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The peritoneum and disease morbidity in childhood acute appendicitis

Tzu-Chieh Yu
ABSTRACT

AIM To describe the pathophysiology and disease morbidity of peritoneal inflammation in childhood appendicitis and to determine whether this understanding can be applied to current management strategies to improve patient outcome.

METHODS An observational study was conducted, using multivariate regression modelling, to describe disease morbidity from appendicitis-related secondary peritonitis, identify predictors of patient outcome, and evaluate current treatment strategies. Adequately powered, prospective clinical trials investigated the impact of interventions targeting appendicitis-related peritoneal inflammation and its clinical manifestations. A comparison cohort study investigated the use of clinical criteria representative of resolving peritoneal inflammation to guide postoperative antibiotic duration in appendicitis-related secondary peritonitis. A double-blinded, randomised, controlled trial (RCT) investigated warm humidified gas insufflation during laparoscopic appendicectomy as a means of preventing pneumoperitoneum-related peritoneal desiccation.

RESULTS In complicated appendicitis, significant morbidity arises from secondary peritonitis. The observational study of 359 participants found a postoperative complication rate of 25% with intraabdominal infections being the most common complication (44 cases, 12%). Late (≥ Day 3) postoperative fever was an independent predictor of intraabdominal infections (odds ratio = 0.35, p = 0.016) while duration of postoperative antibiotic therapy was a key predictor of LOS (F = 215.5; partial $\eta^2 = 0.48; p < 0.001$). The comparison cohort study of 94 participants found that using bedside clinical criteria to determine postoperative
antibiotic duration shortened median LOS (5 versus 6 nights, p = 0.010). Compared to a fixed duration of therapy, readmission rates and the incidence and severity of complications were not significantly different, including intraabdominal infections (6 versus 8 cases, p = 0.562). Altering physical features of laparoscopic gas insufflation affects the peritoneal desiccation, acidosis, hypothermia, and hypoxia associated with conventional carbon dioxide pneumoperitoneum. Warm humidified insufflation gas prevents peritoneal desiccation. However, the RCT of 190 participants found no short-term benefits on postoperative opiate consumption, pain scores, and return to normal activities after laparoscopic appendicectomy in children.

**CONCLUSIONS** Childhood appendicitis is accompanied by peritoneal inflammation and injury from secondary peritonitis and laparoscopic surgery. Management strategies that take into account the contributing factors and clinical consequences of peritoneal inflammation can reduce disease morbidity and improve practice efficiency.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my father and mother, Charles and Nina Yu. They have been an endless source of encouragement and understanding. I feel truly fortunate knowing that they will never cease to support me unconditionally in all of my endeavours. I would also like to thank my sister and brother, Sandy and Tommy Yu. Their insightfulness and sound advice has been so valuable, along with their belief in me.

This work would not have been possible without the guidance and mentorship of Professor Andrew G. Hill. I feel extraordinarily privileged to have him as an academic supervisor and teacher, clinical colleague, and friend.

I would like to sincerely thank the team of research fellows and administrative staff at the South Auckland Clinical School, University of Auckland. It has been immensely memorable and enjoyable to work alongside such dedicated, professional, and wonderful individuals.

The same can be said about the clinicians from the Departments of Paediatric Surgery, Anaesthesia, and Infectious Diseases at Starship Children’s Hospital. I would like to thank them and the nursing teams from Ward 24A, Ward 24B, and the operating theatre for their unwavering efforts to make this research possible.

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GLOSSARY

Symbols

%  Percentage
°C  Degree Celsius
-ve  Negative

A

ABs  Antibiotics
ACh  Acetylcholine
ASBO  Adhesive small bowel obstruction

B

bpm  Beats per minute

C

CI  Conference interval
cm  Centimeters
CNS  Central nervous system
CO₂  Carbon dioxide
CONSORT  Consolidated Standards of Reporting Trials
COX-2  Cyclo-oxygenase 2
CRP  C-reactive protein
CT  Computer tomography
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>dBA</td>
<td>Decibel A-weighting</td>
</tr>
<tr>
<td>DC</td>
<td>Discharge (from hospital)</td>
</tr>
<tr>
<td>DHB</td>
<td>District health board</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene-diamine-tetra-acetic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte colony-stimulating factor</td>
</tr>
<tr>
<td>GALT</td>
<td>Gut-associated lymphoid tissue</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>H2O</td>
<td>Water</td>
</tr>
<tr>
<td>HGF</td>
<td>Hepatocyte growth factor</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamo-pituitary-adrenal</td>
</tr>
<tr>
<td>Hrs</td>
<td>Hours</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>IAI</td>
<td>Intraabdominal infections</td>
</tr>
<tr>
<td>ICAM</td>
<td>Intracellular adhesion molecule</td>
</tr>
<tr>
<td>ICD</td>
<td>International classification of diseases</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL-n</td>
<td>Interleukin-n (e.g. IL-8 = Interleukin-8)</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilodaltons</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>KGF</td>
<td>Keratinocyte growth factor</td>
</tr>
<tr>
<td>L</td>
<td>Litres</td>
</tr>
<tr>
<td>Lap</td>
<td>Laparoscopic</td>
</tr>
<tr>
<td>LED</td>
<td>Light-emitting diode</td>
</tr>
<tr>
<td>LOS</td>
<td>Length of stay</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>LT-B4</td>
<td>Leucotriene-B4</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>m</td>
<td>Meters</td>
</tr>
<tr>
<td>Max</td>
<td>Maximum/Maximal</td>
</tr>
<tr>
<td>MCP-(n)</td>
<td>Monocyte chemoattractant protein-(n)</td>
</tr>
<tr>
<td>MEDD</td>
<td>Mean equivalent daily doses</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>(\mu)g</td>
<td>Micrograms</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MIP-(n)</td>
<td>Macrophage inflammatory protein-(n)</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitres</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetres</td>
</tr>
<tr>
<td>(\mu)m</td>
<td>Micrometres</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimetres of mercury</td>
</tr>
<tr>
<td>MMC</td>
<td>Migrating motor complex</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>mth</td>
<td>Month</td>
</tr>
<tr>
<td>n</td>
<td>Number/frequency</td>
</tr>
<tr>
<td>NA</td>
<td>Not applicable</td>
</tr>
<tr>
<td>(\text{NaHCO}_3)</td>
<td>Sodium bicarbonate</td>
</tr>
<tr>
<td>NHI</td>
<td>National health index</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
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</tbody>
</table>
NTS: Nucleus tratus solitaries

O

O₂: Oxygen
Op.: Operative approach

P

PACU: Post-anaesthesia care unit
PAI: Plasminogen activator inhibitor
PALT: Peritoneum-associated lymphoid tissue
PCA: Patient-controlled analgesia
PCAM: Platelet-endothelial cell adhesion molecule
PDGF: Platelet derived growth factor
PDS: Polydioxone suture
PMN: Polymorphonuclear granulocytes/leucocytes
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

R

RANTES: Regulated on activation normal T cell expressed and secreted
RCT: Randomised controlled trial
RH: Relative humidity

S

SBO: Small bowel obstruction
SD Standard deviation
SE Standard error
SEM Scanning electron microscopy
sICAM-1 Soluble intercellular adhesion molecule-1
STROBE Strengthening Reporting of Observational Studies in Epidemiology

T
TGF-α Transforming/tumour growth factor alpha
TGF-β Transforming/tumour growth factor beta
Th T helper cell
TIMP Tissue inhibitor of metalloproteinase
TNF-α Tumour necrotising factor alpha
tPA Tissue plasminogen activator

U
uPA Uokinase plasminogen activator

V
V Volts
VAS Visual analogue scale
VCAM Vascular cell adhesion molecule
VEGF Vascular endothelial growth factor
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
<td>W</td>
<td>Watts</td>
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<tr>
<td>WBC</td>
<td>White blood cell</td>
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<td>wk</td>
<td>Week</td>
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Chapter 1

INTRODUCTION
1.1 ACUTE APPENDICITIS

1.1.1 Epidemiology

Acute appendicitis is the most common acute abdominal surgical condition in childhood\(^1\) with a lifetime risk of approximately 7% in females and 9% in males.\(^2\) Although described centuries earlier, the term ‘appendicitis’ was first used by Fitz in 1886.\(^3\) Chronologically, the incidence of acute appendicitis rose in Western countries during the end of the 19th century and early part of the 20th century, but since then has had a dramatic decline. This has been most pronounced in the younger age groups\(^4\)\(^-\)\(^7\) and while the reasons are unclear, suggestions include changes in diet, improvement in hygiene, and changing patterns of childhood infection resulting in reduced lymphoid hyperplasia in the gut.\(^8\)\(^,\)\(^9\)

Despite this steady decline in incidence, acute appendicitis remains highest in the paediatric age group with about one third of affected patients being younger than 18 years of age\(^10\) and the peak incidence rate found in those between the ages of 11 and 12. Appendicitis is ultimately diagnosed in 1 to 8% of children presenting to paediatric emergency departments with acute abdominal pain,\(^11\) making it the most common surgical emergency in children and, globally, one of the major causes for hospitalisation among patients aged from 1 to 14 years.\(^1\)

Additionally, not only is the incidence of appendicitis higher in children but acute appendicitis with appendiceal necrosis, gangrene and perforation resulting in secondary peritonitis is also more common in the paediatric population.\(^12\)\(^,\)\(^13\) It affects up to 30-50% of
children with appendicitis\textsuperscript{14,15} and in those less than 5 years of age, the incidence rate can be as high as 74\%. In children less than 2 years of age, the rate has been reported to be 95\%.\textsuperscript{16} There are several arguments for why younger children present more often with complicated appendicitis (see Section 1.1.2). Spread of inflammation to the peritoneum differentiates ‘simple’ from ‘complicated’ appendicitis with the latter being linked to significantly higher complications and is the most common cause of peritonitis and intraabdominal sepsis with Gram-negative septic shock in children.\textsuperscript{17,18}

Generally, a delay in diagnosis of appendicitis and the corresponding perforation rates have been linked to a variety of patient socio-economic factors such as ethnicity, access to healthcare, and health insurance status, as well as clinician factors including patient referral patterns and antibiotic administration.\textsuperscript{19-22} These delays have largely been attributed to the pre-hospital period and it has been shown that a short period of in-hospital observation with repeated physical examination in children with suspected early appendicitis is safe and effective.\textsuperscript{23}

In contrast to the declining incidence of simple appendicitis, the global rate of complicated appendicitis has remained unchanged in the last 3 decades\textsuperscript{24,25} leading to speculation about whether complicated appendicitis is merely the result of nonperforated appendicitis with delayed treatment or whether the two are in fact different disease entities with diverging pathophysologies.\textsuperscript{26,27} Nevertheless, it is clear that predictors of the subsequent disease course and factors shaping patient morbidity are not shared by these two conditions (see Section 1.1.5 on disease outcomes).
1.1.2 Pathophysiology

1.1.2.1 Luminal obstruction

Acute appendicitis develops primarily from direct obstruction of the appendiceal lumen and secondary bacterial infection. This process was first described by van Zwalenberg in 1905 and experimentally confirmed by Wangensteen and Dennis in 1939. A range of mechanisms causes luminal obstruction (Table 1.1) although it is not always clear in individual patients.

Table 1.1 Causes of Appendiceal Lumen Obstruction Leading to Appendicitis

<table>
<thead>
<tr>
<th>Cause</th>
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<tbody>
<tr>
<td>Faecoliths</td>
</tr>
<tr>
<td>Hyperplasia of Appendiceal Lymphoid Follicles as a result of bacterial, viral, or parasitic infections</td>
</tr>
<tr>
<td>Altered Composition of Luminal Mucus Secondary to Cystic Fibrosis</td>
</tr>
<tr>
<td>Carcinoid Tumours</td>
</tr>
<tr>
<td>Foreign Bodies</td>
</tr>
<tr>
<td>Blunt Abdominal Trauma</td>
</tr>
<tr>
<td>Psychological Stress</td>
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<tr>
<td>Heredity</td>
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The natural configuration and physiology of the appendix is self-detrimental when the lumen becomes obstructed. Firstly, the appendix is a blind-ending tube and in contrast to the wall of the caecum, which is able to stretch because of a diagonal, rhomboid-like mesh of collagen fibres, the horizontal collagen fibres of the appendiceal wall only allow for minimal passive luminal expansion. In addition, the foldless appendiceal mucosa does not have sufficient reserve to accommodate swelling. Secondly, the appendiceal mucosa secretes 2 to 3 mL of mucus daily and this normal function does not cease if its lumen...
becomes obstructed and intraluminal pressure increases. In fact, the appendix is able to continue secreting until gangrene and perforation occur. Couple this with the fact that the average appendiceal luminal capacity is only approximately 1 mL, an increase in intraluminal pressure can occur rapidly. Within the lumen, undrained mucus promotes bacterial proliferation.

1.1.2.2 Loss of intestinal barrier integrity

After luminal obstruction, steadily increasing intraluminal pressure starts to impair appendiceal lymphatic and venous drainage, directly resulting in transmural oedema. This congestion subsequently reaches pressures that impede arterial inflow leading to tissue ischaemia, infarction, and gangrene. The integrity of the mucosa becomes impaired allowing for bacterial invasion and translocation. Eventually further breakdown of the appendiceal wall leads to perforation with spillage of infected intraluminal contents and subsequent localised abscess formation or generalised peritonitis.

How quickly the disease progresses to perforation and the degree to which it spreads within the intraperitoneal cavity depends on several factors including the patient’s ability to mount a host defence response and contain the spilled contamination, and the timely administration of antibiotics. Younger children tend to have inferior ability to understand or articulate developing symptomatology compared to adolescents and presentation is therefore often further delayed. Compared to older patients, the thin wall of the appendix in children and immaturity of the omentum that is devoid of fat is believed to also contribute to the rapid spread of intraluminal infection to generalised intraperitoneal disease. It has been found that antibiotic treatment during the early stages of
appendicitis causes regression of lymphoid hyperplasia resulting from bacterial infection which in turn prevents ischaemia and continued bacterial invasion.\textsuperscript{44, 45}

1.1.3 Clinical Presentation

Although many terms have been used to describe varying states of appendicitis including ‘acute’, ‘suppurative’, ‘gangrenous’, and ‘perforated’, these terms are not standardised and can be imprecise because of significant variation between clinicians and institutions. Clinically, the only relevant distinction is between ‘simple’ and ‘complicated’ appendicitis although these terms only describe two points along the continuum of a progressive disease. As mentioned, the terms make a distinction between the absence and presence of peritoneal inflammation and infection and therefore predict subsequent course of disease and patient outcome.\textsuperscript{10} Table 1.2 summarises these two forms of acute appendicitis in terms of differences in their clinical presentation, investigative findings, intraoperative findings, histopathological findings, and postoperative course.
### Table 1.2 ‘Simple’ versus ‘Complicated’ Acute Appendicitis

<table>
<thead>
<tr>
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<th>‘Simple’ Appendicitis</th>
<th>‘Complicated’ Appendicitis</th>
</tr>
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<tbody>
<tr>
<td><strong>Clinical Signs</strong></td>
<td>- Localised right lower quadrant abdominal pain and tenderness</td>
<td>- Generalised signs of peritonism or localized tender intraabdominal mass</td>
</tr>
<tr>
<td></td>
<td>- Low grade fever</td>
<td>- High grade fever and signs of septic shock e.g. tachycardia</td>
</tr>
<tr>
<td><strong>Investigative Findings</strong></td>
<td>- Mildly elevated or normal leucocyte count</td>
<td>- Markedly elevated leucocyte count &amp; serum inflammatory markers</td>
</tr>
<tr>
<td></td>
<td>- Ultrasound shows fluid-filled, noncompressible blind-ending structure with increased diameter and perioappendiceal echogenicity</td>
<td>- In addition to signs consistent with simple appendicitis, ultrasound also shows periappendiceal and pericaecal free fluid or a localised intraabdominal abscess</td>
</tr>
<tr>
<td><strong>Intraoperative Findings</strong></td>
<td>- Acute inflammation of the appendix (erythematos, enlarged and oedematous) with or without reactive serous peritoneal free fluid</td>
<td>- Acute appendicitis and the presence of an intraperitoneal abscess with significant purulent free fluid, appendiceal necrosis and gangrene, and/or gross evidence of perforation (hole in the appendiceal wall)</td>
</tr>
<tr>
<td><strong>Histopathological Findings</strong></td>
<td>- Acute appendiceal inflammation without signs of perforation</td>
<td>- Acute suppurative, gangrenous, or necrotic appendicitis with macroscopic or microscopic perforation</td>
</tr>
<tr>
<td><strong>Postoperative Course</strong></td>
<td>- Low risk of postoperative complications</td>
<td>- Significant risk of postoperative complications</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Prolonged hospitalisation</td>
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The clinical course of appendicitis in its simplest and classic presentation begins with anorexia and vague periumbilical pain that migrates to the right lower quadrant. This is a reflection of the progression of inflammation and infection from structures with visceral afferent innervation to adjacent structures with somatic afferent pathways. Without its own somatic afferent pathways, the appendix, like many intraabdominal organs, is reliant on the adjacent enveloping segment of peritoneum to communicate injury to the central nervous system (CNS) to produce symptoms of appendicitis. Clinical signs of appendicitis elicited by
the examining clinician are also derived from adjacent peritoneal irritation such as percussion tenderness and pain elicited by gentle pressure applied to the left side of the abdomen (Rovsing’s sign) or by mild shaking of the abdomen.

Initially the contraction of the appendix against an obstructed lumen results in activation of visceral afferent fibres that enter the spine at the level of T10, causing vague pain referred to the periumbilical area. As the inflammation becomes transmural and reaches the parietal peritoneum, peritoneal somatic afferent pain fibres become activated causing pain localised to the vicinity of the appendix.\textsuperscript{46} Nausea and vomiting are common symptoms after the onset of pain and are caused by the increasing dilation of the appendiceal lumen and stretching of the serosa.\textsuperscript{10} Transitory diarrhoea, after the onset of abdominal pain, is also often reported and likely the result of terminal ileum and caecal irritation or it may indicate a pelvic abscess.

The most discrete physical finding is tenderness in the right lower quadrant reflecting localised peritoneal irritation but this sign can be affected by various factors including obesity, retrocaecal position of the appendix, omental wrapping, or partitioning loop of small bowel or mesentery. In these cases, the patient may maintain vague symptoms.\textsuperscript{47} Irritation of the psoas and obturator muscles by retrocaecal appendicitis causes demonstrable rigidity of these muscles.

When complicated appendicitis has occurred, localised findings are taken over by signs of generalised peritoneal inflammation and the systemic acute phase response to this including fever and markedly elevated serum inflammatory markers.\textsuperscript{48} A small proportion of
young children present with acute small bowel obstruction secondary to extensive inflammation of the terminal ileum and caecum. Older children are more likely to present with organised abscesses and a boggy tender mass can be felt on examination depending on the degree of abdominal wall rigidity.

1.1.4 Treatment

The standard management of appendicitis has evolved to include both operative and non-operative interventions but the methods can vary significantly among clinicians and medical centres according to the patient’s clinical status, the medical centre’s capabilities, and the physician’s experience and technical expertise. Appendicectomy remains the mainstay of definitive operative treatment but the technical details of this procedure vary considerably. Furthermore, while it is agreed that antibiotic regimens used must provide broad-spectrum coverage of enteric organisms, the best regimen and duration of use is a subject of continued controversy.

Treatment of appendicitis begins with intravenous (IV) fluids to correct fluid and electrolyte imbalances and systemic broad-spectrum antibiotics. The use of antibiotics in treating appendicitis has clear benefits and while patients with simple appendicitis require only a single dose of perioperative prophylactic antibiotics, those diagnosed with complicated appendicitis generally require ongoing postoperative antibiotic therapy to treat accompanying secondary peritonitis until signs of clinical resolution. The length of time required for antibiotic treatment or the mode of delivery has yet to be delineated although there has been a trend toward decreasing the duration of antibiotic therapy.
first part of this thesis addresses the issue of postoperative antibiotic use in the setting of paediatric acute appendicitis complicated by secondary peritonitis.

The second part of this thesis deals with optimising the surgical management of acute appendicitis, particularly the process of setting up laparoscopy. Appendicectomy is the most widely accepted treatment for appendicitis and traditionally it has been considered an emergent procedure. However, more recently the timing of appendicectomy has come under some scrutiny. This is partly due to data suggesting that appendiceal perforation rates and clinical morbidity are not affected if surgery is delayed by 12 to 24 hours. Because of this, most surgeons now consider appendicectomy for uncomplicated appendicitis to be a semi-urgent rather than emergent intervention.

In cases where appendiceal perforation and secondary peritonitis are clinically suspected, most clinicians will proceed to surgery after fluid resuscitation and the commencement of antibiotics. However, some surgeons continue non-operative management if the patient initially remains stable during the first 24 hours and then improves in response to treatment. Interval appendicectomy in 8 to 12 weeks time may or may not then be planned.

Two operative approaches are available for appendicectomy. The open technique via a muscle-splitting right lower quadrant incision has served for over a century while the minimally invasive laparoscopic approach was first described in the early 1980’s. Despite numerous prospective, randomised clinical trials comparing the two approaches, the debate regarding their advantages and disadvantages continues. Reported advantages of
laparoscopic appendicectomy includes those commonly associated with this technique such as reduced hospital stay, decreased postoperative pain, faster recovery time, and improved access in obese patients. In the setting of clinically suspected appendicitis, it has an extra advantage of providing an overall visual inspection of the intraperitoneal space when there is uncertainty about diagnosis. Its disadvantages include higher costs due to extra equipment and time, and a need for operator training and skill development. Despite these, the safety and effectiveness of laparoscopy has seen its utilisation increase dramatically in the last decade. A more detailed analysis of its role in managing appendicitis in children is provided in Section 1.1.6.2.

Two other controversies in the management of appendicitis warrant mentioning. In the setting of simple appendicitis, appendicectomy is straightforward but when surgeons encounter complicated appendicitis, he/she must also contend with the added problem of intraperitoneal contamination and secondary peritonitis. Unfortunately, many of the described and practised surgical strategies such as peritoneal debridement, lavage, and drainage are poorly understood and/or sufficiently researched. There is currently no agreed-upon standard surgical strategy to deal with peritoneal disease found in children with complicated appendicitis. This further reinforces the reliance on postoperative antibiotic therapy and the peritoneum's host defence system to effectively clear residual intraperitoneal microorganisms.

Until recently, appendicitis has been regarded a classic surgical disease and although it is no longer considered an operative emergency due to the efficacy of available antibiotic therapy, appendicectomy remains the most widely accepted treatment strategy.
However, it is becoming clearer that antibiotic therapy may be a safe alternative primary intervention in select cases of early acute appendicitis which eliminates the need for invasive and potentially morbid intraabdominal surgery.\textsuperscript{27, 63}

1.1.5 Outcomes and Complications

Being such a prevalent disease, the postoperative complications of acute appendicitis are well documented and include wound infection, unresolved intraabdominal infection with or without abscess formation, postoperative ileus, adhesive small bowel obstruction (ASBO), and, very rarely, formation of entero-cutaneous fistula.\textsuperscript{10} With the introduction of effective antibiotic therapy, overall rates of infectious complications have dramatically reduced\textsuperscript{64} but the reported total complication rate for children with complicated appendicitis can still be as high as 58\%.\textsuperscript{65} In fact, the incidence, nature, and severity of perioperative complications significantly differ between simple and complicated appendicitis.

Most importantly, while the incidence of postoperative intraabdominal infection in patients with nonperforated appendicitis range from 0 to 3.6\%\textsuperscript{66, 67, 68} rates for complicated appendicitis commonly range between 8 and 20\%.\textsuperscript{66, 69, 70} And while wound infection rates are 3\% in simple appendicitis, the equivalent rate is 8\% in complicated appendicitis.\textsuperscript{46} The latter is also associated with a higher rate of postoperative ASBO.\textsuperscript{71} In most cases of intraabdominal infection, when the patient is stable and there is an absence of residual infective material within the abscess, conservative management with antibiotics will be successful.\textsuperscript{72, 73} Percutaneous or surgical drainage is occasionally required and can contribute to already increased morbidity.
Aside from the degree and spread of intraabdominal infection, a number of other factors can influence patient outcomes from acute appendicitis. The implementation of evidence-based clinical pathways to standardise management has been shown to reduce length of hospital stay, healthcare spending, and readmission rates.\textsuperscript{74, 75} Clinician experience and expertise also seems to be important as reports suggest that young children with appendicitis have improved outcomes when cared for by paediatric surgeons.\textsuperscript{76, 77}

1.1.6 Appendicitis and Peritoneal Inflammation, Injury, and Infection

Disease morbidity arising from acute appendicitis is closely linked to the peritoneum. While secondary peritonitis is undeniably the cause of morbidity in complicated appendicitis, the rise of laparoscopic surgery has brought about a new form of insult to the peritoneum in the setting of simple appendicitis.

1.1.6.1 Secondary peritonitis

Appendicitis leads to peritoneal infection and inflammation via appendiceal gangrene, necrosis, and/or perforation. Activated by bacterial invasion, the peritoneum then mounts a host immune response (see Section 1.2.3.2 and Appendix A). Secondary peritonitis from complicated appendicitis is a mixed polymicrobial infection caused by residents in gut flora. The most commonly isolated species are \textit{Bacteroides fragilis}, \textit{Escherichia coli}, \textit{Pseudomonas} species, \textit{Enterobacter} species, and \textit{Peptostreptococcus}.\textsuperscript{93} The early high mortality rate in peritonitis is caused by Gram-negative aerobes that generate high levels of circulating endotoxins including lipopolysaccharides (LPS). Later stages that involved abscess formation are mediated by anaerobes, particularly \textit{B. fragilis}.\textsuperscript{94}
Simplification and synergism are key processes in bacterial peritonitis as only a few of the several hundred bacterial species making up gut flora actually play a pathological role within the peritoneal cavity. For example, *B. fragilis* enhances lethality of *E. coli* when they are implanted in an intraabdominal fibrin clot. And while encapsulated *B. fragilis* freely form abscesses, unencapsulated strains require the presence of aerobes in order to form abscesses. In return, anaerobes secrete enough succinic acid within abscesses to inhibit polymorphonuclear leucocytes (PMN) function and allow other organisms, in particular *E. coli*, to proliferate. Additionally, intraperitoneal blood and contaminant particles promote peritoneal inflammation and bacterial proliferation.

Acute conclusive management of complicated appendicitis depends on successful surgical control of the infectious source via appendicectomy, lessening of intraperitoneal bacterial load, and complete bacterial elimination by the host’s own peritoneal defence system with the aid of adjuvant systemic antibiotics. Failure at any stage results in recurrent or persistent intraabdominal infections resulting in additional morbidity. Currently, complicated appendicitis is the most common cause of peritonitis and intraabdominal sepsis in children, with morbidity arising from postoperative complications, prolonged LOS, reoperation, and readmission.

Complicated appendicitis is significantly more frequent in children than in adults and delay in presentation is a key factor determining the incidence of complicated appendicitis. This pre-hospital variable is difficult to remedy and with minimal change in its occurrence rate over the last 30 years, complicated appendicitis will continue to be an important disease in healthy children.
Complicated appendicitis is challenging for paediatric and general surgeons\textsuperscript{104} and there remains a lack of high-quality clinical evidence guiding interventions that integrates disease pathophysiology. The predictors of disease outcome also remain poorly described.\textsuperscript{105} To reduce morbidity from complicated appendicitis, key factors influencing outcome need to be identified and subsequent interventions and remedies to improve outcome need to incorporate a thorough understanding of the accompanying secondary peritonitis.

1.1.6.2 Laparoscopy and the pneumoperitoneum

Although curative and reliable, surgery poses potential threats to healthy tissue. As mentioned above, the time-honoured practice of appendicectomy for simple appendicitis has recently been challenged by the finding that selected patients can be treated entirely and conclusively with antibiotics resulting in reduced overall morbidity from avoidance of surgery.\textsuperscript{61, 78} It would seem that, as a significant contributor to patient morbidity, surgery induces injury that surpasses the pathological insult from simple appendicitis.

The realisation that surgery causes additional tissue injury has helped to popularise laparoscopic surgery and aid its acceptance and integration into common surgical practice. In comparison to conventional open surgery, minimally invasive techniques are reported to reduce postoperative pain, shorten hospital stay, improve cosmetic results, and enhance recovery.\textsuperscript{79, 80} As well as appendicectomy, laparoscopic surgery has become widely adopted for managing a variety of other paediatric surgical conditions.\textsuperscript{54, 81}

Laparoscopic appendicectomy was first described by Kurt Semm in 1983\textsuperscript{82} and it has gained significant popularity since then. In the United States, utilization of laparoscopic
appendectomy at academic centres increased more than two-fold between 1999 and 2003. The Morgan Stanley Children’s Hospital in New York is an extreme example of how rapid this trend has occurred: laparoscopic appendicectomies were performed for < 10% of appendicitis cases in 1997 but by 2005, this figure had increased to > 95%. Globally, the same trend is seen in several countries, including the United Kingdom and New Zealand.

The measurable advantages of laparoscopic appendicectomy in children, however, remain debatable. In fact, the latest Cochrane review is inconclusive regarding its benefits to postoperative pain and convalescence. Startlingly, at a molecular level, laparoscopic appendicectomy elicits the same or an augmented postoperative systemic inflammatory response in children compared to open procedures. Is it possible that the minimally invasive feature of laparoscopic surgery reduces trauma to the abdominal wall but also introduces novel entities into the abdominal cavity to explain this phenomenon?

Creation of the pneumoperitoneum increases intraabdominal pressure, exposes the peritoneum to insufflation gases, and is associated with local and core body temperature shifts, all of which have effects on peritoneal integrity and biology. Carbon dioxide (CO₂) gas insufflation in particular is known to have pro-inflammatory effects within the peritoneal cavity. It is naturally an irritant to the peritoneum and causes adverse structural alterations to the mesothelial lining, local pH disturbances, and changes in peritoneal macrophage responsiveness. Compellingly, interventions that limit or prevent the peritoneal inflammatory injury associated with CO₂ pneumoperitoneum may hold potential benefits for patient outcomes after laparoscopic appendicectomy for childhood acute appendicitis.
1.2 THE PERITONEUM

To understand how appendicitis morbidity of arises from associated peritoneum inflammation, injury, and infection, it is important to understand the peritoneum’s anatomy and physiology.

1.2.1 Anatomy

1.2.1.1 Gross anatomy

The peritoneum is the largest and most complexly arranged serous membrane in the body consisting of two layers. In adults, it grows to a total area of approximately 1.8 m² – almost equal to the body surface area of skin.¹⁰⁶ The parietal peritoneum lines the abdominal and pelvic cavities and covers the anterior and posterior abdominal walls and the under-surface of the diaphragm. In certain places, this departs from the posterior abdominal wall, diaphragm, and pelvic floor to form a partial or complete investment for intraabdominal viscera. This layer enveloping viscera is the visceral peritoneum. In males, these two layers form a closed sac but in females, it is accessed by the lateral ends of the Fallopian tubes. The potential space existing between the two layers is known as the peritoneal cavity. The free surface of the peritoneum has a layer of flattened mesothelial cells kept moist by a thin film of serous fluid (< 100 mL),¹⁰⁷ which is essentially an ultra-filtrate of plasma and contains hyaluronic acid-rich glycoproteins, sugars, various enzymes, and resident inflammatory cells.¹⁰⁸
Most of the peritoneal cavity consists of the greater sac with the lesser sac (or omental bursa), situated behind the stomach, making up the remainder. The two compartments communicate via the epiploic foramen (foramen of Winslow). Various folds or reflexions of peritoneum connect viscera to the abdominal walls or to one another and further divide the peritoneal cavity into various compartments. These take the form of the mesentery supporting the small bowel, mesenteries supporting the transverse colon, sigmoid colon, and appendix (transverse mesocolon, sigmoid mesocolon, and mesoappendix), the lesser and greater omenta, and various ligaments associated with the liver, stomach, spleen, and the uterus in females. The peritoneal cavity is also descriptively divided into supracolic and infracolic compartments by the attachment of the transverse mesocolon to the posterior abdominal wall.

The peritoneal cavity and its reflections are vulnerable to infection, inflammatory, neoplastic, and traumatic processes and due to its extensive size and sweeping capacity, localised intraperitoneal disease easily spreads to become generalised intraabdominal sepsis. Fortunately, peritoneal ligaments, mesenteries, and omenta serve as boundaries for disease processes and conduits for disease spread. The potential peritoneal compartments created by various peritoneal reflections, ligaments, mesenteries, omenta, and the natural flow of peritoneal fluid determine the route of spread of intraperitoneal fluid and, consequently, disease processes within the abdominal cavity. This flow is directed by gravity to its most dependent sites. It is also directed in a cephalad direction by the negative intraabdominal pressure generated in the upper abdomen by respiration.
1.2.1.2 Embryology and development

During human development, the intraembryonic mesoderm on each side of the neural groove differentiates into paraxial, intermediate and lateral mesoderm. The lateral mesoderm is continuous with the extraembryonic mesoderm covering the yolk sac and amnion. At the end of week 3 gestation, small spaces appear in the lateral mesoderm and when they fuse, the mesoderm is divided into two layers: the intraembryonic somatic or parietal layer and the intraembryonic splanchnic or visceral layer. The somatic mesoderm and overlying embryonic ectoderm form the embryonic body wall (somatopleure), whereas the splanchnic mesoderm and embryonic endoderm form the embryonic gut wall (splanchnopleure). A continuous mesothelial membrane lines the margin of these two layers and therefore borders the intraembryonic coelom. Between 5 and 7 weeks, the coelom is sub-divided by a process of septation into a future pericardial cavity, two pleural cavities and a peritoneal cavity. In this phase of development, the mesothelial and submesothelial layers of the coelom are referred to as the pericardium, pleura, and peritoneum respectively, and together as serous membranes. In this way, mesothelial cells originate from primitive mesoderm but share characteristics with both epithelial and mesenchymal cells.

1.2.1.3 Blood and lymphatic supply

The parietal peritoneum receives its blood supply from abdominal wall vessels while the visceral peritoneum is primarily supplied by branches of the superior mesenteric artery and vein. Blood vessels within the peritoneum are located in the areolar layer of the mesothelium.
1.2.1.4 Nerve supply

The parietal peritoneum is supplied segmentally by the spinal nerves that innervate the adjacent muscles. For example, the diaphragmatic peritoneum is supplied centrally by the phrenic nerve (C3, C4, C5) and hence, referred pain and hyperaesthesia from this area is perceived at the shoulder tip. The remainder of the parietal peritoneum is supplied segmentally by intercostals and lumbar nerves while in the pelvis, the obturator nerve is main source for the peritoneum.

Until recently, it was thought that the visceral peritoneum did not possess its own afferent nerve fibres and was reliant on those supplying the underlying viscera. Conditions including ischaemia, muscle spasm, and overstretching of the viscera or mesenteric fold would result in pain sensation. It is now understood that a distinct neuro-immuno-humoral axis also exists, activated by the paracrine action of local cytokines on two different types of afferent nerve endings (see Section 1.2.3). The vagal afferents are intramuscular or intraganglionic and their cell bodies lie in the nodose ganglia entering via the brainstem while the spinal afferents are located in the serosa, submucosa, and mesentery of the gastrointestinal tract and their cell bodies lie in the dorsal root ganglion projecting to the dorsal horn of the spinal cord. They follow the same path as autonomic efferent nerves to the gut wall.

1.2.1.5 Histology

The free surface of the peritoneum consists of a single layer of flattened polyhedral-shaped cells, 2.5 to 3 μm thick, termed the mesothelium due to its origin from mesoderm and its resemblance to epithelium. The mesothelium was first described by Bichart in 1827 after he observed that serous cavities were lined by a layer of flattened cells similar to those of
The cells have well-developed cell-cell junctional complexes, including tight junctions and desmosomes. Tight junctions in particular are crucial for the development of cell surface polarity and the establishment and maintenance of a semi-permeable diffusion barrier. Mesothelial cells, in general, contain well-developed cytoskeletons and abundant endoplasmic reticular and Golgi apparatus. The cytoplasm also contains lamellar bodies that store and release surfactant which acts as a lubricant within the peritoneal cavity, minimising friction between adjacent organs. This surfactant also contains phospholipids that work synergistically with the intraperitoneal complement cascade during peritoneal inflammation.

Numerous microvilli project from the apical surface of mesothelial cells and vary in shape, length, and density between adjacent cells and different areas, reflecting their ability to functionally adapt. Microvilli trap water and serous exudates, and also prevent friction injury to the peritoneal mesothelial cells. Below the mesothelial cells is a basement membrane made of fibroelastic tissue and further below is an areolar layer containing blood vessels, lymphatics, resident immune cells, and fibroblast-like cells. The thickness and density of this areolar layer varies in different areas: over expansile parts, it is loose and cellular (e.g. transversalis fascia on the anterior abdominal wall) while over non-expansile parts it is thick (e.g. parietal pelvic fascia).

Although mainly squamous in appearance, cuboidal mesothelial cells can be found at various peritoneal locations including over organs with hollow reservoir function (stomach, rectum, urinary bladder, and uterus) and parenchymal organs that can change their volume considerably (spleen, liver, and ovary), the peritoneal surface of the diaphragm overlying...
the lymphatic lacunae, and the ‘milky spots’ of the omentum (see below).\textsuperscript{108} Termed cubic mesothelial cells, they show ultrastructural differences to squamous-like mesothelial cells including possessing a larger nucleus with prominent nucleoli, abundant mitochondria and rough endoplasmic reticulum, a well-developed Golgi apparatus, microtubules, and a greater number of microfilaments. They are also covered in a relatively denser coat of microvilli.\textsuperscript{122} These features all suggest a more metabolically active state and these cells are thought to promote the flow of pathogenic contamination.\textsuperscript{122} It is interesting to note that at sites of peritoneal tissue injury, mesothelial cells with a cuboidal phenotype have been identified.\textsuperscript{106}

There are two further structural features to the peritoneal mesothelium associated with its host-defence response. The first are stomata and the second are ‘milky spots’. Stomata are micrometer sized ‘gaps’ in between mesothelial cells found on the undersurface of the diaphragm, the omentum, and mesentery, and pelvic peritoneum.\textsuperscript{122} The size of stomata is controlled by actin filaments and can increase from 4 to 10 μm to more than 20 μm during peritoneal inflammation.\textsuperscript{123} This property and the local absence of basal lamina, facilitate their function of draining large contaminant particles and whole cells from the intraperitoneal cavity into underlying terminal lymphatic lacunae.\textsuperscript{124} These lacunae contain valves and those situated under the diaphragm connect up to the substernal lymph nodes that then drain into the main lymphatic ducts. The rapid absorption of particulate matter by stomata has been demonstrated by the recovery of bacteria from the thoracic ducts of dogs within 6 minutes of intraperitoneal contamination.\textsuperscript{125} Consistent with this is the autopsy finding of bacteria within the anterior mediastinal lymphatics of human patients with peritonitis.\textsuperscript{126}
‘Milky spots’ are opaque small white spots found over the otherwise transparent serosa of the greater omentum. Named by the French anatomist Ranvier, these are formed by aggregates of PMNs, macrophages, and lymphocytes. In the setting of intraperitoneal infection, ‘milky spots’ become significantly more prominent and are visible to the naked eye as tiny, cotton wool-like specks. In a non-stimulated state, ‘milky spots’ contain 70% macrophages, 10% B lymphocytes, 10% T lymphocytes, and a small percentage of mast cells. The density of ‘milky spots’ is highest in infancy (20-40 per cm$^2$) and decreases to approximately 5 per cm$^2$ with age. The role of ‘milky spots’ in the setting of intraperitoneal infection is described in more detail in Appendix A.

1.2.2 Mesothelial Physiology

The sole physical function of the peritoneal was traditionally thought to provide a protective, non-adhesive surface to reduce friction and facilitate free movement between abdominal viscera. However, it is now recognised that mesothelial cells of the peritoneum are not passive cells. In fact, the mesothelium is a dynamic cellular membrane that participates in maintenance of peritoneal homeostasis. Their physiological functions include the control of fluid and solute transport between the bloodstream and the serous cavity, immune surveillance and the production of extracellular matrix (ECM) molecules, proteases, cytokines and growth factors that regulate inflammatory processes and wound healing (Table 1.3).
Table 1.3 Functions of the Peritoneal Mesothelium

<table>
<thead>
<tr>
<th>Function</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provides a protective barrier and frictionless interface for the free movement of apposing organs and tissues.</td>
<td></td>
</tr>
<tr>
<td>Provides protection against invading microbes.</td>
<td></td>
</tr>
<tr>
<td>Facilitates transport and movement of fluid and particulate matter across the peritoneal cavity.</td>
<td></td>
</tr>
<tr>
<td>Key regulator of intraperitoneal homeostasis: ability to synthesize a plethora of cytokines, growth factors, and matrix proteins.</td>
<td></td>
</tr>
<tr>
<td>Actively participates in the peritoneum’s immune defence response.</td>
<td></td>
</tr>
<tr>
<td>Initiator and regulator of peritoneal inflammation.</td>
<td></td>
</tr>
</tbody>
</table>

1.2.2.1 Protective non-adhesive barrier

From its anatomic position, the mesothelium firstly acts as a physical barrier at the interface between blood and the serous peritoneal cavity, preventing invasion by foreign particles and injury to the peritoneum from chemical and surgical insult. This function is facilitated by the strong structural integrity between neighbouring mesothelial cells: they are joined by tight junctions, desmosomes, intracellular canaliculi, and tonofilament-like filaments. The protective barrier is further increased by the secretion of glycosaminoglycans, proteoglycans, and surfactant lubricants that prevent attachment and penetration of cells by infective agents and possibly inhibits tumour dessemination.

1.2.2.2 Transport and secretion

Although mesothelial cells form a tight barrier that prevents invasion of particulates across the serosal surface, they also actively facilitate transport of selective cells, fluid, and molecules through the serosa via pinocytic vesicles, intracellular junctions, and stomata. Created by the boundaries of two or more mesothelial cells and generally found in the regions where cuboidal mesothelial cells are present, these openings provide a direct access.
to the lymphatic system allowing rapid removal of fluid, cells, and foreign particles from the peritoneal cavity.

The glycosaminoglycans secreted by mesothelial cells, in particular hyaluronan, proteoglycans including syndecans and biglycan, and surfactant lubricants, also help to provide a slippery non-adhesive surface, protecting the serosal surface from abrasion, infection and possibly tumour dissemination. In addition, mesothelial cells secrete a diverse array of inflammatory mediators and cytokines, growth factors, products of the coagulation cascade, and fibrinolytic agents as part of its humoral response to injury.

1.2.2.3 Host defence response

The molecular basis for peritoneal inflammation is founded on the loop and cascade chemical signals that are passed between peritoneal mesothelial cells and neutrophils, macrophages, lymphocytes, and cells in the submesothelium such as mast cells and fibroblasts. Mesothelial cells not only play a central role in the cell-signalling pathways, they themselves are a potent source of a variety of pro- and anti-inflammatory, and immuno-modulatory mediators. Cytokines and chemokines then interact to determine the vigour of the inflammatory response and the extent of subsequent adhesion formation.

Stimuli such as bacterial products, instilled agents, and tissue injury induce mesothelial cells to release pro-inflammatory cytokines and chemokines including interleukin (IL) 6, IL-1, IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α, and tumour necrosis factor-α (TNF-α), which recruits inflammatory cells to the site of damage within the peritoneal cavity. Furthermore, mesothelial cells are also stimulated by
hyaluronan and macrophage-derived cytokines such as TNF-α and IL-1β, to produce high levels of IL-8, which is a neutrophil chemoattractant. As neutrophils influx into the peritoneal cavity, mesothelial cells control this process by modulating their surface microvilli density and the expression of adhesion molecules. Mesothelial cells express a variety of adhesion molecules including intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and platelet endothelial cell adhesion molecule-1, that bind neutrophils and facilitate their extravasation from the bloodstream and migration into the peritoneal cavity.

The initial influx of neutrophils is replaced by macrophages within 6-12 hours of the onset of peritonitis and this switch is partly regulated by mesothelial cells. Soluble IL-6 receptors shed by infiltrating neutrophils combine with IL-6 produced by mesothelial cells to control chemokine expression and regulate the pattern of cell recruitment. Creation of a chemotactic gradient across the mesothelium is the main determinant of cell trafficking.

Although they are not recognised as professional antigen-presenting cells, mesothelial cells are capable of presenting both soluble and particulate antigens to autologous and major histocompatibility complex (MHC)-compatible allogeneic lymphocytes. They express CD40 protein, a surface receptor whose ligation up-regulates the production of IL-15 and RANTES (regulated on activation normal T cell expressed and secreted), both of which are involved in T cell recruitment and activation. Lastly, mesothelial cells regulate the inflammatory response by secreting anti-inflammatory mediators including prostaglandins, prostacyclin, and IL-6.
1.2.2.4 Mesothelial healing

Peritoneal mesothelial cells are able to release growth factors, including tumour growth factor-β (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor, hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), and members of the epidermal growth factor (EGF) family (e.g. EGF, heparin-binding EGF, and vascular EGF), that initiate cell proliferation, differentiation, and migration of mesothelial and submesothelial cells surrounding a lesion. Given that the deposition of ECM is important for the synthesis of a basement membrane and maintenance of submesothelial connective tissue, mesothelial cells are integral in the production of ECM molecules, including collagen types I, III, and IV, elastin, fibronectin, and laminin, and the regulation of ECM turnover through the production of matrix metalloproteinases and tissue inhibitors of metalloproteinases.

1.2.3 The Peritoneal Neuro-immuno-humoral Axis

The stimulation and activation of peripheral immune cells by a variety of inflammatory agents produces large changes in neural activity and consequent physiological and behavioural responses mediated by the CNS. These include extensive regionally specific alterations in brain monoamine metabolism and the expression of early-immediate genes, physiological changes such as fever, hypothalamo-pituitary-adrenal (HPA) axis activation, hyperalgesia, increased levels of plasma corticosteroid and CNS catecholamines, and a variety of behavioural alterations (somnolence, anorexia, taste aversion, reduced activity, exploration, and social interaction) summarised as “sickness behaviours”.

Although it is clear that the synthesis and release of pro-inflammatory cytokines, including IL-1α and β, IL-6, and TNF-α, from a host of different peripheral cells such as macrophages are the key
messengers to the brain following immune stimulation, the pathway that they use to signal to the brain and initiate the cascade of neural events has not been clearly understood.\textsuperscript{114}

Traditionally, it was presumed that cytokines entering into the bloodstream accumulates and travels to the brain but because pro-inflammatory cytokines are typically large, hydrophilic proteins, they are unlikely to penetrate the blood-brain barrier in significant concentrations. Therefore, alternative mechanisms must be in place for blood-borne cytokines to signal to the brain and a number of these have been discovered including carrier-mediated, saturable, specific transport mechanisms, direct CNS entry via circum-ventricular organs where the blood-brain barrier is weak, and binding to receptors on endothelial cells of brain vasculature leading to release of other mediators within brain parenchyma.\textsuperscript{114} However, there is little evidence that any of these mechanisms mediate the neural and behavioural changes that follow peripheral immune stimulation. The only obvious mechanism is the possibility that, as well as systemic activation, cytokines signal the CNS by activating afferent neurons via paracrine action at the local site.\textsuperscript{114, 145}

In addition, the peritoneal inflammatory response to intraabdominal injury appears to be relatively independent of the systemic response. Firstly, there is a much higher cytokine concentration in peritoneal fluid in comparison to plasma after gastrointestinal surgery suggesting that cytokine production occurs in a compartmentalised manner within the abdominal cavity.\textsuperscript{146-148} Moreover, there is incomplete absorption of peritoneal cytokines into the bloodstream after abdominal surgery, and what is absorbed is broken down by the liver and diluted in plasma.\textsuperscript{149} Differing levels of the same inflammatory cytokines in the peritoneal fluid and serum of patients with peritonitis further suggest that the
intraabdominal immune response to infection is independent of the systemic response.\textsuperscript{150}

These all point to the presence of a direct neural communication between the peritoneum and the brain under the influence of local intraperitoneal inflammatory mediators – i.e. a neuro-immuno-humoral axis.

\textbf{1.2.3.1 Neural innervation}

While the parietal peritoneum is innervated by spinal nerves that supply overlying muscles,\textsuperscript{110} traditionally it was thought that the visceral peritoneum was reliant on afferent fibres supplying the underlying viscera. More recently, it has been discovered that local cytokines can execute paracrine actions on afferent nerve endings servicing the peritoneum and gastrointestinal tract. These afferent fibres mediate the reflexes that control motility, secretion, blood flow, and also modulate immune responses.\textsuperscript{151}

Two types of peripheral afferent nerve endings transmit sensory information to the CNS: vagal afferents and spinal afferents. Vagal afferents have cell bodies in nodose ganglia and enter the brainstem. Spinal afferent have cell bodies located in dorsal root ganglia and project to the dorsal horn of the spinal cord and the dorsal column nuclei. They are broadly divided into splanchnic and pelvic afferents that follow the paths of sympathetic and parasympathetic efferents to the gut wall.\textsuperscript{115}

Peripheral endings of vagal and spinal sensory neurons terminate within the musculature, mucosal epithelium, and ganglia of the enteric nervous system.\textsuperscript{151} Spinal afferents also terminate in the serosa and mesenteric attachments and form a dense network around mesenteric blood vessels and their intra-mural tributaries. Those terminating in the serosa
and mesentery respond to distortion of the viscera during distension and contraction while those endings in the submucosa respond to chemical signals following injury, ischaemia, and infection, and may play a role in generating hypersensitivity to distension and muscle contraction.\textsuperscript{152}

1.2.3.2 Immune response

The peritoneum has a multilayered and complex immune defence system that protects its serosal surface (Table 1.4). Similar to other body surfaces, the peritoneum relies on innate immune mechanisms and then specific immune system that has evolved as a secondary amplification system.\textsuperscript{153} While the innate response is inborn and recognises pathogen-associated motif patterns to react rapidly to fight infection, the acquired specific response develops after birth, improves upon repeated exposure, and usually participates after the initial phase of inflammation. As mentioned, mesothelial cells have a significant part to play in the peritoneum’s host defence response but there are also several other cellular and humoral components.

| Table 1.4 Peritoneal Defence Response\textsuperscript{142} |
|---------------------------------|-------------------------------------------------|
| **Type** | **Description** |
| Innate | Particulate absorption via diaphragmatic stomata |
| | Anti-bacterial activity of peritoneal fluid, complement mediated |
| | Phagocytosis by macrophages and polymorphonuclear leucocytes |
| | Natural killer cells |
| | Abscess Formation |
| Specific | Milky spots, immunoglobulin A antibody production |
| | Influx of memory T lymphocytes |
Essentially, the peritoneal cavity deals with infection in four phases: first, a rapid absorption of bacteria via diaphragmatic stomata; second, the destruction of bacteria via mechanisms generated by innate immune responses including the complement cascade and recruited PMNs. There is then evolution to a third phase involving the acquired specific immune response. It is marked by peritoneal infiltration by mononuclear cells including lymphocytes from the T and B cell lineages as well as macrophages. T and B cells have specific surface receptors that recognise a large variety of antigens. Acquired immunity also has the capability of specific memory for foreign antigens. Lastly, fibrin deposition as a result of coagulation cascade activation and impaired fibrinolysis, leads to the localisation of infection as abscesses. Details of the peritoneal defence system are enclosed in Appendix A.

1.2.3.3 Humoral response

In response to peritoneal injury and infection, mesothelial cells and local peritoneal immune cells secrete a variety of mediators (Table 1.5) responsible for both the local and systemic inflammatory response and subsequent tissue repair with clinical implications including adhesion formation. With both pro- and anti-inflammatory processes, these interactions are complex and understanding them offers the possibility of identifying potential targets for therapeutic intervention during the various phases of peritoneal inflammation. See Appendix B for a summary of the key humoral factors known to mediate peritoneal and systemic inflammatory processes following peritoneal infection and iatrogenic injury.
### Table 1.5 Mediators of Peritoneal Inflammation and Repair

<table>
<thead>
<tr>
<th>Mediator</th>
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</thead>
<tbody>
<tr>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Cytokines</td>
</tr>
<tr>
<td>E.g. TNF-α, IL-1β, IL-6, IL-10, IFN-γ</td>
</tr>
<tr>
<td>Chemokines</td>
</tr>
<tr>
<td>E.g. IL-8, LT-B4, MCP-1</td>
</tr>
<tr>
<td>Growth factors</td>
</tr>
<tr>
<td>E.g. TGF-α, TGF-β, PDGF, VEGF, EGF</td>
</tr>
<tr>
<td>Coagulation factors</td>
</tr>
<tr>
<td>E.g. tissue factor, tPA, uPA, PAI</td>
</tr>
<tr>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>E.g. TIMP1 – TIMP4</td>
</tr>
</tbody>
</table>

**Note:** TNF-α = tumour necrosis factor α, IL-1β = interleukin 1β, IL-6 = interleukin 6, IL-10 = interleukin 10, IFN-γ = interferon γ, IL-8 = interleukin 8, LT-B4 = leucotriene B4, MCP-1 = monocyte chemoattractant protein 1, TGF-α = tumour growth factor α, TGF-β = tumour growth factor β, PDGF = platelet derived growth factor, VEGF = vascular endothelial growth factor, EGF = epidermal growth factor, tPA = tissue plasminogen activator, uPA = urokinase plasminogen activator, PAI = plasminogen activator inhibitor, TIMP = tissue inhibitor of metalloproteinase.

### 1.2.3.4 Clinical implications

With the existence of the neuro-immuno-humoral axis coming to light, its clinical implications are being realised. By serving as a direct neuro-humoral communication pathway between the peritoneum and the brain, essentially the peritoneum, among all of its functions, operates as a diffuse immune sensory organ instantaneously informing the CNS of events such as intraabdominal infection, inflammation, and injury. Its existence not only explains why peritoneal inflammation adds further disease morbidity, but it also implores the clinician to focus therapies on reducing peritoneal inflammation and injury.
associated with infection and surgery, preventing unnecessary activation, and targeting its clinical manifestations.

The central role of peritoneal injury in generating the host of ‘sickness behaviours’ now recognised as postoperative fatigue can be seen by the profound and generalised physiological insult and metabolic derangement associated with abdominal surgery which is significantly greater in magnitude when compared to surgical procedures outside of the abdominal cavity but of similar duration and performed in comparable populations.\(^{156-159}\)

This coordinated response to peritoneal injury results from the fact that the entire peritoneal cavity is linked via transcoelomic spread of immuno-humoral factors within the peritoneal fluid.\(^{160, 161}\) Levels of peritoneal cytokines have been directly correlated to the occurrence of postoperative complications\(^{162, 163}\) and reported postoperative fatigue.\(^{164}\)

There has been considerable interest in preventing this response in order to attenuate surgical stress and enhance recovery.

The perioperative administration of systemic glucocorticoid, known to reduce circulating systemic pro-inflammatory cytokine levels\(^{165}\) and dampen the local peritoneal response after surgery (as measured by reduced peritoneal fluid cytokine concentration)\(^{166}\) has been demonstrated to significantly reduce postoperative fatigue, postoperative pain, and nausea and vomiting. In a meta-analysis, the preoperative administration of systemic glucocorticoid decreased postoperative complications and length of stay after major abdominal surgery, likely the result of attenuated postsurgical inflammation.\(^{167}\)
The existence of a neuro-immuno-humoral axis, propagated by fibres of the vagus nerve, can also be proven by demonstrating in rats, that subdiaphragmatic vagotomy partially blocks the effects of intraperitoneal IL-1β injection on core body temperature and blunts the increasing levels of CNS and systemic catecholamines. The equivalent has been demonstrated in human adults. The administration of intraperitoneal local anaesthetic during and for three continuous days after open colectomy has been shown to effectively act as a temporary chemical afferentectomy, preventing the intraperitoneal response to surgical insult from being communication to the CNS. Within a standardised enhanced recovery after surgery program, this intervention was associated with a blunting of postsurgical systemic cytokines and cortisol. Patients also had significantly reduced postoperative pain and opioid use over and above the effect of an epidural infusion.

1.2.4 Consequences of Peritoneal Inflammation, Injury, and Infection

Peritoneal inflammation is characterised by increased vascular permeability, activation and expansion of the peritoneal macrophage population, recruitment of infiltrating cells to sites of injury, release of pro- and anti-inflammatory mediators, and increased matrix protein synthesis and tissue remodelling. A number of diseases and pathophysiologic conditions can cause peritoneal inflammation including acute peritonitis secondary to gastrointestinal necrosis and perforation (secondary peritonitis), spontaneous bacterial peritonitis, and infection associated with continuous ambulatory peritoneal dialysis. Surgery also causes peritoneal trauma, triggering a series of inflammatory responses that aim to regenerate and repair the injury.
In response to trauma, the primary intraperitoneal inflammatory reaction causes an influx of inflammatory cells but there is also activation of resident mesothelial cells leading to a fibrinous exudate. Depending on the severity of the trauma, this exudate is transient or becomes dense and fibrous depending on the rate of fibrinolysis. Influxing and resident peritoneal cells also produce cytokines and proteinases that play pivotal roles in the inflammatory and regeneration process. In this way, the rate of peritoneal injury determines the rate and extent of the peritoneal inflammatory response; this inflammatory reaction in turn determines normal peritoneal healing and the clinical consequences of impaired healing (Figure 1.1). The pathophysiology of intraabdominal adhesion and abscess formation is closely tied to the equilibrium between coagulation and fibrinolysis in the abdominal cavity.
Figure 1.1 The Peritoneal Inflammatory Response to Injury and Factors that Influence Peritoneal Return to Normal Physiology versus Impaired Healing
Peritoneal inflammation has a number of important consequences, both locally and at a systemic level. It is fitting that clinically, these consequences are the key measures of disease morbidity and treatment efficacy in acute appendicitis.

1.2.4.1 Local consequences

Intraabdominal abscesses

Intraabdominal abscesses are purulent collections covered by a fibrin matrix and/or multiple peritoneal adhesions involving the serosal surfaces of intestine, omentum, and other intraabdominal organs. They are formed when the intraperitoneal inoculum is beyond the peritoneum’s intrinsic capabilities of clearance. And although this ability to ‘wall off’ infections is in fact also part of the peritoneum’s intrinsic immune response, offering improved outcomes and survival, it is also a consequence as they protect bacteria from host defence mechanisms and reduce the effectiveness of systemic antibiotic therapy which has limited penetration into fibrin.

In addition to peritoneal defence mechanisms that interfere with bacterial growth, adherence, and invasion, the pathogenesis of intraabdominal infections is also dependent on bacterial pathogenicity. Bacterial adherence to the serosal lining via microbial ligands and respective peritoneal binding sites appears to be one of the most decisive steps as microbes are then allowed access to the flow of host nutrients. Other virulence factors include growth characteristics, metabolic needs, and intrinsic resistance to antibiotics as well as the capacity to elaborate this resistance. For example, the capsular polysaccharide produced by *B. fragilis* is known to have several functions when inducing abscess formation including preventing phagocytosis and mediating organism binding to mesothelial cells.
Its metabolites such as succinic acid affect neutrophil migration, chemotaxis, and killing, and contribute to virulence and persistence of infection.\textsuperscript{176} In the setting of complicated appendicitis, foreign material such as mucus and faeces, and necrotic tissue further interfere with effective bacterial phagocytosis. The presence of haemoglobin, ascites, and other intraperitoneal fluids also encourage bacterial growth or hamper peritoneal defences.

The interior of the abscess is characterised by hypoxia, acidosis, microbial toxins, and bioactive intermediates generated by responding peritoneal cells. They also contain viable neutrophils and large amounts of chemotactic proteins such as CP-10 and MRP-14. However, \textit{in vitro} studies have found that these activated neutrophils are unable to kill the Gram-negative bacteria that they have engulfed.\textsuperscript{177, 178} Therefore, sequestration of bacteria within neutrophils impairs clearance and aids persistence of infection.

Preoperatively, intraabdominal abscesses are part of the definition for ‘complicated appendicitis’ (ICD-9 code 540.1).\textsuperscript{179} Postoperatively, they occur as a result of insufficient clearance by the peritoneum, by surgical interventions, and/or by the adjuvant systemic antibiotic therapy. They are the most common and clinically significant postoperative complications as they are associated with prolonged LOS, additional antibiotic therapy, and further imaging and invasive interventions.\textsuperscript{180} Their occurrence rate remains the mainstay outcome measure for complicated appendicitis. Risk factors for developing postoperative intraabdominal abscesses are still clear despite a multitude of variables been suggested.\textsuperscript{105, 181, 182}
Peritoneal adhesions

Abdominal and pelvic adhesions are defined as pathologic bonds between the surfaces of the peritoneal or pelvic cavities formed as a result of peritoneal scarring and surface defects caused by surgery, infection, any inflammatory pathology, and chemical irritation. They range from thin films of connective tissue to thick, fibrous bridges containing blood vessels and can cause mechanical intestinal obstruction, infertility, and chronic pain. Adhesions also limit abdominal accessibility, hinder surgical procedures, and can increase the complication rate of the intended surgical procedure. In the setting of acute appendicitis, it is a leading cause for conversion from laparoscopic appendicectomy to an open approach.

The peritoneum mounts an acute inflammatory response to surgery and postoperatively, adhesive small bowel obstruction (ASBO) is a rare but well recognised short- and long-term complication after appendicectomy. It is associated with potentially severe morbidity in the form of prolonged LOS, hospital readmission, and re-operation. Although reported incidence rates have varied partly due to their differing follow-up periods, recent studies published since 2007 involving paediatric populations report figures between 0.7% and 3.1%. Clinically, the risks of developing postoperative ASBO correlate strongly with the degree of peritoneal inflammation associated with appendicitis as invading microorganisms serve as an additional physiological protective mechanism for deposition of fibrin within the peritoneal cavity.

Formed as a result of fibrin deposition during peritoneal inflammation, various strategies have been used to prevent their formation and reformation. These have largely fallen into
five categories: limit or prevent peritoneal inflammation and injury, prevent activation of coagulation cascade and the formation of fibrin within peritoneal serous exudate, remove or dissolve deposited intraperitoneal fibrin, keep apart fibrin-coated healing peritoneal surfaces until mesothelialisation has occurred, inhibit fibroblastic proliferation once it is established.  

**Ileus**

Ileus is a well-known disorder characterised by temporarily abdominal distension, nausea, vomiting, delayed defaecation, bowel distension, and impaired bowel motility. Postoperatively, it is associated with additional patient discomfort, increased morbidity, prolonged hospitalisation, and increased costs of surgical care. Clinicians now understand that normal gastrointestinal (GI) tract motility is dependent upon electrophysiological activity in bowel smooth muscle cells, neural input from enteric and central autonomic nervous systems, coordinated smooth muscle contractions in the form of migrating motor complexes (MMC), and hormonal interactions. The exchanges between these regulatory components are complex and much remains unknown. The role of peritoneal inflammation, infection, and injury in disrupting normal GI motility, however, appears critical.

Although an abundance of mediators have been described as contributing to the development of postoperative ileus, two mechanisms appear to be crucial. Firstly, surgical manipulation of the intestines triggers a neuronal reflex inhibiting motility. Secondly, leucocyte infiltration into the intestinal muscularis layer results in a molecular and cellular inflammation process that further induces and sustains postoperative ileus. The degree of this inflammatory response is directly proportional to the level of GI
hypomotility. The prevention of monocyte, neutrophil, and mast cell recruitment into intestinal muscularis by leucocyte adhesion molecule blocking antibodies successfully averts postoperative jejunal muscle dysfunction.

Controlling inflammation of the gut wall is a complex multidirectional system involving not only immune and inflammatory cells, but also neurons, and smooth muscle cells. It has been demonstrated that electrical vagus nerve stimulation and actions of a selective $\alpha_7$-acetylcholine (ACh) receptor agonist lead to significant acceleration of postoperative gastric emptying and reduction of neutrophils in the muscularis externa. The fact that peripheral gut inflammation is partially regulated via a nerual signalling pathway further provides concrete evidence of the existence of an endogenous intraperitoneal neuro-immuno-humoral axis. Furthermore, when varying components of the peritoneal inflammatory response are altered by gene knockout, cyclo-oxygenase 2 (COX-2) inhibition, iNOS blockade, adrenergic inhibition, and celiac ganglionectomy, bowel dysmotility is improved.

Since gut motility is also guarded via central neurogenic pathways, peritoneal injury that induces a systemic stress response also stimulates inhibitory neural reflexes that result in decreased bowel motility. These reflexes, triggered by pain (from incision and manipulation of skin, peritoneum, and viscera) are transmitted via somatic and visceral fibres. The blockade of somatic and peritoneal/visceral pathways (via thoracic epidural and intraoperative peritoneal instillation of local anaesthetic agents, respectively) have been shown to reduce postoperative ileus after abdominal surgery. Simply bypassing the peritoneum via a retroperitoneal approach to repair an abdominal aortic aneurysm leads to
hastened restoration of gastrointestinal function when compared to a transperitoneal approach.\textsuperscript{157, 211}

1.2.4.2 Systemic consequences

Pain

Pain is defined as the unpleasant subjective sensation of actual or potential tissue damage\textsuperscript{212} and it produces suffering, fear, vital inhibition, as well as a significant systemic stress response.\textsuperscript{213, 214} As described in Sections 1.2.1.4 and 1.2.3.1, the two layers of the peritoneum convey sensory information to the CNS via different afferent pathways: while the parietal peritoneum is innervated by somatic fibres that also supply the overlying fascia, muscle, and skin of the anterolateral abdominal wall, the visceral peritoneum communicates via the vagal and spinal afferents innervating the underlying abdominal organ. Intraperitoneal instillation of local anaesthetic agents during surgery have been shown to reduce overall postoperative pain, shoulder tip pain, nausea and vomiting, and LOS.\textsuperscript{170, 215-217}

Although often not recognised, the visceral pathway via the subdiaphragmatic vagus nerve is not regulated by the spinal cord blockade and as well as transmitting painful sensations, it also conveys a distinctly different form of nociception – one that is activated by intraperitoneal inflammation and injury including that caused by surgery.\textsuperscript{114, 145, 218} The vagus nerve is in fact the largest visceral sensory nerve in the body with approximately 50,000 afferent fibres, most of which are involved in innovation of the peritoneum. With between 80 and 90% of the subdiaphragmatic vagus fibres being afferent in nature, it has a critical role in direct peritoneal to CNS signal transmission, possessing the potential to
modulate systemic physiology and behaviour.\textsuperscript{219} These include feeding and illness behaviours.\textsuperscript{115,220}

\textit{Postoperative fatigue and delayed recovery}

Postoperative fatigue has been defined as “unpleasant and distressing symptoms associated with a major impact on the patient’s quality of life”.\textsuperscript{221} It prevents a patient from returning to normal physical capacity and role function and in children, the impact is on family, school, social commitments, and other activities of daily living such as self-care and sleep.

Postoperative fatigue is thought to result partly from the biological response to surgery as well as the individual’s emotional responses.\textsuperscript{222,223} The complex biopsychosocial aetiology of postoperative fatigue explains why its symptoms last much longer than pain and abdominal wound healing and adult patients report that quality-of-life scores can take 3-6 months to return to population norms after abdominal surgery.\textsuperscript{224}

After abdominal surgery, it is thought that, in addition to the systemic metabolic and inflammatory responses that give rise to a state of catabolism with the loss of skeletal muscle and cardiovascular function,\textsuperscript{225} the locally occurring peritoneal inflammatory responses influence development of fatigue via the neuro-immuno-humoral axis. Locally occurring intraperitoneal cytokines activate the vagus nerve which has direct connections to the nucleus tractus solitarius (NTS).\textsuperscript{219} It has been demonstrated that the NTS becomes strongly activated following stimulation by peripheral sources of inflammation\textsuperscript{226} and go on to mediate sickness responses via monosynaptic projections to various regions of the CNS.\textsuperscript{219} To confirm this, Paddison et al. demonstrated that cytokine concentrations in
peritoneal fluid collected 24 hours after open colorectal correlated to fatigue scores after controlling for age, gender, co-morbidity, and baseline fatigue prior to surgery.\textsuperscript{164}

Furthermore, instillation of intraperitoneal local anaesthetic not only reduces somatic sensations of postoperative pain, it blocked the propagation of visceral injury via the vagus nerve, effectively acting as a temporary chemical vagotomy, leading to enhanced postoperative recovery and reduced systemic stress response markers.\textsuperscript{170, 227}

\textit{Systemic inflammatory response}

The systemic inflammatory response in acute appendicitis arises as a result of the localised intraabdominal infection progressing to intraperitoneal sepsis and the subsequent stress response produced by surgery. Persistent or recurrent postoperative intraabdominal infection can be a continued source generating systemic inflammatory. The role of the peritoneum in instigating systemic inflammatory appears to be fundamental.

Acute appendicitis generally follows a sequence of events that begins with luminal obstruction and impaired blood flow, subsequent dysfunction of the local epithelial barrier and bacterial invasion leading to activation of an immune response. The homing and migration of leucocytes to the target issue then involves an array of different soluble proteins including C-reactive protein (CRP), cytokines, chemokines, adhesion molecules, and proteases, as well as cells such as PMNs, lymphocytes, monocytes, and natural killer cells. Although many of the studies that have attempted to characterise the inflammatory response in appendicitis have generated only inconclusive results,\textsuperscript{228} the understanding thus far points to appendicitis being a localised infection up until the disease process spreads to the peritoneum.
Providing a partial view of the body’s response to injury and infection, cytokine concentrations in different body compartments during appendicitis have been investigated by several studies. Acute appendicitis clearly stimulates a local intraperitoneal inflammatory response that can be demonstrated by significantly higher levels of IL-8, MMP-9, IL-10, and TIMP-1 in the peritoneal fluid of children with histologically proven acute appendicitis compared with children whose appendiceal histology was normal. Furthermore, appendiceal gangrene, necrosis, and/or perforation produced a more intense intraperitoneal inflammatory response as demonstrated by significantly higher peritoneal concentrations of IL-6, IL-8, and IL-10.

However, there are conflicting data regarding serum cytokine levels as markers of acute appendicitis. While Yoon et al. found that both nonperforated and perforated cases of appendicitis in adult patients produced significantly elevated serum levels of IL-1β, IL-2, IL-6, IL-8, and IL-10 compared to those of normal healthy controls, Dalal et al. demonstrated no differences in serum levels of IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), IFN-γ, soluble intercellular adhesion molecule-1 (sICAM-1), MMP-9, and TIMP-1, in 19 children with appendicitis compared to 5 children with normal appendiceal histology. Both studies, nevertheless, went on to show that, as well as significantly elevated peritoneal cytokines, secondary peritonitis in the setting of appendicitis also led to significantly elevated serum cytokine levels compared to appendicitis without peritoneal involvement.

River-Chavez et al. also confirmed that serum levels of IL-6 and IL-10 were significantly higher in adult patients with complicated appendicitis compared to those with simple appendicitis. They also noted that serum IFN-γ and IL-12 levels were lower in those with
complicated appendicitis and that plasma collected from patients with appendicitis appeared to inhibit the IL-8 releasing properties of monocytes primed by exposure to bacterial LPS. Plasma from patients with complicated appendicitis was found to possess greater suppressive potency than plasma from patients with no peritoneal disease. The authors concluded that patients with complicated appendicitis mounted a systemic response to localised intraperitoneal infection but it was one dominated by anti-inflammatory properties.

Surgery elicits an acute-phase metabolic response irrespective of any underlying pathology. This explains why the incidence of early postoperative fever after clean and clean-contaminated procedures is similar to that found after overtly contaminated surgical procedures such as appendicectomies for complicated appendicitis. In abdominal surgery, the extent to which the peritoneum is entered, dissected, and manipulated has significant flow-on effects on systemic inflammation and subsequently, clinical recovery. Laparoscopic surgery, generally regarded as less traumatic compared to open surgery, in fact often offers no benefits with regards to peritoneal and visceral trauma and also exposes the peritoneum to foreign conditions of a pneumoperitoneum.
1.3 SUMMARY

Globally, acute appendicitis is the most common abdominal surgical condition in children and adolescents. Although the associated mortality has been all but eliminated, patient morbidity remains a significant issue given its high frequency and potential to result in severe intraabdominal bacterial infection. Disease outcome is closely linked to accompanying peritoneal inflammation and injury. The close correlation arises from secondary peritonitis associated with complicated appendicitis and also through iatrogenic peritoneal trauma caused by surgery, specifically laparoscopic surgery and creation of the pneumoperitoneum. The latter contributing factor is increasing in significance as minimally invasive surgical techniques become the accepted standard in current practice.

The peritoneum is a serous membrane that plays an active role in maintaining intraperitoneal homeostasis. When traumatised, a series of protective inflammatory responses are activated in an attempt to minimise further injury and regenerate via healing. This inflammatory response is largely generated through resident peritoneal mesothelial cells, an influx of activated immunocompetent cells, and the host of humoral factors that are produced. Although initiated for host defence and repair purposes, peritoneal inflammation also has a significant number of clinical consequences that impair recovery and add to patient morbidity. This is largely due to the existence of the peritoneal neuro-immuno-humoral axis.
The following chapters aim to highlight peritoneal inflammatory injuries sustained in the setting of acute appendicitis and how they affect disease morbidity. This thesis will also illustrate, with examples, how an understanding of peritoneal inflammation can be applied in the clinical setting of childhood acute appendicitis to improve patient outcomes. Using a series of systematic reviews and clinical studies, the following questions will be answered: What is the disease morbidity of complicated appendicitis in children? What disease- and clinician-related factors influence this? How does laparoscopic surgery and creation of the pneumoperitoneum affect peritoneal structure and function? Can patient outcomes be improved when medical and surgical interventions are designed to reduce or target peritoneal inflammation and injury in childhood acute appendicitis?
Chapter 2

METHODS
This thesis is composed of five experiments: two systematic reviews and three clinical studies involving human participants. Below is a detailed account of how these experiments were designed and conducted.

2.1 SYSTEMATIC REVIEW

The two systematic reviews in this thesis (Chapters 4 and 7) provided up-to-date rationale and equipoise for the clinical trials that were conducted. Below is an account of how the systematic reviews were performed to ensure their accuracy and thoroughness.

2.1.1 Research Question

A systemic review was centred on a principal research question that detailed a target population, an intervention of uncertain efficacy, and a primary outcome measure of significant interest. The timeframe within which this primary outcome is to be assessed is also outlined in the research question. The principal research question and other secondary research questions are specified in the Objectives section of each systematic review chapter.

2.1.2 Systematic Literature Search

Each literature search was strategically designed to be of high-sensitivity and low precision. Search terms were independently generated by at least two investigators and a variety of different electronic databases (for example Ovid MEDLINE, Cochrane Central Register of Controlled Trials or CENTRAL, PubMed, EMBASE) formed the main information source. By
using Boolean operators (‘and’ and ‘or’) to form different search term combinations, the search was narrowed or widened. Commonly, the ‘Explode’ function was used when searching the Ovid MEDLINE search engine. When available and appropriate, predetermined ‘limitations’ were used to restrict the search to human participants and English publications. In addition to searching electronic databases, the reference lists of retrieved original and review articles and editorials were hand searched to identify additional potentially relevant trials. To manage the search results, all records (including original research articles, reviews, editorials, and essays) were imported into a bibliography management program (ENDNOTE X5, Thomson Reuters, New York, USA).

2.1.3 Study Selection

After removing duplicate records, two investigators independently screened all titles and abstracts for potentially relevant articles according to predetermined inclusion and exclusion criteria. Records identified through this initial screening were then obtained in full-text for closer inspection. A second round of study selection was then performed involving full-text articles, again with strict adherence to the inclusion and exclusion criteria. These are clearly stated in the appropriate methods section of each systematic review. Disagreements between investigators were resolved through discussion until consensus was reached. Consultation with a third and more senior investigator was sought if consensus could not be reached. The outcome at each step was recorded and presented using the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement flow diagram for literature search and article selection.
2.1.4 Data Extraction and Critical Appraisal

Once the appropriate studies had been selected, data extraction was performed independently by two investigators using a standardised data extraction and critical appraisal instrument built into an electronic spreadsheet (Microsoft Excel Mac OS X 2008, Microsoft Corporation, Redmond WA, USA). Any discrepancies in opinions were identified and consensus was reached through face-to-face discussions and mediation by a third senior investigator. The following components formed the data extraction instrument:

- **STUDY INTRODUCTION**: research objectives and rationale
- **STUDY CONTEXT**: study setting, details of study participants
- **STUDY METHODS and MATERIALS**: study design, participant recruitment and sampling, study duration and follow-up, method(s) of data collection, reliability of data collection instruments, resources utilised, and statistical analysis
- **STUDY RESULTS**: main study findings, measures of functional significance (effect size, proportion of variance, predictive value), attendance and drop-out rates
- **DISCUSSION OF STUDY RESULTS**: significance and practical implications of study findings, study strengths and limitations
- **STUDY CONCLUSIONS**

When appropriate, the quality of prospective randomised studies was appraised using the Cochrane Risk of Bias tool.\(^{234}\) It consists of six components for which there is empirical evidence for their biasing influence on the estimates of an intervention’s effectiveness in randomised trials. These components are sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other sources of bias.
2.1.5 Reporting

Systemic reviews were reported in accordance with the 27-item PRISMA statement checklist.\textsuperscript{233}
2.2 CLINICAL STUDY

This thesis included three clinical studies that involved human participants (Chapters 5, 6, and 8). One was an observational study whose findings provided the stimulus for subsequent experiments while the remaining two studies were interventional comparison trials performed to test hypotheses founded on key findings from the systematic reviews. Aspects of the materials and methods commonly shared by all three studies are described below. Individual methodologies specific to each study are described as well but this is clearly highlighted where applicable.

2.2.1 Setting

The clinical studies were conducted at Starship Children’s Hospital, Auckland, New Zealand. This tertiary teaching institution was opened in 1991, the first purpose-built children’s hospital in New Zealand, and also the largest such facility in the country\(^{235}\) with over 200 inpatient beds and 16 day-stay beds. It provides medical, surgical, and mental health services for children and adolescents residing within the catchment areas of three District Health Boards (Auckland, Counties Manukau, and Waitemata) serving the wider Auckland region. As the most heavily populated region in New Zealand, the total estimated resident population of these catchment areas combined is over 1.5 million.\(^ {236}\) For the 2010/11 financial year, Starship Children’s Hospital treated 118,512 patients\(^ {237}\) including:

- 26,324 inpatient visits (including 14,003 day patients)
- 30,849 children’s emergency visits (of which 7,071 went on to be inpatient visits)
- 68,410 outpatient visits
The Department of Paediatric Surgery and Urology at Starship Children’s Hospital is responsible for the medical and surgical care of all patients suspected to have appendicitis and those diagnosed with appendicitis. Five full-time consultant paediatric surgeons share this responsibility along with six to eight surgical registrars and two house officers.

Figure 3.1 displays the number of ‘appendicectomy’ procedures performed annually at Starship Children’s Hospital between 2005 and 2012. ‘Laparoscopic appendicectomy’ made up 90 to 91% of these procedures per year.

**Figure 2.1** Frequency of ‘Appendicectomy’ Procedures Performed Annually at Starship Children’s Hospital between 2005 and 2012
2.2.2 Participants

2.2.2.1 Participant population

The participant population consisted of New Zealand children (1 to 14 years of age), with New Zealand citizenship or permanent residency, residing within the catchment area of three District Health Boards (DHB) serving the wider Auckland region. These are Auckland DHB, Counties Manukau DHB, and Waitemata DHB (Figure 2.2).

Figure 2.2 Three District Health Boards Servicing the Wider Auckland Region, New Zealand
2.2.2.2 Eligibility criteria and selection

All patients presenting to Starship Children’s Hospital, Auckland, diagnosed preoperatively, intraoperatively, and/or histologically with acute appendicitis during each respective study timeframe between 2005 and 2011 were deemed eligible to participant. Eligibility screening was performed by the primary research investigator (T Yu) or an admitting surgeon (registrar or consultant). Appropriate exclusion criteria were assembled for each of the three clinical studies.

Appendicitis diagnosed pre-operatively was based on patient history, clinical signs, radiological imaging, and laboratory-based investigations including serum inflammatory markers (white blood cell count, neutrophil count, C-reactive protein level) and urine analysis. Once a clinical diagnosis of acute appendicitis has been made, the final decision to proceed with surgical appendicectomy was made by an attending consultant surgeon.

Intraoperatively, macroscopic diagnosis was made by the operating surgeon and documented in text in each operative note with accompanying photographs. ‘Simple’ appendicitis was defined as acute appendiceal inflammation in the absence of peritoneal inflammation and infection. ‘Complicated’ appendicitis was defined as appendicitis extending beyond the appendiceal viscus into intraperitoneal space and development of peritonitis or appendiceal abscess (ICD-9 codes 540.0 and 540.1).179

Histological diagnosis was obtained for all participants based on pathology reports generated by the Department of Pathology, Auckland City Hospital, New Zealand (See Section 2.2.7.5 for the standardised histology procedures used).
2.2.2.3 Participant recruitment and consent process

Before participants were recruited into the randomised clinical trial (Clinical Study C), written informed consent was acquired from the participant and a legal guardian. Details of the study were explained to patients and their family/whānau members by the primary research investigator (T Yu) or the operating registrar and consultant. Adequate time was given for answering questions and discussion with patients and their family/whānau members. When consent is not obtained from an eligible patient and/or respective legal guardian for any reason, the circumstances were documented and subsequently reported in the study write-up.

Participant information sheets explaining the prospective clinical studies (Clinical Studies B and C) were available for prospective participants and accompanying parent/guardian (see Appendices C and E). These were constructed in accordance with guidelines provided by the Northern X Regional Ethics Committee, New Zealand Government Ministry of Health. The nurse specialist from the Children’s Research Centre, Starship Children’s Hospital, reviewed the participant information sheets intended for children to ensure that their content was age-appropriate.

2.2.3 Study Interventions

2.2.3.1 Laparoscopic insufflation gas humidification

To investigate the clinical benefits of warm humidified insufflation gas for laparoscopic appendicectomy in children, a double-blinded, randomised controlled trial was designed and implemented. The device used to warm and humidify the CO₂ gas used for insufflation
in Clinical Study C was the Fisher & Paykel MR860 Laparoscopic Humidification System, Fisher & Paykel Healthcare, Auckland, New Zealand.

The following technical information about the MR860 Laparoscopic Humidification System is obtained from the manufacturer’s manuals.239,240

**Description**

The humidification system is designed to deliver warm, humidified CO\textsubscript{2} to patients undergoing laparoscopic surgery. It is made up of two components: the humidifier device and the delivery system. The humidifier containing the heater base and the electrical adapter and probe make up the capital equipment while the humidification chamber, filter, insufflator connection tubing, and the heated gas outflow tubing are consumables (Figure 2.3).
Humidifier specifications

- **Mechanical** – The humidifier’s dimensions are 140 mm x 173 mm x 135 mm. Its weight without the fitted humidification chamber is 2.54 kg. With the chamber fitted and filled with 30 mL sterile water, the device weighs 2.62 kg.

- **Electrical** – The humidifier operates with a supply voltage of 230 V, a supply current of 1.0 A Max, and a supply frequency of 50/60 Hz. The heater base capacity is 150 W at nominal mains voltage. The heater wire running along the gas outflow tubing operates
at 22 ± 5 V, 2.7 A Max, 60 W Max, and 50 Hz. The maximum temperature cut-off of the heater base is 118 ± 6 °C.

- **Temperature and humidity parameters** – The humidifier requires a warm up time of less than 15 minutes. Designed to provide conditioned insufflation gas close to natural intraperitoneal conditions (37 °C, 100% relative humidity), its normal operating setting is 37 °C. No external adjustment by theatre personnel is required. The humidifier interface has two buttons (Mute & On/Off) and a 3-digit, 14 mm, 7-segment LED screen that displays the temperature within the humidification chamber (Figure 2.4). This can range between 10 and 70 °C, and has an accuracy of ± 0.3 °C in the 25 to 45 °C range. A high temperature visual alarm is activated at a chamber temperature of 43 °C. At below optimal temperatures, an audible alarm (exceeding 50 dBA at 1 metre) is activated after 10 minutes if the temperature stays < 29.5 °C or after 60 minutes if the temperature stays < 35 °C. The humidifier achieves an absolute humidity of > 33 mg/mL at gas flow rates of up to 10 L/minute.

- **Approvals** – The humidifier fulfils the standards of IEC 60601-1, IEC 60601-1-2, EN 60601-1, and ISO 8185, and has received these respective approvals.
**Figure 2.4** Interface of the Fisher & Paykel MR860 Laparoscopic Humidifier (Fisher & Paykel Healthcare, Auckland, New Zealand)

*Delivery tubing*

The heated gas outflow tube delivers heated and humidified insufflation gas to the patient. It is a double-layered, flexible, and insulated tube containing a heater wire. It is the only component of the humidification system that is supplied in sterile condition (gamma-irradiated) and designed for the sterile operating field.
Application

- Physical setup – Figure 2.5 is a schematic diagram of the physical setup and functioning of the humidification system. Carbon dioxide gas leaves the insufflator and flows through the connection tube into the humidification chamber situated over the heater base containing heated sterile water. As it flows through the chamber, dry gas then mixes with vapour above this water and exits via the conduit outflow tube. Its temperature is maintained within this heated outflow tube as it flows towards the patient.

Figure 2.5 Schematic Description of the Fisher & Paykel MR860 Laparoscopic Humidification System (Fisher & Paykel Healthcare, Auckland, New Zealand)
• Heating circuits – The MR860 Laparoscopic Humidification System contains two separate heating circuits. The first is the heater base situated within the humidifier device, a metal plate that heats the water in the humidification chamber. Temperature of the gas at the chamber’s outlet is monitored via the ‘chamber flow probe’ attachment of the 4-in-1 probe (Figure 2.6) and this feedback process controls the temperature of the heater base. The second heating circuit is the encapsulated heater wire running along the outflow heater tube. It functions to maintain the temperature of the humidified gas flowing from the chamber to the patient as any condensation along the tubing will lower the relative humidity of the gas. The humidifier maintains the temperature along the heater tube by controlling the power delivered to the heater wire via the ‘heater wire connector’ of the 4-in-1 probe.

• Humidifier testing – The reliability of the Fisher & Paykel MR860 Laparoscopic Humidification System at variable flow rates commonly used in the surgical setting (2.0 L/minute, 4.0 L/minute, 6.0 L/minute, 8.0 L/minute, and 10.0 L/minute) has been independently tested. At every flow rate, > 98.0% relative humidity was achieved in the chamber after less than 30 seconds of insufflation.
2.2.3.2 Criteria for cessation of postoperative antibiotic therapy

To evaluate the efficacy and safety of using clinical criteria to guide postoperative intravenous (IV) antibiotic duration in children with complicated appendicitis, a comparison cohort study was designed. It tested the hypothesis that using clinical criteria representative of resolved peritoneal infection to determine cessation of postoperative antibiotic would shorten hospitalisation without compromising patient care or increasing the risks of
insufficiently treated infections. The study compared a prospectively recruited cohort of patients treated under this new strategy with a historical control cohort treated with a traditional fixed antibiotic regimen.

After a detailed literature review (Chapter 4), the following clinical criteria were assembled. Together, they represent resolving peritoneal infectious inflammation and once a patient satisfies all four criteria, inpatient intravenous antibiotic therapy is assumed to be redundant and therefore stopped.

1. Core body temperature less than 38.0 °C for 24 consecutive hours
2. Tolerates a light diet over two consecutive meals
3. Mobilising independently (if ambulatory)
4. Oral analgesia provides effective pain control

To determine whether each criterion had been reached, patients were assessed each morning at the bedside during routine ward rounds starting at 7:30 am. Assessment was performed by responsible consultants and registrars who also subsequently made the overall decision to stop IV antibiotic therapy and to discharge from hospital.

**Criterion 1 – Core body temperature less than 38.0 °C for 24 consecutive hours**

Postoperative recording of core body temperature, every 6 hours, was performed routinely by the ward nursing team using an ear thermometer (Welch-Allyn Braun PRO 4000 Thermoscan Type 6021, Braun GmbH, Kronberg, Germany). The frequency of recordings was increased if there was a need for more intense monitoring. By examining the recording chart (Figure 2.7), clinicians determined whether a patient satisfied this criterion.
Figure 2.7 Paediatric Vital Sign Observational Chart, Starship Children’s Hospital, Grafton, Auckland, New Zealand
Criterion 2 – Tolerates a light diet over two consecutive meals

No formal restrictions are placed on postoperative re-feeding and nasogastric tubes were not routinely used. Resuming the patient’s normal diet was established in graduated step-wise increments, starting first with fluids and then advancing to solids guided by patient progress. Patients must have successfully tolerated two consecutive light meals (breakfast, lunch, dinner) and parental corroboration was regularly requested in the case of younger patients when assessing this criterion. A light meal (lunch) served at Starship Children’s Hospital is shown in Figure 2.8.

Figure 2.8 Light Meal on Ward 24B, Starship Children’s Hospital, Grafton, Auckland, New Zealand
**Criterion 3 – Mobilising independently (if ambulatory)**

The ability to comfortably and independently mobilise was assessed at the bedside by direct observation. Again, in younger children, parental corroboration was actively sought to verify that this criterion had been satisfied.

**Criterion 4 – Oral analgesia provides effective pain control**

Simple and opioid analgesia agents were routinely available to study participants and prescribed in individual medication prescription charts (Figure 2.9). Patient-controlled analgesia (PCA), delivering IV morphine or fentanyl, was also available via prescription by the Acute Pain Team. By examining the medication prescription chart, clinicians were able to assess this criterion.
2.2.4 Allocation

2.2.4.1 Randomisation

Clinical Study C was a randomised controlled study. The random allocation of participants to the study intervention and control groups distributes both known and unknown sources of variability equally between the two groups and the individuals in these groups can be considered as unconditionally exchangeable.\(^{242}\) The following steps were taken to facilitate blind and randomised allocation during Clinical Study C. To ensure accurate co-ordination of all these steps, a 1-week rehearsal period just prior to trial implementation, was conducted.
**Sequence generation**

The generation of random numbers was performed by an independent research assistant. All numbers from 1 to 200 were generated in random sequence and arranged into two columns using an open-source computer-based online random number program ([www.random.org](http://www.random.org)). After generating these two columns, the independent research assistant then randomly assigned one column as ‘intervention’ group allocation numbers and the other as ‘control’ group allocation numbers.

**Allocation concealment**

Randomised study allocations were placed into 200 opaque numbered envelopes by the same research assistant. The envelopes were then securely sealed and kept in a central location. They were opened strictly in sequence and only in the operating room just prior to the start of each study procedure.

**Implementation**

Participants were all recruited preoperatively and allocation occurred intraoperatively. Just prior to the start of surgery, an unblinded rotating scrub nurse, appointed by the research investigator, opened an allocation envelope and revealed the group allocation to her or himself. Without disclosing this to anybody, the theatre nurse then ensured that the participant received either the intervention or control treatment depending on the allocation contained in the envelope. This nurse subsequently had no further involvement in the postoperative care of study participants, or in data collection, analysis, and reporting.
2.2.4.2 Propensity scores

Clinical Study B was a comparison cohort study that used propensity scores to match comparable participants from the control group to those from the intervention group. Introduced in 1983 by Rosenbaum and Rubin, propensity scoring offers a scientifically sound alternative to multivariable analyses and for situations when randomisation for research is not possible for ethical and practical reasons.

In its simplest terms, a participant’s propensity score is the probability of them receiving treatment A (the intervention) rather than treatment B (the control) expressed as a percentage, calculated from observed pre-treatment baseline characteristics (potential confounders). The propensity score replaces this collection of multiple baseline characteristics with one single summary score. When they are incorporated into a matching treatment effect model, the study design is then about comparing individual participants who, based on observables have a very similar probability of receiving treatment i.e. closely matched propensity scores, but one of them received the intervention treatment and the other did not.

Propensity scores were calculated by running a logistic regression analysis using the statistical program PASW Statistics for Windows Version 18.0 (SPSS Inc., IBM, Chicago IL, USA) where the ‘dependent variable’ was the patient’s study allocation (intervention or control cohort) and all possible confounding factors included as ‘covariates’. A confounder was defined by three conditions:

1. It is a covariate available prior to the treatment assignment
2. If given the chance, it may influence a clinician’s decision to opt for one treatment over the other

3. It may influence the outcome of the patient

The confounders included in the calculation of propensity scores in Clinical Study B are listed in Chapter 5.

Once propensity scores had been calculated, one-to-one matching of intervention cohort participants to control cohort participants was performed. Firstly, participants in the intervention cohort were listed in random order and the first participant matched to the control participant with the nearest propensity score. Both patients were then removed from consideration in the next round of matching and focus shifted to the second listed participant. Matching was done until all intervention cohort patients had been matched to the closest control cohort participant.

2.2.5 Blinding

In Clinical Study C, the participants, the primary research investigator (T Yu), surgical team, anaesthetist, theatre personnel, and ward nursing staff responsible for intra- and postoperative care of participants were all blinded to group allocations. As described above, one unblinded rotating scrub nurse assisted with randomisation by opening the allocation envelope and setting up the surgical humidifier according to assigned allocation. Prior to participants entering the operating theatre, the unblinded scrub nurse prepared the device away from view of other theatre personnel. When a participant was allocated to the intervention group, 30 mL sterile water was added to the water chamber and the humidifier
was switched on and muted. When participants were in the control group, water was not added and the humidifier was left off. A commissioned opaque plastic cover was designed to conceal the surgical humidifier from view during each study procedure (Figure 2.10). It covered the front LED screen as well as the water chamber so that it was impossible for theatre occupants to tell whether the device was switched on and whether the chamber contained water. At the end of a procedure, the unblinded scrub nurse removed the humidifier from theatre with its cover intact and disassembled it away from view of theatre personnel.

**Figure 2.10** Opaque Plastic Cover Placed Over the Humidifier Conceals its LED Screen and Water Chamber.
The effectiveness of this blinding process was tested at the end of each procedure by asking the primary surgeon to answer “yes” or “no” to the following question: “Is this participant in the Intervention Group (warm humid gas insufflation)?” Individual responses were compared to the actual allocations once these were revealed.

Blind statistical analysis during Clinical Study C was performed by the primary research investigator (T Yu) and facilitated by a distant research assistant with no involvement in the study. This individual prepared the data spreadsheets by concealing study group allocations (Group X and Group Y). Allocations were only revealed at the completion of data analysis.

2.2.6 Fixed Variables

2.2.6.1 Preoperative care

All study participants presented to Starship Children’s Hospital acutely. Preoperative care consisted of resuscitation with intravenous fluids and administration of systemic antibiotics and analgesia. The choice and dosage of these were left to the discretion of admitting surgical registrars but regimens consisted of either cefoxitin (Cefoxitin sodium injection, Mayne Pharma Pty Ltd, Mulgrave VIC, Australia) alone or, if ‘complicated appendicitis’ was suspected, a combination of amoxycillin (Amoxycillin injection, Douglas Pharmaceuticals, Auckland, NZ), metronidazole (Metronidazole BP for infusion, Baxter Healthcare, Old Toongabbie NSW, Australia), and gentamicin (Gentamicin sulfate BP injection, Pfizer Pty Ltd, Bentley WA, Australia). Patients with documented beta-lactam antibiotic anaphylaxis were prescribed with substitutes such as clindamycin or vancomycin. The dosages and dosing regimens of these antibiotic agents are listed below in Table 2.1.
Table 2.1 Dosages and Dosing Regimens for Routinely Used Antibiotic Agents, Starship Children's Hospital, Grafton, Auckland, New Zealand

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dosage/kg</th>
<th>Maximum dose</th>
<th>Frequency of dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin</td>
<td>25-30 mg</td>
<td>2 g</td>
<td>8 hours</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>20-25 mg</td>
<td>1 g</td>
<td>8 hours</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>7.5 mg</td>
<td>500 mg</td>
<td>8 hours</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>6-7.5 mg</td>
<td>360 mg</td>
<td>24 hours</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>20-40 mg</td>
<td>1.2 g</td>
<td>6-8 hours</td>
</tr>
</tbody>
</table>

2.2.6.2 Surgery

Surgery was scheduled according to acuity prioritisation protocols at Starship Children’s Hospital. All participants underwent acute laparoscopic appendicectomy performed either by a consultant paediatric surgeon or a registrar under the direct supervision of a consultant paediatric surgeon. All the technical aspects of the surgical procedure unrelated to research study protocols were left up to the discretion of the surgeon. Below is a description of the basic surgical technique for laparoscopic appendicectomy at Starship Children’s Hospital.

Before transfer to the operating room, patients are asked to urinate if possible. After induction of general anaesthesia, patients are positioned in a supine position and if the bladder is distended, urinary catheterization is performed. Patients are then secured to the operating table with an adjustable strap placed across both upper thighs. The surgical site is prepared with an antiseptic solution (iodine or chlorhexidine) and drapes placed to display the appropriate sterile operating field. Laparoscopic access is standardised to three laparoscopic ports: periumbilical 10 mm port, left iliac fossa 5 mm port, and left/midline suprapubic 5 mm port, inserted in this order.
The periumbilical port is inserted by the open Hasson technique and pneumoperitoneum established with insufflation of medical standard CO$_2$ gas. The technique used to secure the Hasson port varied but included purse-string or ‘figure-of-eight’ configuration sutures. Insufflation pressures did not exceed 14 mmHg. A 5 mm, 30-degree laparoscopic camera is used and inserted via the Hansson port. Once a satisfactory image had been obtained, the appendix and entire peritoneal cavity was inspected. If macroscopic findings are consistent with acute appendicitis and preoperative antibiotics have not yet been administered, single doses of the above agents are given with preferences being dependent on the intraoperative findings. Cefoxitin is administered for simple appendicitis while triple agents administered if complicated appendicitis is found. The suprapubic and left iliac fossa working ports are then placed under direct vision – these are both 5 mm disposable ports (Endopath Xcel Bladeless Trocar, Ethicon Endo-Surgery LLC, Guaynabo, USA). The standard local anaesthetic agent is 0.25% Bupivacaine with Adrenaline (Marcaine 0.25% with Adrenaline 1:400,000 Injection, AstraZeneca Ltd, Auckland, NZ) permitted to the maximum dosage of 1 mL per kg (equivalent to 2.5 mg per kg) for infiltration into subcutaneous tissue and fascia at each laparoscopic port site at the beginning and/or end of each procedure.

If any free intraperitoneal fluid is encountered including free pus, this is suctioned out before any dissection takes place. The patient is then placed in a head-down and left tilt position. An inspection of the right iliac fossa is performed and the appendix freed from omental and small bowel wrapping and peritoneal adhesions. If the appendix is retrocaecal, the lateral peritoneal attachments to the caecum are incised to mobilise the caecum and appendix. If appendiceal perforation is present, attempts are made to limit the spread of faecal contaminant during dissection. Appendicectomy is then carried out by dissecting off
the mesoappendix using a 5 mm L-shaped (‘hook’) monopolar electrode (Karl Storz GmbH & Co., Tuttlingen, Germany) set on coagulation waveform function. The plane of dissection follows the mesoappendix down to the appendiceal base and is continued as close as possible to the appendix. This is aided by maintaining upward traction of the appendix. If the appendiceal artery is encountered during this dissection, it is avoided but if haemorrhage inadvertently occurs, the bleeding point is electrocauterised. Endoclips or ligatures are rarely utilised to control bleeding.

At the appendiceal base, two polydioxone (PDS) sutures (Endoloop PDS II, Ethicon, Somerville NJ, USA) are deployed to secure the appendiceal lumen before the nearest proximal section of the appendix is fastened with a third Endoloop and resection performed with endoscopic tissue scissors. At the discretion of individual surgeons, the appendix is then delivered through the Hasson port or retrieved using an endoscopic plastic retrieval bag (Endopouch Retriever, Ethicon Endo-Surgery, LLC, Guaynabo, USA).

Intraperitoneal debris and blood is typically suctioned out and in the setting of complicated appendicitis, intraperitoneal irrigation with warm isotonic saline is used to reduce the load of infectious material and byproducts. Transperitoneal drains and local instillation of antibiotics (intraperitoneally or into wound layers) are not routinely practiced strategies. At the end of the procedure, insufflation is discontinued and the vents from all three ports opened to allow release of the pneumoperitoneum. Fascial incisions at all three port sites are then closed. Skin closure is performed with subcuticular absorbable sutures only and the wounds are covered with waterproof dressings.
2.2.6.3 Anaesthesia and analgesia

To reliably measure postoperative pain during Clinical Study C, a standardised Anaesthesia and Analgesia Protocol was developed in collaboration with a paediatric anaesthetist and pain specialist from the Department of Anaesthetics, Starship Children’s Hospital. During the study period, the protocol was actively promoted and advertised perioperatively. It is clearly outlined in Chapter 7 and also included in Appendix D. Otherwise, perioperative analgesia and anaesthetic agents including induction and muscle relaxing agents used were left to the discretion of individual anaesthetists and surgical team members. Epidural, spinal, and intrathecal analgesia/anaesthesia were not used. The application of an upper-body forced-air-rewarming blanket and the choice, volume, and temperature of intravenous fluids given were left to the discretion of anaesthetists.

2.2.6.4 Postoperative care

Postoperatively, established antibiotic regimens were continued. All patients found to have complicated appendicitis and spread of infection into the peritoneal cavity were treated with a triple IV antibiotic regimen consisting of amoxycillin, metronidazole and gentamicin. Serum gentamicin trough levels were monitored immediately before administrating the second dose and then subsequently every 3 to 5 days. The accepted trough level was < 0.5 mg/L. Serum creatinine was also used as a surrogate marker for gentamicin renal toxicity. Monitoring of serum inflammatory markers and electrolytes was performed as required based on patient progress.

Patient vital signs, including pulse rate, tympanic temperature, blood pressure and respiratory rate, were monitored at 6-hour intervals or more frequently if required.
were no formal restrictions on postoperative re-feeding and nasogastric tubes were not placed routinely. Re-feeding was initiated in step-wise increments according to individual clinician preferences starting first with sips of clear fluid and progressing to a full fluid diet and then a light solid diet.

Postoperatively, study participants routinely received regular oral paracetamol (Parapaed, AFT Pharmaceuticals Ltd, Auckland, NZ) and a simple non-steroidal anti-inflammatory agent such as ibuprofen (Fedpaed Oral Suspension, AFT Pharmaceuticals Ltd, Auckland, NZ) or diclofenac (Diclofenc Sandoz, Novartis, Auckland, NZ) supplemented by rescue opiate analgesia. Generally, in the first postoperative 24 hours, this was administered intravenously. The standard protocol for intravenous morphine administration at Starship Children’s Hospital is included in Appendix K and summarised here: Participants who weighed less than 50 kg were given boluses of 0.04 mg/kg while those who weighed 50 kg and above were given boluses of 2 mg. Each bolus was followed by patient re-assessment after 5 minutes and subsequent boluses given if required. Participants who required more than five titrations of morphine in a 25-minute period were provided with a PCA pump device to ensure adequate and efficient pain control. Intravenous morphine (Morphine sulphate injection, Biomed Ltd, Auckland, NZ) or fentanyl (Fentanyl injection, AstraZeneca Ltd, Auckland, NZ) were the drugs of choice for PCA administration and all prescriptions were approved by the Acute Pain Service. Oral opioids were generally offered after the initial 24-hour postoperative period or when oral intake was tolerated. They included oral morphine as the preferred first-line therapy (Morphine sulphate, Douglas Pharmaceuticals Ltd, Auckland, NZ) and Tramadol (Tramadol Hydrochloride, AFT Pharmaceuticals Ltd,
Auckland, NZ). Oral morphine was prescribed at a dosage of 0.3 mg/kg and administered every 1 to 2 hours as required.

To reliably measure postoperative pain during Clinical Study C, this protocol was standardised and strictly adhered to. Any deviation from study protocol was recorded by research investigators as ‘protocol non-adherence’ (see Section 2.2.7.6).

2.2.7 Outcomes

All clinical data were collected by the primary research investigator (T Yu) and recorded on standard data collection forms (See Appendices E and J) to ensure that the collection process was standardised throughout each clinical study. During Clinical Study C, additional data collection tools were utilised including a participant questionnaire (Appendix F), distributed at the time of hospital discharge and completed on postoperative Day 10, and a surgeon questionnaire (Appendix G), completed immediately after an operative procedure. All forms used for data collection were designed a priori and approved by the Northern X Regional Ethics Committee, New Zealand Government Ministry of Health (see Section 2.2.10). All study outcomes and variables measured as part of these clinical studies are described in detail in the following sections.

2.2.7.1 Patient baseline characteristics

Baseline characteristics that were recorded were: National Health Index (NHI) number, full name, age in years, gender, ethnicity (self-identified by the participant and his/her legal guardian), weight in kilograms, and past surgical and medical history. The histological
diagnosis after pathology analysis of resected appendiceal specimens were also obtained from formal hospital reports and recorded.

2.2.7.2 Preoperative variables

Details of each participant’s presentation and diagnostic work-up were recorded including: date and time of presentation, method of referral, duration of symptoms (in days), presenting heart rate, blood pressure, core body temperature, level of various serum inflammatory markers (white blood cell count, neutrophil count, CRP, urine analysis), and results of radiology imaging (plain film X-ray, abdominal and pelvic ultrasound scan, computed tomography). Preoperative management strategies were also recorded such as administration of preoperative antibiotics and analgesia.

2.2.7.3 Intraoperative variables

Operative data recorded included date of procedure, seniority of operative surgeon (registrar, fellow, consultant), procedure(s) performed, surgical approach (laparoscopic, conversion to open, directly open), the use of an external upper-body forced-air-rewarming blanket, operation start time (scalpel to skin), operation end time (all wound dressings applied), macroscopic appearance of appendix (normal, acutely inflamed, suppurative, gangrenous, perforated), presence of localised or generalised peritonitis, presence of faecolith(s) within the peritoneal cavity, presence of walled-off peri-appendiceal abscess, and use of intraperitoneal 0.9% saline irrigation.

During Clinical Study C, the following intraoperative variable were also recorded: gas insufflation start and end times, volume of CO₂ insufflation gas used, maximum insufflation
pressure, and core body temperature measured at 10 minute intervals via a 9-French disposable naso-oesophageal temperature probe (General Purpose Probe GP9400, Truer Medical Inc., Orange CA, USA) (Figure 2.11). Furthermore, at the end of each procedure, the operating surgeon completed a questionnaire, rating the severity of laparoscopic camera fogging and technical difficulty of the procedure using visual analogue scales (VAS) consisting of 10 cm lines anchored at both ends with 0 and 10 (0 = no fogging/perfect images, 10 = worst fogging/very poor images; 0 = no difficulties encountered, 10 = worst level of difficulty). Also included in the questionnaire was a question to test the effectiveness of blinding (see Section 2.2.5 and Appendix G). From anaesthetic charts, the dosages of intraoperative analgesia, antiemetics, and antibiotics administered were collected, as well as the type and volume of IV fluid therapy.
2.7.4 Postoperative outcomes and recovery parameters

Postoperative pain

During Clinical Study C, postoperative pain was quantified in two ways. Objectively, it was represented by postoperative requirement for opiate analgesia including usage in the post-anaesthesia care unit (PACU). As well as recording this as a binary outcome, the quantity required by each participant was also recorded and used for comparison. To reliably compare total opiate usage, the Mean Equivalent Daily Dose (MEDD) method was
used. MEDD is defined as ‘the total opioids used over a 24-hour period converted to an equivalent dosage of parenteral morphine’ and calculated using the standard equi-analgesic conversion factors listed in Table 2.2. All opiates administered to each participant via PCA, intravenous, and oral routes during set postoperative periods were converted to an equivalent MEDD and compared. Both the absolute MEDD and MEDD per kilogram of patient weight were compared.

Table 2.2 Mean Equivalent Daily Dose (MEDD) Conversion Factors (MEDD calculation = Dosage x Conversion factor).

<table>
<thead>
<tr>
<th>Parenteral</th>
<th>Enteral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl (mcg)</td>
<td>0.1</td>
</tr>
<tr>
<td>Penthidine (mg)</td>
<td>0.1</td>
</tr>
<tr>
<td>Tramadol (mg)</td>
<td>0.1</td>
</tr>
<tr>
<td>Alfentanil (mcg)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Postoperative pain was also subjectively quantified by asking participants to rate pain intensity at rest and with movement using a VAS at eight time points from when surgery was completed: 2, 4, 6, 8, 10, 12, 24, and 48 hours (See Appendix H). Participants awake at these time points were asked to provide pain scores and instructed that anchors at the ends of the 10 cm line represented “completely no pain” (0) and “the worst imaginable pain” (10). By measuring the distance, in millimetres, from 0 to the mark made by individual participants, pain intensity scores were recorded and compared as a continuous variable.
**Length of stay**

Length of stay (LOS) was measured by the number of nights spent in hospital. It was further classified into index LOS (LOS during index admission), postoperative LOS, and total LOS (index admission plus any readmission LOS). Readmission was defined as representation within 30 days of hospital discharge for reasons that required additional hospital stay up to and exceeding 24 hours.

**Return to normal activities**

During Clinical Study C, return to normal activities was evaluated using a 10-item questionnaire (Appendix F) developed using the Pediatric Quality of Life Inventory (Pediatric QL™) Version 4.0. It invites participants to respond to each item using a five-level Likert scale (Never, Almost Never, Sometimes, Often, and Almost Always), rating the difficulty they have performing or participating in common daily activities including running, lifting something heavy, taking a bath or shower by themselves, helping out around the house, paying attention to television or a book, and sleeping. The questionnaire also asked participants to rate how often they had pain, experienced low energy, was forgetful, and worried about what would happen to them. The questionnaire lists these ten items twice and participants were asked to first rate themselves when they are healthy and well and then rate themselves again on day 10 after their laparoscopic appendicectomy. Participants were provided with the questionnaire and a stamped and addressed return envelope upon hospital discharge.
Postoperative complications were either prospectively or retrospectively recorded up to 6 weeks after surgery. Participants were prospectively followed-up by the primary research investigator (T Yu) via an outpatient check-up at 2 weeks and a telephone call at 6 weeks. Standardised definitions for postoperative complications (Table 2.3) were used and the severity was graded using the Clavien-Dindo classification. 

**Table 2.3 Standard Definitions for Postoperative Complications**

<table>
<thead>
<tr>
<th>Complication</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraabdominal infection</td>
<td>A symptomatic focus of infection within the abdominal cavity associated with clinical signs of intraabdominal and/or systemic sepsis, diagnosed with radiologic imaging (ultrasound or computed tomography) or during subsequent surgical interventions.</td>
</tr>
<tr>
<td>Wound infection</td>
<td>Erythema and swelling with excessive pain or tenderness in wound, +/- visible pus, +/- wound opened spontaneously or by intervention.</td>
</tr>
<tr>
<td>Postoperative ileus</td>
<td>Postoperative inability to tolerate oral intake and vomiting +/- need for insertion of a nasogastric tube but without radiological evidence for mechanical bowel obstruction.</td>
</tr>
<tr>
<td>Chest infection</td>
<td>Pneumonia, lung abscess or empyema associated with radiographic confirmation</td>
</tr>
<tr>
<td>Lobar atelectasis</td>
<td>Radiographic confirmation of collapse of one or more pulmonary lobes</td>
</tr>
</tbody>
</table>

**2.2.7.5 Histopathological diagnosis**

The following information describing the steps taken to process and fix tissue for histological examination was provided by the Department of Pathology, Auckland City Hospital, Grafton, Auckland, New Zealand. It describes the routine way in which appendiceal tissue, collected by surgeons for suspected appendicitis, is processed, embedded, sectioned, and stained so a histopathological diagnosis can be obtained. All appendiceal tissue from Starship Children’s Hospital is prepared and examined by this Department.
Immediately after removal, specimens are placed into 10% neutral buffered formalin for transportation from the operating theatre to the laboratory. It is then left in this solution for overnight fixation. Processing of the specimen starts at the surgical cut-up bench where examination by a pathologist was performed. Macroscopic descriptions of the specimen are recorded and then appropriate sections are taken to confirm the macroscopic abnormalities seen. Chosen tissue sections are placed into labelled cassettes for tissue processing.

Tissue processing is a standardised and automated procedure, involving three distinct steps: dehydration, clearing and infiltration. The final product is tissue that is stiffened by paraffin allowing very thin sections (only a few microns in thickness) to be cut with a microtome. Dehydration replaces water in the specimen with alcohol in order for the non-aqueous embedding media (paraffin) to penetrate into tissue. Clearing involves replacing the alcohol with a clearing agent so that tissue is receptive to infiltration by the embedding medium. Xylene is the clearing agent routinely used. Subsequent infiltration involved replacing the xylene with paraffin.

At the end of the tissue-processing step, cassettes containing the specimens are immersed/embedded in paraffin to make tissue blocks. Then, a microtome is used to cut four-micron thick sections from the surface of the blocks and these tissue slices are placed directly onto glass slides. Staining of the tissue is automated and performed using a staining machine (Leica ST5020 Multistainer, Leica Biosystems, Nussloch, Germany). Haematoxylin and eosin is the routine stain used at the study institution. Haematoxylin stains the cell nucleus dark blue/purple and the cell cytoplasm pink to red. Finally, stained slides are then
cover-slipped, checked via a quality control process and then submitted to pathologists for reporting.

2.2.7.6 Adherence to study protocol

Although study protocols were thoroughly explained and actively promoted prior to and during each clinical study, incidences of protocol non-adherence did occur. These were identified and recorded as part of data collection and subsequently reported alongside the study findings. Events that would count as protocol non-adherence were defined a priori.

2.2.8 Sample Size Determine

After defining null hypotheses, sample sizes in Clinical Studies B and C were calculated a priori on the basis of their primary outcomes. A decision to perform subgroup analysis was also made a priori. The following factors were the key determinants used in power calculations:

1. Precision and variance of the primary outcome within the target population
2. The magnitude of a ‘clinically-significant’ difference in the primary outcome
3. To what degree is a type 1 error to be avoided (α)
4. To what degree is a type 2 error to be avoided (β/power)
5. The statistical test will be used to perform the comparison analysis

For Clinical Study B, the precision and variance of the primary outcome was again obtained from retrospective data collected at the study institution in November 2010. The mean LOS for 302 children who underwent acute surgery for complicated appendicitis at Starship Children’s Hospital between 2005 and 2009 was 5.8 nights (standard deviation = 3.0).
A 2-tailed student’s t-test showed that a reduction in mean LOS to 4.0 nights (reduction of 31%) would require a minimum sample size of 94 ($\alpha = 0.05$, power = 0.8). Each arm of the study would consist of 47 participants.

For Clinical Study C, the precision and variance of the primary outcome was obtained from retrospective data collected at the study institution. The postoperative opiate consumption of 21 children (aged 5-14 years) after laparoscopic appendicectomy facilitated by conventional CO$_2$ gas insufflation in 2006 was examined. In the postoperative period, 15 (71%) children required rescue opiate analgesia in addition to regular simple analgesia. In order to detect a 30% reduction in the number of children requiring postoperative opiate analgesia, a 2-tailed Fisher’s exact test demonstrated that 95 participants would be required in each study arm ($\alpha = 0.05$ and power = 0.8).

### 2.2.9 Statistical Analysis

All data were analysed using PASW Statistics for Windows Version 18.0 (SPSS Inc., IBM, Chicago IL, USA). Statistical significance was defined as a p-value of < 0.05.

#### 2.2.9.1 Parametricity testing and descriptive statistics

The parametricity of continuous variables was tested using the Shapiro-Wilk test or by visually inspecting plotted quantile-quantile curves. Parametric data were described in means and standard deviations (SD) while nonparametric data were described in medians and inter-quartile ranges (IQR).
2.2.9.2 Comparison and correlation analysis

Univariate comparison analysis was conducted using student’s t-test for parametric continuous variables, Mann-Whitney U tests for nonparametric continuous variables, and Fisher’s exact tests or Pearson chi-square tests for categorical variables. Parametric correlation analysis was conducted by calculating Pearson correlation coefficient and nonparametric correlation analysis conducted by calculating Spearman’s correlation coefficient.

2.2.9.3 Regression

Regression analyses were performed in Clinical Study A to identify the perioperative patient- and clinician-factors (independent variables) that predict patient outcomes (dependent variables). More specifically, they helped to understand how important patient outcomes (LOS and occurrence of postoperative complications) were affected when any one of the independent variables was varied while all others were fixed. When the patient outcome was binary in nature, multivariate analysis was carried out using binary logistic regression. When the patient outcome was ordinal in nature, multivariate analysis using general linear modelling was used.

2.2.10 Ethical Approval

All three clinical studies received official ethical approval from the Northern X Regional Ethics Committee, New Zealand Government Ministry of Health, and institutional approval from appropriate clinical and cultural review boards including the Maori Review Board, Auckland District Health Board.
2.2.11 Trial Registration

Clinical Study C was prospectively registered online with ClinicalTrials.gov, US National Library of Medicine, 8600 Rockville Pike, Bethesda MD, USA.

2.2.12 Reporting

Clinical Study B was reported in accordance with the Strengthening Reporting of Observational Studies in Epidemiology (STROBE) statement. Clinical Study C was reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement for randomised controlled trials.
Chapter 3

CLINICAL TRIAL A

Disease morbidity of complicated appendicitis in New Zealand children and predictors key patient outcomes: A retrospective study
3.1 BACKGROUND

Appendicitis is a surgical disease triggered by invasion of the appendiceal wall by intraluminal bacteria\textsuperscript{254} resulting in infectious inflammation. Unfortunately, in a significant proportion of individuals, the infection also spreads to the peritoneal cavity causing secondary peritonitis and significantly increased disease morbidity. This Chapter outlines an observational study conducted to purposely review the disease morbidity associated with secondary peritonitis arising from advanced appendicitis and the predictors of key patient outcomes.

Acute appendicitis has a fairly consistent time-dependent natural history of progression. The continuum of disease starts with luminal obstruction that progresses to suppurative inflammation and gangrene, and ultimately ends with appendiceal necrosis and perforation. Clinically, ‘simple’ appendicitis refers to infection confined to the appendix alone and is associated with minimal morbidity and requires only prophylactic antibiotics to prevent surgical site infections. In contrast, ‘complicated appendicitis’ is distinguished by the presence of intraperitoneal suppurative infection from appendiceal gangrene and perforation and carries a significantly higher risk of postoperative infectious complications.

In children, the total incidence of appendicitis is not only higher than in adults but the rate of complicated appendicitis is also relatively higher.\textsuperscript{12,13} Appendiceal perforation occurs in up to 50% of paediatric patients at the time of their presentation\textsuperscript{14} and in children less than 5 years of age, the perforation rate can be as high as 65%. In those less than 2 years of age,
the rate is 95%. Currently, complicated appendicitis is the most common cause of peritonitis and intraabdominal sepsis in children, and few common paediatric conditions continue to generate as much debate.

In practice, complicated appendicitis is challenging for clinicians because it can be associated with critically ill patients, difficult operating conditions, delayed recovery, and significantly elevated risks of postoperative infectious complications. In children, the reported total complication rate is as high as 58%.

Persistent or recurrent intraabdominal infection is one of the most significant postoperative complication associated with appendicitis and its risks increase greatly when appendiceal perforation is present. While reported rates of postoperative intraabdominal abscess in patients with acute, nonperforated appendicitis range from 0 to 3.6%, rates for perforated appendicitis are between 8 and 20%. In such cases, disease morbidity also arises from prolonged hospitalisation, additional time off school, and disruption to social functioning. Furthermore, there are increased healthcare costs and interruption to the occupational and social commitments of caregivers and other family members. Treatment strategies for complicated appendicitis continue to evolve but the predictors of disease outcome remain ill defined.

If clinical practices and patient outcomes are to be improved, the present disease morbidity caused by complicated appendicitis in the population of interest (New Zealand children, 1 to 14 years of age) needs to be documented and predictors of key patient outcome determined. In particular, the risk factors for prolonged length of stay (LOS) and
development of postoperative intraabdominal infections need exploration. The specific study aims were:

1. Describe the total disease morbidity including incidence and severity of perioperative complications, readmission rate and causes, and LOS
2. Describe the added morbidity of postoperative intraabdominal infections
3. Identify potential risk factors for postoperative intraabdominal infections
4. Identify the predictors of total and postoperative LOS
3.2 METHODS

This study included prospectively recorded data from a clinical trial conducted at the study institution and retrospectively collected data obtained from the institution’s patient records. Ethics approval was granted in September 2010 by the Northern X Regional Ethics Committee, New Zealand Government Ministry of Health (Committee Reference Number: NTX/10/EXP/182).

3.2.1 Setting

This study was set at Starship Children’s Hospital, the single tertiary centre providing paediatric surgical care to the catchment areas of three District Health Boards (DHB) that serve the wider Auckland region (Auckland DHB, Counties Manukau DHB, and Waitemata DHB).

3.2.2 Participants

Study participants were children, between the ages of 1 and 14 years, treated at Starship Children’s Hospital for ‘complicated’ appendicitis who underwent appendicectomy during their index admission. Due to small numbers and significant variation in management strategies, those diagnosed with an appendiceal collection/abscess during the index admission and treated conservatively (systemic antibiotic therapy with or without percutaneous drainage) were excluded.
The study’s prospectively observed cohort was selected from patients recruited into the randomised controlled trial described in Chapter 7. This study was conducted at the research institution between February 2010 and March 2011 and investigated the impact of warm humidified laparoscopic insufflation gas on postoperative pain and recovery after appendicectomy. From a total of 190 participants, those diagnosed with complicated appendicitis by the operating surgeon were included. All data for this trial were prospectively collected by the primary research investigator (T Yu).

The retrospective cohort of participants was identified from the hospital’s operating theatre records between 1 January 2005 and 31 December 2009. All clinical records for patients who underwent ‘appendicectomy’ were identified and screened by investigators. Using a combination of the operation notes and histology reports, all children diagnosed with complicated appendicitis by the operating surgeon and/or with gangrenous and perforated appendicitis via histology were included in the study. Appropriate data were then collected from available clinical documentation.

3.2.3 Data Collection

Anonymised data were collected and classified into several categories including basic patient demographics (age, gender, self-/caregiver-defined ethnicity), presenting symptoms and signs of disease, intraoperative parameters, perioperative management strategies including details of antibiotics prescribed, postoperative patient recovery parameters, and the incidence and severity postoperative complications.
The duration of antibiotic therapy was defined as ‘the number of days a complete 24-hour course had been received’ and LOS was counted as ‘number of nights spent in hospital’. LOS of the index admission was added to the LOS of any subsequent readmissions in order to determine total LOS. Readmission was defined as return to hospital for 24 hours or more within 30 days of discharge. Standardised definitions for perioperative complications were used and each recorded complication was classified according to its origin and severity (as per the Clavien-Dindo classification). Postoperative intraabdominal infection was defined as symptomatic collections of fluid within the abdominal cavity, diagnosed via radiology imaging or at the time of invasive intervention.

3.2.4 Statistical Analysis

All data analysis was performed using PASW Statistics for Windows Version 18.0 (SPSS Inc., IBM, Chicago IL, USA). Statistical significance was defined as a p-value of < 0.05. Continuous variable parametricity was tested using the Shapiro-Wilk test.

Parametric data are reported as means and standard deviations (SD) while nonparametric data are reported as medians and interquartile ranges (IQR). Univariate comparison analysis was conducted first using student’s t-tests for parametric continuous variables, Mann-Whitney U tests for nonparametric continuous variables, and Fisher’s exact tests or Pearson chi-square tests for categorical data. Nonparametric correlation analysis was conducted by calculating Spearman’s correlation coefficient.

To identify independent predictors of postoperative intraabdominal infections, multivariate analysis was then carried out using binary logistic regression involving all patient and
perioperative variables that were either significantly or near significantly ($p < 0.10$) different in univariate analyses comparing patients that were affected by this complication with those who were not. Similarly, multivariate analysis using general linear modelling was used to identify the patient and perioperative variables that independently predict LOS.
3.3 RESULTS

3.3.1 Participant Recruitment

From 190 participants in the randomised controlled clinical trial, 54 (28%) were diagnosed with complicated appendicitis and included in the current study. From the operating theatre records, a total of 1,453 patients underwent ‘appendicectomy’ over the course of 5 years at Starship Children’s Hospital from 1 January 2005 to 31 December 2009. After reviewing the available operation notes and histological reports, 305 eligible patients were identified and included in the review bringing the total number of participants to 359. Twelve patients presented with established appendiceal abscesses and were successfully managed conservatively during the index admission. They were excluded according to the study’s eligibility criteria.

3.3.2 Baseline Characteristics and Treatment Outcomes

Table 3.1 is a summary of patient demographics and perioperative patient variables including duration of symptoms, presenting serum inflammatory markers, intraoperative findings, and final histological diagnoses. Postoperative recovery parameters including incidence and pattern of postoperative fever (core body temperature > 38°C), duration of IV antibiotic therapy, and LOS are also summarised in Table 3.1. Table 3.2 summaries the incidence, severity, and nature of the postoperative complications.
### Table 3.1 Participant Demographics, Perioperative Variables, and Diagnoses

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>[n = 359]</th>
<th>% (n/359)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median in years (IQR)</strong></td>
<td>11 (3)</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>142</td>
<td>39.6</td>
</tr>
<tr>
<td>Male</td>
<td>217</td>
<td>60.4</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand European</td>
<td>169</td>
<td>47.1</td>
</tr>
<tr>
<td>New Zealand Maori</td>
<td>72</td>
<td>20.1</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>59</td>
<td>16.4</td>
</tr>
<tr>
<td>Asian</td>
<td>42</td>
<td>11.7</td>
</tr>
<tr>
<td>Other European</td>
<td>14</td>
<td>3.9</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Weight, mean in kg (SD)</strong></td>
<td>44.6 (18.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of presenting symptoms, median in days (IQR)</strong></td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td><strong>Presentation time between 0600 and 1800</strong></td>
<td>242</td>
<td>67.4</td>
</tr>
<tr>
<td><strong>Presenting WBC Count, mean in xE9/L (SD) [n]</strong></td>
<td>16.8 (5.4) [347]</td>
<td></td>
</tr>
<tr>
<td><strong>Presenting neutrophil count, mean in xE9/L (SD) [n]</strong></td>
<td>13.7 (5.1) [347]</td>
<td></td>
</tr>
<tr>
<td><strong>Presenting CRP, mean in mg/L (SD) [n]</strong></td>
<td>105.8 (90.2) [257]</td>
<td></td>
</tr>
<tr>
<td><strong>Underwent preoperative imaging (USS and/or CT)</strong></td>
<td>90</td>
<td>25.1</td>
</tr>
<tr>
<td><strong>Received preoperative antibiotics</strong></td>
<td>220</td>
<td>61.3</td>
</tr>
<tr>
<td><strong>Received intraoperative antibiotics</strong></td>
<td>272</td>
<td>75.8</td>
</tr>
<tr>
<td><strong>Operation same day as presentation</strong></td>
<td>194</td>
<td>54.0</td>
</tr>
<tr>
<td><strong>Operation start time between 0600 and 1800</strong></td>
<td>189</td>
<td>52.6</td>
</tr>
<tr>
<td><strong>Duration of operation, mean in minutes (SD)</strong></td>
<td>86.4 (34.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Operation Approach</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>285</td>
<td>79.4</td>
</tr>
<tr>
<td>Conversion</td>
<td>41</td>
<td>11.4</td>
</tr>
<tr>
<td>Open</td>
<td>33</td>
<td>9.2</td>
</tr>
<tr>
<td><strong>Seniority of surgeon</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attending surgeon/Consultant</td>
<td>59</td>
<td>16.4</td>
</tr>
<tr>
<td>Resident/Registrar</td>
<td>300</td>
<td>83.6</td>
</tr>
<tr>
<td><strong>Transperitoneal drain</strong></td>
<td>18</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Intraoperative findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppurative Appendicitis</td>
<td>10</td>
<td>2.8</td>
</tr>
<tr>
<td>Gangrenous Appendicitis</td>
<td>75</td>
<td>20.9</td>
</tr>
<tr>
<td>Perforated Appendicitis</td>
<td>274</td>
<td>76.3</td>
</tr>
<tr>
<td><strong>Appendix histology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute inflammation/Suppurative</td>
<td>14</td>
<td>3.9</td>
</tr>
<tr>
<td>Gangrenous</td>
<td>46</td>
<td>12.8</td>
</tr>
<tr>
<td>Perforated</td>
<td>299</td>
<td>83.3</td>
</tr>
<tr>
<td><strong>Postoperative antibiotic therapy duration, median in days (IQR)</strong></td>
<td>5 (1)</td>
<td></td>
</tr>
<tr>
<td><strong>Prescribed oral antibiotics upon discharge</strong></td>
<td>93</td>
<td>25.9</td>
</tr>
<tr>
<td><strong>Postoperative temperature &gt; 38 °C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During entire admission</td>
<td>163</td>
<td>45.4</td>
</tr>
<tr>
<td>Days 0 / 1 / 2</td>
<td>142</td>
<td>39.6</td>
</tr>
<tr>
<td>Days 3 / 4 / 5</td>
<td>62</td>
<td>17.3</td>
</tr>
<tr>
<td><strong>Index LOS, median in nights (IQR)</strong></td>
<td>6 (2)</td>
<td></td>
</tr>
<tr>
<td><strong>Index postoperative LOS, median in nights (IQR)</strong></td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td><strong>Readmission within 30 days</strong></td>
<td>40</td>
<td>11.1</td>
</tr>
<tr>
<td><strong>Total LOS [Index +/- Readmission], median in nights (IQR)</strong></td>
<td>6 (2)</td>
<td></td>
</tr>
<tr>
<td><strong>Patients with complications</strong></td>
<td>90</td>
<td>25.1</td>
</tr>
</tbody>
</table>

**NOTE:** IQR = interquartile range; SD = standard deviation; n = sample size; WBC = white blood cell; CRP = C-reactive protein; USS = Ultrasound scan; CT = Computed tomography.
### Table 3.2 Frequency, Severity, and Nature of Postoperative Complications

<table>
<thead>
<tr>
<th>Complication-related Variable</th>
<th>n</th>
<th>% (n/122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of complications recorded</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Severity of complications (Clavien-Dindo grading)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>42</td>
<td>34.4</td>
</tr>
<tr>
<td>II</td>
<td>61</td>
<td>50.0</td>
</tr>
<tr>
<td>III</td>
<td>19</td>
<td>15.6</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nature of complications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postoperative intraabdominal infection</td>
<td>44</td>
<td>35.2</td>
</tr>
<tr>
<td>Wound infection</td>
<td>10</td>
<td>8.0</td>
</tr>
<tr>
<td>Prolonged ileus</td>
<td>28</td>
<td>22.4</td>
</tr>
<tr>
<td>Acute adhesive small bowel obstruction</td>
<td>6</td>
<td>4.8</td>
</tr>
<tr>
<td>Lung/Pleura-related</td>
<td>10</td>
<td>8.0</td>
</tr>
<tr>
<td>Drug-related</td>
<td>7</td>
<td>5.6</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
<td>16.0</td>
</tr>
</tbody>
</table>

#### 3.3.2.1 Intraabdominal Infections

Of the 44 patients affected by intraabdominal infections, sixteen (36%) were diagnosed and treated during the index admission and 28 were readmitted (of a total of 30 patients readmitted within 30 days). The median time from surgery to diagnosis was ten days (IQR = 8). Ten (23%) patients affected by persistent or recurrent intraabdominal infection were managed with invasive interventions: nine underwent percutaneous or transrectal drainage guided by radiological imaging while the one remaining patient returned to theatre for open drainage. Two of the patients whose infections were initially drained percutaneously subsequently required open drainage and intraabdominal washout via laparotomy. The remaining 34 (77%) patients were successfully managed conservatively by continuing or restarting IV antibiotics. The standard antibiotic regime consisted of triple agents (amoxicillin, metronidazole, and gentamicin).
Postoperative intraabdominal infections had a significant impact on LOS. While children affected had a median index LOS of 8 nights (IQR = 6), those not affected had a median of 5 nights (IQR = 1); \( U = 3192, z = -5.92, p < 0.001 \). This difference became greater when total LOS (index admission plus readmission) was compared: 12 nights (IQR = 6) versus 5 nights (IQR = 1); \( U = 1066.5, z = -9.26, p < 0.001 \).

3.3.2.2 Acute adhesive small bowel obstruction

Acute adhesive small bowel obstruction (ASBO) was the other significant cause for subsequent re-operation. Six cases were recorded and all required laparotomy and adhesiolysis. Prolonged postoperative ileus was the second most common complication after intraabdominal infections (28 cases were recorded). In six cases, intraabdominal infection was also simultaneously present. There were two patients who developed prolonged ileus after open adhesiolysis for acute ASBO. In total, five patients with ileus required parenteral nutrition as part of supportive management.

3.3.3 Predictors of Postoperative Intraabdominal Infections

When univariate analysis was performed comparing patient variables based on the occurrence of postoperative intraabdominal infections, it was found that significant differences existed between the two groups in regards to the duration of presenting symptoms and the occurrence of postoperative fever on Days 3, 4, and/or 5 (Table 3.3). Variables that reached near significant differences \( (p < 0.10) \) included presenting levels of serum white blood cell (WBC) count, neutrophil count, and C-reactive protein (CRP), and the time of day when patients underwent surgery (operation start time between 0600 and 1800 i.e. daytime working hours versus start times outside of these hours). In the multivariate
analysis that included all six of these patient variables, it was found that the presenting level of CRP and the occurrence of postoperative fever on days 3, 4, and/or 5 were independent predictors of persistent or recurrent intraabdominal infections (Table 3.4).
Table 3.3 Comparison of Patient and Perioperative Variables Based on the Occurrence of Postoperative Intraabdominal Infections

<table>
<thead>
<tr>
<th>Patient and Perioperative Variables</th>
<th>Patients Affected [n = 44]</th>
<th>Patients Not Affected [n = 315]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median in years (IQR)</td>
<td>11 (5)</td>
<td>11 (4)</td>
<td>0.842</td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13 (29.5)</td>
<td>129 (41.0)</td>
<td>0.147*</td>
</tr>
<tr>
<td>Male</td>
<td>31 (70.5)</td>
<td>186 (59.0)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand European</td>
<td>22 (50)</td>
<td>147 (46.7)</td>
<td>0.696*</td>
</tr>
<tr>
<td>New Zealand Maori</td>
<td>10 (22.7)</td>
<td>62 (19.7)</td>
<td></td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>8 (18.2)</td>
<td>51 (16.2)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>4 (9.1)</td>
<td>38 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Other European</td>
<td>0</td>
<td>14 (4.4)</td>
<td></td>
</tr>
<tr>
<td>Weight, mean in kg (SD)</td>
<td>47.0 (19.8)</td>
<td>45.3 (18.6)</td>
<td>0.575†</td>
</tr>
<tr>
<td>Duration of presenting symptoms, median in days (IQR)</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.013†</td>
</tr>
<tr>
<td>Presentation time between 0600 and 1800</td>
<td>27 (61.4)</td>
<td>215 (68.3)</td>
<td>0.455*</td>
</tr>
<tr>
<td>Presenting WBC count, mean in xE9/L (SD) [n]</td>
<td>15.2 (6.6) [43]</td>
<td>16.7 (5.1) [305]</td>
<td>0.086†</td>
</tr>
<tr>
<td>Presenting neutrophil count, mean in xE9/L (SD) [n]</td>
<td>12.1 (6.0) [43]</td>
<td>13.6 (4.8) [305]</td>
<td>0.050‡</td>
</tr>
<tr>
<td>Presenting CRP, mean in mg/L (SD) [n]</td>
<td>134.2 (111.2) [34]</td>
<td>100.5 (85.7) [223]</td>
<td>0.099‡</td>
</tr>
<tr>
<td>Underwent preoperative imaging (USS and/or CT) (%)</td>
<td>10 (22.7)</td>
<td>80 (25.4)</td>
<td>0.669*</td>
</tr>
<tr>
<td>Received preoperative antibiotics (%)</td>
<td>30 (68.2)</td>
<td>190 (60.7)</td>
<td>0.339*</td>
</tr>
<tr>
<td>Received intraoperative antibiotics (%)</td>
<td>33 (75.0)</td>
<td>239 (75.9)</td>
<td>0.899*</td>
</tr>
<tr>
<td>Operation same day as presentation (%)</td>
<td>26 (59.1)</td>
<td>168 (53.3)</td>
<td>0.473*</td>
</tr>
<tr>
<td>Operation start time between 0600 and 1800 (%)</td>
<td>29 (65.9)</td>
<td>160 (51.1)</td>
<td>0.066*</td>
</tr>
<tr>
<td>Duration of operation, mean in minutes (SD)</td>
<td>84.1 (41.2)</td>
<td>84.4 (33.3)</td>
<td>0.966‡</td>
</tr>
<tr>
<td>Operation approach (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>38 (86.4)</td>
<td>247 (78.4)</td>
<td>0.304*</td>
</tr>
<tr>
<td>Conversion</td>
<td>2 (4.5)</td>
<td>39 (12.4)</td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>4 (9.1)</td>
<td>29 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Seniority of surgeon (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attending surgeon/Consultant</td>
<td>8 (18.2)</td>
<td>51 (16.2)</td>
<td>0.738*</td>
</tr>
<tr>
<td>Resident/Registrar</td>
<td>36 (81.8)</td>
<td>264 (83.8)</td>
<td></td>
</tr>
<tr>
<td>Transperitoneal drain (%)</td>
<td>1 (2.3)</td>
<td>17 (5.4)</td>
<td>0.710‡</td>
</tr>
<tr>
<td>Intraoperative findings (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppurative Appendicitis</td>
<td>1 (2.3)</td>
<td>9 (2.9)</td>
<td>0.657*</td>
</tr>
<tr>
<td>Gangrenous Appendicitis</td>
<td>7 (15.9)</td>
<td>68 (21.6)</td>
<td></td>
</tr>
<tr>
<td>Perforated Appendicitis</td>
<td>36 (81.8)</td>
<td>238 (75.6)</td>
<td></td>
</tr>
<tr>
<td>Appendix histology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute inflammation/Suppurative</td>
<td>2 (4.5)</td>
<td>12 (3.8)</td>
<td>0.955*</td>
</tr>
<tr>
<td>Gangrenous</td>
<td>6 (13.6)</td>
<td>40 (12.7)</td>
<td></td>
</tr>
<tr>
<td>Perforated</td>
<td>36 (81.8)</td>
<td>263 (83.5)</td>
<td></td>
</tr>
<tr>
<td>Postoperative temperature &gt; 38 °C (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During entire admission</td>
<td>24 (54.5)</td>
<td>139 (44.1)</td>
<td>0.194*</td>
</tr>
<tr>
<td>Days 0 / 1 / 2</td>
<td>22 (50.0)</td>
<td>120 (38.1)</td>
<td>0.130*</td>
</tr>
<tr>
<td>Days 3 / 4 / 5</td>
<td>14 (31.8)</td>
<td>48 (15.2)</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

NOTE: SD = standard deviation; IQR = interquartile range; n = sample size; WBC = white blood cell; CRP = C-reactive protein; * Pearson chi-square test; † Fisher’s exact test (two-tailed); ‡ Student’s t-test; †† Mann-Whitney U test.
Table 3.4 Binary Logistic Regression Analysis: Independent Predictors of Postoperative Intraabdominal Infections

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds Ratio</th>
<th>95% Confidence Intervals</th>
<th>B</th>
<th>S.E.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting WBC count</td>
<td>0.85</td>
<td>-0.40, 0.08</td>
<td>-0.16</td>
<td>0.12</td>
<td>0.168</td>
</tr>
<tr>
<td>Presenting neutrophil count</td>
<td>1.26</td>
<td>-0.01, 0.47</td>
<td>0.23</td>
<td>0.12</td>
<td>0.064</td>
</tr>
<tr>
<td>Presenting CRP</td>
<td>1.00</td>
<td>N/A</td>
<td>-0.00</td>
<td>0.00</td>
<td><strong>0.049</strong></td>
</tr>
<tr>
<td>Duration of presenting symptoms</td>
<td>0.94</td>
<td>-0.31, 0.19</td>
<td>-0.06</td>
<td>0.13</td>
<td>0.634</td>
</tr>
<tr>
<td>Operation start time between 0600 and 1800</td>
<td>0.54</td>
<td>-1.41, 0.19</td>
<td>-0.61</td>
<td>0.41</td>
<td>0.131</td>
</tr>
<tr>
<td>Postoperative temperature &gt; 38 °C on days 3 / 4 / 5</td>
<td>0.35</td>
<td>-1.91, -0.19</td>
<td>-1.05</td>
<td>0.44</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>Constant</td>
<td>15.05</td>
<td></td>
<td>2.71</td>
<td>0.79</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**NOTE:** WBC = white blood cell; CRP = C-reactive protein.

### 3.3.4 Predictors of Length of Stay

Univariate analysis of patient variables identified a number of categorical factors associated with LOS during the index hospital admission (Table 3.5). Furthermore, nonparametric correlation analysis found significant correlations between index admission LOS and the duration of presenting symptoms, level of presenting CRP, duration of operation, and duration of postoperative antibiotic therapy (Table 3.6). These four variables were also significantly correlated with postoperative LOS during the index admission.
Table 3.5 Influence of Categorical Patient and Perioperative Variables on Total and Postoperative Length of Stay

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>Median LOS (IQR)</th>
<th>Median Postop LOS (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>142</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Male</td>
<td>217</td>
<td>6 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.808†</td>
<td>0.831†</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand European</td>
<td>169</td>
<td>6 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>New Zealand Maori</td>
<td>72</td>
<td>5 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>59</td>
<td>5 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Asian</td>
<td>42</td>
<td>6 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Other European</td>
<td>14</td>
<td>6 (2)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>6 (-)</td>
<td>5 (-)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.568^</td>
<td>0.678^</td>
</tr>
<tr>
<td>Presentation time between 0600 and 1800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>244</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>No</td>
<td>115</td>
<td>6 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.210†</td>
<td>0.485^</td>
</tr>
<tr>
<td>Underwent preoperative imaging</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>90</td>
<td>6 (2)</td>
<td>5 (2)</td>
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<td>No</td>
<td>265</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.001†</td>
<td>0.065^</td>
</tr>
<tr>
<td>Received preoperative antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>220</td>
<td>6 (2)</td>
<td>5 (2)</td>
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<tr>
<td>No</td>
<td>137</td>
<td>5 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.009†</td>
<td>0.048^</td>
</tr>
<tr>
<td>Received intraoperative antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>272</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>No</td>
<td>87</td>
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<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.733†</td>
<td>0.775^</td>
</tr>
<tr>
<td>Operation same day as presentation</td>
<td></td>
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<td>Yes</td>
<td>194</td>
<td>5 (1)</td>
<td>5 (1)</td>
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<tr>
<td>No</td>
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<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>&lt; 0.001†</td>
<td>0.307^</td>
</tr>
<tr>
<td>Operation start time between 0600 and 1800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>189</td>
<td>6 (3)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>No</td>
<td>168</td>
<td>5 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.793†</td>
<td>0.040^</td>
</tr>
<tr>
<td>Operation approach</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>285</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Conversion</td>
<td>41</td>
<td>6 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Open</td>
<td>33</td>
<td>6 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.003^</td>
<td>0.026^</td>
</tr>
<tr>
<td>Seniority of surgeon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attending surgeon/Consultant</td>
<td>59</td>
<td>5 (3)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Resident/Registrar</td>
<td>300</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.748†</td>
<td>0.998^</td>
</tr>
<tr>
<td>Transperitoneal drain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>7 (2)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>No</td>
<td>341</td>
<td>5 (1)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.005†</td>
<td>0.004^</td>
</tr>
<tr>
<td>Variables</td>
<td>n</td>
<td>Median LOS (IQR)</td>
<td>Median Postop LOS (IQR)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----</td>
<td>------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Intraoperative findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suppurative Appendicitis</td>
<td>10</td>
<td>3 (5)</td>
<td>2.5 (4)</td>
</tr>
<tr>
<td>Gangrenous Appendicitis</td>
<td>75</td>
<td>5 (3)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Perforated Appendicitis</td>
<td>274</td>
<td>6 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Appendix histology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute inflammation/ Suppurative</td>
<td>14</td>
<td>5 (3)</td>
<td>4.5 (2)</td>
</tr>
<tr>
<td>Gangrenous</td>
<td>46</td>
<td>4.5 (2)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Perforated</td>
<td>299</td>
<td>6 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Postoperative temperature &gt; 38 °C (anytime)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>163</td>
<td>6 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>No</td>
<td>196</td>
<td>5 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Postoperative temperature &gt; 38 °C days 0 / 1 / 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>142</td>
<td>6 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>No</td>
<td>217</td>
<td>5 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Postoperative temperature &gt; 38 °C days 3 / 4 / 5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>62</td>
<td>6 (3)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>No</td>
<td>297</td>
<td>5 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**NOTE:** IQR = interquartile range; LOS = length of stay; † Mann-Whitney U test; ^ Kruskal-Wallis test.
Table 3.6 Nonparametric Correlation Analysis: Total and Postoperative Length of Stay (LOS) versus Patient and Perioperative Variables

<table>
<thead>
<tr>
<th>Spearman’s rho</th>
<th>n</th>
<th>Total LOS Coefficient</th>
<th>p-value</th>
<th>Postoperative LOS Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of presenting symptoms</td>
<td>358</td>
<td>0.272</td>
<td>&lt; 0.001</td>
<td>0.277</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Presenting CRP</td>
<td>257</td>
<td>0.202</td>
<td>&lt; 0.001</td>
<td>0.274</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration of operation</td>
<td>356</td>
<td>0.219</td>
<td>&lt; 0.001</td>
<td>0.225</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration of postoperative antibiotic therapy</td>
<td>359</td>
<td>0.796</td>
<td>&lt; 0.001</td>
<td>0.828</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Multivariate analysis subsequently found that duration of presenting symptoms, timing of operation, occurrence of early and late postoperative fever, and the duration of postoperative antibiotic therapy prescribed were independent predictors of LOS. The duration of presenting symptoms, occurrence of early and late postoperative fever, and duration of postoperative antibiotics prescribed were also independent predictors of postoperative LOS (Table 3.7).
### Table 3.7 Multivariable Linear Model Analysis: Independent Predictors of Total and Postoperative Length of Stay (LOS)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Length of Stay</th>
<th>Partial η²</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of presenting symptoms</td>
<td>Total</td>
<td>0.049</td>
<td>11.928</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.029</td>
<td>6.838</td>
<td>0.010</td>
</tr>
<tr>
<td>Presenting CRP</td>
<td>Total</td>
<td>0.006</td>
<td>1.312</td>
<td>0.253</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.015</td>
<td>3.570</td>
<td>0.060</td>
</tr>
<tr>
<td>Underwent preoperative imaging</td>
<td>Total</td>
<td>0.001</td>
<td>0.178</td>
<td>0.673</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>&lt; 0.000</td>
<td>0.001</td>
<td>0.979</td>
</tr>
<tr>
<td>Received preoperative antibiotics</td>
<td>Total</td>
<td>0.003</td>
<td>0.786</td>
<td>0.376</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.001</td>
<td>0.140</td>
<td>0.708</td>
</tr>
<tr>
<td>Operation same day as presentation</td>
<td>Total</td>
<td>0.036</td>
<td>8.616</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.001</td>
<td>0.258</td>
<td>0.612</td>
</tr>
<tr>
<td>Operation start time between 0600 and 1800</td>
<td>Total</td>
<td>0.004</td>
<td>1.029</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.002</td>
<td>0.571</td>
<td>0.451</td>
</tr>
<tr>
<td>Operation approach</td>
<td>Total</td>
<td>0.005</td>
<td>0.560</td>
<td>0.572</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.008</td>
<td>0.963</td>
<td>0.383</td>
</tr>
<tr>
<td>Duration of operation</td>
<td>Total</td>
<td>0.001</td>
<td>0.165</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>&lt; 0.001</td>
<td>0.003</td>
<td>0.953</td>
</tr>
<tr>
<td>Transperitoneal drain</td>
<td>Total</td>
<td>&lt; 0.001</td>
<td>0.068</td>
<td>0.794</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>&lt; 0.001</td>
<td>0.004</td>
<td>0.951</td>
</tr>
<tr>
<td>Intraoperative findings</td>
<td>Total</td>
<td>0.004</td>
<td>0.404</td>
<td>0.668</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.006</td>
<td>0.724</td>
<td>0.486</td>
</tr>
<tr>
<td>Appendix histology</td>
<td>Total</td>
<td>0.010</td>
<td>1.162</td>
<td>0.315</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.005</td>
<td>0.530</td>
<td>0.589</td>
</tr>
<tr>
<td>Postoperative temperature &gt; 38 °C</td>
<td>Total</td>
<td>0.014</td>
<td>3.148</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.015</td>
<td>3.453</td>
<td>0.064</td>
</tr>
<tr>
<td>Postoperative temperature &gt; 38 °C on Days 0 / 1 / 2</td>
<td>Total</td>
<td>0.026</td>
<td>6.093</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.030</td>
<td>7.195</td>
<td>0.008</td>
</tr>
<tr>
<td>Postoperative temperature &gt; 38 °C on Days 3 / 4 / 5</td>
<td>Total</td>
<td>0.019</td>
<td>4.344</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.023</td>
<td>5.466</td>
<td>0.020</td>
</tr>
<tr>
<td>Duration of postoperative antibiotic therapy</td>
<td>Total</td>
<td>0.484</td>
<td>215.454</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Postoperative</td>
<td>0.508</td>
<td>237.558</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
3.4 DISCUSSION

In this observational study, the demographics, perioperative variables, and total disease morbidity of 359 children who underwent acute surgery for complicated appendicitis, between 2005 and 2011, at a tertiary paediatric hospital in New Zealand are described. Laparoscopic appendicectomy was attempted in 91% (326/359) of cases and successfully completed in 87.4% (285/326) of these. The incidence of postoperative complications was 25.1% (90/359) and hospital readmission rate was 11.1% (40/359). Postoperative intraabdominal infections were the most common complications (44/122, 35.2%) and occurrences significantly prolonged LOS. The duration of presenting symptoms, timing of operation, occurrence of postoperative fever, and duration of prescribed postoperative antibiotic therapy were found to be independent predictors of LOS while the presenting CRP level and occurrence of postoperative fever on Days 3, 4, and/or 5 were independent predictors of intraabdominal infection.

Despite mortality rates associated with complicated appendicitis in children dropping to near zero, its disease morbidity ensures that it remains the most significant paediatric intraabdominal infection. As demonstrated by this study and others in the past, the two most serious postoperative complications from complicated appendicitis are intraabdominal infections and ASBO. The rate of ASBO found in the current study (1.7%) is comparable with those reported by previous studies (0.8 to 1.7%), and its incidence is rare but consistent.
In contrast, there is significant variation in reported rates of postoperative intraabdominal infections after laparoscopic appendicectomy for complicated appendicitis in children. Most recent studies have reported figures between 4.9% and 19.5%.\textsuperscript{251,256-258} These variations may, in part, be due to differing uses of the term ‘complicated’ appendicitis. In the past, studies have commonly regarded gangrenous appendicitis as one form of ‘complicated appendicitis’,\textsuperscript{259,260} treating these patients identically to those with obvious appendiceal perforation.\textsuperscript{70,261} This was due to the belief that the presence of “dead intestine” functions like visceral perforation allowing translocation of bacteria.\textsuperscript{262} However, more recent studies suggest that gangrenous appendicitis is clinically distinct from perforated appendicitis as affected patients appear to have better outcomes than those with perforation when treated under the same protocol.\textsuperscript{70,263,264} When perforated appendicitis is strictly defined as the presence of a hole in the appendix and/or faecolith(s) in the peritoneal cavity, the rates of postoperative intraabdominal abscesses range from 14% to 20%,\textsuperscript{66,69} marginally higher in comparison to the incidence recorded in the current study (12.3%).

The introduction of antibiotic therapy has been one of the key reasons for decreased mortality and morbidity in children affected by complicated appendicitis. However, this study has shown that the duration of prescribed therapy is an important independent predictor of LOS, contributing partly to patient outcome. Reducing duration of inpatient IV antibiotic therapy would shorten LOS if only there were no concerns for inadequate treatment of residual appendicitis-related peritoneal disease. A systematic review in 2004 has concluded that limiting antibiotic therapy to 3 days is unlikely to increase risks of infectious complications\textsuperscript{265} and several more recent retrospective and prospective studies have also questioned the possibilities of shortening postoperative antibiotic therapy\textsuperscript{75,105} or
replacing IV with enteral administration once oral intake has been re-established. The median duration of postoperative therapy in the current study was 5 days and this remains an important area for future investigations and improvement.

The factors influencing LOS after appendicectomy in New Zealand children have previously been described in a Christchurch study in 2000. Retrospectively reviewing the outcomes of 554 patients treated between 1994 and 1998, Foulds et al. found that the main determinant of postoperative hospitalisation was the severity of intraabdominal inflammation associated with acute appendicitis. Appendiceal perforation with peritonitis was demonstrated to double the average length of hospitalisation. Other factors found to influence LOS included the surgical approach taken, use of intraoperative local anaesthesia, type and mode of postoperative analgesia, and age of the child. Furthermore, in keeping with findings of the current study, a longer duration of antibiotic use and presenting symptoms of greater than 24 hours in were also associated with longer LOS. However, in identifying these factors influencing LOS, analysis performed during this earlier study did not account for the severity of appendiceal inflammation with regression analysis.

Numerous factors have been proposed as potential contributors to the development of postoperative intraabdominal infections following appendicectomy in children. These include the female gender, increasing age, increasing weight and body mass index, prolonged duration of presenting symptoms, diarrhoea as a presenting complaint, finding of an intraoperative faecolith, and the occurrence of postoperative fever. The practical usefulness of some of these risk factors has been questioned. For example, diarrhoea is a subjective presenting complaint and patients,
caregivers and healthcare professionals are likely to use different definitions for diarrhoea. Intraoperative faecoliths are relatively rare findings\textsuperscript{105} and this impedes its usefulness as a good prognostic factor.

Similarly, results from this current study question the usefulness of presenting CRP levels as a predictor of subsequent postoperative intraabdominal infections. Even if the demonstrated odds ratio of 1.0 was to be ignored, the difference in means (134.2 versus 100.5 mg/L) is only marginal and not practical for clinical use. Postoperatively, the value of using serum CRP as an indicator to guide duration of antibiotic therapy in children with complicated appendicitis is currently unknown. Chapter 7 provides a detailed discussion of the currently available evidence deliberating on its value.

Early postoperative fever is common in children after appendicectomy for acute appendicitis. Henry et al.\textsuperscript{105} found postoperative fever (temperature $> 38.5 \, ^{\circ}C$) on Day 1 occurs in over 50\% of patients, a significant proportion of whom will not develop subsequent infectious complications. In the current study, postoperative fever at anytime during the index admission occurred in 45.4\% of patients and early postoperative fever (on Days 0, 1, and/or 2) occurred in 39.6\% of patients. These two factors were not found to be predictors of intraabdominal infections but ultimately affected LOS.

Often mismanaged by junior clinicians, early postoperative fever has many causes. Some are not infectious\textsuperscript{268-270} including the acute-phase metabolic response to surgical injury.\textsuperscript{231} This explains why the incidence of early postoperative fever after clean and clean-contaminated procedures is similar to that found after overtly contaminated surgical procedures such as
appendicectomies for complicated appendicitis. Core body temperature rarely varies beyond 0.2 °C of 37.0 °C in humans due tight control by the thermoregulation centre located in the hypothalamus. A number of different circulating pyrogens trigger fever: some are exogenous (e.g. lipopolysaccharide endotoxin produced by Gram-negative bacilli) while the rest are endogenous (mainly cytokines). The principal endogenous pyrogens are tumour necrotising factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-α (INF-α), and macrophage inflammatory protein-1 (MIP-1) and their release is triggered by both infectious and non-infectious causes. During abdominal surgery, increasing levels of circulating IL-6 have been found to correlate logarithmically with postoperative peak in body temperature, with the peak typically occurring 11.1+/− 6 hours after surgery.

In contrast, late postoperative fever (occurring Day 3 or after) occurs less frequently (17.3% of patients in this study) but was found to be an independent predictor of intraabdominal infections. This correlation has already been highlighted by previous studies. Furthermore, Fraser et al. found that both the presence and the degree of postoperative fever, each day after Day 3, increased the chances of a patient developing an intraabdominal infection. A more detailed discussion of postoperative fever as an indicator of ongoing or recurrent intraabdominal infection is presented in Chapter 4.
3.5 CONCLUSION

In summary, this review of 359 children affected by complicated appendicitis treated surgically at a tertiary institution over a 6-year period has found that the overall incidence of postoperative complications was 25.1%. Postoperative intraabdominal infection was the most common complication with an incidence rate of 12.3% and postoperative fever (> 38 °C) on Days 3, 4, and/or 5 was its only independent and clinically relevant predictor. The interpretation of postoperative fever and prescription of postoperative antibiotic therapy were significant clinician-related independent predictors of LOS.
Chapter 4

SYSTEMATIC REVIEW A WITH CRITICAL NARRATIVE

Using clinical criteria to determine duration of postoperative antibiotic therapy in childhood complicated appendicitis
4.1 BACKGROUND

From the findings of the observational study in Chapter 3, it was concluded that clinician practices significantly impact patient outcomes in the setting of appendicitis-related secondary peritonitis in childhood. Instead of patient-related factors determining the duration of hospitalisation, the relatively subjective clinician-related factor of antibiotic duration was a prominent independent predictor for length of stay (LOS). In response, the following chapter focuses on this controversy attached to adjuvant postoperative antibiotic therapy as it is clearly a key determinant of hospital stay, an outcome that affects patient morbidity and practice efficiency.

Administration of antibiotics is complementary to surgery in the management of appendicitis and associated intraperitoneal infection and has improved patient outcomes significantly since introduction in the 1940s. Because secondary peritonitis is a synergistic polymicrobial infection generated by aerobic and anaerobic micro-organisms, experts agree that broad-spectrum single or multi-agent antibiotic therapy is required but there is no consensus on the required duration of postoperative antibiotic therapy.

Patients with complicated appendicitis are especially prone to developing postoperative intraabdominal infections and the risk is increased by incorrect choice or inadequate duration of postoperative antibiotic therapy. To avoid treatment failure, traditional postoperative antibiotic regimens have committed to prolonged periods of therapy, typically between 7 and 14 days. However, prolonged antibiotic treatment does not
convincingly reduce the incidence of persisting or recurrent intraabdominal infections in the postoperative period. In fact, a systematic review in 2004 found that limiting antibiotic duration to 3 days is unlikely to increase risks of infectious complications, meaning that traditional regimens are exposing children to unnecessary inpatient antibiotic treatment.

Furthermore, prolonged administration of broad-spectrum antibiotic agents carries risks and increased costs. These include bacterial resistance and superinfection such as Clostridium difficile colitis, disruption of endogenous flora, and organ injury such as aminoglycoside-related renal toxicity and ototoxicity. Therefore, with the exception of patients with inadequate source control, prolonged administration should be avoided.

Recent guidelines recommend that the resolution of clinical signs of infection should be used to judge the termination point for antimicrobial therapy rather than blind adherence to traditional regimens. The risk of subsequent treatment failure appears to be very low in patients who have no clinical evidence of infection at the time of cessation of antibiotic therapy. These include recent guidelines specific to children with complicated appendicitis sanctioned by The Surgical Infection Society and The American Pediatric Surgical Association.

For practicing clinicians, however, these guidelines pose several problems. First, they vary with regards to which criteria should be included. Second, the guidelines are often vague and do not explain how the selected criteria are to be assessed accurately. Third, the guidelines commonly recommend routine evaluation of serum inflammatory markers, which
need careful consideration in the setting of paediatric patients due to additional blood sampling.

This chapter is dedicated to a systemic review conducted to examine the body of evidence describing the use of clinical indicators of resolved peritoneal infection to determine length of postoperative antibiotic treatment in children with complicated appendicitis. Specifically, through structured review of relevant experimental and observational data, the efficacy of this approach and its therapeutic equivalence to alternative approaches was examined. Following review of the available clinical studies, a critical narrative was provided to examine the physiological mechanisms underlying each individual clinical criterion, aspects of their utility, and evidence of their reliability.
4.2 REVIEW OBJECTIVES

The main objective of this literature review was to determine whether clinical measures of resolving bacterial peritonitis can be safely and effectively used to guide the duration of postoperative antibiotic regimens prescribed for children affected by complicated appendicitis. To do this, the review systematically searched for and critically appraised clinical evidence available to answer the following questions:

1. Which clinical and laboratory criteria have been used to represent resolving or resolved intraperitoneal infection and therefore determine duration of postoperative intravenous (IV) antibiotic therapy?

2. How reliably do chosen sets of clinical criteria reflect resolving or resolved intraperitoneal infection?

3. How does this approach compare in therapeutic efficacy to other strategies?

4. Does this approach to postoperative IV antibiotic therapy have additional benefits?
4.3 METHODS

4.3.1 Search Strategy

A search was conducted in March 2011 of four electronic databases (Ovid MEDLINE, The Cochrane Central Register of Controlled Trials - CENTRAL, PubMED, EMBASE) in order to identify all relevant articles that concerned the use of clinical criteria to determine duration of postoperative antibiotic therapy in children with complicated appendicitis. The following subject heading terms were used: ‘children’, ‘paediatric’, ‘perforated appendicitis’, ‘gangrenous appendicitis’, ‘ruptured appendicitis’, ‘complicated appendicitis’, ‘bacterial peritonitis’, ‘intraperitoneal’, ‘intraabdominal’, ‘antibiotic’, ‘antimicrobial’, ‘antibacterial’, ‘treatment’, and ‘therapy’. Boolean operators (‘and’ and ‘or’) were also used to narrow and widen the search. The ‘explode’ function was used when searching the Ovid MEDLINE search engine and results were limited to studies performed on humans and published in English after 1979. Studies published prior to 1980 were excluded due to significant recent advancements in surgical and medical therapies.

In addition to searching electronic databases, the reference lists of retrieved original and review articles and editorials were hand searched to identify additional potentially relevant trials. To manage the search results, all records (including original research articles, reviews, editorials, and essays) were imported into a bibliography management program (ENDNOTE X5, Thomson Reuters, New York, USA).
4.3.2 Study Selection and Eligibility Criteria

After removing duplicate hits, two investigators independently screened all titles and abstracts for potentially relevant articles and these were obtained in full-text for closer inspection. Disagreements were resolved through discussion until consensus was reached.

Clinical studies were included if they described the use of any number of clinical measure(s) to determine cessation of postoperative IV antibiotic therapy in children (1-18 years of age) affected by complicated appendicitis requiring acute appendicectomy. Both observational and comparison (randomised and non-randomised) trials were included owing to the small number of studies anticipated.

The following exclusion criteria were used:

1. Participants excluded paediatric patients (1-18 years of age);
2. Antibiotic therapy not described or not consistent with recommended and acceptable broad-spectrum antimicrobial agents (in the correct dosages) for paediatric patients affected by complicated intraabdominal infection such an aminoglycoside-based regimen, a carbapenem, a beta-lactam/beta-lactamase-inhibitor combination, or an advanced-generation cephalosporin with metronidazole;¹²⁸
3. Outcomes reported were not stratified according to severity of appendicitis;
4. Outcomes reported did not include the incidence of postoperative intraabdominal infections.
4.3.3 Data Collection and Analysis


Data extraction was performed independently by two individuals – the primary research investigator (T Yu) and a distant research assistant. Any discrepancies in opinions were identified and consensus was reached through face-to-face discussions and mediation by a third and more senior investigator. When patients with complicated appendicitis made up only a subset of study participants, the data collected was specific to only this subset. Similarly, data referring to patient outcomes (complication rates, LOS etc.) collected for this review were from only those patients whose postoperative antibiotic therapy was stopped on the basis of pre-defined clinical measures.

The negative predictive value of using clinical criteria to indicate resolved intraabdominal infection, as a critical measure of the performance of a diagnostic method, was calculated whenever data were available using the following formula:
Participants with resolved intraabdominal infection

Negative predictive value = [n] Participants satisfied criteria for antibiotic cessation

i.e. it represented the proportion of participants who met all clinical criteria for stopping IV antibiotic therapy and subsequently did not represent with intraabdominal infections.

4.3.4 Study Quality

The quality of included comparison studies was appraised by using the Cochrane Risk of Bias tool. It consists of six components for which there is empirical evidence for their biasing influence on the estimates of an intervention’s effectiveness in randomised trials. These components are sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other sources of bias.

4.3.5 Reporting

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement.
4.4 RESULTS

This systematic review is reported in two parts. The first part is reported as a systematic review examining the clinical studies that have described the application and efficacy of postoperative antibiotic regimens where the duration of therapy was based on clinical measures of resolving disease. The second part lists the clinical measures that have been described as representing disease resolution, explains the peritoneal anatomy and physiology that underpins each clinical trait, and discusses the reliability and practicality of using the clinical trait to guide duration of postoperative antibiotic therapy in childhood complicated appendicitis.

4.4.1 Literature Search and Article Selection

Figure 4.1 is a PRISMA Statement flow chart summarising the literature search and article selection process undertaken for this systemic review. The search of four electronic databases generated a total of 1,624 references. After removal of duplicated titles, the remaining 594 citations underwent screening. From these, 171 articles were obtained as full-texts for closer inspection. Manual search of reference lists uncovered an additional citation that was excluded after review of its abstract. Two further studies were excluded during data extraction leaving the review with 13 included articles.
Figure 4.1 PRISMA Statement Flow Diagram Summarising Results of Literature Search and Article Selection

- Ovid MEDLINE: 282 Citation(s)
- PubMed: 587 Citation(s)
- EMBASE: 563 Citation(s)
- CENTRAL: 192 Citation(s)
- Hand-search: 1 Citation(s)

594 Non-duplicate citations screened

Inclusion/Exclusion criteria applied

423 Articles excluded after title/abstract screen

171 Articles retrieved as full-text

Inclusion/Exclusion criteria applied

156 Articles excluded after full-text screen
2 Articles excluded during data extraction

13 Articles included
4.4.2 Overview of Studies

With descriptions of using clinical criteria to guide duration of postoperative antibiotic in children with complicated appendicitis being their only common feature, there was significant heterogeneity among the 13 selected studies. Table 4.1 summarises their key characteristics and findings. No meta-analyses were possible owing to the scarcity of studies with a prospective comparison design and the degree of heterogeneity among the selected studies.
<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Participants</th>
<th>Study Design</th>
<th>Op.</th>
<th>Antibiotic Agents</th>
<th>Criteria for stopping IV ABs</th>
<th>? DC Criteria</th>
<th>Oral ABs</th>
<th>% IAI</th>
<th>% Wound infections</th>
<th>-ve predictive value for IAI</th>
<th>Follow-up after D/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraser et al. 2010</td>
<td>N = 102 [50]; mean age 9.7 yrs; % male: 60</td>
<td>Prospective randomised</td>
<td>Lap 100%</td>
<td>Ceftriaxone &amp; metronidazole</td>
<td>Tolerating a regular diet, afebrile 12 hrs</td>
<td>Yes</td>
<td>Yes</td>
<td>N = 10/50 (20%)</td>
<td>Not reported</td>
<td>Indetermin. 2-4 wks (100%)</td>
<td></td>
</tr>
<tr>
<td>Bensard et al. 2008</td>
<td>N = 72 [20]; mean age 10.6 yrs; % male: 62.5</td>
<td>Prospective observational</td>
<td>Lap 80%</td>
<td>Combination not specified</td>
<td>Afebrile 24 hrs, normal WBC, eating a normal diet</td>
<td>Yes</td>
<td>No</td>
<td>N = 2/20 (10%)</td>
<td>Not reported</td>
<td>90% (18/20) 3 mths</td>
<td></td>
</tr>
<tr>
<td>Ong et al. 2008</td>
<td>N = 82; mean age 9.8 yrs; % male: 65</td>
<td>Retrospective observational</td>
<td>Lap 82-85%</td>
<td>Ceftriaxone &amp; metronidazole</td>
<td>Afebrile &gt; 24 hrs</td>
<td>Yes</td>
<td>Yes</td>
<td>N = 5/82 (6%)</td>
<td>N = 1/82 (1.2%)</td>
<td>NA</td>
<td>Not reported</td>
</tr>
<tr>
<td>Adibe et al. 2008</td>
<td>N = 149; median age 9 yrs; % male 57%</td>
<td>Retrospective observational</td>
<td>Lap 83.9%</td>
<td>Ampicillin-sulbactam ± gentamicin</td>
<td>Afebrile (&lt; 38.0 °C), able to tolerate oral intake, asymptomatic</td>
<td>Yes</td>
<td>Yes</td>
<td>N = 2/47 (4.2%)</td>
<td>N = 0</td>
<td>Indetermin. Not reported</td>
<td></td>
</tr>
<tr>
<td>Emil et al. 2006</td>
<td>N = 397 [85]; % male: 56 &amp; 63</td>
<td>Retrospective observational</td>
<td>Open 73-79%</td>
<td>Ampicillin, gentamicin &amp; metronidazole</td>
<td>Resolution of ileus, afebrile 24 hrs, normal WBC (performed only once other criteria reached)</td>
<td>Yes</td>
<td>Yes, if ↑ WBC</td>
<td>N = 2/85 (2.4%)</td>
<td>N = 4/85 (4.8%)</td>
<td>(100%) 60 days</td>
<td></td>
</tr>
<tr>
<td>Emil et al. 2003</td>
<td>N = 648 [227]; mean age 10.1 yrs; % male: 55 &amp; 58</td>
<td>Retrospective observational</td>
<td>Open 98.5%</td>
<td>Ampicillin, gentamicin &amp; metronidazole or clindamycin</td>
<td>Resolution of ileus, afebrile for 24 hrs, normal WBC (performed only once other criteria reached)</td>
<td>Yes</td>
<td>Yes, if ↑ WBC /fever etc.</td>
<td>N = 10/227 (4.4%)</td>
<td>N = 6/227 (13.6%)</td>
<td>Indetermin. 2 wks (98.4%)</td>
<td></td>
</tr>
<tr>
<td>Author, Year</td>
<td>Participants</td>
<td>Study Design</td>
<td>Op.</td>
<td>Antibiotic Agents</td>
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<td>Follow-up after D/C</td>
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<tr>
<td>Gollin et al. 2002</td>
<td>N = 80</td>
<td>Prospective observational</td>
<td>Lap 52%</td>
<td>Ampicillin, gentamicin &amp; metronidazole</td>
<td>Tolerates enteral intake regardless of fever (&gt; 37.8 °C) &amp; leucytosis</td>
<td>Yes</td>
<td>Yes</td>
<td>N = 2/80 (2.5%)</td>
<td>N = 7/80 (8.8%)</td>
<td>100% (74/74)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Rice et al. 2001</td>
<td>N = 26 [16]; mean age 12.3 yrs; % male: 62</td>
<td>Prospective randomised</td>
<td>Open</td>
<td>Ampicillin, gentamicin &amp; clindamycin</td>
<td>Return of GI function</td>
<td>?</td>
<td>Yes</td>
<td>N = 0</td>
<td>N = 1/16 (6.3%)</td>
<td>Indetermin.</td>
<td>4-6 wks</td>
</tr>
<tr>
<td>Hoelzer et al. 1999</td>
<td>N = 33; mean age 8.5 yrs; % male: 76</td>
<td>Prospective observational</td>
<td>Open</td>
<td>Ampicillin, gentamicin &amp; clindamycin or sulbactam; or gentamicin &amp; metronidazole</td>
<td>Eating, aferebrile (&lt; 38.0 °C) 24 hrs, normal WBC with immature neutrophil counts (bands) ≤ 3%</td>
<td>Yes</td>
<td>No</td>
<td>N = 4/33 (12%)</td>
<td>Not reported</td>
<td>96.7% (29/30)</td>
<td>2 mths</td>
</tr>
<tr>
<td>Keller et al. 1996</td>
<td>N = 56; mean age 9.6 yrs</td>
<td>Retrospective observational</td>
<td>Open</td>
<td>Ampicillin, gentamicin &amp; clindamycin; or Piperacillin/ Tazobactam</td>
<td>Afebrile (&lt; 37.5 °C) 24 hrs, normal WBC count with no left shift or band cells, normal bowel function, no signs of ongoing abdominal sepsis</td>
<td>Yes</td>
<td>No</td>
<td>N = 1/56 (1.8%)</td>
<td>N = 1/56 (1.8%)</td>
<td>100% (50/50)</td>
<td>1 mth</td>
</tr>
<tr>
<td>Firilas et al. 1999</td>
<td>N = 92 [42]</td>
<td>Prospective observational</td>
<td>-</td>
<td>Ampicillin, gentamicin &amp; clindamycin</td>
<td>Postop day 3, aferebrile (&lt; 38.5 °C) 24 hrs, tolerates regular diet, transitioned to oral analgesia, WBC &lt; 14,000</td>
<td>Yes</td>
<td>Yes</td>
<td>N = 7/42 (17%)</td>
<td>N = 1/42 (2.4%)</td>
<td>Indetermin.</td>
<td>Not reported</td>
</tr>
<tr>
<td>Author, Year</td>
<td>Participants</td>
<td>Study Design</td>
<td>Op.</td>
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</tr>
<tr>
<td>Neilson et al. 1990</td>
<td>N = 420 [117]; mean age 10.3 yrs</td>
<td>Prospective observational</td>
<td>Open</td>
<td>Ampicillin, gentamicin &amp; clindamycin</td>
<td>Postop day 3 (gangrenous) or 5 (perforated), afebrile 24 hrs, WBC &lt; 10,000</td>
<td>Yes</td>
<td>No</td>
<td>N = 2/117 (1.7%)</td>
<td>N = 2/117 (1.7%)</td>
<td>Indetermin.</td>
<td>7-14 days (97%)</td>
</tr>
<tr>
<td>Birken et al. 1986</td>
<td>N = 87; mean age 9.0 yrs; % male: 69</td>
<td>Prospective observational</td>
<td>Open</td>
<td>Ampicillin, gentamicin ± clindamycin</td>
<td>Postop day 3, resumed normal GI function, no apparent complications, afebrile 48 hrs, normal WBC</td>
<td>?</td>
<td>No</td>
<td>N = 3/87 (3.4%)</td>
<td>N = 2/87 (2.3%)</td>
<td>100% (75/75)</td>
<td>3 mths</td>
</tr>
</tbody>
</table>

**NOTE:** N = total number of participants, [ ] = number of participants with complicated appendicitis whose postoperative IV antibiotic therapy stopped on the basis of clinical measures, DC = discharge, IAI = intraabdominal infections, Op. = operative approach, Lap = laparoscopic, hrs = hours, GI = gastrointestinal, WBC = white blood cell, ABs = antibiotics, Indetermin. = indeterminable, -ve = negative, NA = not applicable, wk = week, mth = month.
4.4.2.1 Description of study settings, participants, objectives, and outcomes

Settings
The studies were all set in North American specialist children’s hospitals apart from one study that was conducted at KK Women’s & Children’s Hospital in Singapore. Two studies were multi-centre while the remainder were all single-centre studies.

Participants
Study participants ranged from 1 to 18 years of age although one study did not report this demographic. Gender distribution was reported in 8 studies with 55 to 76% of the study participants being male. Information regarding participant ethnicity was reported by one study.

Study total sample sizes ranged from 26 to 648 participants but only eight studies exclusively recruited patients diagnosed with complicated appendicitis. In the five remaining studies, patients with complicated appendicitis made up between 28 and 50% of participants in the intervention group. ‘Complicated’ appendicitis was commonly defined as gangrenous or perforated appendicitis diagnosed at the time of surgery. Alternatively, it was defined as the presence of peritonitis, or frank intraabdominal pus, abscess, or faecolith. 

Study objectives
Overall, there were four different study objectives among the 12 selected studies. To determine the safety, efficacy, and/or therapeutic equivalence of stopping postoperative IV antibiotic therapy on the basis of clinical and laboratory indicators of resolved infection was
the main research objective of six studies. To investigate the efficacy and cost-effectiveness of standardised clinical pathways for paediatric appendicitis where duration of postoperative IV antibiotic therapy was determined by clinical and laboratory indicators was the main research objective of five other studies. Of the two remaining studies, Ong et al. aimed to compare the therapeutic efficacy of different postoperative antibiotic agents and Birken et al. aimed to determine if an inpatient observation period was necessary after the cessation of postoperative antibiotic therapy in children treated for perforated appendicitis.

Patient outcomes

As mentioned, this review focused on the incidence of postoperative persistent or recurrent intraabdominal infections in children with complicated appendicitis whose IV antibiotic therapy was stopped according to clinical measures of adequate therapeutic response. Reported rates varied between 1.7 and 20%. In comparison, when reported, the incidence of wound infections varied between 1.2 and 13.6%. Aside from reporting infectious complications, three studies also described antibiotic-related complications including C. difficile colitis. The remaining studies did not mention this outcome.

The two prospective randomised trials in this review both compared the therapeutic equivalence of a fixed duration of postoperative IV antibiotic therapy to that of a course of IV therapy changed to oral administration as soon as a set of clinical criteria were reached. No difference was found in the incidence of intraabdominal and wound infections between the study groups in both trials.
Given that the selected set of clinical criteria for determining duration of postoperative IV antibiotic therapy also served as the discharge criteria in 11 of the 13 studies, LOS was an important outcome to examine. Of these, two studies examined the impact of having clinical criteria to guide duration of antibiotic therapy and hospitalisation on LOS. Fraser et al.\textsuperscript{266} found that LOS was significantly shortened when the duration of antibiotic therapy was guided by clinical measures rather than being a fixed arbitrary period (4.48 days +/- 2.36 versus 6.06 days +/- 2.00, \( p = 0.01 \)). In comparison, Emil et al.\textsuperscript{259} found that treating patients according to a standardised clinical pathway involving clinical criteria to determine both termination of antibiotic therapy and in-hospital treatment resulted in significantly longer LOS in comparison to treating patients on a case-by-case basis as per individual surgeon discretion (5.96 days +/- 0.36 versus 4.89 days +/- 0.19, \( p < 0.01 \)).

Without describing how participants were allocated to their respective study groups, Adibe et al.\textsuperscript{292} reported that 47 patients who were discharged to complete a course of oral antibiotics after termination of inpatient IV therapy on the basis of clinical measures had an average LOS of 4.7 days. This was significantly shorter in comparison to the average LOS of 102 patients who were discharged on the basis of identical clinical measures but completed their course of postoperative IV antibiotics, totalling 14-days, as outpatients (9.1 days; \( p = 0.001 \)).

Readmission rates were reported by seven studies and for patients with complicated appendicitis, these ranged from 0 to 10%. Fraser et al.\textsuperscript{266} reported the average number of hospital visits (3.1 ± 1.2) during the study rather than a readmission rate. This figure may
include the routine follow-up visit which all patients attended 2 to 4 weeks after discharge or after the completion of outpatient antibiotic therapy for postoperative complications.

Data required for calculation of negative predictive values were reported by five studies.\textsuperscript{75, 293, 295, 296, 299} These were all $\geq 90\%$. While the respective figures were not actually reported by Emil et al.,\textsuperscript{259} these authors found that none of the complicated appendicitis patients in their study whose antibiotic therapy was stopped on the basis of clinical criteria (resolution of ileus, afebrile for 24 hours, and normal leucocyte count) went on to develop postoperative complications. This can be interpreted as a negative predictive value of 100\%. Three other studies\textsuperscript{293, 296, 299} also reported this identical result using slightly differing sets of clinical criteria.

4.4.2.2 Description of the methodological quality of the studies

Study design and patient recruitment

There were eleven observational studies in this review, six of which involved prospective data collection.\textsuperscript{75, 293, 295, 297-299} The remaining five reported retrospectively collected data obtained from hospital records.\textsuperscript{70, 259, 291, 292, 296} They were all single-centred studies although one study did make comparisons between a prospective study group and historical controls recruited from both the study institution and the Pediatric Health Information Systems (PHIS) database.\textsuperscript{297} Two prospective non-blinded randomised trials made up the remaining review studies.

Participant recruitment methods varied depending on study design and exclusion criteria were listed by seven studies.\textsuperscript{70, 259, 266, 291, 292, 294, 298} Sample size calculation was performed
a prior in one study, although recruitment of participants was terminated before this figure was reached. The authors made this decision after the interim analysis when they found that the primary outcome variable was identical between the two study groups. Instead of sampling 150 patients, the study was terminated after recruitment of 102 participants.

Intervention design

Core body temperature and gastrointestinal (GI) function were the two most commonly used clinical measures for determining when postoperative IV antibiotic therapy can be stopped. The studies, however, did not agree on how each one should be assessed and what thresholds constitute resolution of intraabdominal infection. For example, the upper limit of body temperature used to define fever varied between 37.5 and 38.5 °C. The antibiotic regimens described by three studies imposed a minimum duration of treatment before they could be terminated on the basis of clinical criteria. None of the studies provided scientific justification for why the clinical measure(s) were selected. Each of the clinical measures found in this review are examined in more detail in the critical narrative section of this chapter.

Data collection

The studies predominantly favoured the use of computerised tomography (CT) for diagnosis of postoperative intraabdominal infections. Five studies nominated this as the imaging modality of choice while only one study reported the use of abdominal ultrasound scans ahead of CT. In total, nine studies described the duration and method of patient follow-up post hospital discharge, and/or the proportion of participants who
completed study follow-up. The longest follow-up period was 3 months\textsuperscript{75, 299} and the shortest was 7 days.\textsuperscript{298}

*Risks of bias*

Risks of bias were assessed in the two studies that used randomised allocation to assign participants to comparison groups. Sequence generation was performed using computer randomisation in one of the studies\textsuperscript{294} and neither described the methods used to conceal study allocation. Furthermore, neither study used blinded outcome assessors.

**4.4.3 Critical Narrative of Individual Criterion**

4.4.3.1 *Fever*

Widely recognised as a “cardinal sign” of infection, elevated body temperature likely has important biological significance, correlating to improved survival rates following infection.\textsuperscript{300, 301} Core body temperature is closely controlled by the thermoregulation centre located in the pre-optic area of the hypothalamus.\textsuperscript{302} Fever is generated following stimulation by circulating pyrogens that can be endogenous or exogenous. Endotoxin (lipopolysaccharides) produced by gram-negative bacilli is a well-known exogenous pyrogen, which like others, does not trigger fever directly but, instead, induces the production and release by mononuclear phagocytes of endogenous mediators, in particular the following cytokines: TNF-\(\alpha\), IL-1\(\beta\), IL-6, INF-\(\alpha\), and MIP-1. These are considered the principal endogenous pyrogens.\textsuperscript{272}

When the intraabdominal inflammatory response to bacterial infection reaches a certain level, mediators, including those listed above, reach the systemic circulation triggering a
systemic response known as the acute phase reaction. It is characterised by changes in plasma metal levels, induction of acute-phase proteins, leucocytosis, and fever. This last feature is thought to rely on cytokine penetration into the brain either through the organum vasculosum laminae terminalis, which lacks the blood-brain barrier, and/or their transport via a saturable mechanism.\textsuperscript{303, 304} In addition, as mentioned in Chapter 1, the peritoneal neuro-immuno-humoral axis, conveyed via the vagus nerve, is potentially an alternative route for which pyrogenic messages from the intraperitoneal cavity can reach the brainstem.\textsuperscript{305} Experimental studies have demonstrated that sub-diaphragmatic vagotomised rats do not mount a febrile response to intraperitoneal administration of cytokine, while sham-operated animals do. Furthermore, both groups mounted a similar systemic response, as measured by serum cytokine levels.\textsuperscript{168}

Postoperative fever is a common phenomenon that can stem from infectious and non-infectious causes.\textsuperscript{268, 306} Because of this, timing and persistence of fever are important features to consider. Early postoperative fever, generally defined as elevated temperature within 48 hours of surgery, has been shown to have similar incidences after clean, clean contaminated, and contaminated surgical procedures\textsuperscript{232} and has poor predictive value for postoperative infectious complications.\textsuperscript{269, 275} This is echoed by the findings of the observational study in Chapter 3 where fever prior to postoperative Day 3 was not found to be an independent predictor for the late occurrence of intraabdominal infections.

Lennard et al.\textsuperscript{276} highlighted the importance of persisting postoperative fever as a predictor of intraabdominal infection in a study of 65 adults who exhibited a clinical response to postoperative antibiotic treatment for intraabdominal sepsis. Of the 14 patients who were
still febrile when antibiotics were discontinued (average treatment duration of 10 days),
11 developed infectious complications of which 8 (57%) were of intraabdominal origin. In all
11 patients, fever either initially responded to antibiotics but increased again during therapy
or did not respond at all.

As demonstrated by the collection of studies in this review, a range of temperatures are
used as cut-off values to define ‘fever’, reflecting the fact that normal temperature resides
within a range between 36.5 and 37.5 °C.\textsuperscript{307} In the postoperative setting, however, one
single cut-off point for fever is ideal to guide the initiation of investigations. In a prospective
study of 284 patients (and 2,282 postoperative temperature measurements), the diagnostic
value of body temperature for determining infection after surgery was examined.\textsuperscript{308}
Increasing by increments of 0.5 °C from 38.0 °C to 39.0 °C, the positive predictive value of
body temperature for determining infection after surgery fell from 8% to 0%. By contrast, its
negative predictive value plateaued at 93% from 90%. Beyond the cut-off figure of 38.0 °C,
the value of monitoring body temperature is variable. However, when recorded on at least
one occasion, a postoperative temperature of > 38 °C had a sensitivity of only 37% and
specificity of 80% for diagnosing infectious complications. The authors concluded that fever
alone is of limited value in early detection or diagnostic exclusion of postoperative infection.
It should form part of the diagnostic armamentarium of clinical symptoms and signs
available to physicians. This opinion is echoed by other studies.\textsuperscript{277}

Moreover, to be of value, body temperature needs to be accurately evaluated. Axillary
measurements are affected by changes in skin perfusion, aural measurements can be
inconsistent,\textsuperscript{309} and rectal measures, although well-established and more accurate,\textsuperscript{310} are
impractical and not suitable in older children and adolescents. In a clinical research setting, it is therefore important that a single technique and instrument is consistently used by experienced staff in accordance with standardised procedures.

4.4.3.2 Gastrointestinal function

As early as 1906, postoperative intestinal motility dysfunction was recognised as a phenomenon and divided into subgroups according to its primary pathophysiology: mechanical, septic, and adynamic.\textsuperscript{311} Traditionally, two models of experimental ileus are commonly used: one based on abdominal surgery (generally involving laparotomy followed by caecal manipulation), and the other based on peritoneal irritation (often produced by intraperitoneal administration of a pro-inflammatory agent).\textsuperscript{312} Both processes contribute to postoperative GI dysfunction in the setting of complicated appendicitis.

Clinicians now possess a much greater understanding of the processes that lead to the development and maintenance of postoperative ileus at the molecular and cellular level, including the role of the vagus nerve. These processes have been detailed in Section 1.2.4.1. In the case of postoperative intraabdominal infection, additional mechanisms delay return of bowel function including bowel wall oedema from inflammation.\textsuperscript{313} Furthermore, generalised sepsis has been associated with a form of GI disability characterised by abnormal luminal absorption, motility and transit.\textsuperscript{206,314-316} Systemic inflammation from sepsis also causes fluid shifts that can lead to electrolyte abnormalities. Aberrations in electrolytes correlate with altered GI neuromuscular function.\textsuperscript{317} Early postoperative small bowel obstruction due to adhesions, with or without intraabdominal abscesses, can also cause delayed return of GI function.
Gastrointestinal function was one of the most commonly used clinical measures in this review. However, the studies did not agree on how they assessed this. A range of different symptoms and signs are commonly used as surrogate markers of gastric, intestinal, and colonic motility, including passage of flatus and/or stool, presence of bowel sounds, and restoration of oral intake. These signs, however, vary in their reliability as markers of restored function, have poor correlation to each other, and usually represent only one single segment of the GI tract. This is significant because postoperative recovery of GI hypomotility differs by segments. Small bowel motility is usually transient, recovering within several hours of surgery. In contrast, gastric motility typically recovers within 24 to 48 hours after surgery and colonic motility can take 48 to 72 hours to recover, making it the limiting factor in resolving postoperative ileus. Recovery of the left colon can take up to 7 days.

The primary markers for returning GI motility after abdominal surgery are the return of flatus and bowel movement, indicating recovery of the colon. However, frequency of stool passage has been found to be a poor surrogate for colonic and whole-gut transit times. Propagating colonic contractions measured postoperatively also have no correlation with passage of flatus and first bowel movement. Bowel sounds are thought to arise from the movement of an air-water interface in the upper GI tract and their presence has not been found to coincide with recovery of small and large bowel motility.

Additional indicators include the patient’s ability to tolerate oral intake without nausea or vomiting, the return of appetite, and an absence of other symptoms of ileus such as
abdominal distension, feeling bloated, and abdominal cramps.\textsuperscript{327, 328} Postoperative re-feeding practices remain a controversial area of surgical care but early ingestion has been associated with enhanced recovery of bowel motility. Both bowel sounds and the passage of flatus correlate poorly with patient tolerance of oral intake and are unreliable indicators for when patients can resume feeding.\textsuperscript{327, 329} Reestablishment of oral intake is largely a patient’s subjective perspective and can be difficult to quantify. It is however a clinical pre-requisite to hospital discharge unlike passage of flatus and stool.

4.4.3.3 Leucocytosis

Eight studies in this review made use of serum white blood cell (WBC) count as an indicator for resolved intraabdominal infection. As a continuous variable, the diagnostic accuracy of WBC count strongly depends on the chosen cut-off values.\textsuperscript{330} Two studies provided cut-off values although these differed.\textsuperscript{297, 298} Aside from indicating that normal WBC count was a prerequisite for discontinuing postoperative antibiotic therapy, Birken et al.\textsuperscript{299} also found that of the 75 children who recovered uneventfully, serial leucocyte counts decreased rapidly and in most cases returned to normal by postoperative Day 3. In contrast, all 12 children who developed complications during their postoperative course demonstrated persistent, significantly elevated leucocytosis. Ciftci et al.\textsuperscript{289} also found that in children affected by perforated appendicitis, the mean postoperative duration of elevated WBC (> 10 x 10\textsuperscript{9}/L) ranged between 2.3 and 2.9 days. Lastly, Fraser et al.\textsuperscript{181} found that an increased WBC on postoperative Day 5 in children with perforated appendicitis was highly predictive for intraabdominal abscesses.
The leucocyte component of peripheral blood consists of five basic cell types: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Neutrophils, eosinophils, and basophils can also be referred to as granulocytes or polymorphonuclear leucocytes (PMNs). Among neutrophils there are both segmented and band neutrophil types. The total serum leucocyte count is performed on EDTA-anticoagulated blood via electrical impedance methods or light-scatter techniques.\(^{331}\)

Leucocytosis is mediated by several molecules, released or upregulated in response to stimulatory events that include growth or survival factors (e.g. granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, c-kit ligand), adhesion molecules (e.g. CD11b/CD18), and various cytokines (e.g. IL-1, IL-3, IL-6, IL-8, tumour necrosis factor). The mechanism that causes leucocytosis can be of several forms: an increased release of leucocytes from bone marrow storage pools, decreased margination of leucocytes onto vessel walls, decreased extravasation of leucocytes from the vessels into tissues, or an increase in number of precursor cells in the marrow.\(^{332}\) Because the leucocyte count is composed predominantly of PMNs, any change in the total WBC typically reflects a change in the total PMN count.\(^{332}\)

The use of leucocyte count in the setting of postoperative surgical infection comes about via several early studies involving adult patients. Of 2,567 adult patients, the WBC count was slightly more accurate than body temperature for predicting recurrent sepsis in patients treated for surgical infection at the time of antibiotic therapy termination.\(^{277}\) Counts above the normal range did have a prediction rate of 67% for sepsis exacerbation. However, 6% of
the patients experienced recurrence of the original or a related infection despite a normal WBC count.

Although commonly used for the diagnosis and monitoring of infection, leucocytosis by itself is neither highly sensitive nor specific for an acute or chronic infectious process. In other words, an elevated WBC should not be considered as evidence of an infectious process unless there is clinical evidence of such and, conversely, a low WBC does not rule out an infectious process when clinical findings favour an infectious process.

Leucocyte count is therefore commonly used in conjunction with other clinical measures representative of persistent or recurrent infection. Its pairing with fever has been extensively studied in adult patients after abdominal surgery. After observing that persistent leucocytosis in afebrile patients exhibiting a clinical response to therapy for intraabdominal sepsis is associated with an increased risk of developing postoperative infectious complications, Lennard et al. conducted a larger study involving 65 patients. In this study, antibiotics were stopped when patients exhibited a clinical response to treatment for intraabdominal sepsis and the presence and absence of both leucocytosis and fever were recorded. Of the 51 patients who were afebrile when antibiotics were stopped, 21 had persisting leucocytosis. Of these patients, 7 (33%) went on to develop intraabdominal infections. Of the 30 patients who had normal WBC counts at the end of antibiotic treatment, none developed intraabdominal infections (p < 0.005).

To identify predictors of bacterial infection in patients developing postoperative fever, Mellors et al. prospectively observed 434 adults post abdominal surgery. Using logistic
regression analysis, they identified 3 features associated with postoperative bacterial infection: abnormal leucocyte count, elevated blood urea nitrogen level, and fever occurring after postoperative Day 2. When used together as a predictive index, the occurrence of all three features was associated with 100% (3/3) likelihood of postoperative infection. On the other hand, the absence of all three features was associated with 2% (1/50) chance of postoperative infection. In patients with one or two of the index features, the risks were 14% (12/88) and 45% (10/22), respectively.

The percentage of immature granulocytes offers improved accuracy in predicting persistent or recurring sepsis. In the absence of any immature granulocytes, sepsis never occurred in 256 adult patients discharged from hospital following antibiotic treatment for surgical infections. The study also showed that a finding of < 3 immature granulocytes per 100 WBCs counted was associated with a 0.2% likelihood of recurrent infection. On the other hand, the presence of > 4 immature forms was associated with 54% risk of recurrence.

The sum of evidence in the paediatric population is less substantial and not specific to postoperative intraabdominal infection. As a marker of disease severity and the effectiveness of antibiotic therapy in children, leucocyte count has also been investigated in the setting of generalised sepsis, lower respiratory tract infection, septic arthritis, soft tissue infections, and meningitis. Its reliability, however, has largely been shown to be inferior to alternative inflammatory markers such as C-reactive protein (CRP) and procalcitonin. A large systematic review found that, when attempting to identify serious infections in febrile children, measuring WBC count was less useful for ruling in serious
infection when compared to other serum inflammatory markers, and not useful for ruling out serious infection.\textsuperscript{340}

An important practical consideration needs to be examined before any serum inflammatory markers are used for the monitoring of postoperative antibiotic therapy. Venipuncture and IV cannulation procedures are a routine part of modern clinical practice. Unfortunately, these procedures also cause pain and distress, which can have significant impact on children. Children report IV line placement as the leading cause of procedure-related pain in the hospital, on par with postsurgical pain.\textsuperscript{341} A significant proportion of children undergoing venipuncture also experience moderate or severe pain\textsuperscript{342} and elevated levels of pre-procedural and procedural distress.\textsuperscript{342, 343} The rationale for performing serial postoperative blood tests needs to be balanced with these considerations for added patient anxiety and stress.

4.4.3.4 Resolution of abdominal symptoms

The resolution of abdominal symptoms, including pain, is naturally a pre-requisite for termination of therapy, expected not only by clinicians but also by patients and their families. Abdominal pain is also an indirect marker impaired recovery of GI motility. Although it does not feature prominently in the review studies, several adult studies have described its utilisation. Smith et al.\textsuperscript{344} believed that a satisfactory response allowing for the discontinuation of antimicrobials for appendicitis-related peritonitis required the patient to demonstrate all three major conditions as well as two of the four minor conditions. The major conditions were: oral temperature < 38 °C (or < 38.5 °C rectally) during the previous 24 hours, reduction in the initial WBC count by 10% or more, and passage of flatus. The
minor determinants of success were: absence of rebound tenderness, absence of clinical or radiological evidence of intraabdominal abscesses, absence of wound infection, and negative blood culture. Complication rates were not reported in full but of the 24 patients with gangrenous or perforated appendicitis treated by this protocol, the mean number of antibiotic doses received was 12.72 ± 2.35.

More recently, in a comparison study, Taylor et al. defined a set of clinical criteria for discontinuing postoperative IV antibiotics that included resolution of fever, improved abdominal signs and symptoms, and return of bowel function. When 79 patients treated under this protocol were compared to 74 patients treated with a minimum of 5-days postoperative IV antibiotics, the authors found that complication rates were no different but length of antibiotic therapy and hospital stay were significantly shorter for the intervention group.
4.5 DISCUSSION

This systematic review retrieved 13 studies in total describing the use of clinical criteria, representing resolved intraabdominal infection, to determine the duration of postoperative antibiotic therapy in children with appendicitis-related secondary peritonitis. Of these, eleven were observational studies and two were intervention comparison studies. The most commonly used clinical measures were elevated body temperature, a functioning GI tract, and a normal or normalising WBC count. Although the studies were all small, collectively, the negative predictive values of the criteria they describe for ruling out postoperative intraabdominal infection were all greater than 90%. The interventional studies respectively found that, in comparison to setting a fixed duration of antibiotic treatment, using clinical measures to determine when postoperative antibiotics can be terminated reduced both LOS and antibiotic administration.

Although, this review has dealt with the research questions, it has also raised several issues for discussion. The first concerns the discharge of patients following antibiotic cessation. Among the 12 review studies, there was almost uniform agreement that once a patient has satisfied all clinical criteria for termination of IV antibiotic treatment, they were also ready for hospital discharge. After performing a multicenter case-control study involving four tertiary paediatric hospitals and analysing data from 265 children with appendicitis, Henry et al. found that a period of inpatient observation was unnecessary. They compared the outcomes of 37 children who were discharged on or before postoperative Day 3 with 21 children who were also afebrile and tolerating a diet at the time but remained in
hospital. There were no significant differences between the 2 groups. None of the early-discharge group developed intraabdominal abscesses, while two of those remaining in hospital were affected ($p = 0.06$).

Aside from antibiotic administration, postoperative hospital care involves control of postoperative pain, monitoring for early complications, and discharge planning. Following appendicectomy, discharge criteria for children have included tolerance of liquids and semi-solid foods, mobility without marked discomfort,$^{345}$ and tolerance of oral analgesia.$^{261}$ Fishman et al.$^{261}$ also outlined social criteria for discharging patients home to continue outpatient IV antibiotics via a percutaneously inserted central catheter (PICC). These included functioning home telephone, family willingness, continuous caretaker availability, insurance coverage for homecare, and the absence of other social impediments. Social conditions, such as these, did not feature in any of the included review studies.

Another issue of disagreement among the studies is the designation of a minimal duration of antibiotic therapy before clinical criteria are used to terminate treatment. Three of the review studies required patients to have received at least 3 or 5 days of antibiotic treatment before clinical criteria could be assessed. The total duration of antibiotics received by patients in these studies, however, were not reported so it is not possible to determine whether this prolonged therapy in comparison to patients in the remaining studies. In a study of 235 adult and paediatric patients, three days of IV cefoxitin, in comparison to five days, was found to be adequate for prevention of postoperative wound infections but not intraabdominal infections.$^{346}$ Wound infection rate was found to be 10% and the rate of intraabdominal infections 12%. 


The role of oral antibiotics after termination of IV therapy was disputed among the studies in this review, perhaps because there was no distinction between whether clinical criteria represented ‘resolved’ or ‘resolving’ intraabdominal infection. The first would not require further treatment while the latter may possibly. Five studies did not utilise oral antibiotics, five studies did consistently, and the remaining two studies prescribed them only in certain conditions after the termination of IV antibiotics. These were an elevated WBC count and/or fever.\textsuperscript{70, 259}

The evidence for the use of oral antibiotics when IV antibiotics have been discontinued is conflicting. The American Pediatric Surgery Association’s most recent guidelines\textsuperscript{279} recommend the use of oral antibiotics in paediatric patients whose postoperative IV therapy was administered for less than 5 days. The justification for this is evidence from one single study.\textsuperscript{266} Included in this systematic review, the study was not powered adequately nor blinded. A study of 110 adult patients found that when postoperative IV antibiotics were discontinued (approved when abdominal pain, tenderness, and distension have resolved, fever has settled, bowel function improved, and WBC count reduced), the addition of a 7-day course of outpatient oral antibiotics did not decrease postoperative infectious complications.\textsuperscript{347} The mean duration of IV antibiotic therapy for those with advanced appendicitis was 4.3 days.

This review is subject to several important limitations. As already mentioned, there are few randomised controlled trials on antibiotic duration on which this review could be based. Also the studies in this review were undertaken for related, but not identical, purposes. Furthermore, although this study did address the issue to antibiotic agents, it did not tackle
the dosage of each antibiotic used. Instead, the review relied on individual investigators to use appropriate and widely accepted paediatric dosages.

Finally, the period of time covered by this review coincided with the rising popularity of laparoscopic appendicectomy and this trend can be seen when the studies are examined in chronological order. Operative approach is important to consider as it has been shown to affect rates of postoperative intra-abdominal infections. A 2010 Cochrane systematic review and meta-analysis of studies comparing open to laparoscopic appendicectomy found that intra-abdominal abscesses were more common after laparoscopic appendicectomy, confirming the findings of an earlier meta-analysis published in 2007. This latter analysis of 34 studies also included a subgroup analysis of pre- versus post-2000 studies and found that the risk of intra-abdominal abscesses was increased in studies published after 2000. A number of reasons were put forward including the rising popularity of laparoscopy in cases of complicated appendicitis.
4.6 CONCLUSION

In summary, the most commonly described clinical measures used to represent resolved or resolving postoperative intraabdominal infection in children with complicated appendicitis are core body temperature, GI function, and WBC count. When they are used in various combinations to determine appropriate discontinuation of postoperative antibiotic therapy, the negative predictive value for excluding postoperative intraabdominal infections is >90%. Available studies, however, were mostly small, single-centred observational experiments and the therapeutic equivalence of this treatment strategy in comparison to alternative strategies could not be clearly demonstrated. More solid evidence provided by an adequately-powered intervention comparison study is needed to convincingly support the current recommendation of using clinical criteria to determine length of IV antibiotic treatment for peritonitis in complicated appendicitis.
Chapter 5

CLINICAL STUDY B

Duration of postoperative antibiotic therapy in childhood complicated appendicitis: A propensity score-matched comparison cohort study
5.1 BACKGROUND

Patients with complicated appendicitis are especially prone to developing postoperative intraabdominal infections and the risk is increased by incorrect choice or duration of postoperative antibiotic therapy. The duration of inpatient intravenous (IV) therapy remains a particularly controversial issue but is a determinant of postoperative hospital stay as demonstrated by the multivariate regression analysis in Chapter 3. Although the most recent guidelines, provided by a number of expert organisations such as the Surgical Infection Society, Infectious Diseases Society of America, and the American Pediatric Surgical Association, advocate using certain clinical signs representative of resolving peritoneal infection to guide the duration of therapy, the safety and efficacy of this strategy is unclear and supporting evidence comes from small single-centred observational studies as demonstrated by the systematic review in Chapter 4.

A comparison cohort study, using propensity-scores to match comparable patients, was therefore designed to evaluate the efficacy and apparent safety of using a set of clinical criteria representing resolution of intraperitoneal inflammation to guide postoperative IV antibiotic duration in children with complicated appendicitis. We hypothesized that tailoring the duration of antibiotic administration to each individual patient would improve clinical practice efficiency by shortening inpatient therapy and hospitalisation without compromising patient care or increasing the risks of insufficiently-treated infections.
5.2 METHODS

This comparison cohort study compared the outcomes of a prospectively observed cohort treated under a new study protocol with outcomes of a historical control cohort treated prior to the introduction of the protocol. Propensity scores, calculated from pre- and intraoperative variables, were used to match participants from the two cohorts. Ethics approval was granted by the Northern X Regional Ethics Committee, New Zealand Government Ministry of Health, in May 2011 (Reference no. NTX/11/05/040).

5.2.1 Participants

Study participants were children (5 to 14 years) treated at Starship Children’s Hospital, the single tertiary centre providing paediatric surgical care to the catchment areas of three District Health Boards. The prospectively observed study cohort consisted of consecutive children presenting to Starship Children’s Hospital, diagnosed between 1 August 2011 and 2 February 2012 with ‘complicated appendicitis’ requiring acute surgery. Diagnosis was made by the operating surgeon and included appendiceal gangrene, visible appendiceal perforation, intraperitoneal abscess formation, and/or appendicitis-related secondary peritonitis. Patient eligibility was assessed within the first 24 hours after surgery by the primary research investigator (T Yu). Those not assessed within this timeframe were excluded to ensure that all prospectively recruited patients received the standardised postoperative care outlined in the study protocol. Participant information sheets were available for participants as well as family/whānau (Appendix C).
The historical control participants were identified from a retrospective review of clinical records at Starship Children’s Hospital in November 2010. The review covered a 5-year period between 1 January 2005 and 31 December 2009 and included all patients diagnosed macroscopically or histologically with gangrenous and/or perforated appendicitis associated with generalised peritonitis or appendiceal abscess. Propensity scores, calculated using pre- and intraoperative covariates, were used to match control group participants to the closest equivalent prospective cohort participants. The following exclusion criteria were applied during participant selection for both study groups:

- Known immunosuppression
- Pre-existing and severe co-morbidity likely to impact postoperative recovery
- Diagnosed with concurrent cause for secondary peritonitis other than appendicitis
- Latrogenic/surgical appendiceal perforation with peritoneal contamination only

5.2.2 Intervention

The following aspects of perioperative care are routine at Starship Children’s Hospital and provided to all study participants.

5.2.2.1 Preoperative care

All participants presented to Starship Children’s Hospital and proceeded to acute surgery after the diagnosis of appendicitis was made clinically or with the aid of radiological investigations. Preoperative care consisted of resuscitation with isotonic IV fluids and the administration of analgesia and IV antibiotics. Antibiotic choice was decided by admitting residents but regimens consisted of either cefoxitin (25 mg per kg, every 8 hours) alone or, if ‘complicated appendicitis’ was suspected, a combination of amoxycillin (20 or 25 mg per kg,
maximum 1 g per dose, every 8 hours), metronidazole (7.5 mg per kg, maximum 500 mg per
dose, every 8 hours), and gentamicin (6 to 7.5 mg per kg, maximum 320 mg per dose, every
24 hours). Patients with documented beta-lactam antibiotic anaphylaxis were discussed
with the Infectious Diseases Team who authorized substitutes such as clindamycin or
vancomycin.

5.2.2.2 Intraoperative care and surgery

Acute surgery was scheduled according to acuity prioritisation protocols at Starship
Children’s Hospital. All procedures were performed under the supervision of consultant
paediatric surgeons. While the technical aspects of each procedure were left to the
discretion of operating surgeons, available strategies include peritoneal irrigation with
warm isotonic saline to reduce contaminated intraperitoneal debris, and laparoscopic
retrieval bags and antiseptic wound irrigation to minimize wound contamination.
Transperitoneal drains were not routinely used. If preoperative antibiotics had not yet been
administered, single doses of the above agents were given with preferences being
dependent on the intraoperative findings. Local instillation of antibiotics (into the peritoneal
cavity or wound layers) was not routinely practiced.

5.2.2.3 Postoperative care

Postoperatively, established antibiotic regimens were continued. All patients found to have
complicated appendicitis and spread of infection into the peritoneal cavity were treated
with a triple IV antibiotic regimen consisting of amoxicillin, metronidazole and gentamicin.
Serum gentamicin trough levels were monitored immediately before administrating the
second dose and then subsequently every 3 to 5 days. The accepted trough level was
< 0.5 mg/L. Serum creatinine was also used as a surrogate marker for gentamicin renal toxicity. Monitoring of serum inflammatory markers and electrolytes was performed as required based on patient progress.

Patient vital signs, including pulse rate, tympanic temperature, blood pressure and respiratory rate, were monitored at 6-hour intervals or more frequently if required. Appropriate analgesia was prescribed by the Acute Pain Team to facilitate early mobilisation. There were no formal restrictions on postoperative re-feeding and nasogastric tubes were not placed routinely. Re-feeding was initiated in step-wise increments according to individual clinician preferences starting first with sips of clear fluid and progressing to a full fluid diet and then a light solid diet. At the study institution, light meal items are selected and prepared to aid easy digestion. Breakfast generally consisted of cereal, toast, fruit, juice, and milk. Lunch typically consisted of sandwiches, clear soup or juice, and fruit while dinner consisted of a main meal (meat, starch, vegetables), dessert (fruit, gelatine, custard), and a cold or hot drink.

Postoperative analgesia consisted of regular paracetamol and a simple non-steroidal anti-inflammatory agent such as ibuprofen or diclofenac. Oral morphine and Tramadol were also available to inpatients but very rarely prescribed to patients at time of discharge.

5.2.2.4 Prospective and control cohorts

Prior to 1 August 2011, all children diagnosed with complicated appendicitis were treated routinely with a triple agent IV antibiotic regimen (amoxycillin, metronidazole and gentamicin) for a minimum of five postoperative days as part of an agreed departmental
protocol. Patients in the study’s retrospective control cohort were managed under this protocol. At the end of five days, patients were discharged if they had been afebrile consecutively for 24 hours, demonstrated signs of adequate bowel function (tolerance of oral intake and/or passage of flatus or bowel motion), mobilised independently, and had adequate pain control using oral analgesia. Routine prescription of oral antibiotics at the time of discharge was not part of the protocol and left to the discretion of individual surgeons. Patients were followed-up after six weeks in ambulatory outpatient clinic.

From 1 August 2011 to 2 February 2012, an alternative protocol was introduced and the first patients to receive treatment under this protocol were recruited into the study’s prospective cohort. The new protocol did not include five days of postoperative IV antibiotics. Instead, antibiotic therapy was discontinued and patients discharged from hospital as soon as a set of objective bedside clinical criteria were satisfied. Assessed each morning, these were:

1. Core body temperature < 38.0 °C for 24 consecutive hours
2. Tolerates a light diet over two consecutive meals
3. Mobilising independently (if ambulatory)
4. Oral analgesia provides effective pain control

The new protocol also consisted of a number of supplementary features, including recognition of patients who fail to reach the criteria for cessation of IV antibiotic therapy due to postoperative complications. Ongoing postoperative fever by Day 4 or 5 warranted investigation for possible infectious complications at the surgeon’s discretion and appropriate management was instigated which may or may not have included continuing
antibiotic therapy. Similarly, if postoperative ileus was suspected, an intraabdominal
infectious pathology was excluded (based on clinical assessment, serum inflammatory
markers, and radiology as required) and IV antibiotic therapy replaced with supportive
measures as soon as possible.

In accordance with currently available guidelines, the new protocol included a course of
oral antibiotics (amoxycillin and clavulanic acid, 20 mg / 5 mg per kg, every 8 hours) for
children who did not receive five days of IV antibiotics. This was prescribed to make up a
total postoperative course of seven days. Finally, all patients managed under the new
protocol were invited to attend an outpatient appointment for review two weeks after
discharge. Those who did not attend were followed-up by telephone.

5.2.3 Objectives

The study objective was to test the hypothesis that tailoring the duration of antibiotic
administration to each individual patient based on clinical markers of resolving peritoneal
inflammation and infection would improve clinical practice efficiency by shortening
inpatient therapy and hospitalisation. Furthermore, the study aims to determine whether
this strategy would compromise patient care by increasing the risk of insufficiently treated
infections.

5.2.4 Outcomes

Study data were all collected by the primary investigator (T Yu) using a standard data
collection instrument (Appendix D).
5.2.4.1 Primary outcomes

The primary study outcome was length of stay (LOS) during the index hospital admission. In addition, the postoperative LOS during the index admission, LOS during subsequent readmissions, and total LOS (index admission combined with readmission) were recorded. Readmission was defined as return to hospital for 24 hours or more within 30 days of discharge.

5.2.4.1 Secondary outcomes

Secondary study outcomes included the duration of IV antibiotics received during the index admission, frequency and severity of perioperative complications, and clinician adherence to the new antibiotic protocol. The duration of antibiotic therapy was defined as ‘the number of days a complete 24-hour course had been received.’ Postoperative complications were recorded prospectively in the prospective cohort for up to 30 days after surgery and severity was graded using the Clavien-Dindo classification. Complications were defined using standardised definitions. For example, ‘ileus/functional bowel obstruction’ was defined as an inability to tolerate oral intake in the postoperative period associated with nausea and vomiting with or without a need for nasogastric tube drainage but without radiological evidence for mechanical bowel obstruction. Due to the extensive geographic coverage area of the study institution, postoperative complications that required hospitalisation in either study cohorts would have been known to study investigators. Lastly, adherence to the new protocol was prospectively evaluated. Non-adherence was defined as ‘deviation from the protocol instructions without apparent reason to do so.’
5.2.5 Sample Size

The power calculation was based on reduction of hospital LOS and performed a priori using data from the retrospective review conducted in November 2010. The mean LOS for 302 children who underwent acute surgery for complicated appendicitis at Starship Children’s Hospital was 5.8 nights (standard deviation = 3.0). A two-tailed Student’s t-test showed that a reduction in mean LOS to 4.0 nights (reduction of 31%) would require a minimum sample size of 94 (alpha = 0.05, power = 0.8). Each arm of the study would consist of 47 participants. This reduction is an effect size (Cohen’s d) of 0.59.

5.2.6 Propensity-Score Modelling

In order to match historical control patients to the prospectively observed cohort so that meaningful comparisons could be made, propensity score analysis was performed. This is a post hoc statistical method used to account for confounding factors that influence outcome when study subjects are not randomly assigned to prospective and control groups. The propensity score – the probability that an individual study patient would be managed under the new antibiotic protocol – was calculated by logistic regression with the ‘dependent variable’ being the patient’s study allocation (prospective or control cohort) and all possible patient confounding factors included as ‘covariates’.

The following pre- and intraoperative variables were used to calculate propensity scores for patients in both the prospective and retrospective cohorts: age, gender, ethnicity, weight, duration of presenting symptoms, white blood cell (WBC) and neutrophil counts at presentation, duration of operation, seniority of primary surgeon performing appendicectomy, surgical approach taken (laparoscopy, conversion to open, direct open),
intraoperative findings/diagnosis, presence of an appendiceal mass, and whether or not pre- and intraoperative IV antibiotics had been administered.

Once the propensity scores had been calculated, one-to-one matching was used to identify control patients who are best ‘matched’ to each patient in the prospective cohort on the basis of pre- and intraoperative covariates. To do this, prospective and control cohort patients were randomly ordered into separate lists and the first prospective patient matched to the control patient with the nearest propensity score. Both patients were then removed from consideration for the next round of matching and attention shifted to the second prospective patient. Matching was done until all prospective patients had been matched to respective control patients with comparable propensity scores.

5.2.7 Statistical Analysis

All data analysis was performed using PASW Statistics for Windows Version 18.0 (SPSS Inc., IBM, Chicago IL, USA). Continuous variable parametricity was tested by visually inspecting plotted quantile-quantile curves. Parametric data were presented as means and standard deviations while nonparametric data were presented as medians and inter-quartile ranges. Parametric continuous variables were compared using Student’s t-tests while nonparametric continuous variables were compared using Mann-Whitney U tests. Categorical variables were compared using the Fisher’s exact test or Pearson chi-square test. Statistical significance was defined as a p-value of < 0.05.
5.3 RESULTS

5.3.1 Recruitment and Numbers Analysed

Over the 6-month period between 1 August 2011 and 2 February 2012, 54 children aged between 5 and 14 were diagnosed with ‘complicated appendicitis’ and assessed for eligibility to participate in the prospective arm of the study. Of these, 47 were recruited and managed under the new antibiotic protocol. Six excluded patients were under the care of one surgeon who was away on sabbatical during finalisation of the study protocol and the initial 4 months of participant recruitment. One patient was excluded because eligibility to participate was not assessed by study investigators within the first 24 hours after surgery.

Retrospective review of clinical records identified 303 patients operated on acutely who were diagnosed with macroscopic peritonitis as well as gangrenous or perforated appendicitis on the basis of histology. Of these, 290 (96%) patients were between the ages of 5 and 14 at time of presentation and, subsequently, 285 of them were found to have complete clinical records containing the required pre- and intraoperative data points for propensity score calculation. One-to-one propensity score matching identified 47 patients to make up the control cohort.

The study’s comparison analysis involved 94 participants and data for the primary study outcome was available for all participants. Of the 47 participants in the prospective group, 43 (91%) attended the follow-up outpatient clinic 2 weeks post discharge where
complications were assessed and recorded. A further 2 participants were followed-up via telephone resulting in a total study follow-up rate of 96% (45/47).

5.3.2 Participant baseline characteristics

At baseline, the prospective and control groups were well matched in regards to demographics, duration of presenting symptoms and severity of presenting disease, represented by serum inflammatory markers, intraoperative finding and subsequent histological diagnoses (Table 5.1).

5.3.3 Length of Stay

Significant differences were found when various measures of LOS were compared between the prospective and control cohorts (Table 5.2). As well as significant differences in median LOS, there was also a significant difference between the mean LOS during the index admission of prospective subjects (4.9 nights, SD = 2.1) and that of control subjects (5.8 nights, SD = 1.9); t(92) = -2.23, p = 0.028. A significant difference was also found between the mean postoperative LOS during the index admission of prospective subjects (4.5 nights, SD = 2.0) and that of control subjects (5.5 nights, SD = 1.9); t(92) = -2.55, p = 0.013.
<table>
<thead>
<tr>
<th>Table 5.1 Participant Baseline Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intervention Cohort [n=47]</strong></td>
</tr>
<tr>
<td>Age, median in years (IQR)</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>New Zealand European</td>
</tr>
<tr>
<td>New Zealand Maori</td>
</tr>
<tr>
<td>Pacific Islander</td>
</tr>
<tr>
<td>Asian</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Weight, mean in kg (SD)</td>
</tr>
<tr>
<td>Duration of presenting symptoms, median in days (IQR)</td>
</tr>
<tr>
<td>Presenting WBC count, mean in xE9/L (SD)</td>
</tr>
<tr>
<td>Presenting neutrophil count, mean in xE9/L (SD)</td>
</tr>
<tr>
<td>Presenting CRP level, mean in mg/L (SD) [n]</td>
</tr>
<tr>
<td>Received preoperative antibiotics</td>
</tr>
<tr>
<td>Received intraoperative antibiotics</td>
</tr>
<tr>
<td>Operation duration, mean in minutes (SD)</td>
</tr>
<tr>
<td>Seniority of surgeon</td>
</tr>
<tr>
<td>Attending surgeon/Consultant</td>
</tr>
<tr>
<td>Resident/Registrar</td>
</tr>
<tr>
<td>Conversion to open operation</td>
</tr>
<tr>
<td>Intraoperative macroscopic findings</td>
</tr>
<tr>
<td>Normal appendix, no peritonitis</td>
</tr>
<tr>
<td>Acute appendicitis +/- suppuration</td>
</tr>
<tr>
<td>Suppurative/gangrenous appendicitis</td>
</tr>
<tr>
<td>Perforated appendicitis</td>
</tr>
<tr>
<td>Appendiceal mass present</td>
</tr>
<tr>
<td>Appendiceal histological diagnosis</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Acute inflammation +/- suppuration</td>
</tr>
<tr>
<td>Gangrenous</td>
</tr>
<tr>
<td>Perforation</td>
</tr>
</tbody>
</table>

**NOTE:** SD = standard deviation; IQR = interquartile range; n = sample size; WBC = white blood cell; * Pearson chi-square test; † Fisher’s exact test (two-tailed); ‡ Student’s t-test; # Mann-Whitney U test.
5.3.4 Postoperative Complications

There were 33 postoperative complications occurring in 25 patients (Table 5.2). No statistical differences were found in the number of participants with complications, the total number of complications, or the severity of complications between the two study cohorts. Fourteen patients had recurrent or persistent intraabdominal infections and twelve (86%) were managed conservatively with continued antibiotic therapy and follow-up imaging. Two patients underwent placement of percutaneous drains, one from the prospective cohort and one from the control cohort. There was no significant difference in the number of patients who developed intraabdominal infections between the two cohorts; \( \chi^2 \) (1, \( N = 94 \)) = 0.34, \( p = 0.562 \).

Four drug-related complications were recorded. One patient in the prospective cohort developed a severe anaphylactic reaction after receiving the first doses of amoxycillin and metronidazole. She was treated immediately with adrenaline, antihistamine, and a short-acting \( \beta_2 \)-adrenergic receptor agonist delivered via an inhalation nebulizer. There were two reported incidences of antibiotic-related candidiasis infection (oral and vaginal). Both patients were in the control cohort and required topical antifungal treatment. One other patient in the control cohort developed severe pruritus secondary to IV morphine.
Table 5.2 Postoperative Recovery Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intervention [n=47]</th>
<th>Control [n=47]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of postoperative IV antibiotics, median in days (IQR)</td>
<td>3 (2)</td>
<td>5 (1)</td>
<td>&lt; 0.001†</td>
</tr>
<tr>
<td>Prescribed oral antibiotics upon discharge</td>
<td>40 (85%)</td>
<td>13 (28%)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Received &lt; 5 days postoperative IV antibiotics</td>
<td>36 (77%)</td>
<td>18 (38%)</td>
<td>&lt; 0.001‡</td>
</tr>
<tr>
<td>Index admission LOS, median in nights (IQR)</td>
<td>5 (3)</td>
<td>6 (1)</td>
<td>0.010</td>
</tr>
<tr>
<td>Index admission postoperative LOS, median in nights (IQR)</td>
<td>4 (2)</td>
<td>5 (2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Index admission postoperative LOS &lt; 5 nights</td>
<td>28 (60%)</td>
<td>12 (26%)</td>
<td>0.002‡</td>
</tr>
<tr>
<td>Representation within 30 days</td>
<td>8</td>
<td>9</td>
<td>0.789*</td>
</tr>
<tr>
<td>Readmission within 30 days</td>
<td>6</td>
<td>7</td>
<td>0.765*</td>
</tr>
<tr>
<td>Total LOS [Index admission +/- Readmission], median in nights (IQR)</td>
<td>5 (4)</td>
<td>6 (3)</td>
<td>0.009†</td>
</tr>
<tr>
<td>Patients affected by perioperative complication</td>
<td>13</td>
<td>12</td>
<td>0.815*</td>
</tr>
<tr>
<td>Severity of complication (Clavien-Dindo Grade)</td>
<td></td>
<td></td>
<td>0.362†</td>
</tr>
<tr>
<td>I</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nature of complication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraabdominal infection (+/- collection)</td>
<td>6</td>
<td>8</td>
<td>0.562*</td>
</tr>
<tr>
<td>Wound infection</td>
<td>1</td>
<td>0</td>
<td>1.000*</td>
</tr>
<tr>
<td>Ileus/Functional bowel obstruction</td>
<td>4</td>
<td>2</td>
<td>0.677*</td>
</tr>
<tr>
<td>Mechanical bowel obstruction</td>
<td>1</td>
<td>0</td>
<td>1.000*</td>
</tr>
<tr>
<td>Drug-related</td>
<td>1</td>
<td>3</td>
<td>0.753*</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>3</td>
<td>1.000*</td>
</tr>
<tr>
<td>Total frequency of complications</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** SD = standard deviation; IQR = interquartile range; n = sample size; IV = intravenous; LOS = length of stay; * Pearson chi-square test; ‡ Fisher’s exact test (two-tailed); † Mann-Whitney U test.
5.3.5 Adherence to Study Protocol

There were four instances where clinical practice deviated from the study protocol. Two patients were not discharged from hospital despite satisfying criteria for discharge and received additional doses of IV antibiotics. Each patient’s LOS was extended by one night and they were discharged the next day without event. Another patient was given an additional 7-day course of oral antibiotics upon discharge after already receiving 7 days of IV antibiotics. One patient had IV antibiotic therapy ceased on postoperative Day 2 when protocol criteria had not yet been achieved. The patient received oral antibiotics for 24 hours before reaching discharge criteria on Day 3.
This comparison cohort study investigated the efficacy of using a set of bedside clinical parameters to determine duration of postoperative inpatient IV antibiotics for children with appendicitis-related secondary peritonitis. Using propensity scores, the study matched a prospective intervention cohort to comparable participants from a control cohort of historical patients and found that, in contrast to administering antibiotics for a fixed duration of 5 days, the use of selective clinical parameters to guide duration of therapy significantly reduced LOS, improving clinical efficiency. No differences were found in the frequency or severity of postoperative complications suggesting that the intervention strategy did not compromise patient safety.

As detailed in Chapter 4, a series of retrospective and prospective studies involving paediatric participants have previously examined the strategy of using a selection of clinical parameters to guide duration of postoperative antibiotic therapy in patients with complicated appendicitis. A number of adult studies have also described their use.

Adibe et al. retrospectively reviewed outcomes of 149 children with perforated appendicitis, 102 of whom received IV antibiotics alone and 47 who received a shortened course of IV antibiotics followed by oral agents upon discharge. The criteria for discharge were: afebrile (temperature < 38 °C), asymptomatic, and able to tolerate oral intake. Although the duration of antibiotic therapy was not reported, the authors found that LOS
and treatment costs were reduced significantly in the second group while the incidence of postoperative intraabdominal abscess formation was no different.

In contrast, Fraser et al.\textsuperscript{266} conducted a randomised controlled trial comparing the outcomes of 102 patients allocated to receiving either a minimum course of 5-days IV antibiotics or treatment with IV antibiotics up to the time they were able to tolerate a regular diet and been afebrile for the previous 12 hours. Patients were then switched to oral amoxycillin/clavulanate and discharged to complete a course to make up 7 days of antibiotic therapy in total. Patients in the first group were only discharged if the WBC count on Day 5 was normal. If not, IV antibiotics were continued for a minimum of 2 additional days. When postoperative LOS was compared, patients who received oral antibiotics had significantly shorter mean duration (4.48 days) compared to patients who received a 5-day course (6.06 days). The authors noted that 42% of patients were discharged safely prior to postoperative Day 5. The rates of postoperative intraabdominal abscesses in this study were no different between the IV antibiotic group (19%) and oral antibiotic group (20%).

Taylor et al.\textsuperscript{290} conducted a randomised controlled clinical trial involving 94 adult patients diagnosed intraoperatively with perforated or gangrenous appendicitis. Group 1 patients received a minimum of 5-days IV antibiotics (ampicillin/sulbactam) while Group 2 patients were treated on the basis of clinical criteria with no minimum IV antibiotic requirement. The clinical criteria for discontinuing IV antibiotics in both groups include resolution of fever, improved abdominal signs and symptoms, and return of bowel function. Improving leucocytosis was also used but inconsistently and all patients were also given a course of oral cephalexin upon cessation of IV therapy. Investigators found that participants in
Group 1 received longer durations of IV antibiotic therapy but there were no significant differences in the rate of infectious complications encountered between the two groups.

From the results of these studies as well as the current study, it would seem that using clinical parameters to determine postoperative IV antibiotic duration safely reduces LOS and antibiotic administration in childhood complicated appendicitis. However, two questions remain unanswered. The first concerns the necessity of an additional course of oral antibiotics after cessation of IV treatment. The second relates to the specificity and sensitivity of individual clinical parameters, including selected serum inflammatory markers, for predicting the resolution of intraperitoneal infection.

The feasibility of early conversion to oral antibiotic therapy had been investigated not just in complicated appendicitis \(^{293,350}\) but also for a variety of different intraabdominal infections.\(^{351,352}\) In the setting of perforated appendicitis in children, Rice et al.\(^ {294}\) were the first to evaluate prospectively the feasibility of switching from a 10-day course of IV antibiotics to a combined course of IV-oral antibiotics. Included in the systematic review reported in Chapter 4, this randomised controlled trial showed that switching to oral antibiotics as soon as gastrointestinal function has returned was cost-effective and did not confer increased morbidity. Subsequent prospective studies\(^ {293,347}\) have also suggested that patients with a combined IV-oral regime have shorter hospital stays without added risks of complications. There has not yet been a study comparing treatment with and without oral antibiotics after cessation of IV therapy based on selected clinical parameters and this remains an important focus for future studies.\(^ {266,353}\) Although it was not examined by the current study, patient and parental compliance to outpatient oral antibiotics should be
measured as it can vary significantly in the paediatric age group and is often overestimated by clinicians.$^{354-357}$

The use of serum inflammatory markers, such as WBC count, as indicators for resolving intraabdominal infection in children has been both recommended$^{70, 358-360}$ and dismissed by differing authors.$^{293, 361}$ They were not utilised in the current study since our patients are not routinely subjected to postoperative blood sampling and without convincing evidence that regular monitoring of serum inflammatory markers has practice benefits, it was inappropriate to include unpleasant investigations in a research protocol likely to be inducted into routine practice. Furthermore, to be useful, acute phase proteins such as C-reactive protein (CRP) often require serial measurements$^{362}$ amounting to numerous sampling occasions. Similar to the discharge criteria described by Adibe et al.,$^{292}$ this study utilised a set of clinical parameters all easily accessible from the patient’s bedside.

Although WBC count is the most commonly used serum inflammatory marker reported in the context of monitoring patient progress in childhood complicated appendicitis, other serum inflammatory markers may warrant attention. In particular, the value of CRP and procalcitonin has been described in cases of severe lower respiratory tract infections and suspected sepsis.$^{334, 336, 363}$

However, in the setting of childhood complicated appendicitis, postoperative serum CRP monitoring has not yet been found to confer benefits. A retrospective comparison study conducted by van Wijck et al.,$^{251}$ compared the outcomes of 68 patients who received 5 days of postoperative IV amoxicillin/clavulanate with that of 81 patients who received
5 days of postoperative IV amoxicillin/clavulanate and gentamicin and then had the antibiotic therapy continued until serum CRP levels decreased to < 20 mg/L. Results showed that the later approach significantly prolonged the median duration of antibiotic treatment (5 versus 7 days, \( p < 0.0001 \)) without producing a statistically significant difference in the frequency of intraabdominal abscesses (13 versus 16 cases, \( p = 0.95 \)).

It has been suggested that postoperative peritoneal cytokine levels (IL-6, TNF-\( \alpha \), IL-1\( \beta \)) are indicative of peritoneal inflammation even before clinical manifestations develop.\(^{163,364}\) However, the use of inflammatory mediators to assist in the diagnosis of peritonitis, which is often difficult, is still debatable. The diagnostic value of peritoneal cytokine levels is limited in the postoperative setting, and percutaneous puncture to collect fluid for measurement of cytokines is potentially harmful and not justified. Previous studies have intraoperatively placed transperitoneal drains so that peritoneal fluid can be collected postoperative for cytokine analysis,\(^{170,365}\) However, drains are no longer indicated in complicated appendicitis\(^{58,59,366}\) as they have been linked to increased risks of intraabdominal abscess formation and prolonged LOS.

A further area of controversy is the use of a triple IV antibiotic regimen in this study. Traditionally, perforated appendicitis has been managed with multi-agent antibiotic regimes that commonly include an aminoglycoside.\(^{255,367}\) But despite their established reliability and low costs, there are inherent problems associated with aminoglycoside-induced renal and ototoxic adverse effects and requirement for therapeutic drug monitoring. This has prompted some authors to suggest simplification to single or double-agent regimes.\(^{261}\) Broad-spectrum agents, such as ceftriaxone and piperacillin-tazobactam have been
advocated as practical alternatives and several small prospective studies have found comparable patient outcomes.\textsuperscript{69, 261, 289} Future research involving large multi-centred studies powered to detect differences in treatment efficacy should be performed to justify their utility and exclude possible adverse effects such as increased bacterial resistance.

This current study has a number of limitations including non-randomised group allocation design and retrospective data collection. As a study comparing the incidence, severity, and management of perioperative complications, it relies on the accuracy of clinical documentation and data extraction. Missing records and data points resulted in exclusion of five eligible patients as propensity scores could not be calculated. This is one of the potential drawbacks from using propensity scores. Similarly, it is possible for a study’s sample size to be substantially reduced if suitable patient matches cannot be found. Although not a setback encountered by this study, it can significantly affect a study’s final conclusions when only a select subset of patients could be matched. This is the reason why propensity scores are generally reserved for studies with large available sample sizes. Lastly, propensity scores do not adjust for unknown confounders and if many outcomes events (more than 8-10) per confounder are recorded, a well-tailored regression analysis may actually allow for better control of confounders.\textsuperscript{368}

A further point for discussion is the controversy arising from the study’s definition of ‘complicated appendicitis’. The classification of appendicitis currently varies significantly and many descriptions do not sufficiently account for the presence and extent of any associated peritoneal contamination and infection.\textsuperscript{66, 369} Without a widely accepted definition, the authors chose to define it as ‘appendicitis with established intraperitoneal
infection’ and recognise that this led to heterogeneity in histological diagnoses after examination of appendiceal tissue. However, this definition specifies presence of peritoneal disease, the entity targeted by postoperative antibiotics. Perhaps future studies should distinguish between ‘complicated appendicitis’ with ‘perforation’ (hole in appendix or faecolith within the intraabdominal cavity) from those without perforation as the risks of developing postoperative intraabdominal abscesses may differ between the two groups.66

The current study was not powered to detect differences in the rate of postoperative infectious complications and this has been a weakness in a majority of studies to date. Future research should focus on the long-term safety of shortened IV antibiotic therapy and also establish whether it is associated with additional benefits such as lower rates of drug-related toxicity and drug-resistant bacteria.

Finally, the timing of when clinical parameters are evaluated has an impact on optimising protocol delivery and should be further investigated. Instead of assessing patients for discharge eligibility once a day (typically during morning ward round), it may be possible to routinely perform patient reviews more frequently for example introduce standardised afternoon ward reviews. This may further facilitate early hospital discharge and reduce LOS and healthcare costs.
5.5 CONCLUSION

Using a set of objective bedside clinical parameters to determine the duration of postoperative inpatient IV antibiotic therapy in children with appendicitis-related secondary peritonitis improves practice efficiency and reduces patient morbidity by shortening hospital stay without apparent compromise to sepsis management. Future investigations on a larger scale are needed to establish the true safety of this management strategy for a common but morbid childhood surgical disease.
Chapter 6

SYSTEMATIC REVIEW B

Pneumoperitoneum-related peritoneal alterations and the effects of modifying gas insufflation conditions
6.1 INTRODUCTION

The peritoneum is the largest serous membrane in the body and it has a number of important functions including protection of intraabdominal contents and maintenance of intraperitoneal homeostasis by allowing exchange of molecules and production of peritoneal fluid.\textsuperscript{370} However, owing to its delicacy, the peritoneal surface is highly susceptible to surgical trauma and disruption. Principally, mesothelial cells are poorly interconnected through loose intercellular bridges\textsuperscript{371} and the slightest degree of mobilisation results in denudation of peritoneal surfaces. Furthermore, with the introduction of laparoscopy, an additional aspect of surgery now poses harm to the peritoneum.

Laparoscopic surgery has led to shortened postoperative recovery with reduced pain and disability. However, the pneumoperitoneum required for laparoscopy has been associated with a number of concerning features. Mechanical distension of the abdominal wall is known to cause morphological changes to peritoneal microstructure, namely the loss of contact between mesothelial cells favouring translocation of intraperitoneal bacteria.\textsuperscript{372,373} Gas insufflation during laparoscopy is also linked to aerosolisation of cells and microorganisms,\textsuperscript{374} leading to concerns of increased seeding by pathogens and tumour cells. Furthermore, the pneumoperitoneum has been shown to cause marked peritoneal desiccation\textsuperscript{375} and produce a number of local changes directly related to carbon dioxide gas and its properties.
Carbon dioxide (CO$_2$) has become the most widely accepted gas for laparoscopic insufflation due to its safety profile and cost-effectiveness. As well as being inert and highly soluble in water (1.45 mg/L), it has a high exchange capacity in the lungs$^{376,377}$ and does not support combustion. Furthermore, CO$_2$ has less pro-inflammatory effects compared to air.$^{378}$ However, it is an irritant within the intraperitoneal cavity, causing local pH disturbances$^{91,379-381}$ as well as changes in peritoneal macrophage responsiveness.$^{92,382,383}$

A large number of studies have highlighted the extensive structural, metabolic, and immune impairments associated with CO$_2$ pneumoperitoneum and although correlation to clinical implications have not yet been clearly established, concerns have been raised with regards to additional postoperative pain and delayed recovery, impaired peritoneal defence, and intraperitoneal adhesion formation.$^{384,385}$

For these reasons, there has been growing interest in CO$_2$ pneumoperitoneum-associated changes to peritoneal surface structure and physiology. Surgeons have realised that they are capable of manipulating several physical aspects of CO$_2$ insufflation gas including temperature, relative humidity, concentration, and insufflation pressure. Furthermore, modifying these conditions has been shown to have striking effects.$^{385}$ Given the near complete incorporation of laparoscopy into modern-day surgical practice, including in cases of simple and complicated appendicitis, and the increasing complexity and length of laparoscopic procedures, how manipulation of these conditions affects the peritoneal changes associated with pneumoperitoneum deserves attention.
The aim of this review is, therefore, to systematically review how variation of CO₂ insufflation conditions affects the physical (morphologic and chemical) and functional (metabolic and immunologic) characteristics of the peritoneum. The effects of conventional CO₂ pneumoperitoneum will also be described so that results of the reviewed studies can be appreciated.
6.2 METHODS

6.2.1 Systematic Search Strategy

A systematic literature search was conducted in January 2010 of four electronic databases (Ovid MEDLINE, The Cochrane Library, PubMed, and EMBASE) by two research investigators independently. The search was focused on retrieving papers describing the effects of CO₂ gas pneumoperitoneum on peritoneal surface features and intraperitoneal environment, and the impact of altering insufflation physical conditions including gas temperature, relative humidity, concentration, and pressure. The search terms used are outlined in Table 6.1. Searches were limited to papers published in English but no restriction was placed on publication year. All laboratory studies, clinical studies, reviews and comments were retrieved.

Table 6.1 Literature Search Terms Used

<table>
<thead>
<tr>
<th>Database</th>
<th>Search Terms</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVID Medline</td>
<td>(Laparoscop$ OR Endoscop$ OR Coelioscop$ OR Celioscop$ OR Pneumoperitoneum) AND (Peritone$ OR Mesothel$) AND (Morpholog$ OR Structure OR Appear$ OR Change OR Microscopy OR Metaboli$ OR pH OR Hypoxia OR Immun$ OR Macrophage OR Fibrin$ OR Inflamm$).m_titl.</td>
<td>English</td>
</tr>
<tr>
<td>The Cochrane Library</td>
<td>(Laparoscop* [Title] OR Endoscop* [Title] OR Coelioscop* [Title] OR Celioscop* [Title] OR Pneumoperitoneum [Title]) AND (Peritone* [Title] OR Mesothel* [Title])</td>
<td>N/A</td>
</tr>
<tr>
<td>PubMed</td>
<td>(Laparoscop* OR Endoscop* OR Coelioscop* OR Celioscop* OR Pneumoperitoneum) AND (Peritone* OR Mesothel*)</td>
<td>N/A</td>
</tr>
<tr>
<td>EMBASE (1980+)</td>
<td>(Laparoscop$ OR Endoscop$ OR Coelioscop$ OR Celioscop$ OR Pneumoperitoneum) AND (Peritone$ OR Mesothel$) AND (Morpholog$ OR Structure OR Appear$ OR Change OR Microscopy OR Metaboli$ OR pH OR Hypoxia OR Immun$ OR Macrophage OR Fibrin OR Inflamm$).m_titl.</td>
<td>English</td>
</tr>
</tbody>
</table>
In addition to searching electronic databases, the reference lists of retrieved original and review articles and editorials were hand searched to identify additional potentially relevant trials. To manage the search results, all records (including original research articles, reviews, editorials, and essays) were imported into a bibliography management program (ENDNOTE X5, Thomson Reuters, New York, USA) that enabled removal of duplicate records.

### 6.2.2 Study Selection

After removing duplicates, two investigators independently screened all titles and abstracts for potentially relevant articles and these were obtained in full-text for closer inspection. Disagreements were resolved through discussion until consensus was reached. Consultation with a senior research investigator was sought whenever disagreement could not be easily resolved. All study designs were included including randomised controlled trials (RCTs), non-RCTs, case-control studies, case series, and case reports. The following exclusion criteria were used during the final article selection process:

- Not published in English
- An editorial, commentary, or conference abstract
- Study results duplicated in an earlier publication
- Exposure to CO₂ pneumoperitoneum not the single causative factor investigated
- Study materials (cells and/or tissue) not derived from native peritoneum of human subjects or comparable animal models
- Study material (cells and/or tissue) not exposed to CO₂ pneumoperitoneum *in vitro*
- Outcome measures reported did not include local effects on peritoneal surface morphology (microscopic and macroscopic) and function
• Pre-existing peritoneal pathology present at time of CO₂ insufflation
  e.g. endometriosis, peritoneal malignancy, exposure to peritoneal dialysis solution,
  secondary peritonitis

6.2.3 Data Extraction and Analysis

All selected studies were reviewed by at least two investigators who used a standardised
data extraction and critical appraisal instrument. The information extracted from each study
consisted of the following: [1] setting, [2] research subjects/participants and materials,

Reported data were extracted from the text, results tables, graphs, and figures, and
categorised by outcome measures. Animal studies were distinguished from human studies.
No meta-analyses were possible, and study quality was not assessed owing to the
heterogeneity of study design and outcome measures.
6.3 RESULTS

6.3.1 Literature Search and Article Selection

Figure 6.1 is a PRISMA Statement flow diagram summarising results of the literature search and article selection process. The search of four electronic databases generated a total of 1,183 hits. Once duplicate hits were removed, two investigators carefully and independently screened the title and abstract of 639 articles, looking for potentially relevant studies. In addition to the articles identified via electronic databases, the hand-search of reference lists from 15 review articles and relevant studies found 9 additional titles. These were also obtained in full-text for consideration. A total of 127 studies were obtained as full-text for closer inspection and from these, 18 were selected for this review based on the eligibility criteria.
Figure 6.1 PRISMA Statement Flow Diagram Summarising Results of Literature Search and Article Selection

- Ovid MEDLINE: 480 Citation(s)
- PubMed: 181 Citation(s)
- The Cochrane Library: 73 Citation(s)
- EMBASE (1980): 449 Citation(s)
- Hand-search: 9 Citation(s)

639 Non-Duplicate Citations Screened

Inclusion/Exclusion Criteria Applied: 512 Articles Excluded After Title/Abstract Screen

127 Articles Retrieved

Inclusion/Exclusion Criteria Applied: 109 Articles Excluded After Full Text Screen

18 Articles Included

0 Articles Excluded During Data Extraction
Table 6.2 provides a summary of the 18 studies selected for this review. They are organised according to the primary outcome that was measured: peritoneal morphology and structure (microscopic and macroscopic), peritoneal metabolism, and peritoneal immune and inflammatory response. This results table is followed by a description of the studies and their results along with summaries of what is already understood about the effects of conventional CO₂ pneumoperitoneum on peritoneal morphology and physiology/function.
### Table 6.2 Summary of Reviewed Studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study Design</th>
<th>Study Subjects</th>
<th>Exposure CO₂ Insufflation Condition(s)</th>
<th>Control CO₂ Insufflation Condition(s)</th>
<th>Fixed CO₂ Insufflation Conditions</th>
<th>Peritoneal Outcome Measures</th>
<th>Timing of Outcome Measures</th>
<th>Other Outcome Measures</th>
<th>Summary of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peng et al.</td>
<td>2009</td>
<td>Randomised comparison</td>
<td>Wistar rats, n = 160</td>
<td>37 ºC, 95% relative humidity</td>
<td>21 ºC, &lt; 1% relative humidity</td>
<td>9 mmHg; 300 mL/min; 3, 4 &amp; 5 hrs;</td>
<td>1. Microscopic morphology (SEM &amp; light microscopy) 2. Adhesions</td>
<td>Morphology: 6, 24, 48, &amp; 96 hrs postop. Adhesions: 2 wks postop</td>
<td>Core body temperature changes during insufflation</td>
<td>Exposure group showed significantly less microscopic morphologic damage and no formation of adhesions.</td>
</tr>
<tr>
<td>Davis et al.</td>
<td>2006</td>
<td>Randomised single-blinded</td>
<td>Human adults, n = 44</td>
<td>~ 37 ºC or 95% relative humidity or both</td>
<td>Standard (not otherwise specified)</td>
<td>Not reported</td>
<td>1. Histological surface structure, 2. Macrophage activity</td>
<td>Beginning &amp; end of surgery</td>
<td>Intraop core temperature, postop pain &amp; recovery parameters</td>
<td>No significant histological differences were identified between the study groups.</td>
</tr>
<tr>
<td>Erikoglu et al.</td>
<td>2005</td>
<td>Comparison</td>
<td>Male Sprague-Dawley rats, n = 30</td>
<td>40 ºC, 98% relative humidity</td>
<td>21 ºC, 2% relative humidity</td>
<td>10 mmHg; 2 hrs</td>
<td>Microscopic morphology (SEM &amp; light microscopy)</td>
<td>12 hrs after exposure</td>
<td>-</td>
<td>Exposure group demonstrated less peritoneal morphologic alterations.</td>
</tr>
<tr>
<td>Elkelani et al.</td>
<td>2004</td>
<td>Randomised comparison</td>
<td>Female Naval Medical Research Institute mice, n = 100</td>
<td>3%, 6%, 9%, or 12% added O₂, 37 ºC &amp; humidified (Aquapor)</td>
<td>0% added O₂, 37 ºC &amp; humidified (Aquapor)</td>
<td>5, 10, or 20 cm H₂O; 23 mL/min; 10, 30, or 60 min;</td>
<td>Postop macroscopic adhesion formation</td>
<td>7 days postop</td>
<td>-</td>
<td>Addition of 3% O₂ reduced adhesion formation. Addition of O₂ at higher concentrations had no impact on adhesion formation.</td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Study Subjects</td>
<td>Exposure CO$_2$ Insufflation Condition(s)</td>
<td>Control CO$_2$ Insufflation Condition(s)</td>
<td>Fixed CO$_2$ Insufflation Conditions</td>
<td>Peritoneal Outcome Measures</td>
<td>Timing of Outcome Measures</td>
<td>Other Outcome Measures</td>
<td>Summary of Results</td>
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<tr>
<td>Hazebroek et al.</td>
<td>2002</td>
<td>Randomised comparison</td>
<td>Male Brown Norway rats, n = 60</td>
<td>37 °C or humidified or both</td>
<td>Non-heated &amp; non-humidified; gasless abdominal wall lifting</td>
<td>6 mmHg, 2 hrs</td>
<td>Microscopic morphology (SEM)</td>
<td>Immediately postop, 2 &amp; 24 hrs postop</td>
<td>Intraop core body &amp; intraperitoneal temperature</td>
<td>Exposure groups (heated or humidified) demonstrated no significant differences when compared to control groups (cool-dry &amp; gasless).</td>
</tr>
<tr>
<td>Molinas et al.</td>
<td>2001</td>
<td>Comparison</td>
<td>Female Naval Medical Research Institute mice, n = 130</td>
<td>0.5-12% added O$_2$</td>
<td>0% added O$_2$</td>
<td>5-15 cm H$_2$O, 10 or 60 min</td>
<td>Postop macroscopic adhesion formation</td>
<td>7 &amp; 28 days postop</td>
<td>-</td>
<td>Exposure group demonstrated reduced adhesion formation. Increased adhesions were associated with increasing insufflation pressure.</td>
</tr>
<tr>
<td>Molinas &amp; Koninckx</td>
<td>2000</td>
<td>Randomised comparison</td>
<td>Female NZ white rabbits, n = 48</td>
<td>4% added O$_2$</td>
<td>0% added O$_2$</td>
<td>8 mmHg; 25 L/min; 10 or 45 min</td>
<td>Postop macroscopic adhesion formation</td>
<td>7 days</td>
<td>-</td>
<td>Exposure group demonstrated significantly reduced degree of postop adhesion formation.</td>
</tr>
</tbody>
</table>

**Peritoneal Metabolism**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study Design</th>
<th>Study Subjects</th>
<th>Exposure CO$_2$ Insufflation Condition(s)</th>
<th>Control CO$_2$ Insufflation Condition(s)</th>
<th>Fixed CO$_2$ Insufflation Conditions</th>
<th>Tissue O$_2$ tension</th>
<th>Intraop</th>
<th>Summary of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bourdel et al.</td>
<td>2007</td>
<td>Randomised comparison</td>
<td>C57BL6 mice, n = 40</td>
<td>2 mmHg</td>
<td>8 mmHg</td>
<td>2 L/min, 1 hr</td>
<td>Tissue O$_2$ tension</td>
<td>Intraop arterial blood gases, rectal temperature</td>
<td>Exposure group demonstrated significantly higher levels of peritoneal tissue O$_2$ tension ± controlled respiratory support.</td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Study Subjects</td>
<td>Exposure CO\textsubscript{2} Insufflation Condition(s)</td>
<td>Control CO\textsubscript{2} Insufflation Condition(s)</td>
<td>Fixed CO\textsubscript{2} Insufflation Conditions</td>
<td>Peritoneal Outcome Measures</td>
<td>Timing of Outcome Measures</td>
<td>Other Outcome Measures</td>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Wong et al.</td>
<td>2004</td>
<td>Comparison</td>
<td>Pigs, n = 21</td>
<td>37 °C, bubbled through sterile water ± diluted NaHCO\textsubscript{3}</td>
<td>Standard (not otherwise specified)</td>
<td>5-6 mmHg, 3 hrs</td>
<td>Intraop peritoneal surface pH levels</td>
<td>Before insufflation, 5 min after insufflation &amp; then at 15 min intervals</td>
<td>Intraop bowel serosal pH levels &amp; arterial blood gases</td>
</tr>
<tr>
<td>Wildbrett et al.</td>
<td>2003</td>
<td>Comparison</td>
<td>Male BD IX rats, n = 30</td>
<td>20% added O\textsubscript{2}, 0% added O\textsubscript{2}</td>
<td>10 mmHg, 2 hrs</td>
<td>1. Tissue O\textsubscript{2} partial pressure 2. Peritoneal fluid pH levels</td>
<td>Continuous intraop monitoring</td>
<td></td>
<td>Intracellular free calcium levels &amp; pH levels of rat colonic carcinoma cells</td>
</tr>
<tr>
<td>Yavuz et al.</td>
<td>2003</td>
<td>Comparison</td>
<td>Domestic pigs (both genders), n = 15</td>
<td>0, 5, &amp; 10 mmHg (consecutively each for 30 min)</td>
<td>N/A</td>
<td>90 min</td>
<td>Tissue blood flow</td>
<td>After 25 min of insufflation at each pressure</td>
<td>Intraop core body temperature &amp; haemodynamic parameters (pH, cardiac output, BP, HR, PaCO\textsubscript{2})</td>
</tr>
<tr>
<td>Brundell et al.</td>
<td>2002</td>
<td>Comparison</td>
<td>Female domestic pigs, n = 25</td>
<td>4, 8, &amp; 12 mmHg</td>
<td>N/A</td>
<td>30 min</td>
<td>Tissue blood flow</td>
<td>Before &amp; after 30 min insufflation</td>
<td>Renal &amp; hepatic blood flow</td>
</tr>
<tr>
<td>Kuntz et al.</td>
<td>2000</td>
<td>Comparison</td>
<td>Male Wistar rats, n = 65</td>
<td>3, 6, &amp; 9 mmHg</td>
<td>0 mmHg</td>
<td>30-90 min</td>
<td>Intraop intraperitoneal pH</td>
<td>During insufflation</td>
<td>Intraop blood &amp; subcutaneous pH</td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Study Subjects</td>
<td>Exposure CO₂ Insufflation Condition(s)</td>
<td>Control CO₂ Insufflation Condition(s)</td>
<td>Fixed CO₂ Insufflation Conditions</td>
<td>Peritoneal Outcome Measures</td>
<td>Timing of Outcome Measures</td>
<td>Other Outcome Measures</td>
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<tr>
<td>Brokelman et al.</td>
<td>2008</td>
<td>Randomised controlled</td>
<td>Human adults, n = 30</td>
<td>37 °C</td>
<td>Room temperature</td>
<td>Not reported</td>
<td>Tissue tPA, uPA, PAI-1 antigen concentrations, tPA-activity</td>
<td>Immediately after insufflation, after 45 min exposure or at end of procedure</td>
<td>-</td>
</tr>
<tr>
<td>Brokelman et al.</td>
<td>2007</td>
<td>Randomised comparison</td>
<td>Human adults, n = 50</td>
<td>10, 13, &amp; 16 mmHg</td>
<td>N/A</td>
<td>Not reported</td>
<td>Tissue concentrations of total &amp; active TGF-β1</td>
<td>Beginning of surgery &amp; after 45 min</td>
<td>-</td>
</tr>
<tr>
<td>Brokelman et al.</td>
<td>2006</td>
<td>Randomised comparison</td>
<td>Human adults, n = 50</td>
<td>10, 13, 16 mmHg</td>
<td>N/A</td>
<td>Not reported</td>
<td>Tissue tPA, uPA, PAI-1 antigen concentrations, tPA-activity</td>
<td>Beginning of surgery &amp; after 45 min</td>
<td>-</td>
</tr>
<tr>
<td>Margulis et al.</td>
<td>2005</td>
<td>Randomised comparison</td>
<td>Female pigs, n = 15</td>
<td>Provided by Insuflow® device</td>
<td>Standard (not otherwise specified)</td>
<td>Mean 42-70 min; mean gas volumes 41.8-102.7 L</td>
<td>Peritoneal fluid cytokine levels (TNF-α, IL-1β, IL-6)</td>
<td>Start of procedure, and at 1, 4, 24, and 48 hrs postop</td>
<td>Serum cytokine &amp; cortisol levels, intra- &amp; postop core temperature</td>
</tr>
<tr>
<td>Reference</td>
<td>Year</td>
<td>Study Design</td>
<td>Study Subjects</td>
<td>Exposure CO₂ Insufflation Condition(s)</td>
<td>Control CO₂ Insufflation Condition(s)</td>
<td>Fixed CO₂ Insufflation Conditions</td>
<td>Peritoneal Outcome Measures</td>
<td>Timing of Outcome Measures</td>
<td>Other Outcome Measures</td>
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<tr>
<td>Puttick et al.</td>
<td>1999</td>
<td>Randomised controlled</td>
<td>Human adults, n = 30</td>
<td>Room temperature</td>
<td>Mean gas volumes used 85.5-92.1 L; Mean op duration 31.5-32.1 min</td>
<td>Peritoneal fluid cytokine levels (TNF-α, IL-1β, IL-6)</td>
<td>24 hrs postop</td>
<td>Intraop core body &amp; intraperitoneal temperature, postoperative pain &amp; analgesia consumption</td>
<td>Exposure group demonstrated significantly lower levels of peritoneal inflammatory cytokines.</td>
</tr>
</tbody>
</table>

**NOTE:** n = sample size, min = minutes, hrs = hours, SEM = scanning electron microscopy, tPA = tissue plasminogen activator, uPA = urokinase-type plasminogen activator, PAI-1 = plasminogen activator inhibitor-1, NaHCO₃ = sodium bicarbonate, TNF-α = tumour necrosis factor alpha, IL-1β = interleukin 1 beta, IL-6 = interleukin 6.
6.3.2 Description of Study Settings, Subjects, and Objectives

6.3.2.1 Setting

This review was dominated by studies from Europe with 12 out of 18 originating from this region. Three of these studies were set in the same research institutions while a separate group of three studies were conducted by the same research investigators. The remaining studies originated from Australia, Turkey, China, and the United States.

6.3.2.2 Subjects

Animal experiments made up the majority of studies in this review with only five human clinical trials. The total number of human participants in these trials ranged from 30 to 50 while individual study groups consisted of 10 to 15 participants. Two of the studies reported differing primary outcomes obtained from the same group of human study participants. Eligibility criteria used for recruitment included adult patients undergoing elective laparoscopic cholecystectomy and elective laparoscopic Roux-en-Y gastric bypass.

Animal experiments involved mainly rodents (8 studies) and pigs (4 studies). There was also one study involving New Zealand white rabbits. Research subjects per individual study numbered between 15 and 160 and study groups ranged between 5 and 25. A power calculation performed to determine sample size was reported by one study. In general, study subjects were bred and kept in standardised conditions that were well described by study investigators. Weight and age were the most commonly used parameters to standardise selection of comparable subjects. The majority of studies subjected animal
subjects to CO$_2$ insufflation only without mechanical disruption of the peritoneum. Three of the 13 animal studies$^{389,391,392}$ created standardised lesions in the peritoneum, mimicking surgical trauma, to study the effects of CO$_2$ pneumoperitoneum on postoperative adhesion formation. Margulis et al.$^{401}$ subjected their porcine study subjects to laparoscopic nephrectomy.

6.3.2.3 Study objectives

This review consists of a very heterogeneous group of animal experiments and clinical trials. Aside from investigating the effects of altering various physical aspects of CO$_2$ pneumoperitoneum on the structure and function of the peritoneum, a number of other insufflation and operative variables were also investigated including the use of helium$^{391,392,394-397}$ and room air insufflation,$^{397}$ the impact of gasless insufflation$^{390}$ or abdominal wall lift$^{386}$ and laparotomy,$^{380,393,396}$ and the effect of varying laparoscopic light intensities and dissecting devices.$^{399,400}$ To investigate peritoneal acidosis associated with CO$_2$ pneumoperitoneum, Wong et al.$^{380}$ also examined the effects of bubbling heated CO$_2$ insufflation gas through diluted sodium bicarbonate solution before intraperitoneal installation. Lastly, the impact of pneumoperitoneum duration was investigated by five review studies.$^{386,389,391,392,397}$

6.3.3 Description of Study Methodologies

6.3.3.1 Study design

The five human clinical studies were all prospective randomised trials that used sealed envelopes to conceal group allocations. Davis et al.$^{387}$ also described how the randomisation sequences were generated. Of the 13 animal experiments, randomised group allocation was
utilised by six studies\textsuperscript{386, 389, 390, 392, 393, 401} although the manner in which this was achieved was not well described.

In contrast, on the whole, the studies provided detailed descriptions for how experimental CO\textsubscript{2} insufflation conditions were produced, administered, and verified. Furthermore, the standardisation of anaesthesia and operative procedures were clearly defined. To test the effects of warm +/- humidified CO\textsubscript{2} pneumoperitoneum, most investigators used a variety of commercially available devices to heat and humidify insufflation gas. In contrast, Peng et al.\textsuperscript{386} used a newly patented device with control and regulation functions to both heat and humidify CO\textsubscript{2} insufflation gas and Erikoglu et al.\textsuperscript{388} used a previously constructed and tested device consisting of a heater-humidification chamber placed downstream from the gas source.\textsuperscript{403} Wong et al.\textsuperscript{380} humidified CO\textsubscript{2} insufflation gas by heating it to 37 °C and then bubbling it through sterile water before administering it into the intraperitoneal cavity. The humidity of this gas was not evaluated or monitored.

Investigators varied intraabdominal insufflation pressure during CO\textsubscript{2} pneumoperitoneum using standard laparoscopic insufflators. In addition, using rats as test subjects, Kuntz et al.\textsuperscript{397} also connected the abdominal cavity to a large airtight box to equalise pressure fluctuations during insufflation. Furthermore, a fluid manometer was used to control the pressure indicated by the insufflator.

Four studies investigated effects from reducing the hypoxic impact of pure CO\textsubscript{2} pneumoperitoneum by adding a small fraction of oxygen (O\textsubscript{2}) to the insufflation gas. The percentage of added O\textsubscript{2} ranged between 0.5 and 20%. To achieve the target gas mixture,
some investigators utilised a commercial insufflator capable of adding 0-12% O$_2$ to either CO$_2$ or helium gas while others operated two separate insufflators, one for CO$_2$ and one for O$_2$.\textsuperscript{392} To obtain a homogeneous mixture, the output from both insufflators was mixed in a chamber that was fitted with a water valve to limit insufflation pressure to 10 cm H$_2$O.

6.3.3.2 Outcome measures

This review consisted of studies reporting a heterogeneous group of outcomes describing peritoneal surface structure, physiology, and function. Microscopic morphological changes secondary to CO$_2$ exposure were documented using light microscopy and scanning electron microscopy (SEM) and the methods of specimen staining and fixation used were appropriately described. Macroscopic structural changes in the form of postoperative peritoneal adhesions were reported by four animal studies.\textsuperscript{386, 389, 391, 392} Animals were sacrificed at either 7 or 14 days after CO$_2$ pneumoperitoneum exposure to assess both the quality (extent, type, and tenacity) and quantity (based on number and length of individual adhesions). All four studies utilised one or two blinded assessors who scored each aspect of adhesion quality and quantity.

To investigate how various aspects of CO$_2$ pneumoperitoneum (insufflation pressure, temperature etc.) affects peritoneal metabolism, study investigators examined intraoperative intraperitoneal pH levels, peritoneal tissue blood flow, and tissue O$_2$ tension/partial pressures. Wong et al.\textsuperscript{380} also recorded a pre-insufflation baseline level of peritoneal pH while Brundell et al.\textsuperscript{396} obtained reference blood samples prior to insufflation in order to count the radio-labelled marker used to calculate tissue and organ blood flow. The remaining studies recorded respective study outcomes during insufflation at 5 to 15
minute intervals or up to 30 minutes into the procedure. Alternatively, Yavuz et al. measured regional peritoneal blood flow and cardiac output using the coloured microsphere technique where spheres of three different colours are injected into the systemic circulation, 25 minutes into insufflation, and an ultraviolet/visible spectrophotometer is then used to detect the photometric absorbency of prepared specimens given that specific absorbance is determined by colour.

Intraperitoneal pH was measured using three different methods. Wong et al. used a pH probe inserted into the abdominal cavity via one of the laparoscopic trocars while Wildbrett et al. used aspirated peritoneal and wound fluid for pH measurements. Kuntz et al. used tonometry to analyse regional tissue CO₂ partial pressure in gastric mucosa and then calculated tissue pH using a given formula.

The peritoneum’s immune and inflammatory response to CO₂ pneumoperitoneum and how this is altered by various insufflation conditions was investigated by five studies. Peritoneal fluid was sampled before and after CO₂ insufflation to analyse pro-inflammatory cytokine concentrations, indicative of intraperitoneal inflammation severity in test subjects that included pigs and humans. Alternatively, peritoneum biopsies, sampled before and after CO₂ insufflation, were used to ascertain tissue levels of growth factors and key fibrinolytic components involved in peritoneal healing. Commercially available kits deploying enzyme-linked immunosorbent assay (ELISA) techniques were used for these analyses. Lastly, myeloperoxidase and CD68 immunohistochemical assays were used for detection of increased macrophage activity in peritoneal tissue.
A number of secondary outcomes were also investigated by these studies. Of the eight studies that heated insufflation gas to body temperature, five investigated perioperative core body temperature alongside local peritoneal effects. Intraperitoneal temperature during insufflation was monitored by two studies. Animal experiments also examined intraoperative organ perfusion, arterial blood gases, and other markers of systemic haemodynamic functioning (blood pressure, cardiac output, heart rate) while clinical trials involving human subjects measured postoperative recovery parameters and postoperative pain and analgesia consumption.

6.3.4 Review Findings and Overview of Pneumoperitoneum-related Peritoneal Alterations

6.3.4.1 Morphologic alterations

As described in Chapter 1, the peritoneum comprises of a single continuous layer of mesothelial cells, 2.5 to 3 μm in thickness, separated by a basement membrane from a layer of connective tissue composed of collagen fibre bundles, fibroblasts, and free cells such as macrophages, granulocytes, and mast cells. Blood and lymphatic vessels supplying the peritoneum also run in this submesothelial connective tissue layer. Healthy mesothelial cells are densely covered in microvilli.

Peritoneal surface integrity is visibly disrupted by laparoscopic surgery. Volz et al. conducted one of the earliest studies documenting the effects of conventional CO₂ pneumoperitoneum on peritoneum surface morphology. Using murine peritoneum and scanning electron microscopy, what is now recognised as the characteristic changes induced by CO₂ pneumoperitoneum, were evident 2 hours after exposure to 30 minutes of
insufflation. Involving nearly the entire peritoneal cavity, these included bulging of mesothelial cells to take on a spherical appearance and widening of the intercellular junctions with subsequent exposure of the peritoneal basement membrane.\textsuperscript{90}

Two hours after insufflation, the investigators also noted the appearance of peritoneal macrophages in the mesothelial intercellular gaps and lymphocytes on the peritoneal surface. Their numbers increased after 24 hours.\textsuperscript{90} Regeneration cells appeared after 48 hours and by 96 hours after exposure, the intercellular gaps had become much smaller or disappeared, and the mesothelial surface was nearly a confluent layer of microvilli-covered cells. These changes were also documented by Rosario et al.\textsuperscript{404} and Suematsu et al.\textsuperscript{405}, the latter of whom found that CO\textsubscript{2}, helium, and air pneumoperitoneum all caused bulging of murine mesothelial cells. Intercellular clefts, however, occurred to differing extents depending on the gaseous agent used. Regeneration of mesothelial cells occurred after 72 hours and intercellular clefts were only persistent in the helium pneumoperitoneum group.

There is limited evidence describing the peritoneal changes during laparoscopic surgery in humans. Liu et al.\textsuperscript{406} described the peritoneal morphology of 40 patients exposed to 30 minutes of pneumoperitoneum. Mesothelial cells demonstrated bulging immediately at initiation of insufflation while intercellular clefts appeared after 30 minutes of surgery. The underlying basement membrane could be seen at 1 hour and inflammatory cells found in the intercellular clefts at 2 hours.
The review’s literature search identified four studies that investigated effects from altering the physical conditions of CO$_2$ gas insufflation. Three were animal studies using rat peritoneum while the remaining study was a human clinical trial. After exposure to 120 minutes of CO$_2$ insufflation, Hazebroek et al.\textsuperscript{390} found recognisable retraction and bulging of the mesothelial cells, loss of their hexagonal shape, and reduction in the number of covering microvilli. The underlying basal lamina also became obvious and intercellular clefts were visible 24 hours after exposure. These changes were independent of the temperature and humidity of CO$_2$ used for insufflation.

In contrast, Erikoglu et al.\textsuperscript{388} found using both light and electron scanning microscopy that 2 hours of exposure to heated-humidified (40 °C, 98% relative humidity) CO$_2$ gas insufflation resulted in less peritoneal surface alterations compared to standard CO$_2$ gas insufflation (21 °C, 2% relative humidity). While the latter resulted in extreme mesothelial cell desquamation, appearance of macrophages, loss of microvilli, and clearly exposed areas of basement membrane, conditioned gas insufflation caused mesothelial cell retraction and intercellular cleft creation but the basement membrane was not exposed. Depression of microvilli was also only partial. Similarly, Peng et al.\textsuperscript{386} found that heated-humidified CO$_2$ gas insufflation averted the full extent of peritoneal surface changes associated with conventional CO$_2$ pneumoperitoneum. Again using both light and electron scanning microscopy but with prolonged pneumoperitoneum durations of 3, 4, and 5 hours, heating and humidifying CO$_2$ insufflation gas resulted in less mesothelial cell desquamation and diminished intraperitoneal inflammatory response.
Davis et al. conducted a randomised clinical trial investigating the impact of heated, humidified, and heated-humidified CO₂ gas insufflation for laparoscopic Roux-en-Y gastric bypass in 44 adult patients. Outcome measures included peritoneal biopsies to identify structural damage and macrophage activity. These were obtained at the beginning and end of the procedures from three patients in each of study group which including the control group who underwent surgery facilitated by standard CO₂ insufflation. In the intervention groups, CO₂ gas was heated to ~ 37 °C and humidified to ~ 95% relative humidity. After haematoxylin and eosin and Mason-trichrome histologic staining, biopsies showed no structural differences among the four study groups. Macrophage activity was assessed using myeloperoxidase and CD-68 immunohistochemical assays and increased activity was found in only one biopsy sample from the heated-humidified group. The remaining biopsies showed no changes in comparison to those from the control group.

### 6.3.4.2 Metabolic alterations

In addition to structural alterations, laparoscopy also induces metabolic changes in the peritoneum. Principally, CO₂ pneumoperitoneum affects local peritoneal O₂ levels, which may induce metabolic changes including acidosis and hypercapnoea secondary to absorption of CO₂ across the peritoneal surface. Local peritoneal acidosis also occurs as a result of carbonic acid formed from the reaction between CO₂ and water.

Furthermore, pneumoperitoneum reduces abdominal wall perfusion as a result of increased compartmental pressure within the abdomen, leading to build up of the end products of hypoxic metabolism. Using insufflation gases that are void of O₂ likely further exacerbates peritoneal hypoxia. Peritoneal acidosis is not limited to the peritoneal surface, extending into the underlying connective tissue layer where CO₂ insufflation-related acidosis
has been associated with disturbances in the electrical surface charge and release of various inflammatory mediators such as endotoxin.\textsuperscript{412}

This review found one study that investigated the effects of heated and humidified CO\textsubscript{2} gas insufflation on peritoneal acidosis. Wong et al.\textsuperscript{380} compared peritoneal pH levels in pigs subjected to standard CO\textsubscript{2} gas laparoscopy and heated and humidified CO\textsubscript{2} gas laparoscopy. They observed severe peritoneal cavity acidosis (pH = 6.59-6.74) in both groups after induction and there were statistically significant differences found. In comparison, pigs that underwent laparotomy did not demonstrate peritoneal pH changes.

In the search for a non-hypoxic gaseous medium, several studies have investigated the effects of mixing CO\textsubscript{2} insufflation gas with small amounts O\textsubscript{2}. This review identified four studies that have documented the local effects of this intervention on peritoneal surface structure and physiology. All four were experimental animal studies and three of them assessed post-pneumoperitoneum macroscopic adhesion formation, a macroscopic structural outcome. All three studies\textsuperscript{389, 391, 392} reported a reduction in postoperative adhesion formation with the addition of a small percentage of O\textsubscript{2} to CO\textsubscript{2} insufflation gas. Molinas et al.\textsuperscript{391} also found that adhesion formation significantly increased as insufflation pressure was increased.

The last study, conducted by Wildbrett et al.,\textsuperscript{394} monitored abdominal wall and peritoneal tissue O\textsubscript{2} partial pressures as well as peritoneal fluid pH in rats exposed to 2 hours of continuous pneumoperitoneum created using pure CO\textsubscript{2}, helium, and a gas mixture of 80% CO\textsubscript{2} and 20% O\textsubscript{2}. While CO\textsubscript{2} and helium both caused significant reduction in tissue O\textsubscript{2} partial
pressure (from 23 to 5 mmHg and from 25 to 6.5 mmHg; \( p < 0.001 \)), insufflation using the gas mixture of \( \text{CO}_2 \) and \( \text{O}_2 \) caused no differences between the laparoscopy and control groups. Furthermore, four of the 10 measurements taken following insufflation with pure \( \text{CO}_2 \) recorded \( \text{O}_2 \) levels below 0.5 mmHg, the critical cut-off point defining cell hypoxia.\(^{413}\) From this observation, the investigators hypothesised that cells on the peritoneal surface such as macrophages are subjected to acute hypoxia during \( \text{CO}_2 \) pneumoperitoneum. In addition, this study also found that when compared to 100% \( \text{CO}_2 \) insufflation, the non-hypoxic gas mixture caused a significantly smaller pH decrease in peritoneal fluid pH levels (7.36 to 6.39 versus 7.38 to 6.70; \( p < 0.001 \)). On the other hand, helium insufflation was associated with a significant pH increase to 7.58 (\( p = 0.04 \)).

Included in this review, were four studies that investigated the metabolic effects of increasing insufflation pressure associated with \( \text{CO}_2 \) pneumoperitoneum. Using porcine study subjects, Yavuz et al.\(^{395}\) found that, in comparison to 0 mmHg, 25 minutes of insufflation at pressures of 5 and 10 mmHg led to significant increases in parietal peritoneum blood flow. Brundell et al.\(^{396}\) also found that insufflation pressures of 8 and 12 mmHg significantly increased peritoneal blood flow from baseline levels in domestic pigs and a pressure of 4 mmHg did have a similar effect although it was not statistically significant. In a study of mice, an insufflation pressure of 2 mmHg was associated with significantly higher intraoperative levels of peritoneal tissue \( \text{O}_2 \) tension in comparison to insufflation at 8 mmHg.\(^{393}\) Also using a murine study model, Kuntz et al.\(^{397}\) found that increasing pneumoperitoneum insufflation pressures (3, 6, and 9 mmHg) led to dramatic decreases in intraperitoneal pH levels.
6.3.4.4 Immunologic and inflammatory alterations

The healthy peritoneal cavity usually contains less than 300 cells/mm$^3$ but it has a tremendous ability to generate inflammatory cells. Intraabdominal surgery, even in the absence of gross infectious inflammation, elicits a rapid influx of polymorphonuclear leucocytes (PMN) and macrophages. $^{142}$ Degranulation of peritoneal mast cells increases vascular permeability and an inflammatory response is activated with the release of active components including complement factors and opsins. PMN cells also secrete cytokines such as transforming growth factor beta (TGF-$\beta$) and tumour necrosis factor alpha (TNF-$\alpha$). In addition, peritoneal macrophages release cytokines such as interleukin-1 (IL-1), IL-6, and arachadonic acid metabolites. $^{142}$ These acute-phase proteins ultimately lead to the activation of repair mechanisms.

$\text{CO}_2$ gas insufflation also results in an inflammatory response within the peritoneal cavity resulting in an influx of lymphocytes and macrophages. $\text{CO}_2$ is an irritant to the peritoneum, causing the release of inflammatory mediators such as transforming growth factor alpha (TGF-$\alpha$). $^{412}$ In comparison to exposure to air, laparoscopic surgery and $\text{CO}_2$ pneumoperitoneum, has been shown to partially suppress the inflammatory response associated with surgery. $^{414, 415}$ Air exposure triggered a higher transmigration rate of PMN cells from peripheral circulation into the peritoneal cavity and decreased PMN cell apoptosis compared to $\text{CO}_2$. $^{414}$ Clinical and experimental studies have also demonstrated that laparoscopic surgery may preserve the systemic immune system better than open procedures. $^{416-418}$
Locally, peritoneal macrophages are an important aspect of the peritoneum’s first line of defence and CO₂ exposure has been shown to affect functioning of peritoneal macrophages in several conflicting studies. Several experimental studies suggest that CO₂ insufflation gas is associated with a significant impairment of peritoneal macrophage function. Its effects included reduction in cytokine production and diminished anti-tumour cell cytotoxicity. The effects of CO₂ on macrophage function also include impairment of peritoneal cell-mediated immunity. Chekan et al. found that the ability of murine peritoneal macrophages to clear Listeria monocytogenes, 3 days after exposure, is significantly reduced when insufflation occurred using CO₂ instead of helium.

In contrast, Moehrlen et al. found that after CO₂ pneumoperitoneum, when compared to laparotomy, resulted in peritoneal macrophages demonstrating a decreased basal TNF-α secretion rate but enhanced TNF-α release when an additional stimulus in the form of Escherichia coli was introduced. This suggests that CO₂ exposure led to enhanced macrophage immunocompetency.

One of the factors associated with peritoneal macrophage impairment is intraperitoneal hypothermia. During conventional laparoscopy, intraabdominal temperature has been demonstrated to decrease to as low as 27.7 °C. In animal and human studies, reduction in local peritoneal and core body temperature during laparoscopy can be prevented by the use of heated and humidified insufflation gas. Perioperative systemic hypothermia has been associated with increased wound infection, presumed to be related to impaired macrophage function and reduced tissue O₂ tension secondary to thermoregulatory vasoconstriction.
The review found one study that suggests peritoneal macrophage function might be related to peritoneal hypothermia. Puttick et al.\textsuperscript{402} randomised patients undergoing laparoscopic cholecystectomy to CO\textsubscript{2} gas insufflation at room temperature and at body temperature. They found that core body temperature fell significantly more in the patients receiving room temperature gas (0.42 \pm 0.23 \degree C versus 0.24 \pm 0.21 \degree C, p = 0.03). Furthermore, greater levels of peritoneal inflammatory cytokines were detected in the peritoneal fluid samples collected from patients who experienced hypothermic intraperitoneal conditions. These cytokines included TNF-\(\alpha\), IL-1, and IL-6.

This review also identified one other study that reported peritoneal cytokine levels in animal and human study subjects when insufflation gas conditions were altered. Margulis et al.\textsuperscript{401} investigated peritoneal cytokine levels measured in ten pigs randomised to standard CO\textsubscript{2} gas insufflation and to warmed, humidified gas insufflation achieved via the Insuflow\textsuperscript{\textregistered} (Lexion Medical, St. Paul MN, USA) device.\textsuperscript{425} When peritoneal TNF-\(\alpha\), IL-1\(\beta\), and IL-6 levels were compared at several postoperative time points between 1 and 48 hours, no statistically significant differences were found.

Alternatively, Davis et al.\textsuperscript{387} found that neither heating, humidification, or heating and humidification of CO\textsubscript{2} insufflation gas had an effect on peritoneal macrophage activity. Using peritoneal tissue biopsies taken before and after CO\textsubscript{2} pneumoperitoneum from three adult participants in each study group as well as those exposed to conventional insufflation in the control group, the investigators found that there was evidence of increased macrophage activity in only one sample from the heated and humidified group. In all other aspects, the samples showed no changes as compared to the control group.
In addition to mounting a defensive inflammatory response to adverse stimuli, the peritoneum possesses an inherent ability for repair and healing. While various cytokines, growth factors, and adhesion molecules regulate these processes, the peritoneal plasmin system also has a critical role. Tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) are the main plasminogen activators in the peritoneal cavity with plasminogen-activating inhibitors (PAI) regulating their activity.\textsuperscript{426} Intraabdominal surgical intrusion results in a rapid decline in peritoneal fibrinolytic as a result of increased levels of inhibitors and swift production of tPA by the visceral peritoneum.\textsuperscript{427}

A number of experimental and clinical studies have investigated the effects of CO\textsubscript{2} gas exposure during laparoscopy on peritoneal fibrinolysis and their results, overall, point to inhibition of the peritoneal plasmin system, an effect similar to that resulting from conventional open surgery.\textsuperscript{428,429} The magnitude of this hypofibrinolytic effect from laparoscopy, however, seems to be influenced by several factors including duration of operation,\textsuperscript{430} insufflation pressure,\textsuperscript{431} and extent of tissue handling.\textsuperscript{432}

This review identified one study suggesting that decreased intraabdominal temperature from insufflation gas related cooling could also affect peritoneal fibrinolysis. Brokelman et al.\textsuperscript{398} conducted a clinical trial of 30 patients undergoing laparoscopic cholecystectomy where participants were randomised to either CO\textsubscript{2} insufflation at room temperature or at body temperature (37 \textdegree C). Peritoneal biopsies were obtained at the start and at the end of surgery for assessment of tPA antigen, uPA antigen, and PAI-1 antigen tissue concentrations and tPA activity via ELISA. The investigators found that PAI-1 antigen levels at the end of surgery were significantly higher in patients exposed to room temperature gas while tPA
and uPA antigen levels were not significantly different between study groups. They concluded that cooling of the peritoneal cavity affects peritoneal inflammatory physiology via increased inhibition of plasmin activation leading to diminished fibrinolytic activity.

In contrast, the review identified a pair of clinical studies\textsuperscript{399,400} that investigated the effects of increasing intraabdominal pressure on peritoneal inflammation and repair. Analysis of peritoneal tissue obtained from 50 adult human study participants who underwent laparoscopic cholecystectomy facilitated by CO\textsubscript{2} pneumoperitoneum set at a variety of intraperitoneal pressures (10, 13, and 16 mmHg) found that this level of insufflation pressure had no significant effects on tissue concentrations of the growth factor TGF-\(\beta\)\textsubscript{1} and fibrinolytic factors tPA, uPA, and PAI-1.
This review has examined the relationship between CO$_2$ pneumoperitoneum and its effects on peritoneal surface structure and physiology. More specifically, the review summarised how altering the physical conditions of CO$_2$ insufflation gas effects peritoneal structure and physiology (Figure 4.2). Although it is the gas with the best safety profile, CO$_2$ causes several local peritoneal changes including acidosis, desiccation, hypothermia, and hypoxia. They affect peritoneal morphology and structure, metabolism, immune response, and healing processes but are themselves affected by insufflation gas temperature, humidity, composition, and pressure. The prevention of desiccation is associated with attenuation of microscopic structural changes as well as possible changes to the peritoneal inflammatory response. Peritoneal hypoxia induced by CO$_2$ pneumoperitoneum is likely preventable with the addition O$_2$ in small fractions resulting in improved peritoneal healing and reduced adhesion formation. Lastly, changing insufflation pressure likely has localised peritoneal effects that need further investigation. Whilst the majority of studies were based on animal models, a number of important clinical implications were highlighted including postoperative pain and recovery, formation of adhesions, and systemic hypothermia.
One of the challenges of investigating how the peritoneal surface is affected by CO$_2$ pneumoperitoneum is isolating the individual effects of desiccation, acidosis, hypoxia, hypothermia, increased intraabdominal pressure, and temporary mechanical stretching and expansion of the peritoneum. To do this, not only do investigators have to study the effects of other gaseous agents but also alter insufflation temperature to prevent peritoneal cooling, alter relative humidity to prevent desiccation, alter insufflation pressure to avoid excessive divergence from normal intraperitoneal pressure, and add O$_2$ gas to produce a non-hypoxic gaseous environment. Whenever one is altered, the rest must remain constant.
The duration of gas insufflation is another factor that has been highlighted although surgeons may not be so readily able to alter it. Furthermore, insufflation duration may not be as significant as insufflation pressure. Suematsu et al. \(^{405}\) found that intercellular clefs were more evidence immediately after CO\(_2\) pneumoperitoneum at 10 mmHg for 30 minutes than after CO\(_2\) pneumoperitoneum at 5 mmHg for 30 minutes.

It has been hypothesised that one of the key mechanisms by which the pneumoperitoneum damages peritoneal structure and physiology is desiccation\(^{433}\) given that room temperature gas is typically used without humidity conditioning, i.e. at 21 °C with 0-2% relative humidity.\(^{434}\) Furthermore, large volumes of gas are commonly required for a single patient owing to the imperfect seal of the laparoscopic ports and peritoneal absorption of CO\(_2\) gas. The peritoneal cavity, in comparison, is normally at 36 °C with a thin film of fluid covering the surfaces that is 100% relative humidity in a steady state of fluid equilibrium.\(^{384}\) Exposure to relatively cool and dry CO\(_2\) gas in significant volume results in changes desiccation, similar to those observed after open abdominal surgery with ballooning of mesothelial cells, their detachment from the basement membrane, and appearance of denuded areas.\(^{405, 435}\)

In fact, any inflammatory or ischaemic peritoneal injury including surgical trauma or bacterial infection, will cause desquamation of injured mesothelial cells. As described in Chapter 1, this leaves denuded areas and triggers an inflammatory response characterised by cellular infiltration, accumulation of a serosanguinous exudate, and a growth response by the mesothelial cells.\(^{436}\) In normal conditions, mesothelial cells proliferate at a limited speed: only 0.16 to 0.5% of mesothelial cells are in mitosis at any one time. But this rate
increases to 30-60% when the peritoneum is injured, mostly due to increased levels of growth factors and cytokines.

Based on the results of this review, preventing peritoneal desiccation with use of warmed humidified CO₂ insufflation results in significantly attenuated intraperitoneal cytokine production. IL-1 and IL-6 reflect activation of peritoneal macrophages and neutrophils as well as that of mesothelial cells. It has been demonstrated that both serum and peritoneal levels of pro-inflammatory cytokines (IL-6, TNF-α, and IL-1β) increase after surgery and correlate with the magnitude of surgical stress. Raised levels in the postoperative period may also be associated with occurrence of postoperative complications.

TNF-α and IL-1β mediate many non-hepatic manifestations of the acute phase response such as fever, tachycardia, leucocytosis, and activation of a systemic immune response. In contrast, IL-6 is a major modulator of hepatic acute phase protein synthesis. Transforming growth factor-beta (TGF-β1) is a naturally occurring growth factor involved in peritoneal healing, adhesion formation, and dissemination of malignancies. Found in platelet, macrophages, and wound fluid, TGF-β is activated by plasmin during the acute phase of inflammation and, by stimulating fibroblastic production of collagen and fibronectin, it contributes to synthesis of the extracellular matrix (ECM) and also tissue fibrosis. Over-expression of TGF-β has been associated with increased adhesion formation.

While the studies in this review form an incomplete representation of how CO₂ pneumoperitoneum leads to local intraperitoneal acidosis, they do support what is already
known. CO\textsubscript{2} gas is readily absorbed and also dissolves into peritoneal fluid forming carbonic acid.\textsuperscript{412} When used for insufflation, it lowers peritoneal pH significantly compared to other gaseous agents such as helium.\textsuperscript{394} Furthermore, CO\textsubscript{2} pneumoperitoneum also decreases local tissue O\textsubscript{2} partial pressure leading to mesothelial hypoxia and peritoneal acidosis, which to some extent is preventable by the addition of O\textsubscript{2}.\textsuperscript{394} Increasing insufflation pressure contributes to increasing severity of peritoneal acidosis\textsuperscript{397} likely as a result of tension in the abdominal wall leading to reduced blood flow and gas exchange and the accumulation of hypoxic metabolism end-products (hydrogen ion, lactate, and ketones).\textsuperscript{411} Low cardiac output, a systemic haemodynamic change associated with raised intraabdominal pressure, may further decrease peritoneal blood flow.\textsuperscript{444}

The combination of processes affecting peritoneal inflammation is complex and dependent on the balance between several opposing mechanisms. In addition to the disturbances caused by physical changes associated with CO\textsubscript{2} insufflation, the metabolic changes associated with hypoxia also affect the host immune response. In response to a systemic lipopolysaccharide (LPS) challenge, acidification of the peritoneal cavity has been shown to increase serum IL-10 levels and decrease serum TNF-\textalpha levels. Furthermore, the degree of peritoneal acidification correlated with the degree of reduction in systemic inflammatory response.\textsuperscript{445} The attenuation of peritoneal inflammation by CO\textsubscript{2}-related peritoneal acidification has been demonstrated by several other studies.\textsuperscript{414} These effects are not only limited to cytokine production but are also present at the level of cellular function.

With a diverse range of study outcomes, the findings from this review carry several clinical implications. These include formation of postoperative adhesions, enhanced by peritoneal
hypoxia and impaired fibrinolysis, as well as an amplified inflammatory response to surgical trauma. Locally altered peritoneal immunity raises concerns regarding the host's ability to counter tumour cell seeding and bacterial dissemination. This is particularly relevant as laparoscopic procedures are now regularly indicated for various surgical infections such as appendicitis and diverticulitis complicated by secondary peritonitis. Even in the absence of pre-existing intraperitoneal pathology, local effects of CO₂ pneumoperitoneum may have clinical implications for postoperative pain and recovery. The peritoneal neuro-immuno-humoral axis, as mentioned in Chapter 1, is fundamental to this concept, allowing the peritoneum to expressly communicate with the central nervous system (CNS), conveying messages of inflammation via a direct route.

Postoperative pain after laparoscopic surgery has already been extensively investigated and there is evidence to suggest that the dominant source of pain and discomfort is from the peritoneum rather than skin or abdominal wall. The degree of postoperative pain has already been linked to the type of insufflation gas used, the pressure created by pneumoperitoneum, and the temperature of the gas. Furthermore, the humidity of insufflation gas appears to be clinically important as well, to postoperative pain as well as perioperative core body temperature regulation. The fact that intraperitoneal cytokine levels are reduced after humidified insufflation is particularly important given that IL-6, released during abdominal surgery is a sensitive marker of tissue damage induced by mechanical or thermal injury. TNF-α concentrations in peritoneal fluid have been shown to correlate to symptoms of abdominal pain in women affected by endometriosis.
Lastly, it is important to appreciate that physiological changes in the peritoneum can ultimately lead to systemic changes. Hypoxia of the peritoneum during pneumoperitoneum has been linked to systemic acid-base imbalances and the creation of a metabolic acidosis. Changes in insufflation pressure can affect the rate of transperitoneal CO\textsubscript{2} absorption. The resulting hypercapnia has important consequences for systemic haemodynamics and intraabdominal organ blood flow. Studies in this field are, however, difficult to conduct using human subjects and the results from animal studies require further correlation studies in order to achieve applicability. CO\textsubscript{2} pneumoperitoneum pressure correlations between rodents and humans have already been the subject of several studies.

Aside from the limitations of individual studies, the review as a whole was weakened by several collective limitations. As mentioned already, the majority of reviewed studies were animal experiments with restricted applicability to human patients. Sample sizes were also generally small and the heterogeneity amongst studies further diluted the size of common findings. Finally, the effects of a number of CO\textsubscript{2} insufflation features were investigated but on the whole, studies did not seek to determine critical cut-off points at which certain effects on the peritoneum became significant. For example, it remains unknown whether the correlation between peritoneal morphological changes and warming and humidifying CO\textsubscript{2} insufflation gas is a linear or exponential relationship, whether it plateaus, and at what point this occurs.
In conclusion, peritoneal morphology and structure, metabolism, immunity, and healing after laparoscopic surgery are significantly influenced by the physical conditions of CO₂ gas insufflation. Relevant factors include gas temperature, humidity, composition, and pressure. The prevention of desiccation is associated with attenuation of microscopic structural changes as well as possible changes to the peritoneal inflammatory response. Peritoneal hypoxia induced by CO₂ pneumoperitoneum likely contributes to adhesion formation and the addition of O₂ appears to reduce its effects. Finally, in addition to systemic effects, insufflation pressure has localised peritoneal effects that need further investigation. A number of important clinical implications were highlighted by the studies in this review including postoperative pain and recovery, formation of adhesions, and systemic hypothermia. Well-designed and adequately powered clinical trials are now needed to further delineate the association between these issues and peritoneal morphology and function.
Chapter 7

CLINICAL TRIAL C

Warm humidified gas insufflation during laparoscopic appendicectomy for childhood acute appendicitis: A double-blinded randomised controlled trial
7.1 INTRODUCTION

Laparoscopic surgery requires creation of intraabdominal working space typically facilitated by a carbon dioxide (CO₂) pneumoperitoneum i.e. insufflation of CO₂ gas into the peritoneal cavity. Despite its wide acceptance, the CO₂ pneumoperitoneum produces a number of characteristic alterations to peritoneal surface and physiology closely associated with the inherent properties of CO₂ gas and the physical conditions of insufflation. As highlighted in Chapter 6, the desiccating effects of insufflation appear to be particularly important, associated not only with microscopic alterations to peritoneal morphology but also with possible changes to its inflammatory response.

By definition, medical grade CO₂ for insufflation requires impurity of less than 200 parts per million including water vapour so that its relative humidity approaches 0%.\(^\text{457}\) Compressed liquid CO₂ is transported in cylinders at a pressure approximately 40 times greater than atmospheric pressure and when released, its temperature is approximately -90 °C.\(^\text{385}\) As it flows through standard insufflation devices and tubing, its temperature reaches room temperature (19-21 °C) rapidly\(^\text{402}\) but without further active warming and conditioning, it is relatively cold and dry compared to the peritoneal cavity’s natural environment which is moist and at core body temperature (36 °C and virtually 100% relative humidity).\(^\text{384}\) Peritoneal desiccation and local hypothermia stems from this difference in temperature and relative humidity.
In order to avoid exposing the peritoneum to such foreign conditions, warming and humidifying CO₂ gas prior to its delivery into the peritoneal cavity has been investigated with some promising effects. The structural alterations to peritoneal surface morphology can be prevented to an extent if insufflation gas is warmed to body temperature and humidified to near full saturation. Furthermore, conditioning of insufflation gas in this way may also have meaningful effects on peritoneal cytokine response and fibrinolytic pathways.

Clinically, desiccation-related peritoneal injury has been linked to a conscious sensation of pain in patients undergoing awake-laparoscopy. Two meta-analyses have concluded that the use of warm humidified CO₂ insufflation results in reduced postoperative pain and analgesia consumption.

Furthermore, conventional insufflation without conditioning has been linked to peritoneal hypothermia and a drop in core body temperature as a result of evaporative heat loss from intra-abdominal tissue in both animal models and adults. By maintaining the normal moist physiological condition of the intraperitoneal cavity during laparoscopy, warm humidified insufflation gas may also reduce risks of perioperative hypothermia. This is particularly relevant in paediatric surgery owing to the larger body surface area-to-volume ratio of children and infants and a thinner insulating tissue layer, predisposing them to intraoperative hypothermia.
To date, the clinical benefits of warm humidified insufflation gas has not been investigated in the setting of paediatric surgery and acute appendicitis. Thus, this Chapter outlines the implementation and findings of an adequately powered, prospective study, conducted to test the hypothesis that warm humidified CO\textsubscript{2} insufflation gas during laparoscopic appendicectomy would reduce postoperative pain and speed recovery in children by reducing peritoneal desiccation-related inflammation and injury.
7.2 METHODS

7.2.1 Participants

The study population was New Zealand children, between the ages of 8 and 14 years, residing within or visiting the catchment areas of the three District Health Boards (DHB) that serve the wider Auckland region (Auckland DHB, Counties Manukau DHB, and Waitemata DHB) between 10 February 2010 and 12 March 2011. Starship Children’s Hospital is the single tertiary centre providing paediatric general surgical care to this catchment area. During study recruitment, all such patients undergoing laparoscopic appendicectomy for clinically diagnosed appendicitis at Starship Children’s Hospital were assessed for eligibility to participate. Once deemed eligible, details of the study were explained to patients and their family/whānau members by the primary investigator (T Yu) or the operating registrar and consultant. Participant information sheets (Appendix E) were also available and written informed consent to participate was obtained.

The study exclusion criteria were installed to ensure that the sample population was a homogeneous group of healthy, volunteer children who were able to co-operate with data collection. They were as follows:

- Diagnosis of mental retardation, developmental delay, neuromuscular impairment, attention-deficit disorder, chronic pain, or any psychiatric illness
- Previous abdominal surgery and/or the presence of any abdominal prosthesis e.g. gastrostomy
- Immunosuppression including chronic use of or dependency on steroids
• Unable to speak and read English
• Partially-sighted or blind
• Appendicectomy not performed as planned
• Significant violation of Study Analgesia and Anaesthetic Protocol including instalment of a regional nerve block or contraindication to morphine
• Written consent not obtained preoperatively from participant and a parent or equivalent legal guardian

All participants who underwent conversion to open appendicectomies were included in the final data analysis. Decision to convert was at the discretion of individual surgeons.

### 7.2.2 Intervention

#### 7.2.2.1 Preoperative care

All study participants presented to Starship Children’s Hospital acutely during the study period. Appendicitis was diagnosed clinically and preoperative care consisted of resuscitation with intravenous (IV) fluids and administration of systemic antibiotics and analgesia. The choice and dosage of these were left to the discretion of admitting surgical residents.

#### 7.2.2.2 Surgery

Surgery was scheduled according to acuity prioritisation protocols at Starship Children’s Hospital. All participants underwent planned laparoscopic appendicectomy under the supervision of consultant paediatric surgeons. Laparoscopic access was standardised to three laparoscopic ports: periumbilical 10 mm port, suprapubic midline 5 mm port, and left
iliac fossa 5mm port. Insufflation pressures did not exceed 14 mmHg. The standard local anaesthetic agent was 0.25% Bupivacaine with Adrenaline (Marcaine 0.25% with Adrenaline 1:400,000 Injection, AstraZeneca Ltd, Auckland, NZ) permitted to the maximum dosage of 1 mL per kg (equivalent to 2.5 mg per kg) for infiltration into subcutaneous tissue and fascia at each laparoscopic port site at the beginning and/or end of each procedure. Skin closure was performed using only absorbable sutures. Technical aspects of laparoscopic appendicectomy that were unrelated to the study’s analgesia protocol were also left to the discretion of operating surgeons.

7.2.3 Anaesthesia and Postoperative Care

A standardised Anaesthesia and Analgesia Protocol (Appendix F) was developed in collaboration with a paediatric anaesthetist and pain specialist from the Department of Anaesthesia, Starship Children’s Hospital. During the study period, this protocol was actively promoted and advertised perioperatively. Verbal agreement to adhere to the protocol was obtained from anaesthetists prior to participants undergoing anaesthetic induction.

For intraoperative analgesia, the protocol permitted administration of morphine (Morphine sulphate injection, Biomed Ltd, Auckland, NZ) up to doses of 0.3 mg per kg and fentanyl (Fentanyl injection, Boucher & Muir NZ Ltd, Auckland, NZ), at 2 µg per kg, titrated as required. A single dose of IV paracetamol (Perfalgan 500 mg Solution for Infusion, Bristol-Myers Squibb Australia, Noble Park VIC, Australia), dosed at 15 mg per kg, was permitted if it had not been administered preoperatively. Prophylactic antiemetic was standardised to IV ondansetron (Zofran injection, GlaxoSmithKline, Boronia VIC, Australia) dosed at 0.15 mg per kg. Aside from morphine and fentanyl, the protocol asked anaesthetists to avoid other
forms of intraoperative analgesia including non-steroidal anti-inflammatory agents, alternative opioids, and dexamethasone. Epidural, spinal, intrathecal, and regional analgesia were not permitted as part of the study analgesia protocol. Choice of induction and muscle relaxant agents was left to the discretion of anaesthetists. Ambient temperature in the operating theatre was set to 20 °C at the start of all procedures. Use of upper-body forced-air-rewarming blankets and the choice, volume, and temperature of IV fluids given were left to the discretion of anaesthetists.

Postoperatively, study participants received standardised analgesia that included regular oral paracetamol (Panadol, GlaxoSmithKline, Auckland, NZ) and diclofenac sodium (Voltaren, Novartis, Auckland, NZ) supplemented by rescue opiate analgesia. In the first postoperative 24 hours, this was administered intravenously if required. The study institution’s morphine protocol was used to standardise administration of IV morphine (Appendix K). Participants who weighed less than 50 kg were given boluses of 0.04 mg per kg while those who weighed 50 kg and above were given boluses of 2 mg. Each bolus was followed by patient re-assessment after 5 minutes and subsequent boluses given if required. Participants who required more than five titrations of morphine within a 25-minute period were provided with a patient controlled analgesia (PCA) pump device to ensure adequate and efficient pain control. Intravenous morphine or fentanyl was the drug of choice for PCA administration and prescriptions were all approved by the institution’s Acute Pain Service.

Oral opioids were offered to study participants after the initial 24-hour postoperative period. They included oral morphine as the preferred first-line therapy (Morphine sulphate,
Douglas Pharmaceuticals Ltd, Auckland, NZ) and Tramadol (Tramadol Hydrochloride, AFT Pharmaceuticals Ltd, Auckland, NZ). Oral morphine was prescribed at a dosage of 0.3 mg per kg and administered every 1 to 2 hours as required.

In addition to analgesia, all other aspects of postoperative care were standardised. These included regular monitoring of vital signs, early re-feeding and mobilisation, and daily review of wounds. Cefoxitin (Cefoxitin sodium injection, Mayne Pharma Pty Ltd, Mulgrave VIC, Australia) was administered every 8 hours at doses of 25 to 30 mg per kg (up to a maximum of 2 g per dose) to participants diagnosed with non-gangrenous, nonperforated appendicitis. Participants with gangrenous or perforated appendicitis were treated with antibiotics for 5 days or more depending on response to therapy and surgeon preference.

7.2.2.4 Intervention group

Intervention Group participants received warm (37 °C), humidified (98% relative humidity) medical grade CO₂ insufflation gas (BOC Ltd, Auckland, NZ) delivered by the Fisher & Paykel MR860 Laparoscopic Humidification System (Fisher & Paykel Healthcare, Auckland, NZ). This device was specifically designed for this purpose and its effectiveness has been independently confirmed.²⁴¹ Its water chamber is filled with 30 mL sterile water and warmed by a heating plate. As gas flows from the insufflator through the chamber, it transports the sterile water vapour. The temperature of the humidified gas is maintained as it is delivered via a custom-built heating tube from the chamber to the laparoscopic port and into the patient’s peritoneal cavity. Both the temperature and flow rate of insufflation gas are constantly monitored by the humidification device via a sensor probe attached to
the chamber’s outlet. The set temperature of the insufflation gas is maintained via this feedback loop mechanism controlling the temperature of the heating plate.239

7.2.2.5 Control group

Participants in the Control group received standard carbon dioxide medical gas for insufflation (BOC Ltd, Auckland, NZ) delivered at room temperature (20-21 °C) and 0% relative humidity.

7.2.3 Objectives

The study objective was to determine whether warm humidified CO₂ insufflation gas for laparoscopic appendicectomy in children with suspected appendicitis would confer clinical benefits by reducing postoperative pain and enhancing recovery.

7.2.4 Outcomes

Data were all collected by the primary investigator (T Yu) to ensure standardisation. She remained blinded until all data collection and analysis were completed. A data collection form was created for this study (Appendix G).

7.2.4.1 Baseline characteristics

Baseline data collected from all participants included age, gender, self-identified ethnicity, weight in kilograms, duration of symptoms in days, presenting vital observations (tympanic temperature, heart rate, blood pressure) and serum inflammatory markers (white blood cell count, segmented neutrophil count, C-reactive protein). Usage of opiate analgesia preoperatively was recorded and converted to Morphine Equivalent Daily Dosage (MEDD)
for comparison. The histological diagnoses made by pathologists examining the appendiceal specimens were also recorded.

7.2.4.2 Primary outcome

The primary study outcome was postoperative requirement for opiate analgesia during the index hospital admission including usage in the Post-Awesia Care Unit (PACU). The number of patients requiring postoperative opiate analgesia in each group was recorded as well as quantities required by each participant. Opiate usage was converted to MEDDs for comparison. Both the absolute MEDD and MEDD per kg of patient weight were compared.

7.2.4.3 Secondary outcomes

- **Pain intensity scores** – Intensity of postoperative pain at rest and on moving was assessed using a VAS at 8 time points: 2, 4, 6, 8, 10, 12, 24, and 48 hours (Appendix J). Participants awake at these time points were asked to provide pain scores. They were familiarised with the VAS and instructed that anchors at the ends of the 10 cm line represented “completely no pain” (0) and “the worst imaginable pain” (10).

- **Intraoperative core body temperature** – Core body temperature was measured intraoperatively at 10 minute intervals using a naso-oesophageal temperature probe (General Purpose Probe GP9400, Truer Medical Inc., Orange CA, USA) placed by the anaesthetist. Baseline temperature was recorded at the start of the procedure, just prior to insufflation of the peritoneal cavity. Absolute change in temperature from the start of the procedure to the end, as well as the maximum, minimum, difference between maximum and minimum, and the mean temperatures were recorded or calculated for comparison between intervention and control groups.
• Postoperative recovery and return to normal activities – Discharge criteria were clinical: afebrile consecutively for 24 hours, return of bowel function suggested by adequate oral intake and passage of flatus or bowel motion, independent mobilisation, and adequate pain control with oral analgesia. The day that these were met and the actual day of hospital discharge were both recorded. The number of nights participants spent in hospital postoperatively was compared and total number of nights in hospital for the index admission was also compared. Readmission was defined as return to hospital for 24 hours or more within 30 days of discharge. Quality of postoperative recovery and return to normal activities was evaluated using a 10-item questionnaire (Appendix H) developed using the Pediatric Quality of Life Inventory (Pediatric QL™) Version 4.0. It invites participants to respond to each item using a five-level Likert scale (Never, Almost Never, Sometimes, Often, and Almost Always), rating the difficulty they had performing or participating in common daily activities including running, lifting something heavy, taking a bath or shower by themselves, helping out around the house, paying attention to television or a book, and sleeping. The questionnaire also asked participants to rate how often they had pain, experienced low energy, was forgetful, and worried about what would happen to them. The questionnaire lists these ten items twice and participants were asked to first rate themselves when they are healthy and well and then rate themselves again on day 10 after their laparoscopic appendicectomy. Participants were provided with the questionnaire and a stamped and addressed return envelope upon hospital discharge.

• Perioperative complications – Complications up to 6 weeks after surgery were prospectively recorded during the index hospital admission, during subsequent readmissions, and also via a follow-up telephone call made to participants.
Standardised definitions for perioperative complications were used and severity was graded using the Clavien-Dindo classification.\textsuperscript{250}

7.2.4.4 Other variables recorded

- \textit{Operative data} – Collected intraoperative data included date of operation, operation performed, surgical approach (laparoscopic or laparoscopic converted to open), macroscopic diagnosis made by operating surgeon (normal, acutely inflamed, gangrenous, or perforated appendix), operation start (scalpel to skin) and end (all wound dressings applied) times, gas insufflation start and end times, volume of insufflation gas used, maximum gas flow rate (L/hour), maximum insufflation pressure (mmHg), presence of peritoneal infection and/or contamination with pus and/or faeces, use of peritoneal 0.9\% saline washout, use of forced-air-rewarming blankets, and dosages of opiate analgesia administered. At the end of each procedure, the operating surgeon rated the severity of laparoscopic camera fogging and technical difficulty of the procedure using visual analogue scales (VAS) consisting of 10 cm lines anchored at both ends with 0 and 10 (0 = no fogging with perfect images, 10 = worst fogging with very poor images; 0 = no difficulties encountered, 10 = worst level of difficulty) (see Appendix I).

- \textit{Adherence to study protocol} – The study’s Analgesia and Anaesthetic Protocol was actively promoted prior to and during the study and adherence was prospectively recorded. Protocol non-adherence included the administration of intraoperative analgesia other than morphine and fentanyl, administration of dexamethasone, and failure to administer regular doses of simple analgesia in the postoperative period.
7.2.5 Sample Size

Power calculation was performed \textit{a priori} using retrospective data collected from the study institution. The postoperative opiate consumption of 21 children (aged 5-14 years) who underwent laparoscopic appendicectomy facilitated by conventional CO$_2$ insufflation was examined. In the postoperative period, 15 (71%) children required rescue opiate analgesia in addition to regular simple analgesia. In order to detect a 30% reduction in the number of children requiring postoperative opiate analgesia, a 2-tailed Fisher’s exact test demonstrated that 95 participants would be required in each study arm with alpha of 0.05 and power of 0.8.

Subgroup analysis was planned \textit{a priori} in order to determine whether benefits associated with warm humidified CO$_2$ insufflation varies with the severity of appendicitis. Patients not found by operating surgeons to be affected by appendicitis-related secondary peritonitis (macroscopic appendiceal perforation and/or abscess, and/or free pus in the peritoneal cavity) were included in a subgroup comparison analysis of the study’s primary outcome: postoperative opiate consumption. No interim analyses were conducted.

7.2.6 Randomisation

7.2.6.1 Sequence generation

The generation of random numbers was facilitated by an independent research assistant. All numbers from 1 to 200 were generated in random sequence and arranged into two columns using an open-source computer-based online random number program (www.random.org). Study allocations were based on these randomly sequenced numbers.
7.2.6.2 Allocation concealment

Randomised study allocations were placed into 200 opaque numbered envelopes by the same research assistant who had no subsequent involvement in this study. The envelopes were then securely sealed and kept in a central location. They were opened strictly in sequence and only in the operating room just prior to the start of each study procedure.

7.2.6.3 Implementation

Participants were recruited preoperatively by the primary investigator (T Yu) or the operating surgeon. Written consent for participation was obtained from both the child and one legal adult guardian (see Appendix E for Participant Consent Forms). Once recruited, an unblinded rotating theatre nurse appointed by study investigators assisted with the randomised allocation of each participant into Intervention or Control groups immediately prior to the start of surgery. This nurse was instructed not to disclose the participant’s group allocation to anybody and they had no subsequent involvement in the postoperative care of study participants, or in data collection, analysis, and reporting.

7.2.7 Blinding

The patient, study investigators, surgeon, anaesthetist, theatre personnel, and ward nursing staff responsible for intra- and postoperative care of participants all remained blinded to group allocations. As described above, one unblinded rotating scrub nurse assisted with randomisation by opening the allocation envelope and setting up the surgical humidifier according to assigned allocation. Prior to participants entering the operating theatre, the unblinded scrub nurse prepared the device away from view of other theatre personnel. When participants were in the Intervention Group, 30 mL sterile water was added to the
water chamber and the humidifier was switched on then muted. When participants were in the Control Group, water was not added and the humidifier was left off. A commissioned opaque plastic cover was designed to conceal the surgical humidifier from view during each study procedure. It covered the front LED screen as well as the water chamber so that it was impossible for theatre occupants to tell whether the device was switched on and whether the chamber contained water. At the end of a procedure, the unblinded scrub nurse removed the humidifier from theatre with its cover intact and disassembled it away from view of theatre personnel. To ensure accurate co-ordination of all the above steps, taken to accomplish double-blinded and randomised group allocation, a 1-week rehearsal period was conducted just prior to trial implementation.

Data collection and analysis was carried out by the primary research investigator (T Yu). Blind statistical analysis was facilitated by a distant research assistant who had no further involvement in the study. This individual prepared data spreadsheets with concealed study group allocations (Group X and Group Y). Allocations were then only revealed at the completion of data analysis. The effectiveness of blinding was tested at the end of each study procedure by asking the primary surgeon to answer “yes” or “no” to the following question: “Is the participant in the Intervention Group (warm humid gas insufflation)?” (see Appendix I). Individual responses were compared to actual study allocations once these were revealed.

7.2.8 Statistical Analysis

Data were analysed using PASW Statistics for Windows Version 18.0 (SPSS Inc., IBM, Chicago IL, USA). Continuous variable parametricity was tested using the Shapiro-Wilk test. Data that
are parametric in nature are presented as mean and standard deviation and those that are nonparametric are presented as median and inter-quartile range. Parametric continuous variables were compared using student’s t-tests while nonparametric continuous variables were compared using Mann-Whitney $U$ tests. Remaining categorical variables were compared using either the Fisher’s exact test or Pearson chi-squared test. Statistical significance throughout the study was defined as a $p$-value of $< 0.05$.

**7.2.9 Ethics Approval and Trial Registration**

Ethics approval was granted by the Northern X Regional Ethics Committee, New Zealand Government Ministry of Health, in October 2009 (Committee Reference number NTX/08/03/019). Approval was also granted by the study institution’s Research Review Committee. The trial was prospectively registered online with ClinicalTrials.gov (Trial Identifying Code: NCT01027455, US National Library of Medicine, 8600 Rockville Pike, Bethesda MD, USA).
7.3 RESULTS

7.3.1 Participant Selection and Recruitment

The flow of study participants through each stage of the trial is detailed in Figure 7.1 in concordance with the CONSORT statement. Over a 13-month period, from February 2010 to March 2011, a total of 257 consecutive patients between the ages of 8 and 14 years were screened for eligibility. Of these, 195 were randomised but 5 subsequently excluded from data analysis due to major protocol violation and the study’s exclusion criteria.
Figure 7.1 CONSORT Statement Diagram Detailing Participant Recruitment and Numbers Analyzed

Enrollment

Assessed for eligibility (n=257)

Excluded (n=62)
- Not meeting inclusion criteria (n=18)
- Previous abdominal surgery (n=1)
- Did not read or speak English (n=8)
- Chronic pain syndrome (n=4)
- Allergy to or contra-indication for morphine or NSAIDs (n=3)
- Dependency on steroids (n=2)
- Declined to participate (n=10)
- Investigators not available (n=31)
- Enrolled but not randomised (n=3)

Randomized (n=195)

Allocation

To INTERVENTION (n=97)
- Received INTERVENTION (n=97)

To CONTROL (n=98)
- Received CONTROL (n=98)

Follow-Up

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Analysis

Analysed (n=95)
Excluded from analysis (n=2)
- Alternative diagnosis made (retrograde menstruation, mesenteric lymphoid hyperplasia); appendix not removed (n=2)

Analysed (n=95)
Excluded from analysis (n=3)
- Alternative diagnosis made (infected Meckel’s diverticulum, haemorrhagic ovarian cyst); appendix not removed (n=2)
- Transversus abdominis plane regional block administered (n=1)
7.3.2 Participant Number Analysed

Data analysis included 190 participants equally divided between the two study groups. Data collection for the primary study outcome was completed for all study participants. Data for other study variables measured during the index hospital admission were complete for 164 (86%) study participants. A total of 146 (77%) Day 10 Postoperative Recovery Questionnaires were returned: 78 (82%) from participants in the Intervention Group and 68 (72%) from participants in the Control Group. Participants were followed-up 6 weeks post-discharge via a telephone call and data collection was completed up to this point for 178 (94%) participants.

7.3.3 Baseline Characteristics

At baseline, the Intervention and Control Groups were well matched (Table 7.1). Preoperative opiate analgesia usage by all participants was converted to MEDD for the 24 hours prior to surgery, and when compared, no significant differences were found between the Intervention and Control Groups.
## Table 7.1 Participant Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Intervention [n=95]</th>
<th>Control [n=95]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median in years (IQR)</td>
<td>12 (3)</td>
<td>12 (3)</td>
<td>0.380†</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.764*</td>
</tr>
<tr>
<td>Male</td>
<td>61 (64.2%)</td>
<td>59 (62.1%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>34 (35.8%)</td>
<td>36 (37.9%)</td>
<td></td>
</tr>
<tr>
<td>Weight, mean in kg (SD)</td>
<td>49.6 (16.4)</td>
<td>50.3 (16.1)</td>
<td>0.761‡</td>
</tr>
<tr>
<td>Duration of symptoms, median in days (IQR)</td>
<td>2 (1)</td>
<td>1 (1.5)</td>
<td>0.715†</td>
</tr>
<tr>
<td>Presenting tympanic temperature, mean in °C (SD)</td>
<td>37.1 (0.7)</td>
<td>37.0 (0.6)</td>
<td>0.808‡</td>
</tr>
<tr>
<td>Presenting heart rate, mean in bpm (SD)</td>
<td>89.5 (16.6)</td>
<td>90.4 (17.9)</td>
<td>0.720‡</td>
</tr>
<tr>
<td>Presenting WBC, mean in x10⁹/L (SD)</td>
<td>13.1 (5.1)</td>
<td>12.5 (5.1)</td>
<td>0.466*</td>
</tr>
<tr>
<td>Presenting neutrophil count, mean in x10⁹/L (SD)</td>
<td>10.2 (4.9)</td>
<td>9.5 (5.3)</td>
<td>0.356‡</td>
</tr>
<tr>
<td>Presenting CRP, mean in mg/L (SD) [n]</td>
<td>35.3 (58.7) [86]</td>
<td>38.8 (69.1) [84]</td>
<td>0.721‡</td>
</tr>
<tr>
<td>Macroscopic diagnosis</td>
<td></td>
<td></td>
<td>0.948*</td>
</tr>
<tr>
<td>Normal</td>
<td>14 (14.7%)</td>
<td>13 (13.7%)</td>
<td></td>
</tr>
<tr>
<td>Acute inflammation +/- suppuration</td>
<td>54 (56.8%)</td>
<td>55 (57.9%)</td>
<td></td>
</tr>
<tr>
<td>Gangrenous</td>
<td>11 (11.6%)</td>
<td>9 (9.5%)</td>
<td></td>
</tr>
<tr>
<td>Perforation</td>
<td>16 (16.8%)</td>
<td>18 (18.9%)</td>
<td></td>
</tr>
<tr>
<td>Presence of peritoneal infection</td>
<td>25 (26.3%)</td>
<td>29 (30.5%)</td>
<td>0.489*</td>
</tr>
<tr>
<td>Histological diagnosis</td>
<td></td>
<td></td>
<td>0.829*</td>
</tr>
<tr>
<td>Normal</td>
<td>17 (17.9%)</td>
<td>21 (22.1%)</td>
<td></td>
</tr>
<tr>
<td>Acute inflammation +/- suppuration</td>
<td>58 (61.1%)</td>
<td>50 (52.6%)</td>
<td></td>
</tr>
<tr>
<td>Gangrenous</td>
<td>4 (4.2%)</td>
<td>4 (4.2%)</td>
<td></td>
</tr>
<tr>
<td>Perforation</td>
<td>15 (15.8%)</td>
<td>19 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>Other than appendicitis</td>
<td>1 (1.1%)</td>
<td>1 (1.1%)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** n = number of participants included; IQR = inter-quartile range; SD = standard deviation; bpm = beats per minute; WBC = white blood cell count; CRP = C-reactive protein; * Pearson chi-square test; ‡ Student’s t-test; † Mann-Whitney U test.
Appendicectomies were performed by 20 different surgeons (5 consultant surgeons, 15 registrars). The operative duration, gas insufflation duration, volume of CO₂ gas used, and conversion rates were statistically similar (Table 7.2). Total opiate analgesia usage by each study participant was converted to MEDD and found to be statistically similar when compared. The operative technical difficulty, based on ratings by operating surgeons for 170 of the study procedures, was also found to be similar. Severity of laparoscopic camera lens fogging was not found to be statistically different. Intraoperative core body temperature variations were not significantly different between the Intervention and Control groups.

### Table 7.2 Intraoperative Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intervention [n=95]</th>
<th>Control [n=95]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conversion to open</td>
<td>5 (5.3%)</td>
<td>4 (4.2%)</td>
<td>0.493‡</td>
</tr>
<tr>
<td>Operating time, mean in minutes (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total duration</td>
<td>69.8 (31.3)</td>
<td>71.6 (29.2)</td>
<td>0.685‡</td>
</tr>
<tr>
<td>Insufflation duration</td>
<td>43.3 (17.9)</td>
<td>44.6 (22.8)</td>
<td>0.670‡</td>
</tr>
<tr>
<td>Volume of CO₂ used, mean in litres (SD)</td>
<td>54.9 (35.8)</td>
<td>60.7 (37.6)</td>
<td>0.278†</td>
</tr>
<tr>
<td>Technical difficulty of procedure by VAS, mean (SD) [n]</td>
<td>4.3 (2.6) [85]</td>
<td>4.3 (2.5) [85]</td>
<td>0.931†</td>
</tr>
<tr>
<td>Severity of camera lens fogging by VAS, mean (SD) [n]</td>
<td>3.8 (2.3) [83]</td>
<td>3.4 (2.4) [81]</td>
<td>0.329‡</td>
</tr>
<tr>
<td>Forced-air-warming blanket used</td>
<td>22 (23.2%)</td>
<td>30 (31.6%)</td>
<td>0.255‡</td>
</tr>
<tr>
<td>Core Body Temperature, mean in °C (SD) [n]</td>
<td>36.9 (0.7)</td>
<td>36.8 (0.6)</td>
<td>0.201‡</td>
</tr>
<tr>
<td>Start of procedure</td>
<td>37.0 (0.7)</td>
<td>36.9 (0.7)</td>
<td>0.378‡</td>
</tr>
<tr>
<td>End of procedure</td>
<td>37.2 (0.7)</td>
<td>37.1 (0.6)</td>
<td>0.349†</td>
</tr>
<tr>
<td>Absolute difference between Start and End</td>
<td>0.34 (0.34)</td>
<td>0.38 (0.34)</td>
<td>0.463‡</td>
</tr>
<tr>
<td>Maximum during procedure</td>
<td>36.7 (0.6)</td>
<td>36.6 (0.6)</td>
<td>0.204‡</td>
</tr>
<tr>
<td>Minimum during procedure</td>
<td>36.7 (0.6)</td>
<td>36.6 (0.6)</td>
<td>0.204‡</td>
</tr>
<tr>
<td>Difference between Maximum to Minimum</td>
<td>0.46 (0.32)</td>
<td>0.48 (0.32)</td>
<td>0.637‡</td>
</tr>
<tr>
<td>Mean during procedure</td>
<td>36.9 (0.7)</td>
<td>36.8 (0.6)</td>
<td>0.623†</td>
</tr>
</tbody>
</table>

**NOTE:** n = number of participants included; SD = standard deviation; VAS = visual analogue scale; # Fisher’s exact test (two-tailed); ‡ Student’s t-test; † Mann-Whitney U test.
7.3.5 Opioid Consumption and Pain Intensity Scores

Sixty-two (65.3%) participants from the Intervention group required postoperative opiate analgesia compared to 65 (68.4%) in the Control group (Table 7.3). There were also no significant differences in quantity of opiate usage within all of the elected postoperative time periods (Table 7.3). Pain perceived at rest and on moving measured by VAS was no different at any of the elected time points in this study (Figures 7.2 and 7.3).
Table 7.3 Perioperative Opiate Usage presented as Morphine Equivalent Daily Dose (MEDD)

<table>
<thead>
<tr>
<th></th>
<th>Intervention [n=95]</th>
<th>Control [n=95]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDD, mean (SD)</td>
<td>2.8 (4.4)</td>
<td>2.5 (4.6)</td>
<td>0.608†</td>
</tr>
<tr>
<td>MEDD per kg, mean (SD)</td>
<td>0.06 (0.08)</td>
<td>0.05 (0.08)</td>
<td>0.401‡</td>
</tr>
<tr>
<td><strong>Intraoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDD, mean (SD)</td>
<td>12.2 (4.7)</td>
<td>12.9 (5.4)</td>
<td>0.290‡</td>
</tr>
<tr>
<td>MEDD per kg, mean (SD)</td>
<td>0.26 (0.09)</td>
<td>0.26 (0.09)</td>
<td>0.675‡</td>
</tr>
<tr>
<td><strong>Postoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required opiate analgesia</td>
<td>62 (65.3%)</td>
<td>65 (68.4%)</td>
<td>0.644*</td>
</tr>
<tr>
<td>Required opiate analgesia in PACU</td>
<td>26 (27.4%)</td>
<td>22 (23.2%)</td>
<td>0.504*</td>
</tr>
<tr>
<td>Required opiate analgesia after PACU</td>
<td>35 (36.8%)</td>
<td>43 (45.3%)</td>
<td>0.238*</td>
</tr>
<tr>
<td>Required patient-controlled opiate analgesia</td>
<td>7 (7.4%)</td>
<td>8 (8.4%)</td>
<td>0.788*</td>
</tr>
<tr>
<td>PACU MEDD, mean (SD)</td>
<td>0.9 (2.1)</td>
<td>0.7 (1.6)</td>
<td>0.524‡</td>
</tr>
<tr>
<td>PACU MEDD per kg, mean (SD)</td>
<td>0.02 (0.05)</td>
<td>0.01 (0.03)</td>
<td>0.347‡</td>
</tr>
<tr>
<td>First 12 hours MEDD, mean (SD)</td>
<td>3.3 (7.2)</td>
<td>3.3 (5.6)</td>
<td>0.991‡</td>
</tr>
<tr>
<td>First 12 hours MEDD per kg, mean (SD)</td>
<td>0.07 (0.14)</td>
<td>0.07 (0.13)</td>
<td>0.772‡</td>
</tr>
<tr>
<td>Day 1 MEDD, mean (SD)</td>
<td>6.6 (14.0)</td>
<td>7.2 (11.1)</td>
<td>0.737‡</td>
</tr>
<tr>
<td>Day 1 MEDD per kg, mean (SD)</td>
<td>0.14 (0.28)</td>
<td>0.15 (0.24)</td>
<td>0.739‡</td>
</tr>
<tr>
<td>Number of doses Day 1, median (IQR)</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>0.506‡</td>
</tr>
<tr>
<td>Day 2 MEDD, mean (SD)</td>
<td>2.2 (5.8)</td>
<td>2.8 (8.9)</td>
<td>0.557‡</td>
</tr>
<tr>
<td>Day 2 MEDD per kg, mean (SD)</td>
<td>0.05 (0.14)</td>
<td>0.07 (0.25)</td>
<td>0.598‡</td>
</tr>
<tr>
<td>MEDD during admission, mean (SD)</td>
<td>3.7 (6.1)</td>
<td>4.2 (6.8)</td>
<td>0.557‡</td>
</tr>
<tr>
<td>MEDD per kg during admission, mean (SD)</td>
<td>0.08 (0.13)</td>
<td>0.09 (0.15)</td>
<td>0.614‡</td>
</tr>
<tr>
<td>Dosing frequency during admission, median (IQR)</td>
<td>1 (4.5)</td>
<td>2 (6)</td>
<td>0.822‡</td>
</tr>
<tr>
<td>Total usage during admission, mean as parenteral morphine equivalent (SD)</td>
<td>10.7 (23.4)</td>
<td>14.8 (38.7)</td>
<td>0.512‡</td>
</tr>
<tr>
<td>Total usage per kg during admission, mean as parenteral morphine equivalent (SD)</td>
<td>0.25 (0.58)</td>
<td>0.30 (0.82)</td>
<td>0.647‡</td>
</tr>
</tbody>
</table>

**NOTE:** n = number of participants included; IQR = inter-quartile range; SD = standard deviation; PACU = post-anaesthesia care unit; * Pearson chi-square test; † Student’s t-test; ‡ Mann-Whitney U test.
**Figure 7.2** Mean of Self-reported Postoperative Pain Scores at Rest, measured by Visual Analogue Scale (VAS)
Figure 7.3 Mean of Self-reported Postoperative Pain Scores with Movement, measured by Visual Analogue Scale (VAS)
7.3.6 Postoperative Recovery Parameters

There were no significant differences in the recovery parameters measured in this study (Table 7.4). Perioperative complications were prospectively recorded during the index hospital admission of all participants and prospectively recorded up to 6 weeks post-discharge for 92 (97%) participants in the Intervention Group and 86 (91%) in the Control Group. There were no statistical differences in the number of participants affected by complications, total number of complications recorded, and the severity grading of complications between the two study groups. Data describing self-evaluated postoperative recovery did not demonstrate statistically significant differences in participant baseline functioning or differences in postoperative Day 10 return to baseline functioning. Table 7.5 outlines reported baseline functioning scores and postoperative Day 10 functioning scores and the difference between them.
Table 7.4 Postoperative Recovery Parameters

<table>
<thead>
<tr>
<th></th>
<th>Intervention [n=95]</th>
<th>Control [n=95]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postoperative length of stay, median in nights (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>To meet discharge criteria</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0.849†</td>
</tr>
<tr>
<td>To actual discharge</td>
<td>2 (3)</td>
<td>1 (3)</td>
<td>0.988†</td>
</tr>
<tr>
<td>Overall length of stay, median in nights (IQR)</td>
<td>2 (2)</td>
<td>2 (3)</td>
<td>0.683†</td>
</tr>
<tr>
<td>Patients with complications</td>
<td>24 (25.3%)</td>
<td>20 (21.1%)</td>
<td>0.492*</td>
</tr>
<tr>
<td>Total number of complications</td>
<td>29</td>
<td>22</td>
<td>0.667*</td>
</tr>
<tr>
<td>Readmission within 30 days</td>
<td>5 (5.3%)</td>
<td>4 (4.2%)</td>
<td>1.000†</td>
</tr>
<tr>
<td>Severity of complications (Clavien-Dindo Grade)</td>
<td>0.270*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>17 (58.6%)</td>
<td>13 (59.1%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>9 (31.0%)</td>
<td>9 (40.9%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3 (10.3%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: n = number of participants included; IQR = inter-quartile range; * Pearson chi-square test; # Fisher’s exact test (two-tailed); † Mann-Whitney U test.
Table 7.5 Self-reported Postoperative Recovery and Day 10 Return to Normal Daily Activities

<table>
<thead>
<tr>
<th>“How much of a problem was this for you?”</th>
<th>Intervention (n=78)</th>
<th>Control (n=68)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>At baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0.072</td>
</tr>
<tr>
<td>Lifting something heavy</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0.068</td>
</tr>
<tr>
<td>Taking a bath or showering yourself</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>0.527</td>
</tr>
<tr>
<td>Helping out around the house</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>0.385</td>
</tr>
<tr>
<td>Hurting or had pain</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>0.628</td>
</tr>
<tr>
<td>Low energy</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.364</td>
</tr>
<tr>
<td>Paying attention to TV or to reading a book</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>0.718</td>
</tr>
<tr>
<td>Sleeping</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.097</td>
</tr>
<tr>
<td>Forgetting things</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.231</td>
</tr>
<tr>
<td>Worrying about what would happen to you</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.805</td>
</tr>
<tr>
<td>Postoperative Day 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td>3 (2)</td>
<td>3 (2)</td>
<td>0.429</td>
</tr>
<tr>
<td>Lifting something heavy</td>
<td>3 (2)</td>
<td>3 (2)</td>
<td>0.975</td>
</tr>
<tr>
<td>Taking a bath or showering yourself</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>0.371</td>
</tr>
<tr>
<td>Helping out around the house</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.468</td>
</tr>
<tr>
<td>Hurting or had pain</td>
<td>3 (1)</td>
<td>3 (2)</td>
<td>0.462</td>
</tr>
<tr>
<td>Low energy</td>
<td>3 (1)</td>
<td>3 (3)</td>
<td>0.779</td>
</tr>
<tr>
<td>Paying attention to TV or to reading a book</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.938</td>
</tr>
<tr>
<td>Sleeping</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.734</td>
</tr>
<tr>
<td>Forgetting things</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0.491</td>
</tr>
<tr>
<td>Worrying about what would happen to you</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>0.218</td>
</tr>
<tr>
<td>Return to Baseline (difference between postoperative and baseline scores)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>0.897</td>
</tr>
<tr>
<td>Lifting something heavy</td>
<td>2 (2)</td>
<td>1 (2)</td>
<td>0.188</td>
</tr>
<tr>
<td>Taking a bath or showering yourself</td>
<td>0 (1)</td>
<td>0 (2)</td>
<td>0.802</td>
</tr>
<tr>
<td>Helping out around the house</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0.932</td>
</tr>
<tr>
<td>Hurting or had pain</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>0.392</td>
</tr>
<tr>
<td>Low energy</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0.569</td>
</tr>
<tr>
<td>Paying attention to TV or to reading a book</td>
<td>0 (1)</td>
<td>0 (0)</td>
<td>0.656</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0.324</td>
</tr>
<tr>
<td>Forgetting things</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.147</td>
</tr>
<tr>
<td>Worrying about what would happen to you</td>
<td>0 (1)</td>
<td>0 (1)</td>
<td>0.259</td>
</tr>
</tbody>
</table>

**NOTE:** 5-level Likert Scale Anchors: 0 = Never, 1 = Almost Never, 2 = Sometimes, 3 = Often, 4 = Almost Always; † Mann-Whitney U test.
7.3.7 Adherence to Study Protocol

Forty-seven incidences of protocol non-adherence occurred during this study. There were 19 during the perioperative care of participants in the Intervention Group and 28 during the care of participants in the Control Group (p = 0.130). Included were 12 incidences where study participants received intraoperative IV dexamethasone from the anaesthetist for prophylaxis against postoperative nausea and vomiting. Five participants were from the Intervention Group and 7 from the Control Group (p = 0.767).

7.3.8 Subgroup Analysis

Comparison analysis was performed on the subgroup of participants who were not diagnosed with macroscopic appendicitis-related secondary peritonitis: 70 from the Intervention Group and 66 from the Control Group. Forty-two (60%) Intervention participants required postoperative opiate analgesia compared to 41 (62%) Control participants and this was not statistically different (p = 0.800). There was also no statistical difference in total opiate usage, expressed as MEDD, between the groups (Table 7.6).
Table 7.6 Subgroup Analysis of Perioperative Opiate Usage Presented as Morphine Equivalent Daily Dose (MEDD)

<table>
<thead>
<tr>
<th></th>
<th>Intervention (n=70)</th>
<th>Control (n=66)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, <em>mean in kg</em> (SD)</td>
<td>50.0 (13.4)</td>
<td>50.6 (15.9)</td>
<td>0.822*</td>
</tr>
<tr>
<td>Pre-operative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDD, <em>mean</em> (SD)</td>
<td>2.6 (4.5)</td>
<td>2.0 (4.3)</td>
<td>0.440†</td>
</tr>
<tr>
<td>MEDD per kg, <em>mean</em> (SD)</td>
<td>0.05 (0.08)</td>
<td>0.04 (0.07)</td>
<td>0.285†</td>
</tr>
<tr>
<td>Intraoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDD, <em>mean</em> (SD)</td>
<td>11.4 (4.2)</td>
<td>12.3 (5.2)</td>
<td>0.303†</td>
</tr>
<tr>
<td>MEDD per kg, <em>mean</em> (SD)</td>
<td>0.23 (0.08)</td>
<td>0.25 (0.08)</td>
<td>0.450†</td>
</tr>
<tr>
<td>Received dexamethasone</td>
<td>3 (4.3%)</td>
<td>6 (9.1%)</td>
<td>0.315*</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required rescue opiate</td>
<td>42 (60.0%)</td>
<td>41 (62.1%)</td>
<td>0.800*</td>
</tr>
<tr>
<td>Required rescue opiate in PACU</td>
<td>20 (28.6%)</td>
<td>18 (27.3%)</td>
<td>0.866*</td>
</tr>
<tr>
<td>PACU MEDD, <em>mean</em> (SD)</td>
<td>1.1 (2.3)</td>
<td>0.9 (1.9)</td>
<td>0.637†</td>
</tr>
<tr>
<td>PACU MEDD per kg, <em>mean</em> (SD)</td>
<td>0.02 (0.06)</td>
<td>0.02 (0.03)</td>
<td>0.435†</td>
</tr>
<tr>
<td>First 12 hours MEDD, <em>mean</em> (SD)</td>
<td>3.3 (7.2)</td>
<td>2.9 (5.8)</td>
<td>0.763†</td>
</tr>
<tr>
<td>First 12 hours MEDD per kg, <em>mean</em> (SD)</td>
<td>0.07 (0.14)</td>
<td>0.06 (0.13)</td>
<td>0.878†</td>
</tr>
<tr>
<td>Day 1 MEDD, <em>mean</em> (SD)</td>
<td>5.4 (10.1)</td>
<td>5.1 (7.9)</td>
<td>0.825†</td>
</tr>
<tr>
<td>Day 1 MEDD per kg, <em>mean</em> (SD)</td>
<td>0.12 (0.23)</td>
<td>0.10 (0.16)</td>
<td>0.682†</td>
</tr>
<tr>
<td>Day 2 MEDD, <em>mean</em> (SD)</td>
<td>1.5 (5.2)</td>
<td>0.5 (1.6)</td>
<td>0.129†</td>
</tr>
<tr>
<td>Day 2 MEDD per kg, <em>mean</em> (SD)</td>
<td>0.03 (0.11)</td>
<td>0.01 (0.02)</td>
<td>0.084†</td>
</tr>
<tr>
<td>MEDD during admission, <em>mean</em> (SD)</td>
<td>3.9 (6.2)</td>
<td>3.9 (6.1)</td>
<td>0.988†</td>
</tr>
<tr>
<td>MEDD per kg during admission, <em>mean</em> (SD)</td>
<td>0.08 (0.13)</td>
<td>0.08 (0.12)</td>
<td>0.880†</td>
</tr>
<tr>
<td>Total morphine equivalent, <em>mean</em> (SD)</td>
<td>7.7 (17.9)</td>
<td>5.5 (8.4)</td>
<td>0.379†</td>
</tr>
<tr>
<td>Total morphine equivalent per kg, <em>mean</em> (SD)</td>
<td>0.17 (0.43)</td>
<td>0.11 (0.17)</td>
<td>0.291†</td>
</tr>
<tr>
<td>Morphine equivalent per dose during admission, <em>mean</em> (SD)</td>
<td>1.1 (1.3)</td>
<td>1.5 (2.1)</td>
<td>0.256†</td>
</tr>
</tbody>
</table>

NOTE: * Pearson chi-square test; # Fisher’s exact test (two-tailed); † Student’s t-test.
7.3.9 Effectiveness of Blinding

Blinding of primary surgeons was tested at the conclusion of 170 (89%) study procedures. On seven of these occasions, the operating surgeon declined to answer, citing that they did not know and did not wish to make a guess. On 39 (24%) occasions, the primary surgeon correctly thought that the participant was in the Intervention and on 31 (19%) occasions they correctly speculated that the participant was in the Control Group. On the remaining 93 (57%) occasions, the primary surgeon’s response was not matched to the participant’s actual group allocation.
7.4 DISCUSSION

This double-blinded, randomised, controlled trial investigated the clinical impact of using a commercial insufflation humidifier to warm and humidify CO\textsubscript{2} gas during laparoscopic appendicectomy for suspected childhood acute appendicitis. It found that this intervention does not impart any statistically significant benefit on postoperative pain. It also had no effects on intraoperative core temperature and postoperative recovery parameters. And finally, the severity of laparoscopic camera lens fogging reported by operating surgeons was not significantly affected by operating the insufflation humidifier.

In the last two decades, a number of clinical studies have been conducted to investigate patient-centred outcomes from warm humidified laparoscopic gas insufflation in adults after general surgical and gynaecological procedures. Results from individual studies, however, have been conflicting and limited by small sample sizes and methodological flaws\textsuperscript{434,460}. Most recently, a Cochrane meta-analysis compiled from 16 adult studies found that warming insufflation gas, with or without additional humidification, had no clinical impact. Like two earlier meta-analyses, it was limited by the inclusion of inadequately blinded and powered studies and significant inter-study heterogeneity\textsuperscript{466}. The reviewers concluded by highlighting the need for adequately powered randomised controlled trials to conclusively examine the impact of warm humid laparoscopic gas insufflation on postoperative pain.
Pain after laparoscopic appendicectomy arises from multiple sources: skin incisions, pneumoperitoneum, appendicectomy, and appendicitis-related secondary peritonitis if present.\textsuperscript{467} Two outcomes are commonly used to quantify and compare acute postoperative pain: opioid consumption and pain scores.\textsuperscript{468} Although both can be used to calculate power and determine sample size, pain intensity perceived by children can vary widely as a result of age, gender, cognitive and linguistic development, previous experience of pain, learning, mood, environmental influences, separation from parents, and the child’s understanding of illness and medical procedures.\textsuperscript{469} Therefore, the sample size in this current study was calculated using retrospective data of postoperative patient opiate consumption at the study institution. Establishing this \textit{a priori} was ultimately crucial as negative studies are regularly scrutinised for a lack in power.\textsuperscript{470}

A statistically significant difference was not demonstrated in this clinical trial with respect to the primary outcome because the $P$ value, obtained after applying an appropriate statistical hypothesis test, was greater than 0.05. This allows an assumption that any differences found between the intervention and control groups can be explained by chance alone. But reporting $P > 0.05$ is not the same as providing evidence of true equivalence between the intervention and control groups. A number of alternative possibilities need be considered too. These include the real difference between study groups being less than hypothesised, the variance of the data were greater than anticipated leading to an inaccurate \textit{a priori} power calculation, the presence of confounding factors during study conduction leading to a smaller difference being demonstrated in comparison to the true difference, and the occurrence of a type 2 error i.e. the one in five chance that a false negative outcome is found if power was set at 0.8. When a clinical trial demonstrates negative results, it is
therefore important to consider the utility of confidence intervals, equivalency testing (for example the Two One-Sided Tests Procedure), and post hoc power calculations.\textsuperscript{470}

In theory, the ideal procedure for investigating benefits of conditioning laparoscopic insufflation gas should be long in duration to expose the peritoneum to prolonged insufflation but minimally disruptive to the peritoneum so that outcomes assessed are not the result of physical trauma. Laparoscopic appendicectomy is minimally disruptive to the peritoneum but relatively short in duration. It is also occasionally associated with secondary peritonitis as a result of appendiceal perforation. In these patients, advantages of warm humidified insufflation gas to postoperative pain and recovery may be masked by the magnitude of morbidity associated with infectious peritonitis.

For this reason, a priori subgroup analysis was planned and performed for the current study. Commonly perceived to be an exercise in data dredging and almost always underpowered,\textsuperscript{471, 472} the practice is however justifiable in a select number of scenarios when pathophysiological principles exist.\textsuperscript{473} These include situations where there are potentially large differences between groups at risk of a poor outcome with or without intervention, there are practical questions about when to treat, there are doubts about benefit in specific groups, and when there is potential heterogeneity of intervention treatment effect in relation to pathophysiology such as the circumstances present in this current study.\textsuperscript{474} However, it should be noted that a diagnosis of peritonitis is only be made once the pneumoperitoneum has been established i.e. once the peritoneum has been exposed to insufflation gas. Should the subgroup analysis have demonstrated statistically significant results, clinicians would be faced with a dilemma given that whether or not the
gas is conditioned is a decision that has to be made preoperatively when the presence of peritonitis has not yet been confirmed.

The participants’ quality of recovery and return to normal daily activities during the immediate postoperative period (<2 weeks) were notable secondary outcomes in this study and their evaluation requires further discussion. Along with patient satisfaction, these parameters are important patient-orientated outcomes and have been shown to correlate closely with postoperative pain. Poor pain management can adversely impact these outcomes by limiting or decreasing basic physical functioning (e.g. ambulation, self-care), quality of sleep, energy/fatigue, and overall mental and emotional health. Furthermore, as described in Chapter 1, localised peritoneal inflammation and injury is known to have a wide variety of physiological and behavioural consequences. The later of these include somnolence, anorexia, reduced activity, and social interaction and are commonly referred to as ‘sickness behaviours’.

Validated patient-questionnaires are the most commonly used method of evaluating the above patient-orientated postoperative outcomes and obtaining baseline values is critically important. More rarely, objective evaluation methods have also been described. In the paediatric age group, ‘uptime’ (time spent upright) is one such measure. Using an activity monitor with mercury tilt switches that was attached to the lateral aspect of the participant’s right thigh, Eldridge et al. recorded time spent upright by children aged 8 to 15 years after open or laparoscopic appendicectomy. They were able to obtain these recordings for 61 participants and subsequently also recorded values one month after surgery to determine the children’s normal baseline level of activity. Their results showed...
that regardless of the surgical approach taken, it can take up to 10 days for children to return to normal uptime. Based on this finding, it was decided that patient quality of recovery and return to normal daily activities should be assessed on postoperative Day 10 using a self-reported questionnaire.

Postoperative follow-up in the current study was completed at 6 weeks and therefore its outcomes can only account for the immediate and short-term clinical effects of warm humidified gas insufflation. The theoretical long-term benefits of attenuated peritoneal inflammation such as reduced adhesion formation have not been investigated. The value of preventing abdominal adhesions is imperative when operating on paediatric patients who must endure the consequences from childhood into old age. Although adhesion formation is multi-factorial, the inflammation associated with mesothelial cell desiccation is likely a significant contributor. Recent experimental studies confirm that prevention of peritoneal desiccation with warm humidified CO₂ gas insufflation prevents processes that facilitate adhesion formation after laparoscopy. Future follow-up of participants from this study may shed light on this.

The following limitations should be considered when interpreting results of the current study. Firstly, evaluation of the study’s primary outcome, postoperative opiate consumption, was restricted by each participant’s duration of index admission. During this study, the duration of postoperative hospital stay was a median of one night for patients with simple appendicitis and two nights overall. Although study participants were discharged when they no longer required regular opiate analgesia, some of these agents can be obtained over-the-counter at pharmacies or prescribed by practitioners in the
community. The study does not account for the use of such additional opiate analgesia. Furthermore, pain intensity was scored 24 and 48 hours postoperatively but participant availability at these time points was limited by hospital discharge. This is worthwhile recognising given that the Cochrane meta-analysis found significantly lower postoperative morphine usage on Day 2 by patients who had received warm humidified insufflation gas despite no differences at 6 hours and on Day 1.  

Lastly, use of intraoperative external warming measures was not standardised during the current study and this is a weakness shared by several earlier clinical studies. The current study is however unique as it includes participants whose underlying infection elicits a preoperative systemic inflammatory response that alters body temperature making it difficult for investigators to standardise intraoperative measures that maintain normothermia.
7.5 CONCLUSION

This double-blinded randomised controlled trial has demonstrated no clinical benefits associated with warm humidified CO₂ gas insufflation for laparoscopic appendicectomy in children. In this setting, this intervention cannot be recommended as a strategy for reducing postoperative pain and opiate usage.
Chapter 8

CONCLUSIONS AND FUTURE DIRECTIONS
8.1 SUMMARY OF RESULTS

The aims of this thesis were to describe peritoneal inflammatory injuries contributing to disease morbidity in childhood acute appendicitis, and to investigate the effects of applying this understanding of current management strategies. The following research questions were posed: What is the disease morbidity of complicated appendicitis in children? What disease- and clinician-related factors influence this? How does laparoscopic surgery and creation of the pneumoperitoneum affect peritoneal structure and function? Can patient outcomes be improved when medical and surgical interventions are designed to reduce or target peritoneal inflammation and injury in childhood acute appendicitis?

The thesis started by summarising important background concepts in the Introduction Chapter and providing a conceptual framework for the significance and implications of this body of research. Clinically, acute appendicitis presents in two different forms. ‘Simple’ appendicitis involves appendiceal inflammation without peritoneal disease and ‘complicated’ appendicitis involves infection extending beyond the appendiceal viscus with development of secondary peritonitis and/or intraperitoneal abscesses (ICD-9 codes 540.0 and 540.1). Simple appendicitis is the most common cause for abdominal surgery in children and complicated appendicitis is the most prevalent cause of intraabdominal Gram-negative sepsis with septic shock in this age group. Despite evolving management strategies, appendicitis continues to challenge clinicians and peritoneal inflammation is key to disease morbidity.
As a metabolically active organ, its unique anatomical position, structure, and physiology, means that the peritoneum is inadvertently part of the disease morbidity associated with acute appendicitis. In ‘simple’ appendicitis, peritoneal injury predominately results from iatrogenic insult associated with laparoscopic surgery and carbon dioxide (CO₂) gas pneumoperitoneum, while in ‘complicated’ appendicitis, bacterial invasion causes secondary peritonitis. Once injured, peritoneal host defence inflammatory response and subsequent healing are initiated and controlled by a number of complex immunological cascades. Excessive inflammation, failed clearance of infection, and delayed or defective healing all have far-reaching consequences including intraabdominal abscesses, adhesion formation, prolonged ileus, metabolic disturbances, needless pain perception, poor quality of recovery, and prolonged hospitalisation. Clinically, this means that reduced surgical disturbance to the peritoneum has a potential to improve postoperative patient outcomes. Furthermore, an appreciation for and an understanding of appendicitis-related peritoneal injury can reduce the disease morbidity.

The experimental part of this thesis starts by examining the disease morbidity caused by appendicitis-related peritonitis and constructing a multivariate regression analysis model to find the predictors of key patient outcomes. An observational study was conducted to describe the outcomes of 359 children (1-14 years), treated at Starship Children’s Hospital for complicated appendicitis between 2005 and 2011. Severity of perioperative complications was graded using the Clavien-Dindo classification. Logistic regression modelling was used to identify predictors of outcome. The median age of the study population was 11 years (IQR = 3) and 60% were male. Histological appendiceal perforation was found in 299 (83%) patients. The main surgical approach was laparoscopy (79%).
Median length of stay (LOS) was 6 nights and readmission rate was 11%. Ninety (25%) patients were affected by postoperative complications, the most frequent being intraabdominal infections, affecting 40 (12%) patients. Early (< Day 3) or late (Day 3 and after) postoperative fever (temperature > 38 °C) and duration of prescribed antibiotic therapy were found to be significant predictors of LOS. However, only late postoperative fever was an independent predictor of intraabdominal infection (odds ratio = 0.35, p = 0.016). The study concluded that complicated appendicitis with secondary peritonitis remains a morbid childhood disease. Late postoperative fever is an independent predictor of unresolved or recurrent intraabdominal infections while the prescribed inpatient antibiotic regimen is an important clinician-related predictor of LOS.

As a result of the above findings, the second study was a systematic review investigating the efficacy and safety of a strategy addressing the issue of postoperative antibiotic therapy in complicated appendicitis. Its thirteen studies described the use of select clinical criteria, representative of resolving or resolved intraabdominal infection, to determine the duration of postoperative antibiotic therapy for appendicitis-related secondary peritonitis. Eleven were observational studies and two were intervention comparison studies. The most commonly used clinical criteria were trends in core body temperature, functioning of the gastrointestinal tract, and normal or normalising white blood cell count. Although the studies were all small, collectively, the negative predictive values of the criteria clusters they described for ruling out postoperative intraabdominal infection were all greater than 90%. The interventional studies respectively found that, in comparison to setting a fixed duration of antibiotic treatment, using clinical measures to determine when postoperative antibiotics can be terminated reduced both LOS and antibiotic administration.
Chapter 5 described a clinical trial conducted to add strength to the findings of the above systematic review and applies the evidence gathered by the critical narrative in Chapter 4. Over a six-month period, this comparison cohort study prospectively recruited 47 children (aged 5-14 years) diagnosed with complicated appendicitis and treated them with postoperative IV antibiotics until each satisfied a set of bedside clinical parameters suggesting resolved intraperitoneal infection (core body temperature < 38 °C for 24 hours, tolerated two consecutive meals, mobilising independently, requiring only oral analgesia). Postoperative recovery parameters were recorded and compared to those of 47 historical control patients, matched by propensity scores, who had received fixed 5-day antibiotic regimens. Sample size was determined by a priori power calculation based on reduction in LOS. Severity of postoperative complications was graded using the Clavien-Dindo classification.

In terms of results, the univariate comparison analysis found no significant differences between the study cohorts with regard to demographics, duration of presenting symptoms, severity of presenting disease, preoperative antibiotic therapy received, duration of operation, seniority of operating surgeon, surgical approach taken, and intraoperative findings. The prospective cohort had a significantly shorter median LOS compared to the control cohort (5 versus 6 nights, \( p = 0.010 \)) while readmission rates and the incidence and severity of complications were similar, including incidence of postoperative intraabdominal infections (6 versus 8 cases, \( p = 0.562 \)). The study concluded that bedside clinical parameters, indicative of resolved intraperitoneal inflammation, could be used to safely tailor the duration of postoperative IV antibiotics for children with complicated appendicitis to improve clinical efficiency without compromising patient outcomes.
To address the issue of potential laparoscopy-related peritoneal injury in the setting of childhood appendicitis, a systematic review was performed summarising the effects of CO\textsubscript{2} pneumoperitoneum on peritoneal structure and function with a particular focus on how manipulating the physical conditions of insufflation impacts these changes. A highly sensitive literature search was performed in order to identify relevant studies. Eighteen full-text articles were finally selected and reviewed, including thirteen animal experiments and five human clinical trials.

Study investigators examined the effects of a diverse group of insufflation conditions and in turn, a heterogeneous group of study outcomes were measured. CO\textsubscript{2} gas insufflation produces peritoneal acidosis, desiccation, hypothermia, and hypoxia, and these affect peritoneal morphological structure, metabolism, immune response, and healing. Varying gas temperature, relative humidity, composition, and pressure can impact the extent to which these changes occur. Most importantly, desiccation prevention by using warm humidified gas is associated with attenuation of microscopic structural changes as well as potential changes to peritoneal inflammatory response. Peritoneal hypoxia is preventable with the addition O\textsubscript{2} in small fractions whilst changing insufflation pressure had convincing localised metabolic effects on tissue oxygen tension and peritoneal blood flow. Although the majority of these studies were based on animal models, a number of important clinical implications were highlighted including postoperative pain and recovery, formation of adhesions, and systemic hypothermia.

The final clinical trial of this thesis was conducted to investigate one such clinical implication. Warm humidified insufflation gas, more closely resembling the natural
conditions of the intraperitoneal cavity, is known to reduce the structural and inflammatory changes associated with peritoneal desiccation. In comparison to conventional gas insufflation, numerous clinical trials suggest that this intervention leads to decreased postoperative pain, presumably from reduced desiccation-related inflammation.

To investigate the impact of warm humidified CO₂ insufflation gas in the setting of laparoscopic appendicectomy, a double-blinded, randomised, controlled trial was conducted. The Intervention Group (n = 95) received warmed, humidified insufflation CO₂ (37 °C, 98% RH) delivered using the Fisher & Paykel MR860 laparoscopic humidification system (Fisher & Paykel Healthcare, Auckland, New Zealand). The Control Group (n = 95) received standard carbon dioxide gas (room temperature ~20 °C, 0% RH). Sample size was based on a priori power calculation. Randomisation was conducted using computer-generated random numbers and allocations were concealed in opaque numbered envelopes. The patient, study investigators, surgeon, and medical staff responsible for patient care were all blinded to patient allocation. Data analysis was also blinded. Anaesthesia and perioperative analgesia were standardised.

The trial’s primary outcome was postoperative pain, represented by postoperative opiate requirement and consumed quantity determined using Morphine Equivalent Daily Dose (MEDD) conversion factors. Secondary outcomes included pain intensity scores, measured at rest and with movement using visual analogue scores, intraoperative oesophageal temperature measured at 10 minute intervals, camera fogging rated by the operating surgeon, patient recovery parameters based on standardised discharge and complication criteria, and self-reported return to normal daily activities measured using a Day 10
questionnaire. Results showed that study groups were well matched at baseline. Intraoperative core body temperature and mean camera fogging scores were similar. No significant differences were detected in the proportion of participants requiring postoperative opiate analgesia, MEDD usage, pain intensity scores, or any of the recovery parameters measured. This suggests that warm humidified insufflation gas provides no measurable clinical benefits to postoperative pain and recovery in the setting of laparoscopic appendicectomy for childhood acute appendicitis.
8.2 CONCLUSIONS

From the sum of these investigations, this thesis draws the following conclusions.

Childhood acute appendicitis inevitably causes peritoneal injury and inflammation as a result of the natural progression of this disease but also iatrogenically from laparoscopic surgery and creation of a pneumoperitoneum. Appendicitis accompanied by secondary peritonitis remains a morbid childhood disease with postoperative intraperitoneal infection being the most common and significant complication.

Laparoscopy and the carbon dioxide pneumoperitoneum can cause a number of changes to the peritoneal surface including acidosis, hypoxia, desiccation, and local hypothermia. The extent to which these changes occur can be influenced by changing the physical condition of insufflation gas but the theory that warm and humidified gas can improve patient outcome currently remains unsubstantiated.

Nevertheless, an awareness and understanding of peritoneal inflammation still offers clinicians a chance to improve clinical practice. Using a set of objective clinical parameters, representative of resolving intraperitoneal infection, to determine duration of postoperative inpatient antibiotic therapy in children with appendicitis-related secondary peritonitis improves practice efficiency by shortening hospital stay without apparent compromise to sepsis management.
8.3 FUTURE RESEARCH

Interventions that focus on peritoneal injury associated with childhood acute appendicitis should continue to be investigated with the aim of improving patient outcomes from this common and frequently morbid surgical condition. The research outlined in this thesis, and the resulting conclusions reveal several interesting and important areas that require further exploration.

First and foremost, aside from desiccation, the carbon dioxide pneumoperitoneum is associated with a host of other effects on peritoneal structure and function. These include acidosis, hypoxia, and altered host inflammatory response. Although this thesis found that the changes associated with peritoneal desiccation caused by cool, dry insufflation gas were of minimal clinical consequence with regards to postoperative pain and recovery, the direct effects and long-term clinical implications of manipulating this and other insufflation conditions have yet to be investigated in detail. And while animal and experimental studies will continue to have a vital purpose in the pursuit for further understanding, without correlation to human studies and clinical outcomes, the absolute significance of these findings cannot be ascertained.

One of these topics is how the pneumoperitoneum and its associated effects on peritoneal morphology and functioning influence subsequent formation of postoperative intraperitoneal adhesions. Pneumoperitoneum-related alterations to the peritoneal host defence inflammatory response is yet another important area for further investigation, its
significance becoming more and more obvious as clinicians increasingly resort to laparoscopic surgery for management of advanced intraabdominal infections.

Another area that warrants further investigation is clinician practices surrounding the management of secondary peritonitis. As this thesis has highlighted, misguided clinical policies can exacerbate patient morbidity. The prescription of adjuvant antibiotic therapy alone generates significant debate regarding the choice of agent, and route and duration of administration. This thesis has tried to address the last of these issues but, in doing so, asked more questions. One such question relates to the practical value of monitoring serum inflammatory markers postoperatively as an adjunct to the clinical parameters used to indicate clearance of intraperitoneal infection.

Finally, while not suggested by the results of this thesis, several studies have proposed that development of postoperative intraabdominal abscesses does not actually appear to correlate well with the length of postoperative antibiotic therapy given. Perhaps, systemic therapies are less likely to be effective if local control of intraperitoneal infection is not achieved. To date, neither surgical nor medical therapies directly targeting the peritoneum have been paid sufficient attention.

Although the safety and efficacy of laparoscopy for managing complicated appendicitis has been recognised, there is considerable uncertainty surrounding what constitutes optimised intraoperative management of the accompanying peritoneal infection and inflammation. The effectiveness of many traditional surgical techniques remains largely unknown and newer medical therapies remain in the experimental stage. These include
intraperitoneal instillation of antiseptics, antibiotics, and anti-fibrinolytics such as tPA and hyaluronan. Adequately-powered randomised controlled trials should be conducted to address each of these therapies.
APPENDIX A

PERITONEAL IMMUNE RESPONSE TO INJURY
The peritoneum deals with infection in the following ways: the absorption of bacteria into the lymphatics via peritoneal stomata, phagocytosis of bacterial and foreign particles by activated peritoneal macrophages and polymorphonuclear leucocytes (PMNs), and the production of an array factors that act to localise the infection as an abscess. The details of the peritoneum’s immune response to infection and injury is summarised below in four chronological steps.

**STEP 1: Stomata and the diaphragmatic pump**

Fluid within the peritoneal cavity moves in a cephalad direction in response to pressure generated by the diaphragm. It is then absorbed via stomata on the under-surface of the diaphragm into terminal lymphatic lacunae. Running parallel to the muscular fibres of the diaphragm, these lacunae contain valves and drain into the main lymphatic ducts via the substernal lymph nodes. This process takes only minutes from the time contaminants are released into the peritoneal cavity and precedes the arrival of phagocytotic cells. Mesothelial cells and resident macrophages are therefore crucial in initiating and amplifying this early peritoneal response to intra-abdominal infections. Diaphragmatic movement during expiration causes rapid inflow into the lacunae and diaphragmatic contraction during inspiration pushes the contents of the lacunae into the efferent lymphatic system where reverse flow is prevented by the valves within the thoracic lymphatics.

This process is affected by several factors: disruption to breathing patterns by general anaesthesia or abdominal wound pain will decrease peritoneal clearance while positive pressure ventilation, conversely accelerates peritoneal fluid outflow. Excessive accumulation of fibrin and inflammatory exudations in the peritoneal cavity may also
adversely affect this trans-diaphragmatic absorption process. Peritoneal clearance is also facilitated by stomata present in the omental, mesenteric, and pelvic peritoneum.

**STEP 2: Innate immune responses**

**Peritoneal fluid and complement**

The normal peritoneal cavity in adults contains less than 100 mL of serous fluid which is essentially an ultrafiltrate of plasma with a protein concentration below 30 grams per litre. The antibacterial activity of peritoneal fluid is mediated by complement. In patients with cirrhosis and ascites, the concentration of C3 in peritoneal fluid has been found to be an independent predictor for occurrence of spontaneous bacterial peritonitis. Defence of the abdominal cavity against major contamination is triggered by activation of C3a and C5a, which are induced by products of bacteria and inflammation. C3a and C5a then stimulate chemotaxis of neutrophils, and degranulation of basophils and mast cells.

**Neutrophils and monocytes**

The normal peritoneal cavity contains only a small scattering of cells (< 300 cells/mm) that include macrophages, neutrophils, and lymphocytes plus desquamated mesothelial cells and dendritic cells. Abdominal surgery, contamination, or infection elicits a rapid influx of leucocytes which may increase their numbers to more than 3000 cells/mm. Two processes mediate the recruitment and activation of leucocytes: chemotaxis and transmigation.

During the early stages of peritonitis, PMNs and activated mononuclear cells migrate to the abdominal cavity after an upstream gradient of chemo-attractant cytokines including
monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 2 (MIP-2), and IL-8. While cytokine-activated peritoneal mesothelial and endothelial cells are known to be a major source of these chemokines, \textsuperscript{501, 502} other resident peritoneal cells, including mast cells and peritoneal macrophages have also proven to be important participants in chemotactic activity.\textsuperscript{503, 504} In addition, besides chemokines, the arachidonic acid metabolite leucotriene-B4 (LTB-4) is also an important chemo-attractant of neutrophils in peritonitis.\textsuperscript{505}

Adhesions molecules are needed to allow PMNs to transmigrate from the circulation into the peritoneal cavity – they are responsible for contact formation to the blood vessel wall and subsequent neutrophil extravasation.\textsuperscript{506, 507} Selectins are known to participate in the early stages of PMN adhesion (rolling of PMN along the endothelium) with L-selectin expressed on PMN and P- and E-selectins expressed on endothelial cells. During peritonitis, both P- and E-selectin expression are increased in the vasculature of intestines and intra-peritoneal organs.\textsuperscript{508, 509}

Subsequent to selectin engagement, integrins facilitate the firm adhesion of neutrophils to endothelial cells and their migration across the mesothelium. Being transmembrane glycoproteins that mediate cell-cell and cell-matrix interactions, their expression is upregulated after inflammation or cytokine stimulation. During peritonitis, peritoneal mesothelial cells and microvilli constitutively express intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and platelet-endothelial cell adhesion molecule-1 (PCAM-1).\textsuperscript{510}
Monocyte migration into the peritoneal cavity occurs much in the same way as neutrophil transmigration\textsuperscript{511,512} but takes place slightly later (within 24 hours) and seems to occur independent of the mesothelium.\textsuperscript{513}

\textit{Macrophages}

Activated by interferon-$\gamma$ (IFN-$\gamma$), IL-2, and IL-12,\textsuperscript{514} resident peritoneal macrophages are drawn to bacteria by chemotaxis. Also upregulated by IFN-$\gamma$, macrophages and mesothelial cells have the ability to express MHC class II molecules on their surfaces, and therefore acquiring an antigen-presenting function.\textsuperscript{515}

\textit{Natural killer cells}

Natural killer cells are large granular lymphocytes capable of destroying virally infected cells and tumour cells extracellularly by inducing apoptosis. They participate in early local and systemic microbial eradication after activation by IL-12. Together with peritoneal macrophages, they are also an important source of IFN-$\gamma$ during peritonitis.\textsuperscript{516,517} T cells induced by IFN-$\gamma$ exhibit a pro-inflammatory phenotype that has augmented bacterial eradication and local host response capabilities.

\textbf{STEP 3: Specific immune defences}

Resident peritoneal lymphocytes express common phenotypes but rare variants such as the CD5+ B lymphocytes that are very uncommon in other tissues have also been identified.\textsuperscript{518}
**T lymphocytes**

T cells make up the majority of peritoneal lymphocytes and their activation by foreign antigens depends on effective antigen presentation by dendritic cells in lymph nodes, and on presentation in the infected tissue and in the peritoneal fluid by dendritic cells and other antigen-presenting cells including peritoneal mesothelial cells.

T cells are divided into several subpopulations via the expression of T-cell receptor proteins that bind processed antigen. Most human peritoneal T lymphocytes express transmembrane markers of thymus-dependent differentiation corresponding to phenotypes CD2+, CD3+, TcR $\alpha\beta+$, CD4+, or CD8 $\alpha\beta+$ with cells of this last phenotype being the most common. These cells migrate into the peritoneum via ligand adhesion to VCAM-1 expressed on mesothelial cells. However, the existence of cells of the CD7+ phenotype indicates that a thymus-independent pattern of differentiation for peritoneal T lymphocytes also exists and thus, peritoneal involvement in the specific immune system begins with T-cell lymphopoiesis.

T cell subpopulations include CD8+ cells (also known as cytotoxic T lymphocytes) and CD4+ cells (also referred to as T helper cells). While the major roles of CD8+ cells are to kill infected host cells, CD4+ cells act to regulate the cellular and humoral immune response. Other T cell subpopulations include $\gamma\delta$ T cells that potentate inflammation and memory T cells which provide immediate, potent, and long-lasting protection. Lastly, regulatory T cells prevent autoimmunity and help terminate the immunological response.
T helper cells (Th) are further categorised into subsets depending on their secretion patterns. Th1 cells secret IL-2, IFN-γ, and TNF-α, and are involved in directing cell-mediate immunity as these cytokines induce activation and proliferation of lymphocytes and macrophages, and increase cellular adhesion. Th2, in contrast, secrete a host of other interleukins, including IL-4, IL-5, IL-9, IL-10 and IL-13, and are responsible for regulating the humoral immune response. This includes the stimulation of mononuclear cell and lymphocyte proliferation, growth of mast cells, and production of IL-6. Between them, these subsets of T helper cells control both inflammatory and anti-inflammatory activities in order to achieve the balance between combating pathogens and maintaining life-long tolerance for commensal enteric bacteria.

*B lymphocytes*

B cells only represent <5% of peritoneal lymphocytes but like certain T cell phenotypes, a subset of B cells such as Ly1+ B lymphocytes appear to be independent of the thymus and bone marrow for their development. Supporting this is the finding of pre B-cell progenitors in human fetal omentum from 8th to 23rd gestational weeks.

Kasaian and Casali proposed that human B lymphocytes are divided into at least three distinct subsets depending on their expression of surface CD5, CD11b, and CD14, and their differential production of immunoglobulins: (1) B-1a (Ly1+ or CD5+) cells that develop from progenitors in the omentum and have the ability to self-replenish during adulthood; (2) B-1b (Ly1- or CD5-) cells whose progenitors are found in the splanchnic area and adult bone marrow; and (3) B2 (CD5- and CD11b-) cells that arise in the fetal liver and continuously replenished in adult life by progenitors in bone marrow.
There are an equal absolute number of B1a cells in the peritoneum and the spleen, and although little exchange between the spleen and peritoneum has been observed, surgical splenectomy severely decreases the number and repertoire of peritoneal B1 cells.\textsuperscript{525} B2 cells migrate into the peritoneal cavity from the circulation by two pathways. The direct pathway involves specific a4B1 integrin adhesion molecules and the indirect pathway is via omental ‘milky spots’ with the involvement of a4B7 integrins. Surgical omentectomy interestingly results in a 40\% reduction of B2 cell migration from the circulation into the peritoneum but has no effect on movement of B cells out of this compartment.\textsuperscript{526}

Ly1+ B lymphocytes have been reported to produce a number of different antibodies and autoantibodies.\textsuperscript{527} Thomas-Vaslin et al.\textsuperscript{528} found that the bulk of natural immunoglobulin M (IgM) secretion can be attributed to peritoneal Ly1+ B lymphocytes and their progeny, and that secretion is independent of adult bone marrow precursors. In addition, approximately half of IgA plasma cells within intestinal lamina propria are derived from the peritoneal B lymphocyte lineage, suggesting an important role for peritoneum-derived B lymphocytes in the mucosal immune response.\textsuperscript{529,530}

\textit{Peritoneum-associated lymphoid tissue (PALT)}

The peritoneum-associated lymphoid tissue (PALT) includes the omental ‘milky spots’, resident lymphocytes within peritoneal fluid, and the draining lymph nodes.\textsuperscript{127} It is evident that PALT enjoys an intriguing relationship with the peripheral innate immune system and GALT system.\textsuperscript{142} The possible mechanism is passage of peritoneal antigens and/or primed peritoneal lymphocytes via lymphatic ducts to GALT and the peripheral circulation. The association between peritoneal lymphocytes and GALT has firstly been shown by the
induction of both systemic and mucosal immune responses following intra-peritoneal immune priming. Following intra-peritoneal immunisation, subsequent encounters with the same antigen either systemically or enterally, results in an extensive IgA antibody response within the gut. By contrast, primary intra-duodenal immunisation results in a substantially lower IgA response. The important role that peritoneal B lymphocytes play in the GALT system has been further demonstrated by Kroese et al. who found that murine peritoneal B lymphocytes migrate to repopulate recipient intestinal lamina propria of mice to become IgA secreting cells.

In addition, fluorescence-labelled phagocytes have been observed to migrate from the peritoneal cavity into B-cell follicles of Peyer’s patches. The fact that peritoneal antigen-presenting cells are present within Peyer’s patches – the antigen-sampling sites of the gut – indicates a role for these cells in the priming of mucosal lymphoid tissues. Primed tissues mount a faster immune response, possibly aiding the prevention of bacterial translocation through intestinal mucosa.

As previously mentioned, embedded within the omentum are aggregations of cells known as ‘milky spots’ that are an intra-peritoneal source of lymphocytes, polymorphonuclear, and macrophages. Formed by these cells clustered around capillary convolutions (omental glomeruli) that lie directly under the mesothelium. The migration of cells from the ‘milky spots’ into the peritoneal cavity is facilitated by the absence of basal lamina in the sub-mesothelial connective tissue. Macrophages tend to form aggregates near the peritoneal surface of the ‘milky spots’ and are orientated toward the peritoneal cavity while lymphocytes converge in peri-arteriolar locations.
Although they are involved in lymphocyte activation and proliferation, due to a lack of dendritic cells, controversy exists as to whether ‘milk spots’ count as secondary lymphoid organs.\textsuperscript{535} Being important an important source of natural antibodies,\textsuperscript{536, 537} ‘milky spots’ are reactive structures with both unstimulated and stimulated states and so perhaps are better classified as perivascular infiltrates. The cells within ‘milky spots’ are supported by a delicate network of reticular fibres and are infiltrated by non-myelinated nerve fibres. dopamine immuno-reactivity has been shown in the nerve fibres and in a portion of the macrophage population suggesting that ‘milky spots’ are sites of possible neuro-immuno-humoral interactions.\textsuperscript{538}

During inflammation, the number and size of ‘milky spots’ increase dramatically, and some develop lymphocyte germinal centres and produce antigen-specific antibodies as demonstrated by the intra-peritoneal injection of streptococcal antigen which produced such changes with 3 hours.\textsuperscript{130} As well as locally-activated lymphocytes, ‘milky spots’ also come into contact with lymphocytes that have encountered distant antigens and recirculated back. In this way, milky spots form part of the general surveillance route for antigen-activated lymphocytes in search for their antigen.\textsuperscript{539}

**STEP 4: Fibrin and abscess formation**

The abdominal deposition of fibrin, the end product of activation of the coagulation cascade, is one of the macroscopic hallmarks of peritonitis. Its formation is controlled by endotoxin and inflammatory cytokines (IFN-\(\gamma\), granulocyte-macrophage-CFS) that initiate coagulation by the expression of tissue factor on endothelial cells and activated leucocytes in the peritoneal cavity.\textsuperscript{540, 541}
Fibrin deposition has both beneficial and adverse effects during peritonitis. Localised fibrin clots physically entrap bacteria and limit movement through the peritoneal cavity and thus potentially prevent systemic sepsis and reduce mortality.\textsuperscript{542, 543} Fibrin may also have a function in tissue repair and closing or restricting intra-abdominal defects. However, fibrinolysis is impaired during peritonitis and abscesses form when fibrin persists. The central core liquefies while the external perimeter remains impermeable and surrounded by phagocytes. Once an abscess is established, it usually only resolves after drainage.
REFERENCES


APPENDIX B

PERITONEAL HUMORAL RESPONSE TO INJURY
In response to peritoneal injury and infection, mesothelial cells and local peritoneal immune cells secrete a variety of mediators responsible for both the local and systemic inflammatory response and subsequent tissue repair with clinical implications including adhesion formation.\textsuperscript{122, 127, 135} The following section introduces some of the key humoral factors known to mediate peritoneal and systemic inflammatory processes following peritoneal infection and iatrogenic injury.

\textit{Nitric oxide (NO)}

This vasoactive gas is produced by the endothelium of peritoneal vessels in response to peritoneal injury. Its production is regulated by nitric oxide synthase (NOS) whose action is to promote NO production. There are three NOS isoforms (endothelial, neuronal, and inducible) and they are differentially expressed in the peritoneal membrane.\textsuperscript{544} Inflammatory cytokines and bacterial lipopolysaccharide (LPS) down regulate eNOS\textsuperscript{545} and prevents NO production to generate vascular responses to inflammation: vasoconstriction, activation of coagulation cascade, and smooth muscle proliferation.\textsuperscript{546} Within the enteric nervous system, NO is produced via the activity of nNOS and acts as the main inhibitory neurotransmitter monitoring intestinal motility. Surgical manipulation of bowel enhances the expression of nNOS.\textsuperscript{547} While neuronal and endothelial isoymes are constitutive isoforms whose activity is controlled by intracellular calcium ion levels, iNOS is quiescent until its transcription is activated by cytokines and/or LPS.

\textit{Cytokines}

Cytokines are a family of polypeptides and glycoproteins with molecular weights ranging from 8 to 30 kD. They are produced locally by damaged cells at the site of injury or by
systemic immune cells and function via paracrine and autocrine mechanisms. Usually short-lived, they play critical roles in the acute phase response to peritoneal injury from both intraperitoneal infection and abdominal surgery. Rather than being synthesised constitutively, they are induced in response to cellular activation.

Among the pro-inflammatory cytokines, tumour necrosis factor-α (TNF-α) is a primary mediator of sepsis. The injection of endotoxin into healthy volunteers results in the appearance of TNF-α in plasma and this leads to the activation of other pro-inflammatory cytokines. In the setting of peritonitis, TNF-α is one of the first cytokines to be produced by peritoneal mesothelial cells in response to a varied range of stimulants and acts as an important upstream pleiotropic cytokine. Ongoing peritoneal production of TNF-α has also been suggested as a useful early clinical indicator for postoperative intraabdominal complication.

Interleukin-6 (IL-6) is a 26 kDa protein considered to be another mediator of the acute phase response to intraperitoneal injury possessing both pro- and anti-inflammatory properties. It is released early in response to endotoxin challenge and stimulates the production of most acute-phase proteins. Systemically, it causes hepatocytes to synthesise several plasma proteins such as fibrinogen that contribute to the acute-phase response. Produced mainly by the endothelium of peritoneal vessels, as well as activated T cells, fibroblasts, and mononuclear phagocytes, peritoneal levels of IL-6 start to increase within the first hour of injury and significantly rise after 4 hours. Significantly elevated serum levels are then detected 6 hours post injury and persist for as long as 10 days. In adult patients with intraabdominal sepsis, serum levels are elevated and correlate to poor outcomes.
while in paediatric patients, persistently elevated postoperative serum IL-6 levels in those diagnosed with peritonitis correlate to prolonged LOS and increased morbidity.\textsuperscript{557}

During acute peritonitis, IL-6 is a key mediator involved in the regulation of leukocyte transmigration into the peritoneal cavity, in particular, the switch from an initial rapid influx of neutrophils to the eventual accumulation of mononuclear cells, monocytes, macrophages, and lymphocytes.\textsuperscript{139,558}

Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced by activated macrophages and helper T cells. Its major functions include inhibiting activated macrophages, limiting the production of several pro-inflammatory cytokines, and down regulating functional properties of other immunocompetent cells.\textsuperscript{162} Working in conjunction with the anti-inflammatory actions of mesothelial cell glucocorticoid receptors and endogenous TNF-\(\alpha\)/IL-1 antagonists,\textsuperscript{559,560} the actions of IL-10 maintain homeostatic control of innate and cell-mediated immune reactions.

\textit{Chemokines}

Stimulation of peritoneal mesothelial cells by activated complement or cytokines results in the release of a number of different chemokines that direct the transmigration of neutrophils and monocytes into the peritoneal cavity including interleukin-8 (IL-8), leukotriene-B4 (LT-B4), and monocyte chemoattractant protein-1 (MCP-1).\textsuperscript{502}

IL-8 is an 8 kDa protein that is serves as a highly selective neutrophil chemoattractant, produced by activated monocytes/macrophages. Its effects on neutrophils are not restricted
to inducing the influx of neutrophils into inflamed tissue; at high concentrations, it also activates neutrophils to degranulate their intracellular stores, thereby promoting the inflammatory reaction.\textsuperscript{561} Given their unique properties, it comes as no surprise that in the setting of acute appendicitis, peritoneal mesothelial cells appear to be the main source of IL-8 production.\textsuperscript{229, 562}

\textit{Growth factors}

The peritoneum has a unique property of being able to rapidly re-mesothelialise after trauma. Irrespective of the size of injury, peritoneal re-mesothelialisation is complete within 5-7 days.\textsuperscript{563} Several mechanisms are responsible including migration of adjacent mesothelial cells, metaplasia of subperitoneal connective tissue cells, transformation of peritoneal cells into mesothelial cells, and mesothelial cell replication. Growth factors such as TGF-\textalpha, TGF-\textbeta, and platelet-derived growth factor (PDGF) are known to play important roles in peritoneal regeneration.\textsuperscript{440, 564} For example, under normal conditions, mesothelial cell proliferation is low with 0.16-0.5\% of cells in mitosis at any one time. During peritoneal repair, increased levels of growth factors and cytokines drive the rate of mitosis up to 30-60\%.\textsuperscript{437}

The interplay between growth factors and the fibrinolytic pathways also drive the formation of peritoneal adhesion.\textsuperscript{440} Vascular endothelial growth factor (VEGF) is a potent angiogenic growth factor and vascular permeability-inducing agent known to be an important marker\textsuperscript{565} for and mediator\textsuperscript{566} of postoperative peritoneal adhesions.
Coagulation factors

The main roles of the coagulation cascade in peritoneal inflammation and repair is fibrin formation and its reverse, fibrinolysis. Injury to the peritoneum from surgery, infection, or irritation elicits a local inflammatory response that results in the accumulation of a serosanguineous, fibrin-rich exudate as part of the haemostatic process and this is an essential component of normal tissue repair. But if it is not resolved in a timely fashion, the fibrin deposits provide a matrix for invading fibroblasts and new blood vessels. Eventually with the deposition of collagen and vascular ingrowth, these organise into fibrous, permanent adhesions.

Fibrin formation is the result of activation of the coagulation cascade during haemostasis, inflammation, and tissue repair. The product from activation of the coagulation cascade is thrombin (factor IIa), formed from prothrombin (factor II). Thrombin then triggers conversion of fibrinogen into fibrin monomers that interact with one another and polymerise forming fibrin clots. But fibrin is meant to fulfil only a temporary role in tissue repair and must be resolved when normal tissue structure and function is restored.

Fibrinolysis is driven by the enzyme plasmin, produced from plasminogen – the inactive proenzyme – via conversion by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA).\textsuperscript{567} tPA is the principal physiological plasminogen activator in peritoneal tissue during peritonitis and is mainly derived from endothelial cells but has also been isolated from virtually all tissues, including peritoneal mesothelial cells and macrophages.\textsuperscript{568} To regulate fibrinolysis, the actions of tPA and uPA are inhibited by plasminogen activator inhibitor-1 (PAI-1) which is also produced and released by a variety of cells including
endothelial cells, mesothelial cells, macrophages, platelets, and fibroblasts. It has been suggested that PAI-1 is an acute-phase reactant protein\textsuperscript{569} whose production and release is enhanced after peritoneal inflammation and surgery. Therefore, the fibrinolytic activity of inflamed or damaged peritoneum is lower than that of normal tissue.

\textit{Matrix metalloproteinases}

This family of 28 enzymes are primarily responsible for turnover and remodelling of the peritoneal ECM and basement membrane components in both normal and pathological processes.\textsuperscript{570} Collectively, they can degrade all the components of the ECM. Each of these zinc-dependent and tightly-regulated endopeptidases are secreted in zymogen form and activated by proteolytic cleavage of the pro-peptide portion. During peritoneal inflammation and repair, to accommodate extensive ECM remodelling, the expression of MMPs is significantly increased.\textsuperscript{570} The potentially hazardous activity of MMPs is controlled through binding to tissue inhibitor of metalloproteinases (TIMPs 1-4). The balance between MMPs and TIMPs is important during normal physiologic events such as tissue repair and embryogenesis, as well as during pathological processes such as tumour invasion.\textsuperscript{571}
REFERENCES


28. diZerega GS, Campeau JD. Peritoneal repair and post-surgical adhesion formation. 
   Hum Reprod Update 2001;7:547-55.

29. Holmdahl L, Kotseos K, Bergstrom M, Falk P, Ivarsson ML, Chegini N. Overproduction 
   of transforming growth factor-beta1 (TGF-beta1) is associated with adhesion 

30. Fukasawa M, Yanagihara DL, Rodgers KE, DiZerega GS. The mitogenic activity 

31. Mutsaers SE, Whitaker D, Papadimitriou JM. Stimulation of mesothelial cell 
    proliferation by exudate macrophages enhances serosal wound healing in a murine 

    expression in surgical postoperative peritoneal adhesions. World J Surg 

33. Cahill RA, Wang JH, Soohkai S, Redmond HP. Mast cells facilitate local VEGF release 
    as an early event in the pathogenesis of postoperative peritoneal adhesions. Surgery 
    2006;140:108-12.

34. Hellebrekers BWJ, Kooistra T. Pathogenesis of postoperative adhesion formation. Br 

35. Vipond MN, Whawell SA, Thompson JN, Dudley HA. Peritoneal fibrinolytic activity 

    cell line HepG2 is increased by cytokines—evidence that the liver contributes to 

37. Cohen PA, Gower AC, Stucchi AF, Leeman SE, Becker JM, Reed KL. A neurokinin-1 
    receptor antagonist that reduces intraabdominal adhesion formation increases 

APPENDIX C

CLINICAL STUDY B: PARTICIPANT INFORMATION SHEETS
Determining Duration of Antibiotic Therapy for Children with Appendicitis-Related Intra-abdominal Infection

Participant Information Sheet – Parent/Caregiver

Principal Investigator:
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We would like to tell you and your child about a study currently running at Starship Children’s Hospital that aims to look carefully at a treatment strategy for children affected by appendicitis-related intra-abdominal infections. We are running this study together with clinical researchers from the University of Auckland.

To help you understand our study, this information sheet contains a comprehensive summary of the study. The operating surgeon will also explain the study and answer questions for you and your child. Please read this information sheet carefully and approach the operating surgeon if you, your child, or your family/Whanau members have any questions.

We have also prepared a separate study information sheet designed especially for your child.

Your child’s participation in this study will be stopped if any harmful effects appear or if we feel that it is not in your child’s best interest to continue.

Why are we interested in your child?
Your child has been diagnosed with acute appendicitis (Figure 1), a surgical disease that is common in children. It is caused by bacterial infection of the appendix and can progress to become a serious infection in the abdominal cavity. Once the disease spreads beyond the appendix, the peritoneum (an extensive membrane lining the abdominal cavity and enclosing the solid organs) becomes infected and this is known as peritonitis. It requires an operation to remove the appendix followed by treatment with intravenous antibiotics (administered by injection through a drip).
Why are we conducting this study?
Antibiotic therapy for appendicitis-related infections has been very effective since its introduction 50 years ago. At Starship Children’s Hospital, children with this intra-abdominal infection have traditionally been treated with antibiotics for a minimum of 5 days. This required children to stay in hospital for a minimum of 5 days to receive repeated injections. There is good evidence now to show that this treatment strategy is not ideal.

International experts recommend that treatment for appendicitis-related abdominal infections in children should be individualized and based on the patient’s response to therapy. Instead of treating all children for a fixed length of time, they recommend using of a standardized set of guidelines to determine the required length of antibiotic therapy for each child.

These guidelines are a set of carefully selected symptoms and signs that represent severity of ongoing infection. Once a child no longer shows these symptoms and signs, we can safely assume that the infection in their abdomen has been successfully treated and antibiotic therapy can be stopped.

Paediatric surgeons and Infectious Diseases Specialists at Starship Children’s Hospital believe that the recommended treatment method is superior to the traditional treatment method. We have adopted these recommendations hoping to improve the health outcomes of children affected by appendicitis-related infections.

To confirm that the new treatment method is effective and safe, we are conducting a study to observe the first group of children managed this way. We will be comparing their treatment outcomes with those of a similar group of children treated in the past. It is hoped that the new treatment method will reduce length of hospital stay and improve treatment outcomes.

What happens during the study?
We will assess how suitable your child is for this study either prior to their operation or within the first 24 hours after their operation.
The new treatment method for antibiotics will not affect your child’s operation in anyway. After their operation, the appropriate antibiotics will be administered to your child to help their body fight the infection. The duration of their antibiotic therapy in hospital will be determined by the severity of their infection and by your child’s response to therapy.

Antibiotic therapy will be stopped once your child satisfies the following clinical criteria:

1. No fever continuously for 24 hours
2. Tolerates 2 consecutive light meals
3. Pain is controlled only with the use of oral pain-killers
4. Walking independently without assistance (if previously able to do so)

We will be observing your child’s recovery on the ward, from the time of their operation to the time that they are discharged. We will be recording this information and using it in a study comparing your child’s treatment outcomes with the outcomes of a group of similar children treated in the past.

We will also be inviting you and your child back to Starship Children’s Hospital for a follow-up visit 2 weeks from the time your child is discharged.

Risks and Benefits
Antibiotics have significantly helped to improve the treatment of children affected by intra-abdominal infections from advanced appendicitis. They work alongside the body’s natural defence system to prevent spread of infection and to help eradicate bacteria from the body.

The ideal length of antibiotic therapy is a balance between under-treatment and over-treatment. Under-treatment can lead to insufficient clearance of the infection while over-treatment is associated with increased risks of bacterial resistance and development of antibiotic-related adverse effects. It is also associated with longer hospital stay.

International experts suggest that the length of antibiotic therapy should be based on individual patient response. This should effectively prevent over-treatment and under-treatment in children with appendicitis-related abdominal infections.

Results
We expect that recruitment of study participants will require 12 to 15 months. After completion of this study, we plan to share our findings with doctors internationally by publishing the results in a widely-read medical journal.

Compensation
Due to the study’s low risks, we can confidently say we do not anticipate any physical harm to your child as a result of the study. However, in the unlikely event of a physical injury or an adverse outcome in your child as a result of participation in this study, ACC Coverage under the Injury Prevention, Rehabilitation and Compensation Act may be available. Please note: ACC cover is not automatic, thus your child’s case will be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If the claim is accepted by ACC, your child still might not get any compensation. This depends on various factors including whether you are an earner or non-earner.
ACC usually provides only partial reimbursement of costs and expenses; there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.

Confidentiality
All collected information will be securely stored by the investigators at Starship Children’s Hospital and at the University of Auckland in password-protected computers. No data material that could be linked to the identity of your child will be included in any publications of this study.

Contacts
If you have any queries or concerns regarding your child’s rights as a participant in this study you can contact an independent Health and Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Telephone (NZ wide): 0800 555 050
Free Fax (NZ wide): 0800 2787 7678 (0900 2 SUPPORT)
Email (NZ wide): advocacy@hdc.org.nz

For Maori health support at ADHB or to discuss any concerns or issues regarding this study, please contact Mata Forbes RGON, Maori Health Services Co-ordinator/Advisor, 5th Level, GM Suite, Auckland City Hospital. Tel: (09) 3074949, extension 23939 or Mobile 021 348 432.

For all other inquires about this study, please contact the Principal Investigator or the Co-Investigator:

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THIS STUDY HAS RECEIVED ETHICAL APPROVAL FROM THE NORTHERN X REGIONAL ETHICS COMMITTEE (20/06/2011).
We are sorry that you are not feeling well and need an operation for a sick appendix.

We would like you to help us find out more about the way children get better after this operation.

**What happens?**

Your operation will be done by doctors in the usual way. After the operation you will be given medicines to help you fight the infection. The nurses will check your temperature to see if you have any fevers.

The medicines will be stopped once the doctors and nurses can see that you are much better.

**What do we want to know?**

- To check that you are much better, doctors and nurses will look at your temperature, how sore your tummy is, when you are first able to eat a full meal, and also walk without help.

The doctors will use all this information to decide when they can stop your medicines and send you home.

To help doctors understand more about the way children get better after operations, we will carefully collect information about how you get better after your operation.

This information will be into a computer together with information from other children. It will help us understand more about how children get better after operations for a sick appendix.

**Why do we need your help?**

Lots of children and young people get a sick appendix (appendicitis) and need an operation to remove the appendix. Then they need medicines to help them fight the infection.

We would like your help to understand more about how much medicine is right for children after the operation.

All the stuff we learn will be kept private – other people will not told who took part in this study.

If you have any questions, talk to your parents (they have some more information) or talk to the doctors and nurses.

Thank you for helping us out 😊

Antibiotic Therapy for Intraabdominal Infections in Children – Version 2 June 20th 2011
APPENDIX D

CLINICAL STUDY B: DATA COLLECTION FORM AND CHECKLIST
IV ABs Study Investigator’s Checklist

Recruitment
☐ Ethnicity: NZ European / Maori / PI / Asian / Other: ________________
☐ Weight: ___________ (Kg)
☐ Date of Presentation: ________________ Time of Presentation: ________________
☐ Presenting HR: ___________ bpm
☐ Presenting Temp (Tympanic / Rectal / other): ___________ °C
☐ Presenting BP: ___________ mmHg
☐ Presenting Bloods: CRP ___________ WBC ___________ Seg. Neut. ___________
☐ Presenting Creatinine: ___________
☐ Duration of Presenting Symptoms: _______________________
☐ Main Presenting Symptom: ____________________________

Set-up
☐ Preoperative antibiotics:

<table>
<thead>
<tr>
<th>Type</th>
<th>Dosage (mg per kg)</th>
<th>Number of doses during admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
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☐ Date of Operation:
☐ Name of Procedure:
Intraoperative

☐ Bear hugger: YES / NO
☐ Seniority of Surgeon: __________________________

<table>
<thead>
<tr>
<th>OT Start Time (Knife to Skin):</th>
<th>OT End Time (Dressings applied):</th>
</tr>
</thead>
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Macroscopic appearance of appendix:

Peritonitis?
- ☐ Acute inflammation of the peritoneum
- ☐ Purulent or faecal exudates (not including intra-operative iatrogenic contamination)
- ☐ Frank viscus perforation
- ☐ Localised abscess
- ☐ Residual fibrinous deposits
- ☐ Unresectable tissue necrosis

☐ Any Contamination of Peritoneal Cavity with Faeces: YES / NO
☐ Intraoperative Iatrogenic Contamination: YES / NO
☐ Transperitoneal Drain?

Peritoneal irrigation? YES / NO

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Volume</th>
<th>Temperature</th>
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Intraoperative IV fluid type, temperature and volume given:

<table>
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<tr>
<th>Fluid</th>
<th>Volume</th>
<th>Temperature</th>
</tr>
</thead>
</table>

☐ Intraoperative antibiotics:
**Postoperative**

**PACU and DAY 0**
- Tympanic temp in PACU:
- Shivering in PACU? **YES / NO**
- Postoperative fever in PACU?
- PCA used: **YES / NO**

- Postoperative Antibiotics:

<table>
<thead>
<tr>
<th>Type</th>
<th>Dosage (mg per kg)</th>
<th>Number of doses during admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DAY 1**
- **DATE:**
  - Afebrile (Temp < 38.0 °C) for 24 hours
  - Tolerating Light Diet for 2 consecutive meals
  - Pain adequately managed with oral analgesia
  - Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments

**DAY 2**
- **DATE:**
  - Afebrile (Temp < 38.0 °C) for 24 hours
  - Tolerating Light Diet for 2 consecutive meals
  - Pain adequately managed with oral analgesia
  - Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments
**DAY 3**  
- Afebrile (Temp < 38.0°C) for 24 hours  
- Tolerating Light Diet for 2 consecutive meals  
- Pain adequately managed with oral analgesia  
- Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments

**DAY 4**  
- Afebrile (Temp < 38.0°C) for 24 hours  
- Tolerating Light Diet for 2 consecutive meals  
- Pain adequately managed with oral analgesia  
- Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments

**DAY 5**  
- Afebrile (Temp < 38.0°C) for 24 hours  
- Tolerating Light Diet for 2 consecutive meals  
- Pain adequately managed with oral analgesia  
- Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments

**DAY 6**  
- Afebrile (Temp < 38.0°C) for 24 hours  
- Tolerating Light Diet for 2 consecutive meals  
- Pain adequately managed with oral analgesia  
- Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments
DAY 7

- Afebrile (Temp < 38.0°C) for **24 hours**
- Tolerating Light Diet for 2 consecutive meals
- Pain adequately managed with oral analgesia
- Mobilising Independently (if ambulatory)

- Protocol Non-adherence?

- Comments

DAY 8+

- Comments
**COMPLICATIONS:** Severity as per Clavien-Dindo Classification

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>GRADE</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

**DISCHARGE DETAILS**  
Date of Discharge:

- Date Discharge Criteria achieved: ________________
- Postoperative nights in hospital:
- Total nights in hospital:

- **Discharging Authority:** Consultant / Fellow / Registrar / House Officer

- Oral AB’s to make up 7 days in total
HISTOLOGY RESULT

2-Week Follow-up

- GP Visits:

- Re-admission within 30 days post-discharge requiring hospital stay > 24 hours:

- Significant conditions affecting recovery:

- Further interventions:

Other issues
i.e. Non-adherence to study protocol

Postoperative blood tests results?
APPENDIX E

CLINICAL STUDY C: PARTICIPANT INFORMATION SHEETS AND CONSENT FORMS
Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial – WARMIST Study

Participant Information Sheet – Parent/Caregiver

Principal Investigator:
Mr James Hamill – Paediatric Surgeon
Starship Children’s Hospital
Private Bag 92 024, Grafton
Work phone no. 09 379 7440 ext. 6381
Emergency phone no. 021 753 081
Email jamesh@adhb.govt.nz

We would like to invite your child to participate in a study to find out if there is a better way to remove the appendix for acute appendicitis during key-hole surgery (also known as laparoscopic surgery). This study will investigate whether this common operation can be performed with reduced risk of hypothermia (abnormally low body temperature), less pain, and faster recovery. Current conventional method is to inflate the abdominal cavity with cold, dry carbon dioxide (CO₂) gas to achieve adequate visualisation of the organs. This can lead to cooling down of core body temperature and cause drying out of the abdominal cavity lining. Both events can cause unnecessary pain and slow the child’s recovery. Our new approach is to use warm and humidified CO₂ gas to minimise cooling of the body and maintain moisture of the abdominal cavity.

To assist you in making the best informed choice for your child, this information sheet contains a comprehensive summary of our study. The operating surgeon will also explain the study and answer questions for you and your child. Please read this information sheet carefully and approach the operating surgeon if you or your child have any questions.

In order for your child to participate, we will ask give both you and your child to give formal consent. We have prepared a separate participant information sheet designed especially for your child.

Your child’s participation in this study is entirely voluntary. You are welcome to involve family/Whanau, friends, and Whanau Support staff to discuss details of the study and help you make an informed decision. Your child or you may decide to withdraw from the study at any time. Your child will not need to give a reason. After reading the study information and meeting the operating surgeon, if your child and you decide not to take part, your child will receive the usual best treatment and care. This study will be stopped should any harmful effects appear or if the doctor feels it is not in your child’s best interest to continue.
Why are you and your child being asked?
Your child has been diagnosed with acute appendicitis, a condition which requires an urgent operation to remove the inflamed and infected appendix. The majority of children with appendicitis are treated with laparoscopic surgery.

FIGURE 1. Laparoscopic appendicectomy

Laparoscopic removal of the inflamed appendix (appendectomy) is performed by inflating the abdominal cavity with CO₂ gas then inserting a video camera and instruments into the abdomen through three small incisions. Usually, this gas is non-humidified (“dry”) and non-heated (“cool”). During the last decade, studies like this one have been conducted and repeated many times in adults and animal. The majority of them have shown that using warm and humidified gas can reduce the risk of hypothermia, reduce post-operative pain and speed up recovery. There is enough evidence to indicate that this new approach may also be beneficial for children. Using warm and humidified gas is very safe and there are no added risks of complications.

The study will investigate whether warm humidified gas for insufflation (blowing gas into the abdomen) will reduce the risk of your child developing hypothermia during the operation and reduce their post-operative pain. It will also investigate whether this helps them recovery more quickly after the operation.

What happens during the study?
We will use a computer generated ‘flip-of-the-coin’ method known as ‘Randomisation’ to allocate study participants to each of the two groups. Half of the study participants will have dry cool CO₂ for abdominal cavity inflation during their operation and the other half will have warm humidified CO₂ gas. With the exception the CO₂ gas used during the operation, all other aspects of medical care including anaesthesia, surgery, and post-operative care will be identical for all children.

During the operation, doctors will monitor your child closely, including changes in their core body temperature. After the operation, your child will be monitored by Recovery Room nurses who will measure pain using a scoring diagram, as well as record heart rate, blood pressure, breathing rate, temperature and level of consciousness.
Once on the ward, nurses will continue to measure and record your child’s pain scores, and monitor their progress to recovery. We will be interested in how soon after the operation your child feels hungry, first passes wind, first eats a full meal, and first passes a bowel motion. We will also want to know how soon after the operation your child is able to walk independently and how soon they venture outside of their room. Lastly, we will be recording the total amount of pain-relief medications your child requires from the time of the operation to the time of discharge from hospital.

Your child will be discharged from hospital once he or she is comfortable and tolerating enough food. We will continue to monitor their progress to full recovery by asking your child to fill out a questionnaire on Day 10 after the operation. They will not have to answer all the questions. A copy of this Questionnaire is available for you and your child to read before consenting to participate in this study.

To remind you and your child about this questionnaire, we ask for your permission to contact you or your child by phone on Day 8 or Day 9 after the operation. If the questionnaire has been accidentally displaced, we will ask you and your child to help us complete the questionnaire over the phone.

We aim to enrol 190 children in our study and anticipate that it will be run over the duration of 15 months.

**Risks and Benefits**

Laparoscopic surgery in children for removal of the appendix has become a common procedure over the last two decades, noted for its safety and early recovery. CO\(_2\) gas is the choice of gas for inflating the abdominal cavity because it is safe, inflammable, and the body can easily absorb it. Warming and humidifying CO\(_2\) gas used to inflate the abdominal cavity has recently been shown to benefit patients by reducing risks of hypothermia (abnormal body temperature), reducing pain after surgery and speeding up recovery. Studies to prove this have so far involved adults and animal models.

With extensive studies already been done in humans, we do not anticipate ill-effects in any of the children from changes made in temperature and humidity of CO\(_2\) gas for the purpose of this study. By taking part in this study, your child will play a crucial part in discovering potentially groundbreaking innovation to improve current practice in paediatric laparoscopic surgery.

**Compensation**

Due to the study’s low risks, we can confidently say we do not anticipate any physical harm to your child as a result of the study. However, in the unlikely event of a physical injury or an adverse outcome in your child as a result of participation in this study, ACC Coverage under the Injury Prevention, Rehabilitation and Compensation Act may be available.

ACC cover is not automatic, thus your child’s case will be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If the claim is accepted by ACC, your child still might not get any compensation. This depends on various factors including whether you are an earner or non-earner.

ACC usually provides only partial reimbursement of costs and expenses; there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury.

If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.
Confidentiality
All collected information will be stored under strict security by the investigators in the Department of Paediatric Surgery at Starship Children’s Hospital. No data material that could be linked to the identity of your child will be included in any publications of this study.

Results
We anticipate participant recruitment for this study will need 15 months. After completion of the study, we plan to share our findings with surgeons internationally by the results from this study in a widely read medical journal. Study participants and their families can request for a copy of the study’s results.

Contacts
If you have any queries or concerns regarding your child’s rights as a participant in this study you can contact an independent Health and Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Telephone (NZ wide): 0800 555 050
Free Fax (NZ wide): 0800 2787 7678 (0900 2 SUPPORT)
Email (NZ wide): advocacy@hdc.org.nz

For Maori health support at ADHB or to discuss any concerns or issues regarding this study, please contact Mata Forbes RGON, Maori Health Services Co-ordinator / Advisor, 5th Level, GMSuite, Auckland City Hospital. Tel: (09) 3074949, extension 23939 or Mobile 021 348 432.

For all other inquires about this study, please contact the Principal Investigator or the Co-Investigator:

Dr Tzu-Chieh (Wendy) Yu
Research Fellow/Lecturer
Department of Surgery
University of Auckland

South Auckland Clinical School
Private Bag 93311
Middlemore Hospital
Otahuhu, Auckland

Work phone no. 09 276 0076
Fax no. 09 276 0066
Emergency no. 02102559841
Email: wendy.yu@auckland.ac.nz

THIS STUDY HAD RECEIVED ETHICAL APPROVAL FROM THE NORTHERN X REGIONAL ETHICS COMMITTEE (01/10/2009).
We would like to invite you to take part in our study (experiment) to find out if there is a better way to remove the appendix for acute appendicitis during key-hole surgery (also called laparoscopic surgery).

We want to find out whether this operation can be done with less risk of:

1. hypothermia (abnormal cooling down of body temperature) during the operation
2. extra pain after the operation
3. slow recovery after the operation

To help you decide if you want to take part in this study, please read all of the information carefully together with your family/Whanau. The doctor who will carry out your operation (the surgeon) will also talk to you and answer any questions. For you to take part in this study, we need written consent (an okay!) from you and an adult from your family/Whanau.

Taking part in this study is entirely voluntary (your choice). You can talk to your family/Whanau and your friends about this study to help you make your decision. You can stop taking part in the study at any time and you do not need to tell us why. The study will be stopped if doctors find that the treatment is harmful to you or other children. After reading this information and meeting the doctors, if you and your family/Whanau decide that you do not want to take part in the study, you will receive all the usual best treatment and care.
Why are we asking you?
We think your tummy pain is caused by **acute appendicitis**. This is when your appendix is **infected** and **inflamed**. To get you better, the infected appendix in your abdomen will be removed by the surgeon. Most children are treated with an operation call **laparoscopic appendicectomy**.

**FIGURE 1.** Laparoscopic (key-hole) removal of the appendix.

![Diagram of normal anatomy and laparoscopic appendicectomy](image)

During laparoscopic appendicectomy, your abdomen is blown up with cold and dry **carbon dioxide** (CO₂) gas to create a working space for the doctors. This cold gas can cause your body to cool down and its dryness can damage the inside lining of your abdomen. We think that it causes children extra pain and they need more time to get better after the operation.

A new way of blowing gas into the abdomen is to **warm** it up and make it **humid** (‘moist’). During the last decade, studies similar to our study in adults and animals have shown that warm and humid CO₂ gas reduces the risk of abnormal cooling of the body (**hypothermia**) during surgery and the pain after surgery. They also show that using warm and humid gas speeds up recovery after surgery and that it is very safe.

We want to find out if warm and humid gas for **insufflation** (blowing gas into the abdomen) will reduce your risk hypothermia and your pain after surgery, and help children like you get better more quickly after their operation.

**What happens during the study?**
We will use a computer-generated ‘flip-the-coin’ method called **randomisation** to decide whether a study participant gets **warm moist** gas or **cool dry** gas. Half of the children taking part in this study will have warm moist gas blown into their abdomen during their operation and the other half will have cool dry gas. All children will receive the same operation and exactly the same care in hospital. Doctors and nurses will **not** know what kind of CO₂ gas was used during your operation until the end of the study.
During the operation, doctors will monitor your temperature closely. After the operation, nurses who will ask you to rate your pain from 0 to 10 (10 is the worst pain you can imagine and 0 is no pain). They will ask you to rate your pain when you are resting and when you are moving about.

We want to find out how much medications you need to treat your pain after the operation. We also want to know how quickly your bowels start to work again after the operation. We will ask you to try to remember when you first feel hungry, when you first eat a full meal, when you first pass wind, and when you first pass a bowel motion.

Finally, we want to know if using warm and moist gas during key-hole surgery helps you recover quicker after the operation. To help us collect this information we need you to fill out a questionnaire on Day 10 after your operation. You and your family will have a chance to look at this Questionnaire before deciding whether you want to take part in this study. You will not have to answer all the questions.

To remind you about this questionnaire, we would like to give you a telephone call a day or two beforehand. If the questionnaire has been accidently lost, we will ask you to help us complete the questionnaire over the phone.

We hope to ask 190 children to take part in this study. Our findings will be shared with other doctors in New Zealand and around the world when they read the report we write.

What else do you need to know?
Carbon dioxide gas is the safest gas for laparoscopic surgery and it widely used in New Zealand and overseas. So far we know from studies in adults and animals that using warm humid gas during laparoscopic surgery helps to keep patients warm, lessen their pain, and make them get better more quickly. We do not expect any bad effects in either group from the type of CO₂ gas used.

This study is the first study to look at the use of warm moist gas for key-hole surgery in children. Because using warm moist gas is very safe in adults, we know it will be safe in children.

Confidentiality
All information that is collected will be stored under strict security by the study investigators. We will not use your name in any part of this study and no information that can be linked to your identity will be used in any publications of this study.

Compensation
The safe nature of this study enables us to confidently say we do not expect any physical injury to you. However, in the highly unlikely event of something unexpected happening to you as a result of taking part in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. In such an event, we will work with your parents or caregivers and other experienced advisers to give you the best care and support. If you have any questions about ACC, please contact your nearest ACC office or the study investigators.

Contacts
If you have any queries or concerns regarding your child’s rights as a participant in this study you can contact an independent Health and Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Telephone (NZ wide): 0800 555 050
Free Fax (NZ wide): 0800 2787 7678 (0900 2 SUPPORT)
Email (NZ wide): advocacy@hdc.org.nz
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For all other inquiries about this study, please contact the Principal Investigator or the Co-Investigator:

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Otahuhu, Auckland

Work phone no. 09 276 0076
Fax no. 09 276 0066
Emergency no. 02102559841
Email: wendy.yu@auckland.ac.nz

THIS STUDY HAD RECEIVED ETHICAL APPROVAL FROM THE NORTHERN X REGIONAL ETHICS COMMITTEE (01/10/2009).
We are sorry that you are not feeling well and need to have an operation to take out your appendix.

We are asking if you would like to help us when you have your operation.

What happens

You have the operation exactly the same way as they normally do it, except some kids will have the air warmed & moistened and the others have it cool & dry.

The doctors won’t know which type of gas was used (moist or dry) (so he can’t favour one more than the other) but will write down how things went on a special form.

Then What....

After the operation, nurses will check to see if your tummy is sore and give you pain medicine just the same way as they always do. They write down how sore it was and what medicine you needed on a special form.

Doctors and nurses will also ask you to try and remember when you first eat a full meal, pass wind (fart!), pass a bowel motion, and walk without help. This is to find out how quickly you recover after the operation. A questionnaire for you to fill out will also help us find out how quickly you got back to doing normal activities.

All the information is put in a computer, and then they work out if one way was better (kids were not so sore) than the other. If it is - that will become the way they do it all of the time.

Why do we need your help?

When they do your operation the doctors put some gas in your abdomen (tummy) to help them see in there better. Some doctors like to make the gas warm and moist while others use it dry and cool.

We are wondering if this difference keeps kids warmer during the operation, make them less sore after the operation and help them get better more quickly.

All the stuff we learn is kept private – other people are not told who took part in the study.

You don’t have to take part, and if you say no, it will make no difference to how you are looked after.

If you have any questions, talk to your folks (they have a paper with even more stuff about it) or talk to the doctor or nurse.

Thank you for thinking about it.

If you think it is ok to be in our study – here is a place to write it.
I agree to be in the study.

Your name ___________________ Date ________________

Laparoscopic Humidification (WARMIST) Study: Child Information sheet - Version 2  01/10/09
Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial – WARMIST Study

Consent Form – Parent/Caregiver

REQUEST FOR INTERPRETER

<table>
<thead>
<tr>
<th>Language</th>
<th>Request for Interpreter</th>
<th>Circle One</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter.</td>
<td>Circle One</td>
</tr>
<tr>
<td>Maori</td>
<td>E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.</td>
<td>Yes No</td>
</tr>
<tr>
<td>Cook Island</td>
<td>Kai inangaro au i tetai tangata uri reo.</td>
<td>Yes No</td>
</tr>
<tr>
<td>Fijian</td>
<td>Au gadreva me dua e vakadewa vosa vei au</td>
<td>Yes No</td>
</tr>
<tr>
<td>Niuean</td>
<td>Fia manako au ke fakaaga e taha tagata fakahokohoko kupu.</td>
<td>Yes No</td>
</tr>
<tr>
<td>Samoan</td>
<td>Ou te mana’o ia i ai se fa’amatala upu.</td>
<td>Yes No</td>
</tr>
<tr>
<td>Tokelaun</td>
<td>Ko au e fofou ki he tino ke fakaliliu te gagana Peletania ki na gagana o na motu o te Pahefika</td>
<td>Yes No</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oku ou fiema’u ha fakatonulea.</td>
<td>Yes No</td>
</tr>
</tbody>
</table>

I have read and I understand the information sheet dated 01/10/09 for parents/caregivers of volunteers taking part in this study designed to investigate the benefits of warm humidified gas for insufflation during laparoscopic (key-hole) surgery to remove the appendix. My child and I have had the opportunity to discuss this study. We are satisfied with the answers we have been given.

We have had the opportunity to talk to family/Whanau and friends, and also to Whanau Support staff to help us ask questions and understand the study.
We have had time to consider whether my child should participate in this study.
We understand the study involves my child completing a Day 10 Postoperative Questionnaire. We have had a chance to look at this Questionnaire before my child agrees to participate in this study.

We understand that taking part in this study is voluntary and that my child may withdraw from the study at any time if he/she wishes. This will not affect his/her continuing health care. We understand that his/her participation in this study is confidential and that no material which could identify him/her will be used in any reports on this study.

We understand that the treatment being studied will be stopped if it should appear to be harmful.
We understand the compensation provisions for this study.

My child and I know who to contact if my child has any side effects to the study or if anything occurs which I think he/she would consider a reason to withdraw from the study. We know whom to contact if we have any questions about the study.

This study has been given ethical approval by the Northern X Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.
I/my child would like a copy of the results of the study. ☐ Yes ☐ No

I hereby consent to my child taking part in this study.

Signed: ____________________________ Date: ____________

Printed name: ____________________________

Relationship to participant: ____________________________

Address for results: ____________________________

Health Professional Obtaining Consent

Signed: ____________________________ Date: ____________

Printed name: ____________________________

Designation: ____________________________

Interpreter

Signed: ____________________________ Date: ____________

Printed name: ____________________________

Language: ____________________________
Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial – WARMIST Study

Consent Form – Child/Young Person

REQUEST FOR INTERPRETER

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<tr>
<td>Tongan</td>
<td>Oku ou fia ma’u ha fakatonulea.</td>
<td></td>
</tr>
</tbody>
</table>

I have read and understood the study information sheet dated 01/10/09 about this study designed to find out if using warm and moist gas for laparoscopic (key-hole) appendix surgery helps to lessen pain and speed recovery in children. I have had the chance to ask questions about this study. I am happy and satisfied with the answers I have been given.

I have had the study explained to me by ____________________________.
I have had the chance to talk with family/Whanau and friends, and also Whanau Support staff to help me ask questions and understand the study.
I have had time to think about my choice to take part in the study.
I understand the study involves me completing a Day 10 Postoperative Questionnaire. I have had a chance to look at this Questionnaire before agreeing to participate in this study.
I understand that taking part in this study is voluntary (my choice) and I can stop taking part at any time. I understand that not taking part in the study will not change the care I get in hospital. I understand that the use of warm moist gas will be stopped if it should be harmful to me.

If I take part in this study, I understand that information about me will be kept confidential (private). Information that can help people to recognise me will not be used in this study’s report. I understand the compensations provisions for this study.

My parent/caregiver and I know who to talk to if I have any side effects from the study.
My parent/caregiver and I know who to talk to if we have any questions about the study.

This study has been given ethical approval by the Northern X Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.
I would like a copy of the results of the study.  

☐ Yes  ☐ No

I hereby consent to taking part in this study.

Signed:  

Date:  

Printed name:  

Address for results:  

Health Professional Obtaining Consent

Signed:  

Date:  

Printed name:  

Designation:  

Interpreter

Signed:  

Date:  

Printed name:  

Language:  


APPENDIX F

CLINICAL STUDY C: ANAESTHESIA AND ANALGESIA PROTOCOL
Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial – WARMIST Study

ANAESTHESIA & ANALGESIA PROTOCOL

Preoperative Protocol:
- Perioperative IV antibiotics will be given according to Starship Children’s Health Clinical Guidelines for Treatment of Suspected Appendicitis, available online: http://www.starship.org.nz/Clinical%20Guideline%20PDFs/Appendicitis.pdf
- Preoperative Analgesia: Paracetamol: 20 mg/kg, Q6 hourly/PRN
  ✗ No preoperative sedation medications permitted.

Intraoperative Protocol:
- Induction and muscle relaxant agents given at the discretion of the anaesthetist
  ✗ No Dexamethasone permitted
  ✗ No Paracoxib or other NSAIDs permitted
- Oesophageal temperature probe: Placed by anaesthetist, after anaesthetic induction and before the start of surgery. Core body temperature to be recorded by Investigators at baseline and at 10-minute intervals during surgery.
- Room temperature of theatre: 20-22 ºC
- Please document whether upper-body is covered by a forced-air-rewarming blanket (Bair-Hugger)
- Prophylactic antiemetic: one single dose IV Ondansetron 0.15 mg/kg

Standardised intraoperative analgesia:
- IV Morphine at 0.1-0.3 mg/kg with up to 2 µg/kg IV Fentanyl titrated as required. No basal infusion of morphine permitted.
- Paracetamol: one single dose 20 mg/kg IV if it was not given preoperatively.
- Type, volume, and temperature of intraoperative IV fluids given at the discretion of the anaesthetist.
- Bupivacaine (Marcain 0.25%) – 1 mL/kg maximum allowed for infiltration in subcutaneous tissue around laparoscopic port sites at the end of the procedure.
Postoperative Analgesia Protocol:

REGULAR simple analgesia in the first 48 hours

1/ Paracetamol
Oral 20 mg/kg, Q6 hourly
If vomiting, IV 15 mg/kg, Q6 hourly

2/ Diclofenac – (if not contraindicated)
Oral 1 mg/kg (50 mg if over 50 kg), Q8 hourly, whole or dissolvable tablets
If vomiting, suppository 1 mg/kg (50 mg if over 50 kg), Q8 hourly

After the first postoperative 48 hours, paracetamol and diclofenac can be given as required.

Nurse-led opiate analgesia for the duration of hospital stay
Any opiate analgesia required in the first 24 hours postop is given INTRAVENOUSLY to counter variations in gastrointestinal absorption and PONV.

1/ IV Morphine or Fentanyl
Given according to the Starship Clinical Guideline for Morphine Administration, available online:

Patient-controlled analgesia (PCA) infusions may be required and will be prescribed by the Acute Pain Team or on-call anaesthetist.

× PCA should not be routinely started in PACU

2/ Oral Morphine as required
Available after the first postoperative 24 hours at 0.3 mg/kg, Q1-2 hourly/PRN

Postoperative antiemetics and IV fluids can be given at the discretion of ward medical staff.

PLEASE CONTACT STUDY INVESTIGATORS – DR YU (021 02559841) OR MR HAMILL (021 753081) – IF THERE ARE ANY CONCERNS REGARDING THIS STUDY.

THANK YOU
APPENDIX G

CLINICAL STUDY C: DATA COLLECTION FORM AND CHECKLIST
WARMIST Investigator’s Checklist

Attach Patient Sticker Here

Recruitment

☐ Consent from child and parent
☐ Ethnicity: NZ European / Maori / PI / Asian / Other: ____________

☐ Height: ____________ (cm)

☐ Weight: ____________ (Kg)

☐ BMI: ____________kg/m²

☐ Presenting HR: ____________ bpm

☐ Presenting Temp (Tympanic / Rectal / other): ____________ °C

☐ Presenting BP: ____________ mmHg

☐ Presenting Bloods: CRP __________ WBC __________ Neut __________

☐ Duration of Presenting Symptoms: ________________

☐ Date of Presentation: ________________  Time of Presentation: ____________

Set-up

☐ Inform Anaesthetist – Give Study Protocol
☐ Set OT room temperature at 20-22 °C
☐ Give unblinded theatre nurse envelope for randomisation
☐ Preoperative analgesia given:

☐ Date of Operation:
☐ Name of Procedure:
**Intra-operative**

- Oesophageal temperature probe placement by Anaesthetist
- Bear hugger: **YES /NO**
- Anti-fogging preparation
- Seniority of Surgeon:

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<tr>
<th>Time:</th>
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**Intra-operative core body temperature: Oesophageal**

<table>
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<tr>
<th>0min</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
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<th>140</th>
<th>150</th>
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<th>190</th>
<th>200</th>
<th>210</th>
<th>220</th>
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<th>240</th>
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<tr>
<th>L/hr</th>
<th>mmHg</th>
<th>L</th>
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</table>

- Macroscopic appearance of appendix:

- Any contamination (pus and/or faeces etc.) within the peritoneal cavity: **YES/NO**

**Peritoneal irrigation?** **YES/NO**

<table>
<thead>
<tr>
<th>Fluid Used</th>
<th>Volume</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Intraoperative IV fluid type, temperature and volume given:

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Volume</th>
<th>Temperature</th>
</tr>
</thead>
</table>

- Intraoperative analgesia:

- Inform PACU nurse of study patient

**Postoperative**

**PACU and DAY 0:**
- Tympanic Temp in PACU:
- Shivering? **YES / NO**

**VAS Scores: (Hours postop) – Unless Child is Asleep**

<table>
<thead>
<tr>
<th>Time</th>
<th>2hr</th>
<th>4hr</th>
<th>6hr</th>
<th>8hr</th>
<th>10hr</th>
<th>12hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Rest</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With movement</td>
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<td></td>
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</tbody>
</table>

- Time + Date patient first requires nurse-controlled PRN IV opiate analgesia:
- Time + Date patient first requires nurse-controlled PRN anti-emetic:

- PCA used? **YES / NO**
DAY 1:

☐ VAS Score at 24 hours
   At rest:  
   With movement:  

☐ Shoulder pain in the first 24 hours? YES / NO

DAY 2:

☐ VAS Score at 48 hours
   At rest: 
   With Movement: 

COMPLICATIONS: Severity as per Clavien-Dindo Classification

<table>
<thead>
<tr>
<th>Description</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
**DISCHARGE DETAILS**  
Date of Discharge:

- Date Discharge Criteria Achieved: _______________________
- Total nights in hospital:

- Discharging Authority: Consultant / Fellow / Registrar / House Officer
- **Day 10 Questionnaire** + pre-paid envelope – Ensure patient’s contact details are correct
- Reminder Telephone call Day 8/9
- **Day 10 Questionnaire completed**

- Any significant medical conditions affecting recovery:

**HISTOLOGY RESULT**

**Record any non-adherence to Study Protocol:**
**Postop Opiate Analgesia Requirement:**

<table>
<thead>
<tr>
<th></th>
<th>First 12 hours</th>
<th>First 24 hours</th>
<th>First 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From:</td>
<td>From:</td>
<td>From:</td>
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<td>To:</td>
<td>To:</td>
<td>To:</td>
</tr>
<tr>
<td>MEDD</td>
<td></td>
<td>MEDD</td>
<td>MEDD</td>
</tr>
</tbody>
</table>

**TOTAL:**
(MEDD)

**Total Opiate Analgesia for duration of hospital stay:** (includes analgesia after initial 48 hours)

---

**Postop Total Anti-emetic Requirements:**

<table>
<thead>
<tr>
<th></th>
<th>First 12 hours</th>
<th>First 24 hours</th>
<th>First 48 hours</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>From:</td>
<td>From:</td>
<td>From:</td>
</tr>
<tr>
<td></td>
<td>To:</td>
<td>To:</td>
<td>To:</td>
</tr>
</tbody>
</table>
6-Week Follow-up

- Any GP visits:

- Any Hospital Representations or Readmissions within 30 days of D/C

- Details/Further Interventions etc.
APPENDIX H

CLINICAL STUDY C: POSTOPERATIVE DAY 10 QUESTIONNAIRE
**Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial (WARMIST)**

**POSTOPERATIVE DAY 10 RECOVERY QUESTIONNAIRE**

This questionnaire helps us find out how quickly you recovered after your operation. Please fill it out the morning of __________ and post it back to Dr Wendy Yu. You do not have to think too long about each question. There are no right or wrong answers. Please ask for help if you do not understand a question and do not put your name on this questionnaire.

*B E F O R E your operation when you were well, how much of a problem were the following for you ....*

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Almost Never</th>
<th>Sometimes</th>
<th>Often</th>
<th>Almost Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. It was hard for me to run</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. It was hard for me to lift something heavy</td>
<td></td>
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</tr>
<tr>
<td>3. It was hard for me to take a bath or to shower myself</td>
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<tr>
<td>4. It was hard for me to help out around the house</td>
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<tr>
<td>5. I was hurting / had pain</td>
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<td>6. I had low energy</td>
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<td>7. It was hard to pay attention to TV or to reading a book</td>
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<tr>
<td>8. I had trouble sleeping</td>
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<tr>
<td>9. I forgot things</td>
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<tr>
<td>10. I worried about what will happen to me</td>
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</table>
**In the last 2 days, how much of a problem has the following been for you ....**

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<th></th>
<th>Never</th>
<th>Almost</th>
<th>Sometimes</th>
<th>Often</th>
<th>Almost</th>
<th>Always</th>
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<tr>
<td>1. It was hard for me to run</td>
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*Adapted from the Pediatric Quality of Life Inventory. Version 4.0.*

Participant Code: ________
APPENDIX I

CLINICAL STUDY C: OPERATING SURGEON QUESTIONNAIRE
Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial – WARMIST

Surgeon Questionnaire

Attach Patient Sticker Here

Date of operation:

Surgeon seniority: Consultant / Registrar

Macroscopic diagnosis:

☐ Normal Appendix
☐ Simple Appendicitis
☐ Perforated Appendicitis

Any intraperitoneal contamination with pus or faeces? YES / NO

Any intraabdominal irrigation used? YES / NO
If YES,
1/ Estimated fluid volume used: __________mls
2/ Room temperature or warmed? (circle)
Please rate the severity of laparoscopy camera lens fogging from 1 to 10. (10 = worst fogging)

Please rate technical difficulty of the operation from 1 to 10. (10 = most difficult)

Do you think this patient is in the warm humidified gas insufflation group? (YES/NO)

Thank you for completing this Questionnaire.
APPENDIX J

CLINICAL STUDY C: POSTOPERATIVE PAIN INTENSITY VISUAL ANALOGUE SCALES (VAS)
Warm Humid Gas Insufflation for Appendix Removal by Minimally Invasive Surgery Trial – WARMIST

Visual Analogue Scales for Recording Post-operative Pain Scores

Attach Patient Sticker Here

Unless the Participant is asleep, please ask them to rate the severity of their pain, at rest and with movement, by marking each 10cm line:

“0 = NO PAIN” and “10 = WORST IMAGINABLE PAIN”

Please label marks as “R” for “At Rest” and “M” for “With Movement”.

OPERATION END TIME + DATE: ______________________

Time From When Operation Ended:

2 hours

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<tr>
<th>Time:</th>
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<tbody>
<tr>
<td>0</td>
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<td>10</td>
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4 hours

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<tr>
<th>Time:</th>
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<td>0</td>
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<tr>
<td>10</td>
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</tbody>
</table>
6 hours  
0 ——— 10

8 hours  
0 ——— 10

10 hours  
0 ——— 10

12 hours  
0 ——— 10

24 hours  
0 ——— 10

48 hours  
0 ——— 10
MORPHINE ADMINISTRATION

- Introduction
- Individualised Administration
- Drug of Choice
- Route of Administration

- Team Management
- Responsibility
- Morphine Protocol Flow Chart

Introduction

A standard for the administration of intravenous Morphine should allow effective relief of pain with an acceptable nursing workload, while increasing patient safety.

Individualised Administration

In a children’s hospital the need to individualise opioid therapy is particularly challenging because of the need to consider developmental issues in terms of both pharmacokinetics and pharmacodynamics. Of particular concern is the immaturity of elimination pathways and of ventilatory control in neonates.

Drug of Choice

Morphine is considered the ‘gold standard’ opioid analgesic for management of pain in children unless contraindicated.

A step-wise approach to managing pain should be considered for optimal pain management.

Route of Administration

- The preferred route for Morphine administration is oral. This route is convenient, administration is painless and a number of preparations are available.

- Many patients who have acute pain will initially require Morphine by injection because either the oral route is contraindicated (e.g. vomiting, fasting, oral ulceration, or because a rapid response is needed.

- IV injections are preferred over intramuscular (IM) or intermittent subcutaneous (SC) injections for management of acute pain. This is because the IV effect is rapid and thus easily titrateable. In addition respiratory depression, if it occurs, will occur rapidly. In contrast, IM and SC administration may result in drug absorption that varies according to peripheral perfusion and the effects may be considerably delayed after administration. Intermittent IM injections to manage pain are painful and children may deny they have pain to avoid the needle. Continuous SC infusions are useful in palliative pain management when the IV or oral route is not appropriate.
MORPHINE ADMINISTRATION

The IV route carries the risk of respiratory depression, but the use of a standardised protocol is expected to minimise the danger.

Morphine Bolus Administration

Administering small boluses of Morphine every five minutes makes it possible to carefully titrate pain relief while observing for the side effects of sedation and respiratory depression.

Usual Dose Range

- Infants < 6 months 0.02 mg/kg at 5 minute intervals
- Child over 6 months but < 50kg 0.04 mg/kg at 5 minute intervals
- Child over 50kg 2 mg at 5 minute intervals

Larger doses may be required at times, but they remove the safety offered by titration and are hazardous in the absence of immediate availability of artificial ventilation.

Initial Morphine Bolus Administration PICU/CED/OR

Larger doses may initially be administered to children over 6 months of age as prescribed by medical staff on an individual basis in the Paediatric Intensive Care Unit (PICU)/ Children’s Emergency Department (CED)/ and Operating Rooms (OR) where there is the immediate availability of artificial ventilation.

- For children over 6 months and less than 50kg the initial administration dose may be 0.1mg/kg.
- For children over 50kg the initial administration dose may be a standard bolus of 5mg. Further doses may then be administered as per IV Morphine protocol.
- Smaller doses are advisable under some circumstances.

Frequent Morphine Bolus Administration Vs PCA/ Continuous Opioid Infusions

The disadvantage of repeated small doses is less adequate pain relief and the time demand upon nursing staff.

If a child requires more than five titrations of morphine within a 25 minute period as described in this protocol and who is likely to have/ has on going pain, then a patient controlled analgesia (PCA) pump or a continuous opioid infusion should be considered.

Usually Morphine is the drug of choice for PCA and continuous opioid infusions unless contraindicated. Alternative opioid analgesic modality prescriptions should be discussed with the Pain Service.

- PCA has a clear safety record provided it is programmed correctly and only the patient presses the button. The inherent safety of PCA arises because a patient will become sedated if the demand button is pressed too often and thus the patient will stop pressing it. PCA, using a non-return valve is the preferred device for children who understand the concept and are physically able to use it. This includes most school-aged children. At times a background infusion may be programmed using this modality. PCA prescription/ modality access is via the
MORPHINE ADMINISTRATION

Pain Service. These children will require observation monitoring as per PCA prescription chart and RBP.

A syringe driver, using a non-return valve is the preferred device for a continuous opioid infusion in children unsuitable for PCA use. Devices such as burettes and simple volumetric pumps are not used for this purpose. Continuous opioid prescription / modality access is via the Pain Service. These children will require observation monitoring as per continuous opioid infusion prescription chart and RBP.

Administration/Monitoring of Neonates and At Risk Patients

Neonates in particular and infants less than 6 months of age have an increased risk of opioid induced respiratory depression. **Infants less than 6 months of age must have continuous respiratory monitoring after opioid administration.**

The preferred monitor is a pulse oximeter. An apnoea alarm is a suitable alternative. **There must be a nurse available to respond to the monitor.**

Monitoring must continue after the last opioid administration. The period of observation should be:

- Infants < 1 month = 9 hours
- Infants >1 month to 6 months of age = 4 hours
- Special consideration should be taken of ex-premature infants with a post conceptual age of less than 60 weeks. These infants will require continuous observation until they have a 24 hour “apnoea free” period.

Caution should also be taken in morphine administration in children with known renal impairment. Children with renal impairment have the potential to accumulate morphine metabolites and therefore have an increase risk of respiratory depression and sedation. It is advisable that consultation with Senior Medical Staff occurs prior to the administration of morphine.

Other children at high risk of respiratory depression that may require continuous monitoring while receiving opioids include:

- central neurological diseases
- sleep apnoea
- pre-existing respiratory failure
- renal impairment
- children receiving sedatives (i.e. diazepam)
MORPHINE ADMINISTRATION

Team Management

The primary team should manage patients receiving Morphine via the Morphine protocol. Patients who require consideration for a PCA/Continuous Opioid Infusion should be referred to the Pain Service after consultation with the primary team.

Responsibility

All Registered Nursing Staff who have completed the competency for Intravenous administration of Morphine and who are currently assessed as competent for IV/medication administration.

Morphine Protocol Flow Chart

Prescribe in the patient’s medication chart as ‘Morphine as per IV Protocol’

The medication will be administered by Registered Nursing Staff who have completed the competency for Intravenous administration of Morphine and who are currently assessed as competent for IV/medication administration.

The flow chart on the following page will enable safe administration of intravenous Morphine.
MORPHINE ADMINISTRATION

Child has moderate / severe Pain?

Only to be administered by an i/IV Registered Nurse who has completed the morphine administration competency or a medical practitioner.

*“Morphine as per IV Protocol” prescribed in medication chart.*

Prepare Morphine in a 10ml syringe: 0.2mg per kg morphine made up to 10mls with 0.9% NaCl.

Child weighs 50kg or over?

Prepare Morphine in a 10ml syringe: 10 mg morphine made up to 10 mlis with 0.9% NaCl.

Child’s age is 6 months or less?

Check patient before administering:
- Rousable to voice
- Respiratory rate > 20 infant
- > 30 neonate
- >20 infant
- Heart rate is appropriate
Do not administer if patient does not meet this criteria.
Check with primary team.

Administer 1 ml IV from the syringe.
1 ml = 0.02mg per kg

Administer 2 ml IV from the syringe.
2 ml = 0.04mg per kg

Wait 5 minutes

Child has moderate / severe pain?

Has 5 cycles of this flow chart been administered in 25 minutes?

Call Primary Team and Pain Service

Check patient before administering:
- Rousable to voice
- Respiratory rate > 15
- Heart rate is appropriate
Do not administer if patient does not meet this criteria.
Check with primary team.

Administer 2 ml IV from the syringe.
2 ml = 0.04mg

Different procedure for Resuscitation Room in Children’s Emergency Department
(refer page 1)

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Author: Pain Service
Editor: Dr Raewyn Gavin
Page: 5 of 5

Service: Pain Service
Date Issued: Reviewed April 2008

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75. Bensard DD, Hendrickson RJ, Fyffe CJ, Careskey JM, Azizkhan RG. Early discharge following laparoscopic appendectomy in children utilizing an evidence-based clinical


205. De Winter BY, Bredenoord AJ, De Man JG, Moreels TG, Herman AG, Pelckmans PA. Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on


249. Varni JW, Seid M, Kuntin PS. PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations. Medical Care 2001;39:800-12.


368. Cepeda MS, Boston R, Farrar JT, Strom BL. Comparison of logistic regression versus propensity score when the number of events is low and there are multiple confounders. Am J Epidemiol 2003;158:280-7.


392. Molinas CR, Koninckx PR. Hypoxaemia induced by CO(2) or helium pneumoperitoneum is a co-factor in adhesion formation in rabbits. Hum Reprod 2000;15:1758-63.


Allen L. The peritoneal stomata. The Anatomical Record 1936;67:89-103.


