ~30 before use).	20min
15	Rinse sections in PBS +FCS	3 x 10 min
16	Chromagen. Incubate sections with Vector ImmPACT DAB peroxidase substrate	15 sec
17	Quench DAB reaction with PBS	5 min
18	Immerse slides in distilled water	
19	Counterstain with Gill's Haematoxylin	1 min
20	Wash in running tap water	5 min
21	Differentiate in 1% acid-alcohol	3 x 3 sec
22	Wash in running tap water	4 min
23	Dehydrate sections in 70% IDA	30 sec
24	95% IMS	30 sec
25	100% IMS	2 min
26	Clear in Xylene 1	2 min
27	Xylene 2	2 min
28	Mount with non-aqueous media and air dry	

Table 4.3: Immunohistochemistry protocol.

Initial testing revealed a significant amount of background staining, therefore the protocol was optimised (Table 4.3) over 11 iterations using a variety of control tissues and test samples. Controls tested were spleen, thymus, pancreas and lymph node. Background staining was minimised by reducing the length of antigen retrieval, increasing washing steps and reducing the chromogen treatment time. Negative and positive controls of rat splenic tissue were finally used with minimal residual background. A test of cross reactivity of the secondary antibody to rat tissue was also performed which revealed a marked reduction in background.

4.2.6 Molecular analysis

4.2.6.1 DNA gel electrophoresis

Decellularised samples were tested for residual DNA using the standardised DNA extraction techniques of GenElute mammalian genomic DNA miniprep kit (Sigma-Aldrich, UK) (*Appendix 5*). Residual DNA was quantified by measuring absorbance in a spectrophotometer (Nanodrop, ND1000, ThermoScientific, Wilmington, USA). The size and quantity of residual DNA was evaluated using agarose gel electrophoresis and visualised against a 1-kb DNA ladder (Q-step 4 quantitative DNA ladder, Yorkshire Bioscience Ltd, York, UK) using ultraviolet transillumination.

4.2.6.2 GAG quantification

GAG content was quantified in decellularised tissue and compared to fresh frozen tissue using the Blyscan GAG assay kit (Biocolor Carrick Fergus, NI, UK). Protein extraction was carried out on 50mg of minced wet tissue using proteinase K as described by Huynh *et al* (157) as opposed to the extraction buffer provided in the kit due to low levels of extraction seen in control tissue. This was carried out in a

Precellys Homogeniser at 56°C. Following this, proteinase K was denatured by increasing the temperature to 90°C and subsequent quantification was then performed according to the kit protocol (*Appendix 6*). The recovered samples were mixed with Blyscan dye reagent and quantified against GAG standard plots using a spectrophotometer.

4.2.6.3 Collagen quantification

The Sircol collagen assay kit (Biocolor, Carrick Fergus, NI, UK) was used to quantify the soluble collagen content in the decellularised and fresh tissue samples (*Appendix 7*). Briefly, 40mg of tissue was measured and dried on blotting paper before being minced and placed in a Precellys homogenisation tube. Acid-pepsin and protease inhibitor were added to the tube and homogenised at 4°C for 18 hours. The samples were centrifuged and the supernatant re-suspended in acetic acid. Absorbance at 555nm was measured using a spectrophotometer to quantify the collagen.

4.2.7 Biomechanical strength and strain testing

Standard sections of decellularised and control tissue (n=4) were cut and mechanical properties were assessed using a tensiometer (Instron Inspec 220 Benchtop Portable tester, Instron, Bucks, UK). The tensiometer loaded the tissue at a constant rate and recorded real time load and elongation. Maximum load (N), extension at maximum load (mm) and testing time (seconds) were automatically recorded. Stress was defined as the load at each metre squared (N/m²). Strain, the rate of sample deformation, was defined as the ratio of sample length at maximal load to initial length. Young's modulus, the measure of a materials ability to withstand change in length, was calculated as the ratio of stress to strain.

4.2.8 Statistical analysis

Data were calculated as mean and standard deviation. Non-parametric data, such as biomechanical testing results, were compared with a Mann-Whitney U test. Comparison of multiple groups, for histological biocompatibility testing, was performed using one-way ANOVA. A p-value of less than 0.05 was taken to be significant.

4.3 Results

4.3.1 Decellularisation and scaffold evaluation

The decellularisation protocol was modified from various existing in-house protocols until adequate clearance was achieved (Table 4.1). Decellularised samples were sterilized using ethanol according to in-house protocols and determined to be sterile at 24 hours, 7 days and 3 months following storage in PBS/1% antibiotic solution (Figure 4.4).

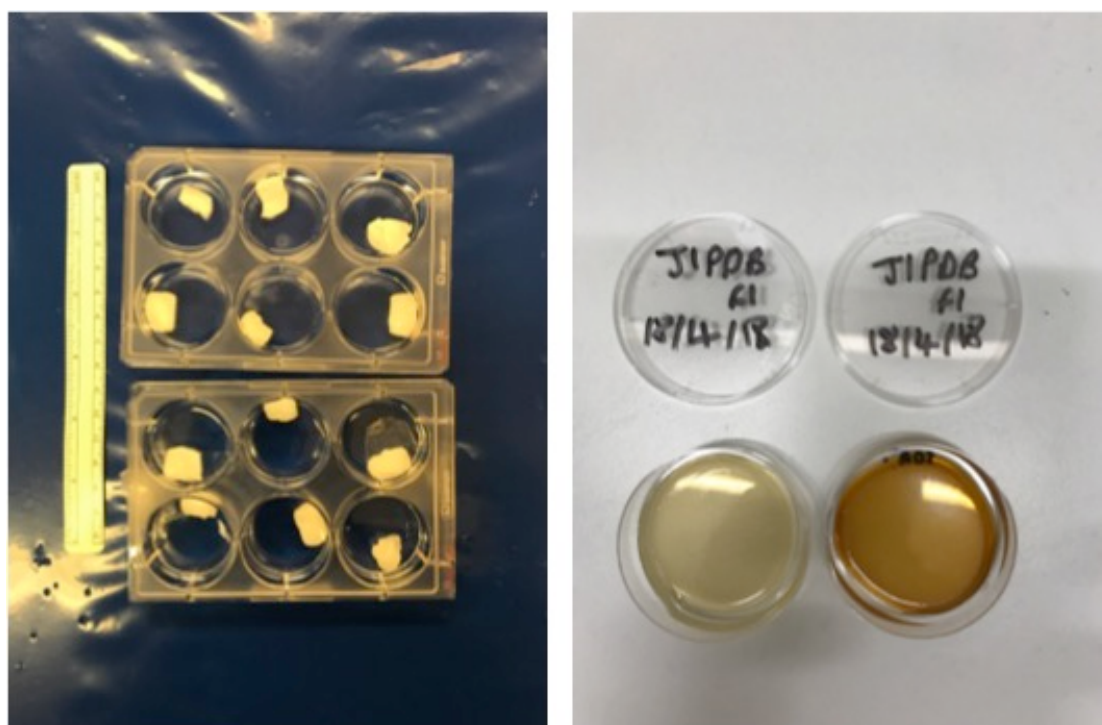


Figure 4.4: An example of decellularised samples and a photograph demonstrating sterility at 1-month post-decellularisation.

H&E staining demonstrated the absence of residual nuclear tissue by comparison to control (Figure 4.5). Comparison of PSR-ME staining demonstrated adequate residual elastin and collagen (Figure 4.6).

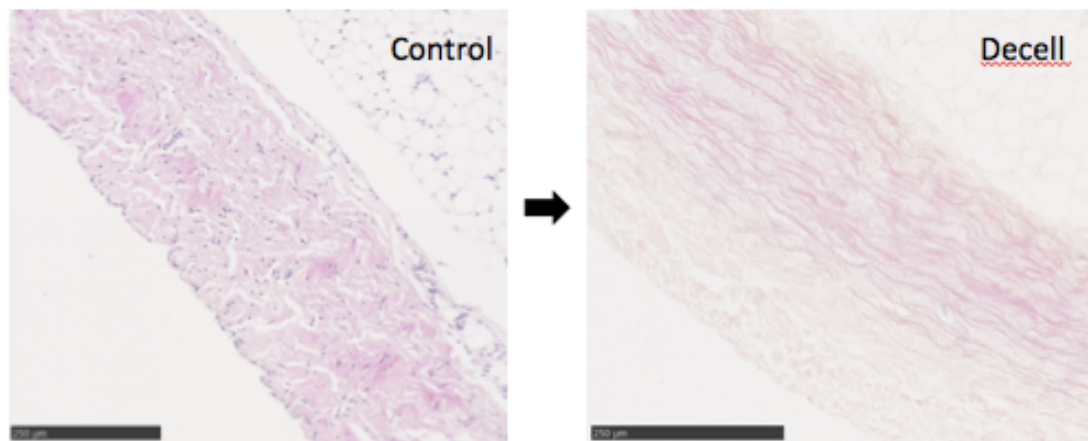


Figure 4.5: H&E staining for residual DNA (black bar - 250μm).

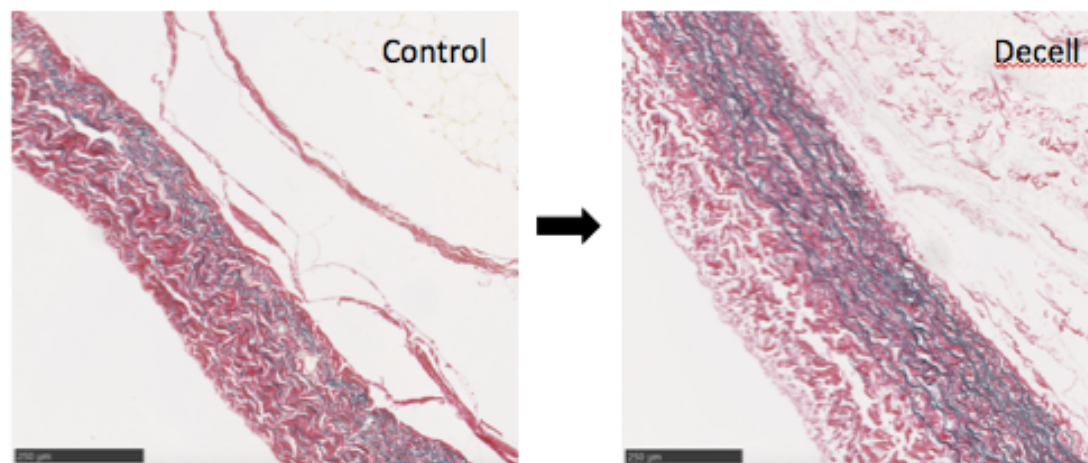


Figure 4.6: PSR-ME staining for residual collagen (black bar - 250μm).

Residual DNA was quantified using DNA gel electrophoresis demonstrating adequate reduction in the level of DNA compared to controls. Some issues were found with overall DNA quantification as reductions from control quantification were not achieved down to the required 50ng/L. The reason for this was thought to be due to the fact low overall levels of DNA were found even in the control tissue compared to other tissue types and therefore there was a significant chance of possible error (Figure 4.7).

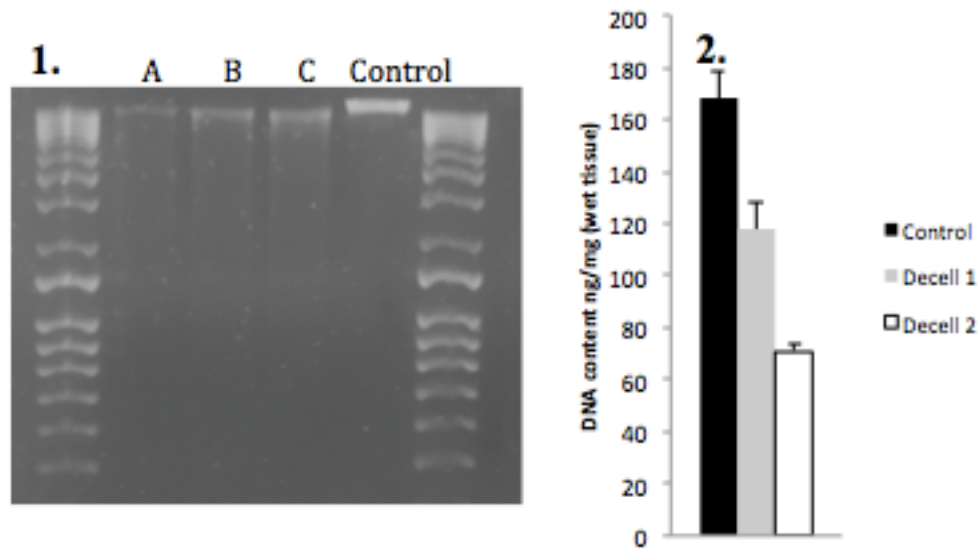


Figure 4.7: 1) DNA gel electrophoresis demonstrating absence of DNA in the decellularised sample (A, B, C) vs control. 2) A graph demonstrating DNA quantification and progressive decrease in residual DNA across subsequent decellularisation protocols.

Comparison was made between samples from an earlier decellularisation protocol (Decell 1) and the final protocol (Decell 2) that demonstrated no visible DNA on H+E staining. This comparison demonstrated a progressive decrease between control and subsequent protocols and was therefore accepted (Figure 4.8).

Residual GAG and Collagen were also quantified demonstrating no significant reduction compared to control tissue (Figure 4.9).

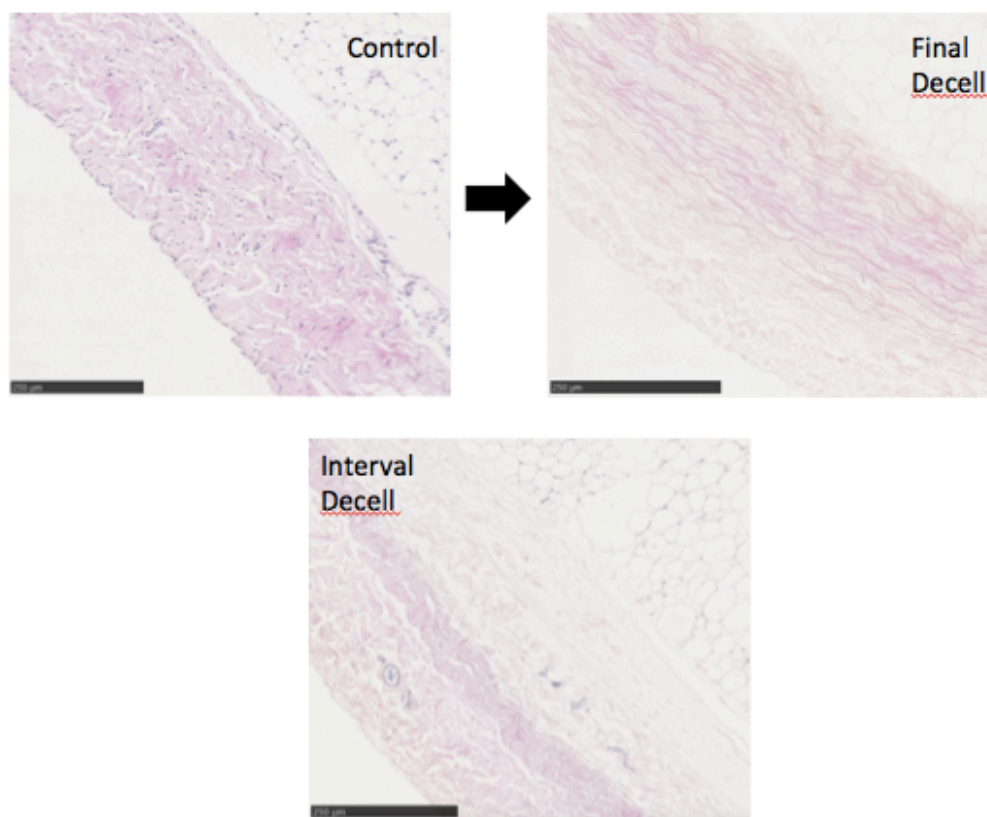


Figure 4.8: H&E staining for residual DNA comparing control with interval decellularisation and final decell protocol (black bar - 250 μ m).

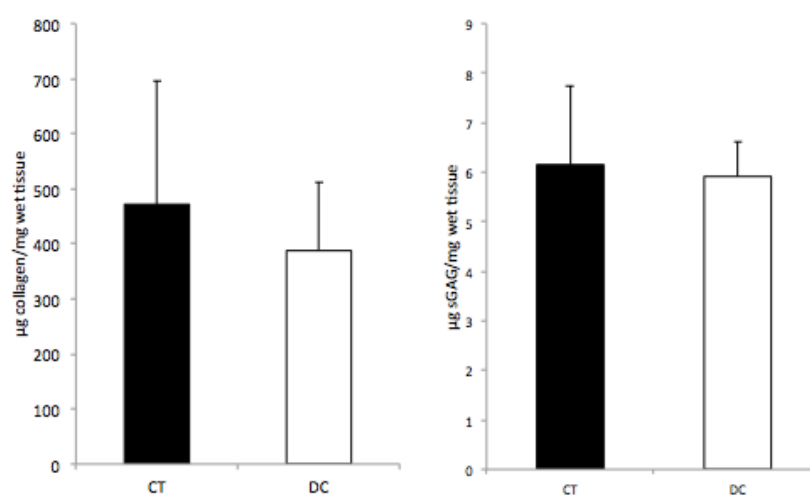


Figure 4.9: Control (CT) vs Decellularisation (DC) quantification of residual GAG and collagen.

4.3.2 Mechanical strength and strain testing

Fresh decellularised samples ($n=3$) following sterilisation were cut into dumbbell shaped pieces to standardise measurements (Figure 4.10). Unfortunately due to a computer error these test runs were deleted from the Instron Inspect tensiometer PDA computer and lost. Therefore residual tissue samples available from previous decellularisation procedures for implant ($n=3$; 1x2cm samples), that had been stored in PBS/1% antibiotic solution for 3 months, were converted for mechanical testing. These were compared to fresh harvested tissue.



Figure 4.10: A demonstration of mechanical strength and strain testing using a tensiometer.

Control tissue was found to have a maximum average force of 24.69N before failure compared to 227.97N for decellularised sterilised tissue. Maximal tensile strength was found to be 24.69N/cm for control tissue and 227.97N/cm for decellularised tissue (Table 4.4). Young's modulus was calculated to be 4x higher in the decellularised tissue, which is thought to be due to strengthening secondary to the

sterilisation process. This could result in a stronger but more brittle scaffold. These values are consistent with those in the published literature for abdominal wall tissue and for currently commercially available biologic meshes (154).

	Control	Decell	p-value
Tensile strength N (cm)	24.69 (1.15)	159.85 (145.45)	0.05
Rupture force N (SD)	24.69 (1.15)	159.85 (145.45)	0.05
Elongation at break % (SD)	58.83 (22.39)	152.6 (65.65)	0.13
Young's Modulus MPa (SD)	487.50 (146.15)	1939.0 (960.6)	0.05

Table 4.4: Results for mechanical strength and strain testing.

4.3.3 In vivo biocompatibility testing

The six rats were successfully implanted with two 1x1cm decellularised samples each. Unfortunately at Day 1 post-operatively the wound from Rat 1 opened and required re-suturing. By Day 3 this wound had reopened and the Rat was terminated as per Home Office guidelines. The remaining rats survived to the completion of the study period. Macroscopically all the samples were visible at explantation. Samples were macroscopically comparable at 4 and 8 weeks however some evidence of degradation was seen at 12 weeks.

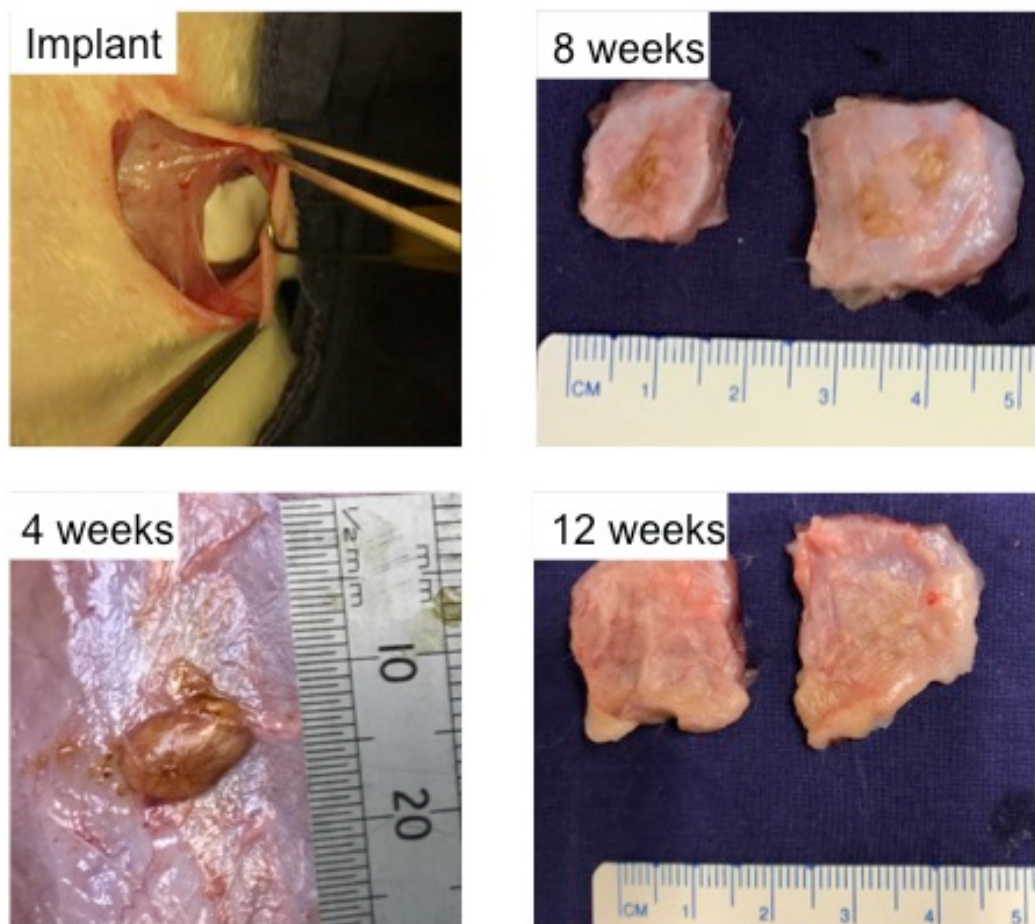


Figure 4.11: Macroscopic appearances of implants at the time of surgery and 4, 8 and 12 weeks post-operatively. The implant is easily seen at 4 and 8 weeks. By 12 weeks there is some visual degradation of the implant particularly in the left sample. The right sample demonstrates better integration with clear signs of vascularity.

Microscopic evaluation was consistent with these findings (Figure 4.12). There was a significant increase in samples degradation and visibility by the 12-week time point consistent with breakdown of some of the sample ($p < 0.0001$ and $p = 0.001$ respectively) (Table 4.5).

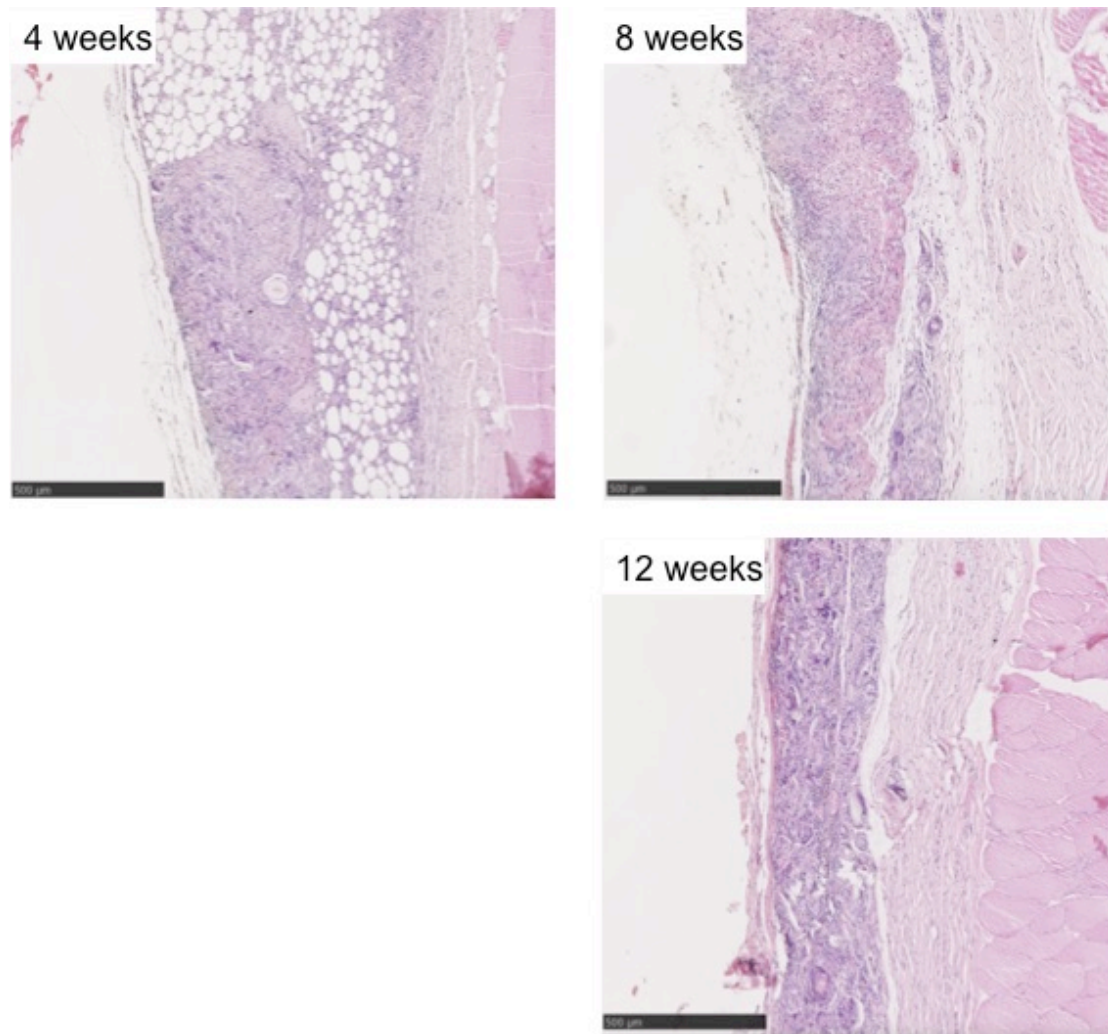


Figure 4.12: Microscopic slides demonstrating implant integration at 4, 8 and 12-week time-points (black bar - 250µm). The implant can be seen separate from native tissue in all examples. At 4 weeks there is some evidence of cellular infiltration that appears complete by 8 weeks. By 12 weeks increased evidence of vascular infiltration is seen.

Excellent cellular infiltration was seen in all samples across all time-points. Minimal inflammatory response was seen at all time points and this was seen to improve with fewer inflammatory cells seen at the later time points ($p=0.001$). A significant improvement in vascular infiltration over the 12-week study period in surviving samples ($p<0.0001$) (Table 4.5).

Histology evaluation	Mean score (95%CI)	F value (dF)	p-value
Mesh visibility			
4 weeks	1.00 (1.00-1.00)	7.76 (2-87)	0.001
8 weeks	0.97 (0.92-1.03)		
12 weeks	0.72 (0.57-0.88)		
Cellular infiltration			
4 weeks	3.00 (3.00-3.00)	1.28 (2-76)	0.28
8 weeks	2.94 (2.86-3.02)		
12 weeks	3.00 (3.00-3.00)		
Inflammatory cells			
4 weeks	1.39 (1.14-1.64)	7.95 (2-76)	0.001
8 weeks	1.46 (1.26-1.65)		
12 weeks	2.00 (1.72-2.28)		
Fibrous capsule			
4 weeks	2.17 (1.86-2.47)	0.48 (2-76)	0.62
8 weeks	2.17 (1.88-2.47)		
12 weeks	2.35 (2.07-2.62)		
Vascularisation			
4 weeks	1.78 (1.38-2.18)	17.67 (2-76)	<0.0001
8 weeks	2.09 (1.80-2.37)		
12 weeks	2.92 (2.81-3.03)		
Degradation			
4 weeks	0.78 (0.28-1.28)	16.68 (2-86)	<0.0001
8 weeks	0.89 (0.61-1.16)		
12 weeks	1.86 (1.62-2.11)		

Table 4.5: Histology scoring results from n=3 evaluators.

4.3.4 Immunohistochemistry

Immunohistochemical analysis was performed staining for CD68, which identifies macrophage invasion into the scaffold. Brown staining demonstrates granulocytes within the scaffold. Very few macrophages were seen at any time-point therefore an example at 4 weeks can be seen in Figure 4.13.

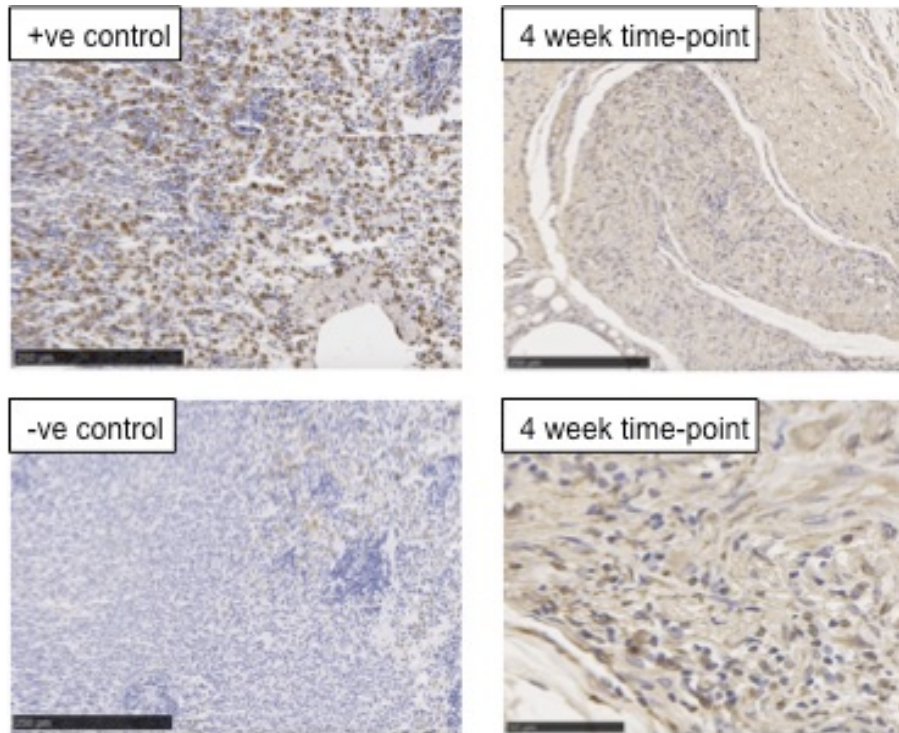


Figure 4.13: Examples of immunohistochemistry for CD68 (black bar - 250µm in left and upper right images; black bar = 50µm in right lower image). The positive control demonstrates the appearance of macrophages. While the 4-week sample demonstrates some brown background staining by comparison to the control very few macrophages can be seen.

4.4 Discussion

No ideal graft for use in abdominal wall reconstruction currently exists. This work aims to develop a novel “like-for-like” biological tissue replacement for abdominal wall defects. Given this scaffold is completely novel, this proof of concept work will need a significant amount of further work to provide clinical benefit however it is a necessary foundation to future development. Successful tissue decellularisation, retention of biomechanical strength and adequate biocompatibility are demonstrated.

Tissue harvest and dissection have revealed that multiple consistent sections of abdominal wall tissue can be used for decellularisation. Samples up to 15cm (approx. 5g of tissue) have been shown to be consistently decellularised in this work however it is possible to create samples up to 30cm which would be comparable to the largest available biologic meshes currently on the market. Further development work to scale-up and commercialise the process would be required to achieve this advance.

The decellularisation protocol initially took time to develop (Table 4.1). Firstly, an increase in time and number of detergent and enzyme steps was introduced, as well as conducting the procedure in a vacuum, additionally Latrunculin B to disrupt the actin and myosin interaction and washing in distilled water were built into the protocol. Failure of the initial protocols was found to be due to significant amounts of residual DNA (approx. 120ng/L). DNA is implicated in provoking an immune/inflammatory response when implanted in a host, so minimising residual DNA is paramount. As demonstrated initial DNA quantification showed levels above what is required, however DNA electrophoresis showed low levels of long-stranded DNA but residual short strands that could lead to a host immune response when implanted. Additional

washing steps, including the addition of Tween 20 washes, were added to clear the remaining DNA leading to adequate levels (approx. 60ng/L).

When a scaffold is to be used in tissue reconstruction, specifically abdominal wall reconstruction, residual strength in the mesh is important. The strength is derived from both the residual collagen content following the decellularisation process and the ability of the host to integrate with the implant. This ability has been tested in this case by demonstrating there has been no significant drop in the levels of collagen and GAGs in the resultant scaffold. Also by showing the scaffold retains mechanical strength on tensiometer testing.

In vivo biocompatibility testing was also performed. Over the course of the 3-month study period a significant reduction in the quantity of inflammatory cells, a significant increase in the amount of vascular infiltration and excellent cellular infiltration in the scaffold demonstrating active tissue integration. Minimal fibrous encapsulation was also seen over the study period, without significant increase, demonstrating a modulated immune response to the scaffold from the host. The main issue seen on *in vivo* testing was some samples were found to be completely degraded/absorbed at the 12-week time-point. This was seen in both animals but interestingly not in both scaffolds in the host. This may affect long-term stability of the graft on implantation and would require further investigation on future development.

The effective integration of any biomaterial depends on a balanced inflammatory response and cell infiltration/migration (158–160). Cell migration into a biomaterial is a complex process and is dependent on the interaction between integrins (trans-

cellular proteins in the host tissue) and proteins in the residual extra-cellular matrix (ECM). Initial interaction between these proteins leads to the development of focal adhesions that link the biomaterial to the host and allow the deployment of actin filaments, known as stress fibres. These fibres can then contract pulling new cells into the material. It is thought that proteins within the residual ECM not only promote cell integration but the specific protein pattern presented to the host will determine what cells are migrated into the tissue and determine future function (161). For this reason using a like-for-like replacement of the abdominal wall may improve future function, for example the presence of residual peritoneal proteins in our scaffold would lead to better intra-abdominal function and fewer adhesions. This feature would be useful in the contaminated setting as there is often need to bridge gaps in the peritoneum with mesh, even when the muscle can be approximated. This theory would need to be tested using immunohistochemistry looking at specific protein and cell integration into the scaffold.

4.5 Conclusion

Herein is demonstrated the successful development of a valid protocol to decellularise porcine abdominal wall tissue to create a scaffold for tissue reconstruction. Molecular analysis reveals adequate reduction in the levels of DNA with maintenance of key structural components and mechanical strength. Initial *in vivo* biocompatibility testing revealed encouraging signs of tissue integration and vascularisation, however some concern must be highlighted by the possible complete degradation of some scaffolds at 12-weeks. This work has adequately demonstrated a proof of concept that decellularisation of porcine abdominal wall is feasible as a future biomaterial. Additional work is warranted to further evaluate biocompatibility and perform pre-clinical full thickness abdominal wall excision animal models.

Chapter 5 – Summary and future directions

The overall aim of this work was to improve the risk profile of patients with complex abdominal wall defects, in the context of contamination, through analysis of why patients develop contaminated defects, evaluation of patients undergoing reconstruction of contaminated defects and analysis of repair techniques and considerations. Historical analysis, via systematic review, revealed hernia recurrence occurred in a quarter of cases (24.3%). Overall the long-term risk of further hernia surgery was higher in patients with contamination at the time of hernia repair by comparison to clean cases (Hazard Ratio 1.79 (95% CI 1.72-1.88); $p < 0.0001$). When combined with analysis of short-term outcomes, by Kanters *et al*, contaminated abdominal wall reconstruction has been shown to have poorer short- and long-term outcomes compared to clean case, even in the presence of other comorbidity, and should therefore be evaluated in isolation.

The Dutch philosopher Erasmus coined the phrase “prevention is better than cure” in the 1500s, however within the field of hernia surgery the focus of prevention of incisional hernia has only become prominent in recent years. Studies have been performed evaluating patient and operative risk factors as well as comparing repair techniques from index operations, however no previous analysis has sought to determine factors associated with the development of contaminated defects. Using local and national data this work has identified a number of patient, procedural and post-operative factors, as associated with the development of contaminated defects. Further work to clarify these findings should be performed, such as the evaluation of factors associated with the development of clean defects and comparing them with contaminated defects. The design of randomised controlled trials controlling for these

factors would be difficult to develop as the prediction and controlling for major surgical complications, including SSI, anastomotic leak and the number of previous abdominal operations, would not be possible. However, accounting for and actively employing measures to reduce these factors should logically reduce the likelihood of the development of contaminated defects. Even if the overall reduction of risk achieved by improving these factors are small, any improvement in risk to prevent patients getting into the worst situations, with significant contaminated defects, would be desirable and should be considered.

When evaluating current repair techniques and outcomes this work was able to demonstrate the development and validation of a risk stratification model for key outcomes following contaminated abdominal wall reconstruction. The Clavien Dindo scale is a validated tool for grading post-operative outcome and more than 40% of patients in this cohort were shown to have an outcome of grade 3 or more. Accurate prediction of the highest risk patients for this outcome is important both in patient counselling, pre-operative preparation and in ensuring adequate post-operative precautions are taken to reduce risk.

Given the paucity of data evaluating this complex group of patients, this work represents the first step along the pathway towards developing robust risk models for all outcomes. The work is limited by the lack of previous evaluation of risk factors for all outcomes in modified-Grade 3 patients. The use of uni-variate analysis to select key risk factors for inclusion in the models could limit its effectiveness, as it is more likely to over-fit the model to these data and make it less applicable to external cohorts. A true external evaluation of the models would require a larger cohort

external to both centres included in the development. Given the two largest European centres for intestinal failure were included in the development of the models the accumulation of a true external cohort was not possible however further work could be performed through the development of national/international databases to collect large scale data on contaminated abdominal wall reconstruction. This would also facilitate the development and validation of more accurate models for other outcomes.

Further work is also required to build other factors, thought to be implicated in predicting outcome in abdominal wall reconstruction, into the models. While CT evaluation of the abdominal wall is becoming a standard method, for much of our retrospective cohort it was not used. The data we have developed has standardised the measurements and highlighted a number of key factors that require further evaluation in a larger cohort before they can be built into the models. Measurements related to body composition appear to be most likely related to Clavien Dindo and surgical site outcomes, including the ratio of lateral muscle to pannus and LSMI. Measurements related to hernia dimension, including defect size and defect percentage, appear to be linked to long-term outcomes, such as hernia recurrence. These data are consistent with previous factors measured in clean hernias and consistent measurement in future cohorts, or inclusion of these measurements in an international database would be recommended.

Factors affecting the decision to perform component separation, and which technique is employed, are numerous. These data demonstrate comparable outcomes between open anterior and posterior component separation with transversus abdominis release suggesting either could be employed in CAWR depending on which is more

appropriate on a case-by-case basis and the skill set of the operating surgeon. Development of a model to predict the need for component separation would be useful to surgeons within the field and would most likely utilise some CT measurements. Given the retrospective nature of the data available and the facts that component separation techniques have changed over time and it was not possible to control for other factors in the decision to perform component separation, it was deemed too unreliable to model for the factors associated with the decision to perform component separation. Also when breaking down the cohort further to evaluate only those with CT and with different types of component separation the number became too small to reliably perform the necessary analysis. Further work would need to be done in larger numbers to evaluate this decision making process while controlling for other factors.

Initial systematic review data demonstrated that hernia recurrence rates remain high despite the use of biologic mesh. Biologic mesh is an expensive adjunct to contaminated abdominal wall reconstruction and the ideal mesh construction has yet to be developed. This work has demonstrated the preliminary steps in developing a novel like-for-like biologic mesh for use in abdominal wall reconstruction. Further work is required, initially to up-scale the process and produce larger mesh scaffolds, as well as, performing pre-clinical full thickness excision models to demonstrate mesh strength over time. The best model for this would be a porcine full thickness abdominal wall excision, as the tissue could be analysed for biocompatibility and because the mechanical forces the mesh is subjected to *in vivo* would be as close to true clinical conditions as possible. Further work could also be done to examine the process of extra-cellular matrix deposition within the mesh, via

immunohistochemistry, as well as characterise and compare this to that within dermal scaffolds. This analysis could provide further incite into the durability of the mesh over time and how to improve integration with the host.

Publications arising from this work

Peer reviews journals

Hodgkinson JD, Oke S, Warusavitarne J, Hanna G, Gabe SM, Vaizey CJ. Incisional hernia and enterocutaneous fistula in patients with chronic intestinal failure: prevalence and risk factors in a cohort of patients referred to a tertiary centre. *Colorectal Dis.* 2019 Jun 20. doi: 10.1111/codi.14735 [Epub ahead of print].

Hodgkinson JD, Leo CA, Maeda Y, Bassett P, Oke S, Vaizey CJ, Warusavitarne J. A meta-analysis comparing anterior component separation with posterior component separation in the repair of midline ventral hernias. *Hernia.* 2018 Aug;22(4):617-626. doi: 10.1007/s10029-018-1757-5.

Hodgkinson JD, Maeda Y, Leo CA, Warusavitarne J, Vaizey CJ. Complex abdominal wall reconstruction in the setting of active infection and contamination: a systematic review of hernia and fistula recurrence rates. *Colorectal Dis.* 2017 Apr;19(4):319-330. doi: 10.1111/codi.13609.

Abstracts

Hodgkinson JD, de Vries FEE, Lung PFC, Claessen JJM, Leo CA, Ilangoan R, Baldwin R, Warusavitarne J, Boermeester MA, Vaizey CJ. Is CT imaging useful in the evaluation of the abdominal wall prior to complex contaminated abdominal wall reconstruction? *Colorectal Dis.* 2018 Sept;20(S4):64.

Hodgkinson JD, Oke S, Warusavitarne J, Gabe SM, Vaizey CJ. An evaluation of the prevalence and factors associated with the development of a “complex” surgical abdomen in a cohort of patients presenting to a national intestinal failure centre for home parenteral nutrition. *Colorectal Dis.* 2018 Sept;20(S4):64.

Hodgkinson JD, de Vries FEE, Leo CA, Maeda Y, van Ruler O, Jaessen JJ, Lapid O, Obdeijin M, Tanis PJ, Bemelman WA, Constantinides J, Hanna G, Warusavitarne J, Boermeester MA, Vaizey CJ. Identifying risk factors for short-term post-operative complications following contaminated complex abdominal wall reconstruction: a 12-year follow-up study from two national referral centres for intestinal failure. *Colorectal Dis.* 2017;19(S2):60.

de Vries FEE, **Hodgkinson JD**, van Ruler O, Jaessen JJ, Lapid O, Obdeijin M, Tanis PJ, Leo CA, Maeda Y, Bemelman WA, Constantinides J, Hanna G, Warusavitarne J, Boermeester MA, Vaizey CJ. A follow-up study of long-term outcomes of a 12-year experience of contaminated complex abdominal wall reconstruction in two national referral centres for intestinal failure. *Colorectal Dis.* 2017;19(S2):20.

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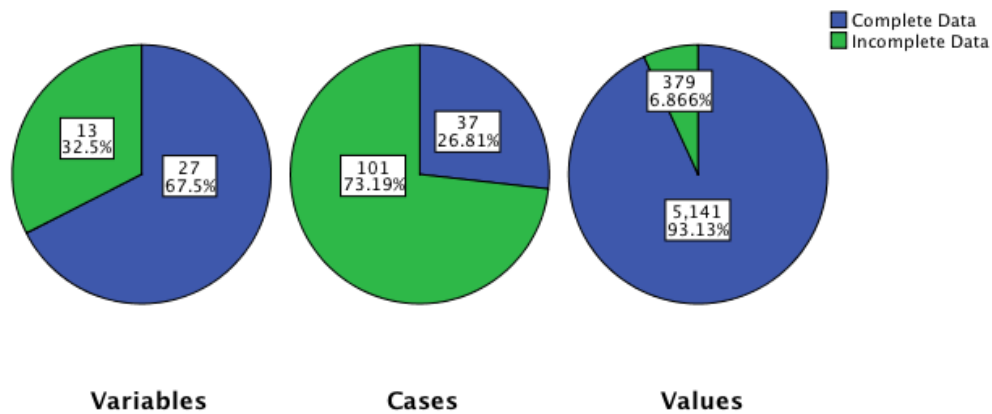
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Appendices

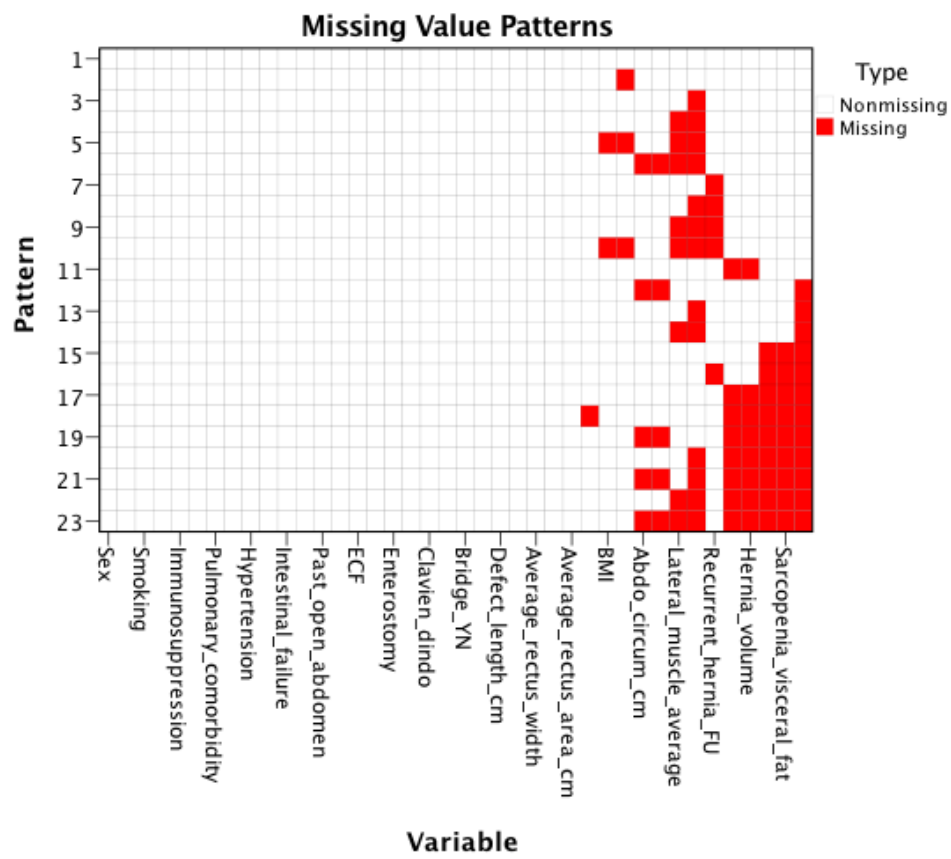
Appendix 1 – Multiple imputation pattern analysis

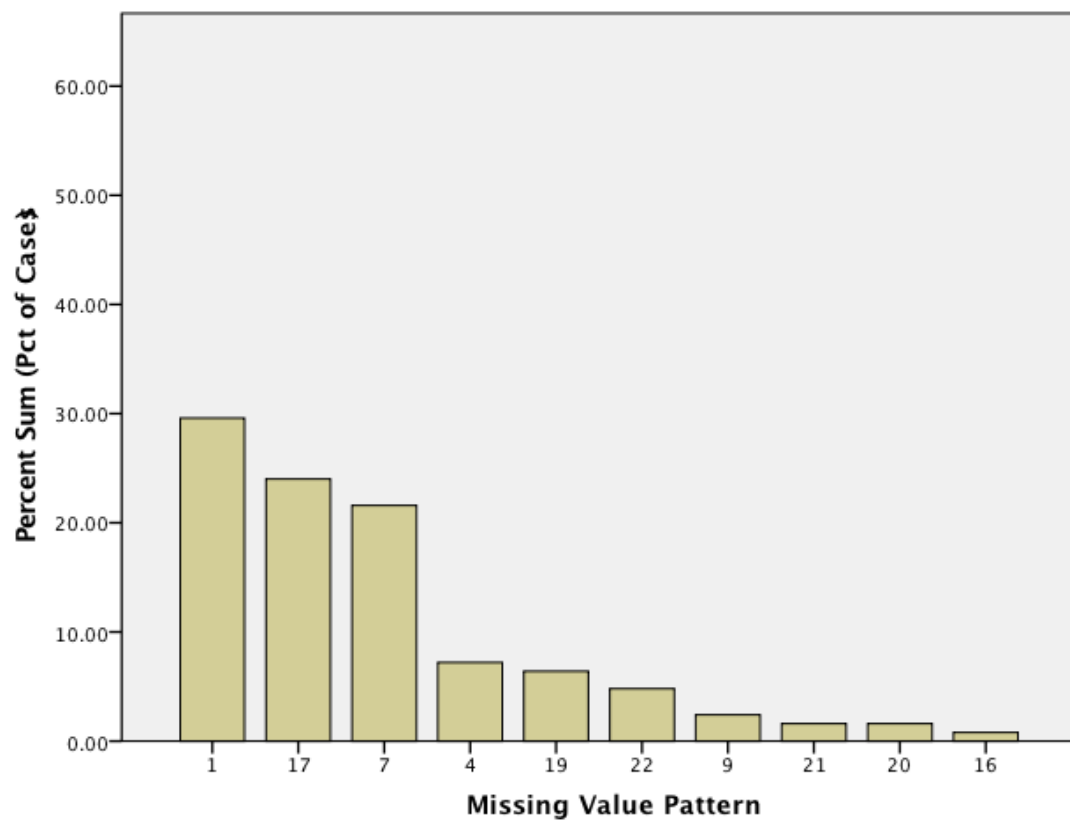
Overall Summary of Missing Values



Variable Summary

	Missing		Valid N	Mean	Std. Deviation
	N	Percent			
LSMI	52	37.7%	86	121.2140	33.88399
Hernia volume	51	37.0%	87	504.9644	580.07965
Intraabdominal volume	51	37.0%	87	8241.84	2384.975
Lateral pannus average	30	21.7%	108	19.4398	12.46804
Lateral muscle average	23	16.7%	115	21.1043	6.60359
Muscle circumference cm	13	9.4%	125	84.4832	10.99867
Abdo circumference cm	13	9.4%	125	102.2912	14.32657





The 10 most frequently occurring patterns are shown in the chart.

Appendix 2 – Scaffold sterilisation protocol

<i>Step</i>	<i>Solution</i>	<i>Duration</i>	<i>rpm</i>	<i>Temp.</i>
1	20% EtOH	15 min	100	25°C
2	40% EtOH	15 min	100	25°C
3	70% EtOH	15 min	100	25°C
4	70% EtOH	15 min	100	25°C
5	40% EtOH	15 min	100	25°C
6	20% EtOH	15 min	100	25°C
7	PBS	15 min + 12 h	100	25°C/4°C
8	PBS + 1% AA	Storage		4°C

Appendix 3 – Standard protocol for Haematoxylin and Eosin staining

1.0 Required Reagents & Apparatus

Gill's III Haematoxylin (Leica Biosystems) (use as supplied)	DPX Mountant (eg Thermo Scientific)
or Harris Haematoxylin (Leica Biosystems) (use as supplied)	Weighing balance & weighing boat
Eosin Yellowish (Pioneer Research Chemicals)	Magnetic stirrer and flea
Hydrochloric Acid (HCl) (Fisher Scientific)	Timer
De-ionised water	Filter Paper
Tap water	Staining troughs
Xylene (Genta Medical) (use as supplied)	Wooden spatula
Absolute Industrial Denatured Alcohol (IDA) (Genta Medical)	1L conical flask
95% IDA	Appropriately-sized beakers
75% IDA	Appropriately-sized measuring cylinders

2.0 Preparation of Specimen for Staining

2.1 Fix specimen as described in SOP 6:1:1

2.2 Process (SOP 6:1:3) and embed (SOP 6:1:4) specimen

2.3 Section wax block containing specimen as described in SOP 6:1:6

3.0 Preparation of Reagents

0.5% Aqueous Eosin (1 Litre)	Add 5g Eosin Yellowish to 1 litre de-ionised water. Mix well on magnetic stirrer. Filter solution using filter paper. Add a few crystals of thymol as a preservative. Transfer ~300ml of the solution to a staining trough ready to use for staining. Transfer remaining solution into an appropriately labelled bottle (SOP 1:1:2). Storage: room temperature. Discard after 1 year.
1% Hydrochloric Acid in 70% IDA (1 Litre)	Add 10ml Hydrochloric acid to 990 ml 70% IDA solution. Agitate container by hand. Storage: At room temperature in labelled bottle. Discard after 1 year.
70% IDA (1 Litre)	Add 300 mls de-ionised water 700 ml Absolute IDA. Storage: room temperature in labelled container. Discard after 1 year.
95% IDA (1 Litre)	Add 50 ml de-ionised water to 950 ml Absolute IDA. Storage: room temperature in labelled container. Discard after 1 year.
Xylene 1 & 2; Absolute IMS	From stock solution, fill appropriately labelled staining troughs.

4.0 Method

- 4.1 Fill appropriately labelled staining troughs with required reagents
- 4.2 Place all slides to be stained in a staining rack.
- 4.3 Follow procedure as detailed in Table 1 by placing staining rack into each of the reagents staining troughs.
- 4.4 Remove any scum from the surface of the Haematoxylin by running a paper towel over the top of the stain or filtration.

Table 1 procedure for H&E stain

Reagent	Time
Xylene 1	5 min (*agitate rack for few seconds)
Xylene 2	2 min
Absolute IDA	2 min *
95% IDA	2 min
70% IDA	2 min
Tap water	2 min *
Gill's III Haematoxylin	1.5 min
<u>or</u> Harris Haematoxylin	<u>or</u> 10min
Tap water	5 min (wash)
1% HCl in 70% IDA - for Gill's	3 dips
- for Harris	10 dips
Tap water	4 min
0.5% Aqueous Eosin	5 min *
Tap water	20 sec (rinse, blot dry) *
70% IDA	30 sec
95% IDA	30 sec
Absolute IDA	2 min
Xylene 1	2 min *
Xylene 2	Until ready to mount with DPX

- 4.5 After staining is complete, coverslip all slides (SOP6:1:8)
- 4.6 Successful staining will show nuclei stained blue and other tissue components stained in shades of red and pink

5.0 Reference

Ellis R. Haematoxylin and Eosin (H&E) Staining Protocol (www.ihcworld.com)

Appendix 4 – Standard protocol for Picrosirius Red staining

1.0 Required Reagents & Apparatus

Potassium Permanganate (Sigma)	95% IDA
Oxalic Acid (Sigma)	75% IDA
Miller's Elastin Stain (Leica Biosystems) (use as supplied)	DPX Mountant (eg Thermo Scientific)
Weigert's Haematoxylin A (Pioneer Research Chemicals)	Weighing balance & weighing boat
Weigert's Haematoxylin B (Pioneer Research Chemicals)	Magnetic stirrer and flea
Sirius Red F3BA (Sigma)	Timer
Aqueous Saturated Picric Acid (Sigma)	Filter Paper
Sulphuric Acid (Sigma)	Staining troughs
Hydrochloric Acid (HCl) (Fisher Scientific)	Staining racks
De-ionised water	Appropriately-sized beakers
Tap water	Appropriately-sized measuring cylinders
Xylene (Genta Medical) (use as supplied)	1L conical flask
Absolute Industrial Denatured Alcohol (IDA) (Genta Medical)	Wooden spatula

2.0 Preparation of Specimen for Staining

2.1 Fix specimen as described in SOP 6:1:1

2.2 Process (SOP 6:1:3) and embed (SOP 6:1:4) specimen

2.3 Section specimen (SOP 6:1:6)

3.0 Preparation of Reagents

3% Sulphuric Acid (1 Litre)	Add 30 ml Sulphuric Acid to 970 ml de-ionised water. Storage: At room temperature in labelled bottle. Discard after 1 year.
0.5% Acid Potassium Permanganate (300 ml)	In staining trough, add 0.75g Potassium Permanganate to 150 ml de-ionised water. Then, add 150 ml 3% Sulphuric Acid solution. Mix well with wooden spatula. Discard solution after use.
1% Oxalic Acid (1 Litre)	Add 10g Oxalic acid to 1 L de-ionised water. Mix well on magnetic stirrer. Storage: At room temperature in labelled bottle. Discard after 1 year.
Weigert's Haematoxylin (300 ml)	Add 150 ml Weigert's Haematoxylin A to 150 ml Weigert's Haematoxylin B. Storage: At 2-10 °C in labelled bottle. Discard after 2 months.
Picro Sirius Red (1 Litre)	Add 1g Sirius Red F3BA to 1 litre Aqueous Saturated Picric Acid solution. Mix well on magnetic stirrer. Filter solution. Storage: At room temperature in labelled bottle. Discard

	after 1 year.
1% Hydrochloric Acid in 70% IDA (1Litre)	Add 10ml Hydrochloric acid to 990 ml 70% IDA solution. Agitate container by hand. Storage: At room temperature in labelled bottle. Discard after 1 year.
70% IDA (1 Litre)	Add 300 mls de-ionised water 700 ml Absolute IDA. Storage: room temperature in labelled container. Discard after 1 year.
95% IDA (1 Litre)	Add 50 ml de-ionised water to 950 ml Absolute IDA. Storage: room temperature in labelled container. Discard after 1 year.
Xylene 1 & 2; Absolute IMS	From stock solution, fill appropriately labelled staining troughs.

4.0 Method

- 4.1 Fill appropriately labelled staining troughs with required reagents
- 4.2 Place all slides to be stained in a staining rack
- 4.3 Follow procedure as detailed in Table 1 by placing staining rack into each of the reagent staining troughs

Table 1 Procedure for Picro Sirius Red with Miller's Elastin Stain

Reagent	Time
Xylene 1	5 min (*agitate rack for few seconds)
Xylene 2	2 min
Absolute IDA	2 min *
95% IDA	2 min
70% IDA	2 min
Tap water	2 min (wash, blot dry) *
0.5% Acid Potassium Permanganate	5 min
De-ionised Water	1 min (rinse) *
1% Oxalic Acid	1 min
De-ionised Water (Fresh)	2 min *
95% IDA	50 sec (rinse)
Miller's Stain	40 min
95% IDA	50 sec (rinse) *
Tap water	2 min (wash, blot dry) *
Weigert's Haematoxylin	10 min
Tap water	1 min (wash, blot dry) *
1% HCl in 70% IDA	3 dips
Tap water	4 min (wash, blot dry) *
Picro Sirius Red	40 min

Tap Water	20 sec (rinse, blot dry) *
70% IDA	30 sec (rinse, blot dry) *
95% IDA	30 sec (rinse, blot dry) *
Absolute IDA	2 min*
Xylene 1	2 min *
Xylene 2	Until ready to mount with DPX

4.5 After staining is complete, coverslip all slides (SOP6:1:8)

4.6 Successful staining will show elastin and mast cell granules stained black/purple, collagen stained red and muscle and cytoplasm stained yellow

5.0 Reference

Ellis R. Picro Sirius Red combined with Miller's Elastic Staining Protocol
(www.ihcworld.com)

Appendix 5 – Standardised DNA extraction protocol from GenElute mammalian genomic DNA miniprep kit (Sigma-Aldrich, UK)

Materials

- Resuspension Solution (P3980)
- Lysis Solution T (B6678)
- Lysis Solution C (B8803)
- Column Preparation Solution (C2112)
- Wash Solution Concentrate (B6553)
- Elution Solution (10mM Tris-HCl, 0.5mM EDTA, pH9.0) (B6803)
- Proteinase K (P2308)
- RNase A Solution (R6148)
- GenElute™ Miniprep Binding Columns in tubes (C9471)
- Collection tubes, 2.0mL capacity (T5449 or T7813)
- 1.5 mL Eppendorf tubes for lysis
- Ethanol (95-100%), (E7148)
- 55 °C water bath
- 70 °C heating block
- Microcentrifuge (2mL tube, rotos-equipped)
- De-ionised water

Preparation

- Set a water bath to 55°C and hot plate to 70 °C

Preparation of reagents

1. Thoroughly mix reagents
2. Dilute wash solution concentrate with 95/50% ethanol as per manufacturer instructions.
3. Dissolve Proteinase K in water to obtain 20mg/ml stock solution as per manufacturers instructions. Store at 2-8 °C for several days or at -20 °C until needed.

Mammalian tissue preparation

1. Prepare tissue samples. Weigh out 25 mg of frozen tissue for each sample, mince thoroughly and place into a 1.5 mL homogenisation tube, keep on ice to protect against degradation.
2. Add 180 µl of Lysis Solution T, followed by 20 µl of the Proteinase K solution to the tissue. Mix by vortexing. Incubate the sample at 55 °C until the tissue is completely digested and no particles remain. Digestion is usually complete in 2 to 4 hours and vortex occasionally. After digestion is complete, vortex briefly.
3. RNase A treatment: add 20 µl of RNase A solution and incubate for 2 minutes at room temperature, then continue with step 3b.
4. Lyse cells: add 200 µl of Lysis Solution C to the sample; vortex thoroughly (15 seconds). A homogenous mixture is essential for efficient lysis. Incubate at 70 °C for 10 minutes.

DNA isolation from all listed sample types

1. Column preparation. Add 500 µl of the Column Preparation Solution to each pre-assembled GenElute™ Miniprep Binding Column and centrifuge at 12,00 x g for 1 minute. Discard flowthrough liquid.
2. Prepare for binding: Add 200 µl of ethanol (95-100%) to the lysate; mix thoroughly by vortexing 5-10 seconds. A homogenous solution is essential.
3. Load lysate: Transfer the entire contents of the tube into the treated binding column from step 1. Use a wide bore pipette tip to reduce shearing the DNA when transferring contents into

- the binding column. Centrifuge at $>6,500 \times g$. Discard the collection tube containing the flow through liquid and place the binding column in a new 2 ml collection tube.
4. First wash: Prior to first use, dilute the Wash Solution Concentrate with ethanol as described under Preparation Instructions. Add 500 μl of Wash Solution to the binding column and centrifuge for 1 minute at $>6,500 \times g$. Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.
 5. Second wash: Add another 500 μl of wash solution to the binding column; centrifuge for 3 minutes at maximum speed (12,000-16,000 $\times g$) to dry the binding column. The binding column must be free of ethanol before eluting the DNA. Centrifuge the column for one additional minute at maximum speed if residual ethanol is seen. Finally, discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.
 6. Elute DNA: Pipette 200 μl of the Elution Solution directly into the centre of the binding column; centrifuge for 1 minute at $>6,500 \times g$ to elute the DNA. To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Solution, then centrifuge.

The eluate contains pure genomic DNA. For short-term storage of DNA, 2-8 °C is recommended. For long-term storage of DNA, -20 °C is recommended.

Appendix 6 – GAG quantification protocol from the Blyscan GAG assay kit (Biocolor, Carrick Fergus, NI, UK).

Materials

- Extraction buffer: 50 mM Tris-HCl, 10 mM NaCl, 3 mM MgCl₂, pH 7.9
 - Add 0.605 g Tris-HCl, 0.584 g NaCl and 0.286 g MgCl₂ to 100 mL distilled water
 - Check the pH=7.9
- Homogenisation tubes (Precellys)
- 1.5 mL Eppendorf
- Proteinase K (Sigma)
- DNase (Sigma)

Preparation

- Set a hot plate to 56°C
- Prepare the extraction buffer

Method

4. Measure out the required volume of extraction buffer and add the appropriate amount of Proteinase K (to make a final concentration 50 µg/mL, stock solution 20 mg/mL = 1/400 dilution), mix well.
5. Prepare tissue samples. Wash control tissue in ice-cold PBS to remove blood before weighing. Dry tissue with blotting paper as much as possible before weighing out 40 mg for each sample, mince and place into a 1.5 mL homogenisation tube, keep on ice.
6. Add 1 mL of extraction buffer containing Proteinase K to each tube.
7. Add 2 µL of protease inhibitor (Sigma) to each tube.
8. Homogenise each sample using the Precellys Homogeniser and place in a heat block set to 56°C to digest the tissue overnight.
9. After overnight digestion is complete inactivate the Proteinase K by increasing the incubation temperature to 90°C for 30 minutes.
10. Allow the samples to cool to RT before adding 1.5 µL DNase (to make a final concentration of 30 U/mL, stock solution 20 KU/mL) to each tube. Incubate overnight at 37°C.
11. Transfer the digest to a new 1.5 mL Eppendorf tube and centrifuge at 13,000 g for 20 minutes.
12. Recover the supernatants to quantify µg GAG per mg wet tissue, using the Blyscan Sulphated Glycosaminoglycan Assay (use protocol supplied with the assay kit).
13. To make up the reagent blanks and the standards as directed in the assay kit manual (see table)
DO NOT use the extraction buffer, use deionised water.
14. Use 50 µL of each test sample with 50 µL deionised water.

sGAG standards

Concentration (µg)	Volume ref. standard (µL)	Volume dH ₂ O (µL)
1.0	10	90
2.0	20	80
3.0	30	70
4.0	40	60
5.0	50	50

Appendix 7 – The collagen quantification protocol from the Sircol collagen assay kit
(Biocolor, Carrick Fergus, NI, UK).

Materials

- Make up 0.5 M acetic acid solution, store at 4°C
 - Add 2.86 mL acetic acid to 97.14 mL water, mix well
- Homogenisation tubes (Precellys)
- 1.5 mL Eppendorf tubes

Preparation

- Prepare the acetic acid solution
- Set up shaker in fridge

Method

15. Measure out the required volume of 0.5 M acetic acid solution and add the appropriate amount of pepsin (Sigma, P7012) (to make a final concentration 0.5 mg/mL), mix well.
16. Prepare tissue samples. Wash control tissue in ice-cold PBS to remove blood before weighing. Dry tissue with blotting paper as much as possible before weighing out 40 mg of each sample, mince and place into a 1.5 mL homogenisation tube, keep on ice.
17. Add 1 mL of acid-pepsin to each tube.
18. Add 2 µL of protease inhibitor (Sigma) to each tube.
19. Homogenise each sample using the Precellys Homogeniser.
20. Leave to agitate gently on a shaker for 18 hours at 4°C.
21. Transfer the contents of each tube to a new 1.5 mL Eppendorf and centrifuge samples at 12,000 g for 10 minutes to separate tissue debris
22. Carefully transfer the supernatant containing protein to a new 1.5 mL Eppendorf.
23. Quantify µg collagen per mg wet tissue, using the Sircol Soluble Collagen Assay (use protocol supplied with the assay kit).
24. Set up the collagen blanks and standards using the same 0.5 M acetic acid solution used for the digestion step (see table).
25. Use 50 µL of each test sample and dilute with 0.5 M acetic acid.
26. Immediately before the measurement step (see Sircol protocol) dilute each test sample 1:5, take 40 µL of test sample and dilute with 160 µL 0.5 M acetic acid. Mix well and transfer to a 96-well plate.
27. Set the micro plate reader to 555 nm and measure the absorbance.

Collagen standards

Concentration (µg)	Volume ref. standard (µL)	Volume 0.5 M acetic acid (µL)
2.5	5	95
5.0	10	90
7.5	15	85
10.0	20	80
15.0	30	70

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