ANALYSIS OF CYTOKINE CONCENTRATION AND THE VISUAL ANALOGUE PAIN SCALE SCORE AS EARLY INDICATORS OF SEPSIS IN GALL STONE DISEASE PATIENTS EVALUATING QUALITY OF LIFE QUESTIONNAIRES TO DETERMINE WHICH PATIENTS WILL EXPERIENCE SIGNIFICANT PAIN AFTER INTERVENTION

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Abstract

Introduction

Sixty thousand cholecystectomies are performed each year (Royal College of Surgeons, 2016). Unplanned admissions occur after 10% procedures secondary to complications and pain (Chandio *et al.,* 2017). This study aimed to identify whether pain was an early indicator of post-procedural sepsis, permitting earlier treatment to reduce morbidity. To successfully do this required identifying these patients, from patients who experienced a lot of pain postoperatively but did not develop sepsis, and were unsuitable for day-case surgery.

Methods

Three hundred and ninety six patients with biliary disease were recruited. Participant's systemic TNF-α, IL-1, IL-6 and IL-10 concentration was measured by ELISA techniques. They were compared to their systemic inflammatory response syndrome (SIRS) markers, and visual analogue score pain assessment. SF-36 and the Gastrointestinal quality of life index were chosen to measure quality of life, after a literature review indicated poorer quality of life scores pre-operatively indicated patients who did not benefit as greatly from cholecystectomy and continued to experience pain.

Results

The VAS score was significantly higher from six hours onwards in those developing sepsis compared to those who did not after ERCP or cholecystectomy. In contrast the inflammatory cytokines peaked at 24 hours in the open and ERCP patients, and at 48 hours in the laparoscopic approach patients developing sepsis. The peak in the SIRS markers coincided with the cytokine peak for each approach.

The quality of life measures permitted us to distinguish a group of patients who experienced a lot of pain post-operatively but did not develop sepsis, from those whose increase in pain was an indicator of sepsis. The group of patients with pain not developing sepsis were unlikely to be suitable for day case surgery, being unlikely to be discharged at 24 hours, and less likely to benefit from cholecystectomy.

Conclusion

Both for laparoscopic and open cholecystectomy pain is an early indicator of potential postoperative sepsis, preempting the rise in cytokines and SIRS. The VAS with the quality of life measures permitted the identification pre-operatively of a patient group unsuitable for day case cholecystectomy.

Earlier recognition and treatment of sepsis would promote improved patient outcome. Heterogeneity of causes of sepsis and small number of cases limits conclusions, and this requires a multi-centre study.

Acknowledgements

I would like to thank Mr. Shehata, Mr. Rigg and Mr. El'Sheikh who kindly let me recruit their patients, and to the patients who participated. The anaesthetic, theatre and endoscopy team, as well as the junior doctors and nursing teams and the laboratory staff. Thank you to the research laboratory team for the bench space for performing the bench work. I would like to apologies that the paperwork for approval by the Research and Ethics Committee, was lost in a fire at the research laboratory at Nottingham City Hospital.

Thank you to Mr. Shehata for his initial support in performing this research work, and to Professor Bown for taking over, and for advising in the writing up of the thesis. To Mr. Williams for his support. I would also like thank you to Miss Taylor and Mr. Chin for the support and time to complete this thesis.

The research work and reading for this thesis has inspired me, with colleagues at Coventry, Bristol and the Royal Marsden to set up a research group examining pain after breast surgery. We are currently recruiting for a national intervention trial examining different approaches to pain management. Having already demonstrated breast surgery patients have significant problems with postoperative pain, and by reducing acute pain there is a demonstrable benefit in reducing chronic pain. We are using the studies to teach the Oxford surgical and anaesthetic and trainees about the research process.

Finally I would like to thank my family who has stood by me through whatever curve ball life has thrown. I am grateful for that support without which I would not be where I am today.

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List of figures

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Materials and Method

Results

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List of abbreviations

ALP – Alkaline phosphatase

ALT – Alanine transaminase

ANOVA – Analysis of variance

APACHE – Acute Physiology and Chronic Health Evaluation

ARDS – Acute respiratory distress syndrome

AST – Aspartate aminotransferase

AU – Adenine uracil base pair

BMI – Basal metabolic index

CARS – Compensatory anti-inflammatory response syndrome

CBD – Common bile duct

Chole - Cholecystectomy

Chi-squared test – Pearson's chi-squared test

circRNAs – Circular RNAs

CO_2 - Carbon dioxide

COX-2 – Cycloxygenase-2

CRP – C reactive protein

DAMPs – Damage associated molecular patterns

DIC – Disseminated intravascular coagulation

EAISA – Enzyme amplified sensitivity immunoassay

ELISA – Enzyme linked immunosorbent assay

ERCP – Endoscopic retrograde cholangiopancreatography

Fi – Fraction inspired

G - CSF – Granulocyte colony stimulating factor

GGT – Gamma-glutamyl transferase

GI – Gastrointestinal

GIQLI – Gastrointestinal quality of life index

GM-CSF – Granulocyte-macrophage stimulating factor

H_2O_2- Hydrogen peroxide

H_2SO_4- Sulphuric acid

HAD – Hospital Anxiety and Depression score

HPFB - Human peritoneal fibroblasts

HPMC – Human peritoneal mesothelial cell

HRP – Horseradish peroxidase

HRQOL – Health related quality of life

Hrs - Hours

ICAM – Intercellular adhesion molecule

IFN - Interferon

IL - Interleukin

IL-1Ra – Interleukin 1 receptor antagonist

iNOS – Inducible nitric oxide synthase

ITU – Intensive care unit

kDa - Kilodalton

Kg – Kilogram

Lap. - Laparoscopic

LFT – Liver function test

lncRNA – Long non-coding RNAs

LPM – Large peritoneal macrophages

LPS - Lipopolysaccharide

M - Male

M – Abs – Monoclonal antibody

mls – millilitres

mL – was used for the singular as the L was confusing for a 1 in lower case

mmHg – Millimeters mercury

MAP – Mean arterial pressure

MAPK – Mitogen activated protein kinase

MARS – Mixed antagonist response syndrome

MCS – Mental component score

Mg/dL – Milligram per decilitre

MHC – Major histocompatibility complex

Mild pain group – VAS less than 4 usually based on enrollment score

Mins - Minutes

MMDS – Mitochondrial distress syndrome

Mmol/L – Milli-mole per litre

MODS – Multiple organ dysfunction syndrome

mRNA – Messenger ribonucleic acid

miRNAs – Micro RNAs

n – Number in a specified group

nm – Nanometers

NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells

NK – Natural killer

NO – Nitric oxide

NSAID – Non-steroidal anti-inflammatory drugs

OTC – On table cholangiogram

P – Patient sample of the EASIA plate

p – value – Calculated probability

Pa – Partial pressure

PAF – platelet activating factor

PAMPs – Pathogen-associated molecular patterns

PCS – Physical component score

pg/mL – Picogrammes/mililitre

Q of L / QoL - Quality of life

RAGE – Receptors for advanced glycation end products

RAND – Research and Development a global policy think tank

RANTES – Regulated on activation, normal T cell expressed and secreted

S – Standard sample on the EASIA plate

SD - Standard deviation

Severe pain group - VAS less than / equal to 7 usually based on enrollment score

SF-36 – Short-form-36

Significant pain experienced – Patient experienced more pain & had poorer QofL

Significant pain manageable - Patient experienced less pain & had better QofL

Significant pain group – VAS 4 to less than 7 usually based on enrollment score

SIRS – Systemic inflammatory response syndrome

SOFA – Sequential Organ Failure Assessment

SPM – Small peritoneal macrophages

sTNFR – Soluble TNF receptors

T-cell – Thymus originated cells

TGF – Transforming growth factor

Th – T helper

TMB - Tetramethylbenzidine

TNF – Tumour necrosis factor

TNFR – Tumour necrosis factor receptor

TRAF – TNF associated factors

TT – Thrombin time

T-Test – Student's T-test

V – Volunteer control on the EASIA plate

VAS – Visual analogue scale

VCAM – Vascular adhesion molecule

VRS – Verbal rating score

Vs - Versus

WCC – White cell count

Introduction

Chapter 1 – Gallstone disease

1.1 Gallstones

Gallstone disease affects 10 - 15% of the adult population. Acute referrals with gallstone disease have increased over the last 10 years. The exact reason for this is unclear Table 1.1.1 highlights possible factors and mortality risk factors. Cholesterol stones are the commonest type of stone; the exact mechanism of formation remains unclear, Table 1.1.2 details proposed factors. Table 1.1.3 demonstrates the commonest presentations.

Reasons for the increasing prevalence of gallstone disease

Possible reasons for increased gallstone disease
Females / multiple pregnancies
Diet / obesity / rapid weight loss
Diagnostic accuracy
Ageing population
Dehydration as incidence increases in the summer
American Indians and Northern Europeans have the highest incidence of stones, with increased prevalence of many of the above factors
Mortality
Rare
Predominantly in elderly due to biliary complications, and surgery to treat complications
Renewed interest in percutaneous cholecystotomy in high risk patients with cholecystitis
Mortality with gallstone pancreatitis 0.7% for mild to moderate disease 1.2% for severe disease

Table 1.1.1: Potential factors responsible for increasing gallstone disease, and the populations where they are most prevalent (Bardiya *et al.,* 2016 and Stinton and Shaffer, 2012). Risk factors for mortality are given as well as treatment (Nesvaderani *et al.,* 2015 and Zarour *et al.,* 2017).

Potential mechanism for cholesterol stone formation

Potential mechanisms of cholesterol stone formation
Imbalance and alteration in secretion of biliary lipids
Biochemical and immunological reactions in the gall bladder producing biliary sludge (mucins)
Changes in the structure of cholesterol (crystallization)
Altered gall bladder and intestinal motility
Cholesterol absorption within the intestine
Maximal stone growth is seen in the first 3 years then stabilises
85% of stones are less than 20mm in diameter

Table 1.1.2: Cholesterol stones are the commonest type; the exact mechanism of formation is unclear. Possible mechanisms are shown in the table and could be interplay between one or more factors (Castro-Torres *et al.,* 2015).

Presentation of gallstone disease

Presentation
Asymptomatic – Majority – 1 – 4% move from this group in the first 5 years after being diagnosed stabilising at 20% by 20 years
Symptomatic - 90% present with pain - Once symptomatic increased risk of complications - Diagnostic dilemma to match symptoms to presence of stones
Complications - Acute cholecystitis - Common bile duct stones with or without pancreatitis / cholangitis - Gallstone ileus - Associated gall bladder cancer 1:1000 patient / year too low to justify cholecystectomy, except in American Indian population or stones over 30mm

Table 1.1.3: Presentation of gallstones in the western population (Stinton and Shaffer, 2012 and Newman *et al.,* 1968).

1.2 Laparoscopic and open cholecystectomy

Comparative data on surgery is limited by patients demand for laparoscopic surgery, bias toward reporting more favourable data on laparoscopic surgery, selection bias of low risk patients for the laparoscopic approach, and absence of data on long term follow up for either modality. Table 1.2.1 compares the two approaches. Variation in anatomy, particularly during the learning curve, is a problem with laparoscopic cholecystectomy. Duct injury frequently goes unrecognised and is seven to eight times more common after laparoscopic surgery (rate 0.1 – 0.2%) (van der Voot *et al.,* 2004, Cope *et al.,* 2015 Bernard and Hartman, 1993). Reduced immune and metabolic response following laparoscopic surgery possibly allows progression toward sepsis prior to homeostatic responses occurring, and may account for later presentations after the laparoscopic approach (Bishoff *et al.,* 1999).

Comparison of the open and laparoscopic cholecystectomy

Open cholecystectomy	Common	Laparoscopic cholecystectomy
First performed over 100 years ago	Common operative steps	First performed in France 1987 86 – 90% procedures now performed by this route in Europe and North America
Kocher's incision	Pre-operative ERCP or intra-operative cholangiogram can be performed	3 or 4, 5 or 10mm incisions
5 day hospitalization and 3 – 6 weeks convalescence		Day case or 1 night and 1 -2 weeks hospitalisation
Morbidity 3.6% With CBD exploration 7% Mortality 0.3% With CBD exploration 1.6%	Overall mortality for ERCP for comparison 0.313%	Morbidity 1.2% With CBD exploration 7.2% Mortality 0.15% With CBD exploration 1.2%
Bowel injury 0.13%		Following the learning curve morbidity and mortality is around access problems 1.2% significant bleeding and 0.7% - Hasson approach 0.02% vascular injury and 0.5% bowel - Verres 0.44% vascular and 0.7% bowel

Table 1.2.1: Table of comparison of open versus laparoscopic cholecystectomy. Men have twice the mortality rate of women, and emergency procedures have four times the mortality of elective procedures, although this is decreasing. Mortality and morbidity figures around access problems, from Aashu *et al.,* 2016 and Krishnakumar and Tambe, 2009 and open Fletcher *et al.,* 1999). Electrocautery injury tends to present later than trocar injury (days as opposed to hours to days) (Alkatout *et al.,* 2012). Perforation and bile spillage is more common in laparoscopic procedures (Ros *et al.,* 2001). Morbidity and Mortality data from Lee *et al.* (2014), Sandblom *et al.,* (2015) Ransohoff and Gracie, (1993), Scollay *et al.,* (2011).

1.3 Endoscopic Retrograde Cholangiopancreatography

Endoscopic retrograde cholangiopancreatography (ERCP) was first performed by Mc Cune in 1968. Classen and Demling from Germany and Kawai from Japan performed the first endoscopic sphincterotomy for biliary and pancreatic disorders in 1972. Procedures now include accessory techniques performed on biliary and pancreatic ducts such as endoprosthesis or stent placement, dilatation of stenotic ducts, basket and balloon stone extraction, and lithotripsy

Common duct stones increase with patient age, with 8 – 15% under 60 years, and 15 – 60% over 60 years having stones within the duct. Investigations are prompted by evidence of jaundice, recent pancreatitis or dilated common duct on imaging studies. In experienced hands the success rate of ERCP approaches 90 to 95%. Due to associated morbidity and mortality ERCP is used selectively, where investigations such as MRCP indicate clinical benefit. Complications of ERCP are detailed in table 1.3.1.

Complications following ERCP

ERCP complications
Asymptomatic hyperamylasaemia Between 25 - 75% patients undergoing ERCP, higher concentrations occurring in therapeutic procedures (Choudhary *et al.,* 2011,Freeman 2007)
Acute pancreatitis Approximately 2 - 4% of patients, usually rapidly resolves, 30% progresses to severe pancreatitis Placement of prophylactic stents decreased the risk of pancreatitis in low and high-risk patients. Stent failure rate of 4 – 10% (Choudhary *et al.,* 2011,Freeman 2007)
Acute necrotizing pancreatitis (ANP) Causes 25% of the ERCP mortality, ANP following ERCP has a higher rate of mortality due to higher rate of infected necrosis and systemic inflammatory response (Wozniak *et al.,* 2001)
Perforation Bile or pancreatic duct or duodenum approximately 0.6% (Fatima *et al.,* 2007, Wu *et al.,* 2006) Erect chest x-ray is a common early investigation, but free air occurs on 13 -29% of films and is not an indication for intervention. Contrast CT can aid diagnosis particularly of retroperitoneal perforations from sphincterotomy or guide wire manipulation. Intraperitoneal perforation occurs from endoscopic trauma or stent impaction (Fatima *et al.,* 2007, Wu *et al.,* 2006).
Biloma Encapsulated collection of bile (biloma) occasionally seen with bile duct perforation (Fatima *et al.,* 2007)
Post-sphincterotomy bleeding 2%, severe bleeding in 0.1 – 0.5% cases. Immediate bleeding occurs in 30%, documented up to 2 weeks post procedure (Szary and Al-Kawas, 2013) Multivariate analysis identified coagulopathy, anticoagulation within 3 days of endoscopic sphincterotomy, cholangitis before ERCP, bleeding during initial endoscopic sphincterotomy, and a lower case volume as risk factors for haemorrhage. Patient factors such as liver cirrhosis, dilated common bile ducts, peri-ampullary diverticulum, precut sphincterotomy, and common bile duct stones appear to increase the risk of post sphincterotomy bleeding (Szary and Al-Kawas, 2013, Ferreira and Baron, 2007).

Table 1.3.1: Commonest complications encountered following ERCP.

Introduction

Chapter 2 - Sepsis

2.1 Inflammatory response

Inflammation is a rapid highly amplified controlled humoral and cellular response. It consists of four parts detailed in Figure 2.1.1, with the classical signs of inflammation and resolution in Figure 2.1.2, in cartoon form.

The Inflammatory process and underlying clinical process

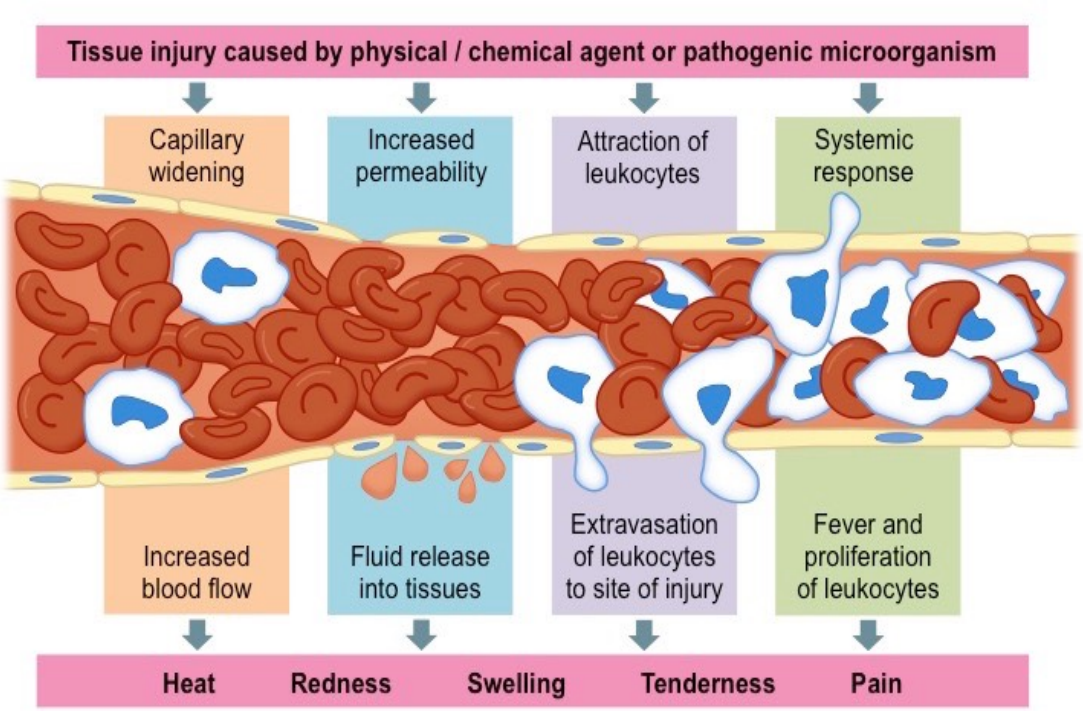


Figure 2.1.1: The response to inflammation of any cause is characterised by the same four processes. The clinical signs seen are an interaction of these four processes (ib.BioNinja.com.au).

Classical signs of inflammation and resolution

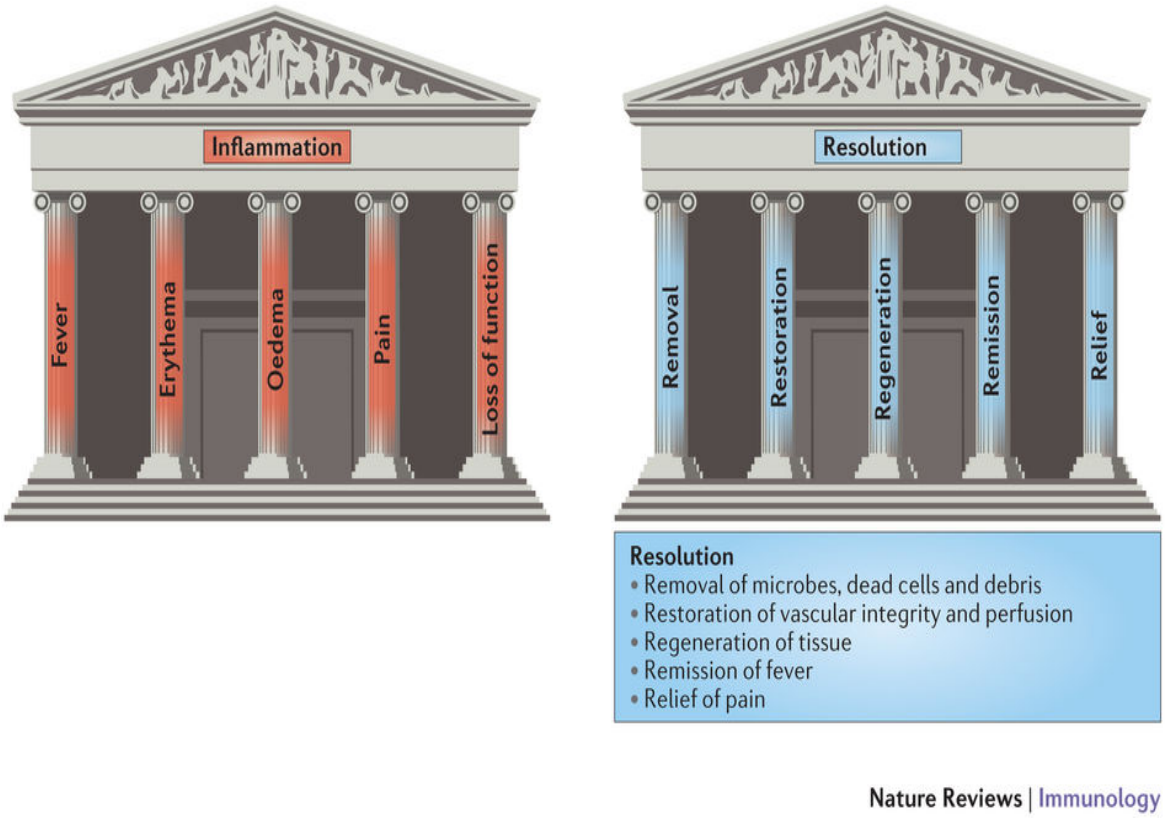


Figure 2.1.2: The four classical signs of inflammation described by Celsus and the fifth added by Virchow as loss of function. The signs of resolution are also described (Basil and Levy, 2016).

The American College of Chest Physicians and Society of Critical Care Medicine, in 1992, introduced definitions for systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock and multiple organ dysfunction syndrome (MODS) Figure 2.1.3 and 2.1.4 demonstrate the inter-relationship (Bone *et al.,* 1992). Table 2.1.5 highlights that SIRS can be elevated by factors other than sepsis, and infection can occur without sepsis. Sepsis triggers a SIRS response, with a compensatory anti-inflammatory response (CARS) and a mixed antagonist response (MARS), which can progress to multiple organ dysfunction (MODS). If there is a secondary insult this can progress to death, with supportive care in the absence of resolution can occur. Table 2.1.6 details the three major flaws in the definitions (Vincent *et al.,* 2013).

The most recent definition of sepsis is as a life-threatening organ dysfunction, caused by a deregulated host response to infection. There is refinement of definitions; the term 'severe sepsis' has been removed, and septic shock has become a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone (Third International Consensus Definitions for Sepsis and Septic Shock (2016)).

The interrelationship between sepsis, systemic inflammatory response syndrome and infection

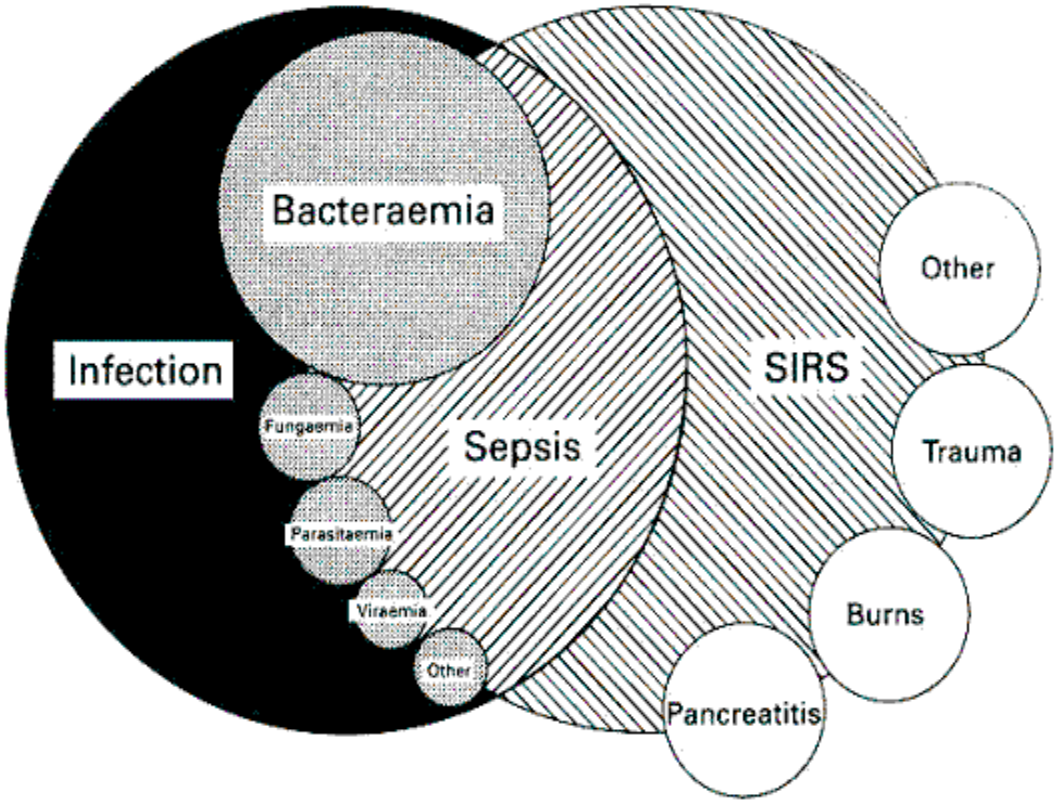


Figure 2.1.3: The inter-relationship between sepsis, systemic inflammatory response syndrome (SIRS) and infection. The diagram highlights that sepsis is the presence of infectious organism with elevated SIRS. The SIRS markers can be elevated due to non-infectious causes, but this is not sepsis (Bone *et al.,* 1992). They have formed the foundation of the Surviving Sepsis Guidelines, the most recent of which was published in January 2017 (De Backer and Dorman, 2017).

The progression from initial septic insult through multi-organ dysfunction to resolution or death

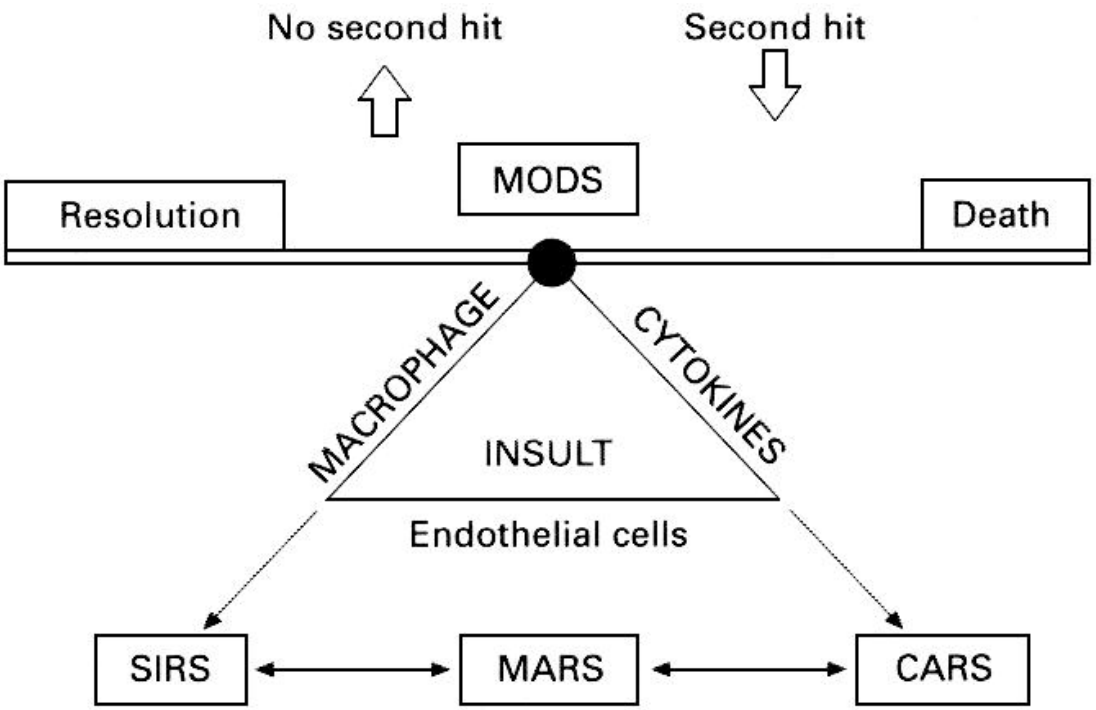


Figure 2.1.4: The pendulum and spectrum of systemic inflammatory response syndrome (SIRS), compensatory anti-inflammatory response syndrome (CARS) and mixed antagonist response syndrome (MARS). Tissue insult / injury triggers a triad of systems encompassing the macrophage cytokines and endothelial cells. This results in SIRS/CARS/MARS, which results in end-organ dysfunction. This can progress to multiple organ dysfunction syndrome (MODS) particularly when aggravated by a second hit (another tissue insult/injury), or can move towards resolution particularly when second hits are avoided (Davies and Hagen, 1997).

Systemic inflammatory response syndrome criteria and definition of sepsis

	SIRS criteria – 2 or more of the following	
Heart rate	> 90 bpm	
Temperature	> 38°C or < 36°C	
Respiratory rate **Or PaCO2**	> 20 breaths per minute < 32 mm Hg	
White blood cell count **Band forms**	12 000 / mm3 or < 4 000 / mm3 > 10% band forms	
Definitions		
First criteria	**With**	**Is**
2 or more **SIRS** criteria	Suspected or present form of infection	**Sepsis**
Sepsis	Lactic acidosis or SBP < 90 mm Hg, or SBP drop ≥ 40 of normal, organ dysfunction, hypotension or hypoperfusion	**Severe sepsis**
Severe sepsis	Hypotension despite adequate fluid resuscitation	**Septic shock**

Table 2.1.5: The systemic inflammatory response syndrome (SIRS) criteria and the definition of sepsis, severe sepsis and septic shock from 1992.

Proposal for the a new definition of sepsis and the reasons for change

Major flaws in the 1992 American College of Chest Physicians and Society of Critical Care Medicine catalysing subsequent revision
- The definition is too sensitive, with almost all patients in intensive care units meeting the criteria of the diagnosis
- The definition does not differentiate between the normal beneficial host response and the pathologic host response producing organ dysfunction
- It doesn't distinguish between the role of infection in the inflammatory response and noninfectious insults causing a similar inflammatory response
Proposed changes in the definition of sepsis
Sepsis is the host's deleterious, non resolving inflammatory response to infection that leads to organ dysfunction
Definition of sepsis used in this study from the Sepsis 4 campaign
Sepsis is a life threatening organ dysfunction caused by deregulated host response to infection

Table 2.1.6: The changes in the definition of sepsis and the reasons for the changes (Vincent *et al.,* 2013). This is similar to the definition of severe sepsis and severe SIRS, with worsening organ dysfunction due to over activation of the inflammatory response due to infection or insult. The definition encompasses an entity that is of significant concern. Sepsis is a significant consumer of resources, cause of complications, and significantly impact on patient's lives, and on future mortality. Survivors have an increased mortality for the following 8 years compared with age-matched non-septic critical care survivors (Dreiher *et al.,* 2012). Sepsis 4 definition of sepsis used in this study (Napolitano, 2018).

2.2 Sepsis

The severity of sepsis was described by Hippocrates in 400 BC when he noted ‘in acute diseases, coldness of the extremities is a very bad sign’. Sepsis is the leading cause of death in most intensive care units world wide, despite improvements in antimicrobial therapy and supportive care. In the UK in 2013 - 2014 the incidence of sepsis was 230 cases per 100,000 of the population with a mortality rate of 42,338 / year (The UK Sepsis Trust 2015). The incidence of sepsis increases with age, as does mortality, being 80% in those aged over 65 years. Taking only the most overt costs it is estimated that sepsis in the US in 2011 cost $20 billion dollars (Agency for Healthcare research, 2013). Reason for the increasing rate is due to improvements in diagnosis and earlier recognition, but there is thought to be an increase in the number of cases, for reasons given in Table 2.2.1 (Scottish intensive care society, 2018).

Reasons proposed for the increase in the cases of sepsis

Reasons for the increase in cases of sepsis
Increased use of invasive monitoring devices
Improved reliability in diagnosing sepsis
Improved patient survival of the initial trigger e.g. surgery or trauma
Increased prevalence of patients with iatrogenic or pathological disorders of the immune system
Ageing population
Reasons for the increased mortality
Mortality from the primary diagnosis is less common with advancements in ITU care
Mortality is frequently due to the progressive organ dysfunction
Patients with multiple organ dysfunction and aggressive organ support fail to respond
Sepsis treatment is still, despite advancements mainly supportive
Sepsis more frequently occurs in the immunocompromised including those with chronic illness and older patients, these groups are more common in society and have a higher mortality

Table 2.2.1: Reasons proposed for the increase in sepsis based on the work of Crowe *et al.,* (1998). The increase in mortality with sepsis is related to the increase in sepsis, and is an area of active research (Bone, 1996 a, The UK Sepsis Trust, 2015, Scottish intensive care society, 2018).

Epidemiological studies indicate, gender as an in independent prognostic variable. Up until the menopause females are less susceptible to sepsis and have over twice the survival (Schröder *et al.,* 1998, Kisat *et al.,* 2013). Cytokine analysis has demonstrated a higher early and late pro-inflammatory cytokines in males, whereas females have increased anti-inflammatory cytokines, possibly limiting the inflammatory response (Reade *et al.,* 2009). Wichmann and colleagues*,* (2003), and Scotland's team (2011) demonstrated surgery causes significant depression of immune competent cells in males. Translating into reduced immunological competence of host defences (Newsome *et al.,* 2011). X-chromosome mosaicism diversifies immune response during entoxaemia. Immune cells have sex hormone receptors such as the oestrogen receptor β on immune cells, important in immuno-protection in females (Angele *et al.,* 2014). This is important in preserving the gastrointestinal barrier function in systemic infection, a common cause of morbidity and mortality.

Sexual diamorphism is only important in the most severely injured patients, in this group 60% of males and 24% of females died of multiple organ failure from uncontrolled inflammatory response (Oberholzer, *et al.,* 2000 a). Speculating in the less severely injured patients the immune system has sufficient reserves to control the sexual diamorphism. This is harder to prove in patients where multiple factors interplay (Angele *et al.,* 2014).

From The Surviving Sepsis Guidelines published January 2017 and updated in 2018, there has also been increasing evidence for bundles of care, with early delivery within the first hour improving survival (Levy *et al.,* 2018). This includes the 'Sepsis six' with commencement of resuscitation and management simultaneously, rather than awaiting the outcome of extended resuscitation, particularly in the presence of hypotension (De Backer and Dorman, 2017). This includes sending blood for culture and commencing broad-spectrum antibiotics, correction of hypotension with fluid boluses, and where required the early commencement of vasopressors, and the measurement of lactate. The need to tailor the antibiotic therapy earlier has led to the rapid advance in molecular characterisation of the organism causing sepsis (Mancini *et al.* 2015).

Diagnosis of sepsis can be very difficult, and there are a number of different scoring systems to try and promote early diagnosis. In 2016 in the UK, the National Institute for

Health and Care Excellence (NICE, 2016) has published guidance on the recognition, diagnosis, and early management of sepsis, which includes specific criteria for risk stratification of adults with suspected sepsis, Table 2.2.2. This is the criteria for assessing managing adults in hospital. NICE have also produced criteria for managing children of different ages, and special groups of adults (e.g. pregnant patients), in and out of hospital and adults out of hospital.

The NICE criteria – Sepsis risk stratification tool

Low risk of severe illness or death from sepsis:
Normal behaviour
No history of acute deterioration of functional ability, impaired immunity, or trauma/surgery in the past 6 weeks
Normal respiratory rate (i.e., less than 21 breaths per minute) and no oxygen requirement to maintain saturation
Normal blood pressure (i.e., systolic blood pressure greater than 100 mmHg)
Normal heart rate (i.e., less than or equal to 90 beats per minute; less than100 beats per minute in pregnant women) and no new onset arrhythmias
Normal urine output in the past 18 hours
Normal temperature
No non-blanching rash

Moderate to high risk of severe illness or death from sepsis:
History of new onset of altered behaviour or mental state (reported by patient, friend, or relative)
History of acute deterioration of functional ability
Impaired immunity (e.g., from illness or drugs, including oral steroids)
Trauma, surgery, or invasive procedures in the past 6 weeks
Respiratory rate 21-24 breaths per minute.
Systolic blood pressure 91-100 mmHg
Heart rate 91-130 beats per minute (100-130 beats per minute in pregnant women), or new onset arrhythmia
No urine passed in previous 12 - 18 hours (for catheterised patients, 0.5 - 1.0 mL / kg of urine passed per hour)
Tympanic temperature less than 36°C
Signs of potential infection (e.g., redness, swelling or discharge at surgical site, or breakdown of wound)

Table 2.2.2: Sepsis risk stratification tool from the NICE criteria, continued overleaf.

The NICE criteria – Sepsis risk stratification tool (continued)

High risk of severe illness or death from sepsis:
Objective evidence of new altered mental state
Respiratory rate greater than or equal to 25 breaths per minute
New need for oxygen (greater than 40% FiO2) to maintain saturation greater than 92% (or greater than 88% in known chronic obstructive pulmonary disease)
Systolic blood pressure less than or equal to 90 mmHg, or systolic blood pressure greater than 40 mmHg below normal
Heart rate greater than130 beats per minute
No urine passed in previous 18 hours (for catheterised patients, less than 0.5 mL / kg of urine passed per hour)
Mottled or ashen appearance; cyanosis of skin, lips, or tongue; non-blanching rash of skin

Management
Low risk criteria or 1 moderate to high risk criterion
Clinical assessment and consider bloods
Act on definitive diagnosis
Consultant review within 3 hours and consider antibiotics
1 or more high risk criterion or 2 or more moderate to high risk criterion
Review by senior clinical decision maker
Venous blood: - blood gas for glucose and lactate, blood cultures, FBC, CRP, U & E and creatinine, clotting screen
Intravenous (iv) broad spectrum antibiotics
Lactate < 2 mmol / L – Consider iv fluid bolus within 1 hour 2 – 4 mmol / L – Give iv fluid bolus within 1 hour 4 mmol / L or systolic BP <90 mmHg – Give 500 ml over < 15 minutes and discuss with ITU
Carry out continuous monitoring, if not possible observations every 30 minutes
Consultant review

Table 2.2.2: Sepsis: recognition, diagnosis and early management for adults in hospital (National Institute for Health and Care Excellence, 2016) NICE Guidelines.

Bone (1996 b), proposed a three-stage progression in the development of systemic inflammatory response syndrome (SIRS) Table 2.2.3. This complex interplay has been developed further with the identification that sepsis commences as a process of 'Malignant intravascular inflammation'. This is countered by a rapid protective response to prevent microorganism invasion. If the defensive response is deficient, by being either excessive or poorly regulated this can harm the organism. Figure 2.2.4 demonstrates this in cartoon form.

Systemic Inflammatory Response Syndrome (SIRS)

Stage1
Initially the body releases pro-inflammatory cytokines into the local environment.
Act to promote recruitment of defence cells, and promote wound repair, to limit the proliferation and invasion of pathogenic organisms.
Anti-inflammatory cytokines are then released to limit the local damage.
Stage 2
Local infection cannot be contained small amounts of inflammatory cytokines enter the systemic circulation.
Macrophages, neutrophils, T and B cells, platelets and coagulation factors are recruited, in the acute-phase reaction.
Phase ends with a fall in the pro-inflammatory mediators and an increase in endogenous antagonists, for example IL-1 receptor antagonists. • Cytokine production is tightly regulated by anti-inflammatory cytokines, receptor antagonists, and antibodies. • Concentration of these antagonists are 30-100 000 fold greater than their respective cytokines (Suffredini *et al.,* 1989). • Together they act to decrease production, and counter the effects of cytokines already released, to maintain healing, and clear infection, and ultimately to restore homeostasis. • If homeostasis cannot be restored then stage 3 or SIRS is triggered.
Stage 3
Loss of regulation of the pro-inflammatory response, where the cytokines effect is destructive as opposed to protective.
System is flooded with inflammatory mediators, and the following changes are seen: • Endothelial cell integrity is lost leading to increased microvascular permeability. • Platelets clump blocking the microcirculation, altering blood flow distribution and possibly progress to tissue ischaemia, cellular hypoxia and reperfusion injury. • The coagulation cascade is activated, while the protein C/ protein S inhibitory pathway is down regulated. • Profound vasodilation, fluid transduction, and maldistribution of blood flow results in circulatory malfunction and shock. • Depression of myocardial contractility, probably secondary to paracrine production of nitric oxide, coronary non-occlusive microvascular damage and myocyte injury
Failure to gain control at this point leads to multiple organ dysfunction and ultimately death

Table 2.2.3: Proposed three-stage progression in the development of systemic sepsis (Bone, 1996 b). Figure 2.2.4 demonstrates this in cartoon form.

Cartoon of the stages of sepsis

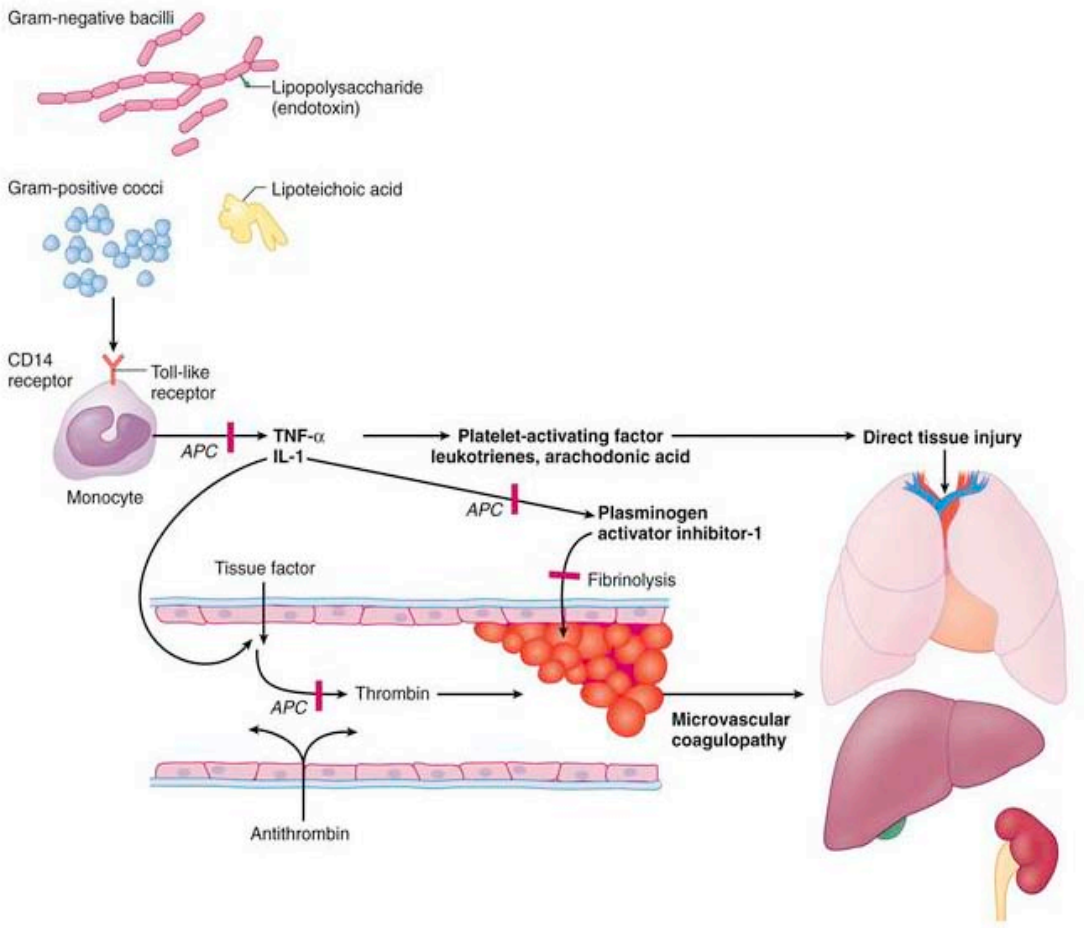


Figure 2.2.4: The cartoon represents the stages of progression to the development of sepsis. The infective organism stimulating the release of pro-inflammatory mediators, to limit the local damage (stage 1), if this continues and cannot be contained then mediators, such as cytokines, are released systemically. These promote the recruitment of white cells and coagulation factors, and steps to dampen down the inflammatory response and restore haemostasis (stage 2). Loss of regulation of the pro-inflammatory response (stage 3), and inflammatory mediators flood the system. Endothelial cell integrity is lost, leading to micro-vascular permeability, platelet clumping causing tissue ischaemia and reperfusion injury, and activation of the coagulation cascade. Profound vasodilation and circulatory malfunction and shock occur, with depression of myocardial contractility, and myocyte injury mediated through nitric oxide. Failure to restore haemostasis leads to multiple organ dysfunction and death. (De Cruz *et al.,* 2009).

Lipid A and other bacterial products stimulate a localised response, with the release of pro-inflammatory mediators. If this cannot be contained, mediators such as pro-inflammatory cytokines are released systemically to recruit monocytes and macrophages to initiate the release of interleukins, tumor necrosis factor (TNF)-α, interferon gamma (IFN-γ), and other colony-stimulating factors within minutes to hours. The inflammatory mediators are tightly regulated with receptor antagonists and antibodies to restore haemostasis. If the pro-inflammatory mediators are not controlled the cytokine effect can become destructive. Lipopolysaccharides, and cytokines such as TNF-α, IL-1 and IFN-γ, act on the inducible form of nitric oxide synthase in endothelium, vascular smooth muscle, macrophages and different parenchymal cells to produce nitric oxide (NO). Excessive NO enhances bacterial destruction, but also profound vasodilation, activation of inflammatory cascades and depression of cardiac function (De Cruz *et al.,* 2009). Figure 2.2.5 details the response initiated.

This ultimately leads to multi organ dysfunction (MODS), associated with widespread endothelial and parenchymal cell injury, but the exact mechanism remains to be elucidated. Four potential mechanisms are proposed shown in Table 2.2.6. Various host factors are important in surviving sepsis; these are detailed in Table 2.2.7.

Infection initiates the release pro-inflammatory mediator and nitric oxide

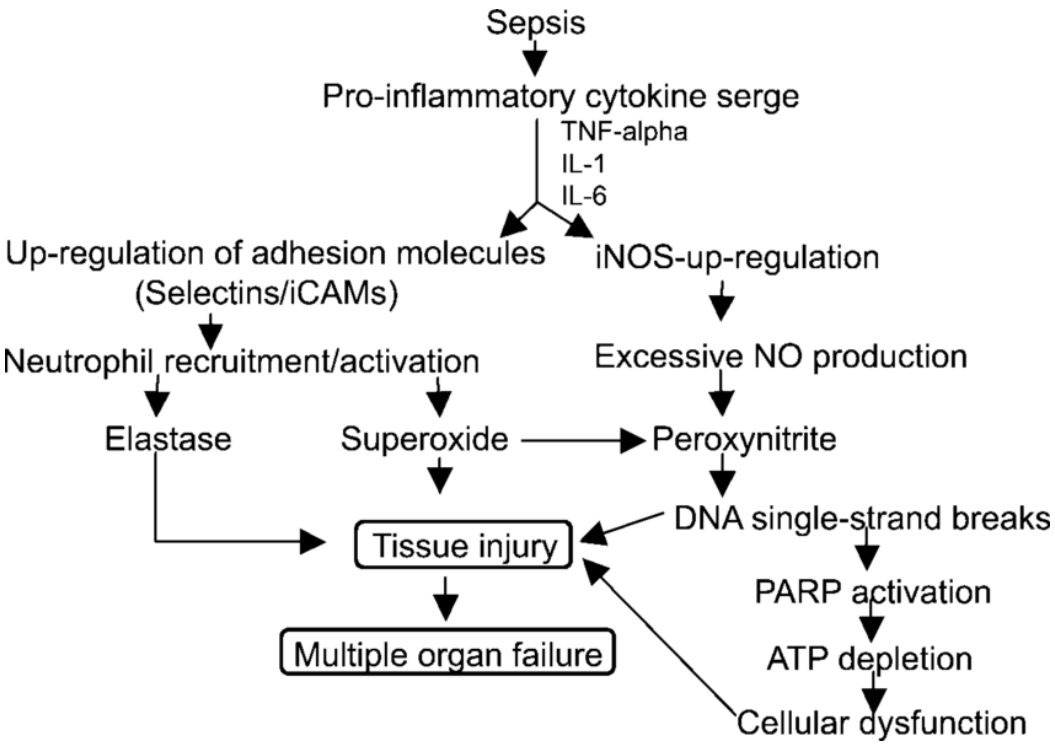


Figure 2.2.5: Infection initiates the release of pro-inflammatory mediators, which if unchecked induce inducible nitric oxide in endothelium, vascular smooth muscle, macrophages and different parenchymal cells to produce nitric oxide (NO). NO enhances bacterial destruction, but also has a profound vasodilation, activation of inflammatory cascades and depression of cardiac function resulting in multi-organ dysfunction (MODS). The coagulation cascade is activated generating fibrin, producing microvascular thrombi in various organs, contributing to MODS. Organ failure can affect any organ, and can be the first sign of sepsis. Mortality increasing as organ failure increases. MODS causes the deregulation of both the pro-and anti-inflammatory pathways. Potentially it is the failure of homeostasis which is the final step of sepsis to MODS, rather than simple hypotension-induced end-organ injury, as may occur with hemorrhagic shock. Survival of MODS requires interventions to reduce the pro- and anti-inflammatory. Hypo-responsiveness of end organs is potentially an adaptive response to overwhelming inflammation, allowing inflammation to clear without permanent end-organ damage (De Cruz *et al.,* 2009).

Potential mechanisms for multi-organ dysfunction

Potential mechanisms for multi-organ dysfunction	
Hypoxic hypoxia	Circulating septic lesion disrupts: - tissue oxygenation, metabolic regulation of tissue oxygen delivery, contributes to organ dysfunction. Micro-vascular and endothelial abnormalities contribute to the septic microcirculatory defect. Reactive oxygen species, lytic enzymes, vasoactive substances (e.g., NO and endothelial growth factors) cause microcirculatory injury, which compounds the altered erythrocyte circulation in septic microcirculation
Direct cytotoxicity	Endotoxin, TNF-α, and NO damage mitochondrial electron transport, leading to disordered energy metabolism, cytopathic or histotoxic anoxia. Causing an inability to utilize oxygen even when it is present
Apoptosis	Usual mechanism by which dysfunctional cells are normally eliminated. Pro-inflammatory cytokines alter apoptosis, delaying it in activated macrophages and neutrophils, and accelerating it in other tissues such as gut epithelium. Together this plays a critical role in the tissue injury of sepsis
Immunosuppression	Interaction between pro-inflammatory and anti-inflammatory mediators leads to an imbalance between them. An inflammatory reaction or an immunodeficiency may predominate, or both may be present

Table 2.2.6: The table details the four potential mechanisms for multi-organ dysfunction that are proposed.

Host factors important in determining the outcome of sepsis

Important host factors in determining the outcome of sepsis
Old age ≥ 65 years
Male
Host genetic characteristics from single nucleotide polymorphism and immune cell variation, and cytokine polymorphism
Noscomial acquisition
Solid tumours or haematological malignancy
Pulmonary, renal and liver disease but not cardiovascular disease
Co-existing infections – Pneumonia, UTI, intra-abdominal infections, pathogens other than *E. coli*
Alcoholism
Neutropaenia
Corticosteroids
Recent surgery
Urinary catheters or other lines for access
Inappropriate antibiotics commenced initially or broad spectrum antibiotics to which organism resistant
Host in severe sepsis or septic shock
Microbial load

Table 2.2.7: Host factors interplay with the pathogen in determining the outcome of sepsis. Initiation of inflammatory responses occurs between pathogen-associated molecular patterns expressed by pathogens, and pattern recognition receptors expressed by host cells at the cell surface. The consequence of the exaggerated inflammatory response is collateral tissue damage and necrotic cell death. This results in the release of damage-associated molecular patterns, so-called danger molecules that perpetuate inflammation at least in part by acting on the same pattern-recognition receptors triggered by pathogens (Angus and van der Poll, 2013).

2.3 Dysfunction of organ systems in SIRS

Circulatory derangement

Commonly found in sepsis is derangement of auto-regulation of the circulation. Mediators like NO, increase vasodilation and micro-vascular permeability at the site of infection, inhibition of vasopressin secretion permits persistence of vasodilatation (Antonucci *et al.,* 2014).

Pro-inflammatory cytokines, and endotoxin are proposed to reset ventricular performance, through the action of NO. During sepsis excess NO causes ventricular dysfunction by three routes; it decreases both calcium trafficking during systole (decreasing contractility), calcium flux during diastole (causing abnormal cardiac filling), together these cause increased left ventricular end diastolic pressure. Finally NO decreases the sensitivity of the myocardium to endogenous adrenergic ligands altering the second messenger systems response. Pre-existing cardiac disease limits this response. Vascular endothelium is highly responsive to inflammatory mediators, and permeability increases, leading to widespread protein-rich tissue oedema. Redistribution of intravascular fluid volume, from reduced arterial tone, diminished venous return from venous dilation, and release of myocardial depressant substances causes hypotension.

At end organ level there is interference with normal distribution of systemic blood flow, reducing oxygen delivery, and causing regional hypo-perfusion. Vasodilator therapies try to overcome this. Failure to do so causes mitochondrial dysfunction and is often associated with reduced mitochondrial trans-membrane potential gradients and decreased aerobic metabolism (Turillazzi *et al.,* 2016). Potentially this is cyto-protective, similar to hibernation, or could be primary mitochondrial pathology due to sepsis (Cimolai *et al.,* 2015). Termed microcirculatory and mitochondrial distress syndrome (MMDS) (Harrois *et al.*, 2009), the sepsis-induced inflammatory auto-regulatory dysfunction persists, and oxygen need is not matched by supply, leading to MODS.

Septic shock and SIRS is characterised by reversible myocardial depression, frequently resistant to catecholamine and fluid administration. TNF-α, IL-1β, and other cytokines, with NO, cause myocardial depression (Antonucci *et al.,* 2014).

Pulmonary dysfunction (Greer, 2015)

Injury to pulmonary vasculature disrupts capillary blood flow. Enhanced micro-vascular permeability, results in interstitial and alveolar oedema, and eventual membrane destruction (Greer, 2015). Neutrophil entrapment within the pulmonary microcirculation initiates and amplifies injury to alveolar capillary membranes resulting in acute lung injury and acute respiratory distress syndrome (ARDS). Occurring in 60% of cases of septic shock (Boontham *et al*, 2003).

Gastrointestinal dysfunction (Spapen *et al.,* 2017)

Gastrointestinal (GI) tract is proposed to drive sepsis-induced MODS. One theory is the hypoperfusion, ischaemia-reperfusion and inflammation, permits 'translocation' and liberation of endotoxins into the systemic circulation. This is associated with GI bacteria becoming increasingly virulent and invasive due to the altered immune regulation. But there is no direct evidence of this occurring (Qin *et al.,* 2011).

Alternatively the 'stressed gut' in sepsis releases nonbacterial pro-inflammatory markers into the systemic circulation through the mesenteric lymph nodes. These protein and lipid mediators stimulate antigen-presenting cells, initiating SIRS and ultimately MODS. In addition pancreatic enzymes penetrate the intestinal wall and drive further damage (Deitch, 2012). The two theories are illustrated in Figure 2.3.1.

Theories for the different mechanisms involved in MODS

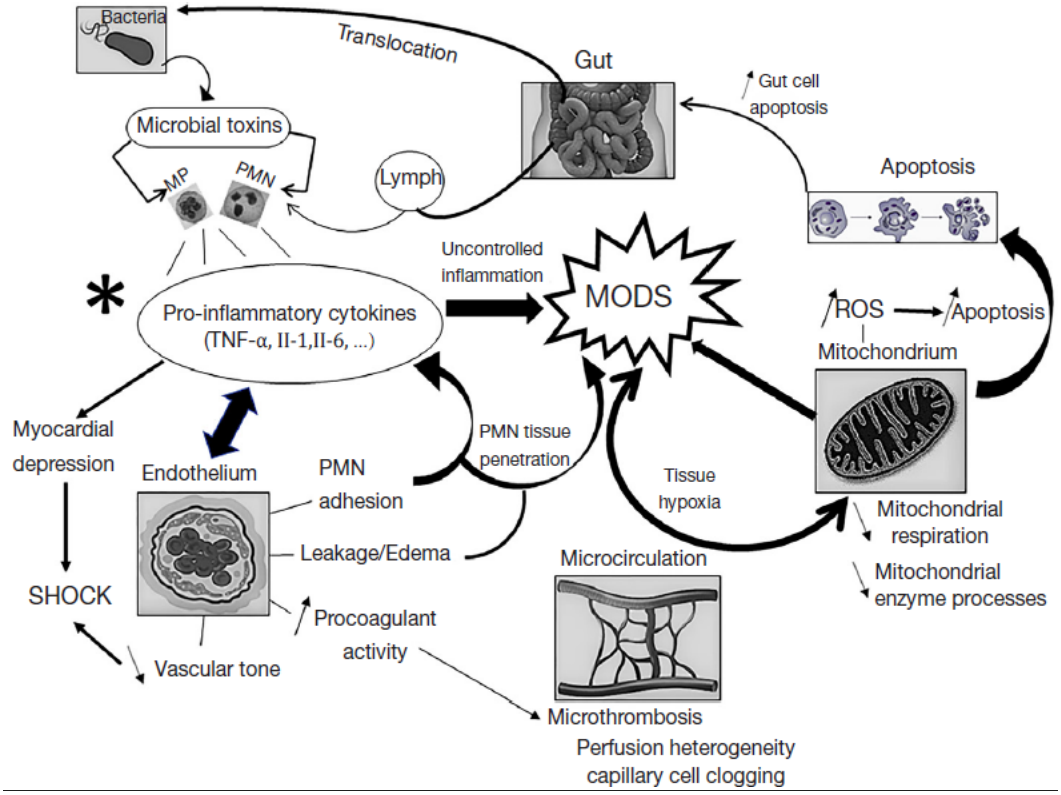


Figure 2.3.1: Theory for the development of MODS and the interaction of systems. MP – macrophage, PMN – polymorphonuclear leukocyte, ROS – reactive oxygen species, * - pattern-recognition (e.g. Toll-like) receptors, together recruit adapter proteins to the cell surface which initiate cytoplasmic enzymatic processes that activate various transcription factors, which in turn, produce and release inflammatory cytokines and chemokines (Spapen *et al.,* 2017).

Liver dysfunction (Wang *et al.,* 2014)

Liver dysfunction contributes to both the initiation and progression of sepsis, and occurs early (Wang *et al.,* 2014). The reticulo-endothelial system acts to clear bacteria and their products. Kuppfer cells secrete cytokines, particularly TNF-α and IL-6. In later sepsis their phagocytic and killing activity becomes impaired, reducing endotoxin and bacteria clearance, with spillover into the systemic circulation (Kennedy *et al.,* 1999).

Pre-existing liver dysfunction increases morbidity and mortality. Parks and colleagues (2003) proposed a two hit phenomenon, sepsis induces a profound alteration in the transport of bile acids and bilirubin to the canaliculi causing cholestasis. The cholestasis triggers an inflammatory response exaggerating the cholestasis. Endotoxins and pro-inflammatory cytokines cause direct impairment of bile flow at a genetic and at a cytoskeletal architecture level (Nesseler *et al.,* 2012). Hyper-bilirubinaemia causes intrahepatic cholestasis, decreased bile flow mucosal atrophy (Assimakopoulos *et al.,* 2007). Bile is bacteriostatic, decreased flow causes overgrowth, with an increase in endotoxin levels and negative feedback on hepatic function (Nesseler *et al.,* 2012).

Haematological dysfunction (Ruf, 2010)

Subclinical coagulopathy is common, seen as a mildly elevated thrombin time (TT) or activated partial thromboplastin time (APTT) or a moderate reduction in the platelet count. Disseminated intravascular coagulation (DIC) though rare occurs, with associated haemmorrhage and microvascular thrombi possibly playing a role in MODS (Ruf, 2010).

Renal dysfunction (Uchino *et al.,* 2005)

Acute kidney injury (AKI) accompanies septic shock in up to 50% of cases. Multifactorial etiologies have been reported (Uchino *et al.,* 2005). Decreased effective intravascular volume from systemic hypotension, direct renal vasoconstriction, release of cytokines, and activation of neutrophils by endotoxins and other peptides, contribute to renal injury. Tubular function is impaired but not evident on histology.

Central nervous system dysfunction (Sharshar *et al.,* 2004)

Sepsis produces encephalopathy and peripheral neuropathy. Pathogenesis is poorly defined, probably being related to systemic hypotension, and hypo-perfusion.

2.4 Intraperitoneal immune function

Peritoneal immune function is complex with surgical factors affecting response. The peritoneal membrane plays a major role in the immunological response to abdominal surgery (Badia *et al,* 1996). Following laparotomy Badia's team (1996) observed sequentially raised cytokine levels in peritoneal fluid, proposing wash over into the systemic and portal circulation at low levels. Supported by Riché *et al.,* (2013) work, who found a significant gradient in cytokine concentration between peritoneal and systemic cytokines, with TNF-α, IFN –γ, IL-6 and IL-10, only being seen in the sickest individuals. The cytokines only being detected in the systemic circulation after a significant peritoneal concentration was reached.

Systemic studies cannot be applied directly to peritoneal function (Badia *et al.,* 1996). Problems obtaining tissue limits *in vivo* studies, and published studies are from multiple different sources of sepsis at different stages of infection.

Anatomy

The peritoneum covers an area of $2m^2$, populated by a small population of predominantly macrophages (Jörres *et al*., 1996). Figure 2.4.1 is a human peritoneal mesothelial cell (HPMC). Up to 72 hours after laparoscopic surgery peritoneal biopsies demonstrate nerve injury and capillary damage, with active inflammation, particularly granulocytes (Narchi *et al*. 1990, Volz *et al.,* 1996).

Human Peritoneal Fibroblasts

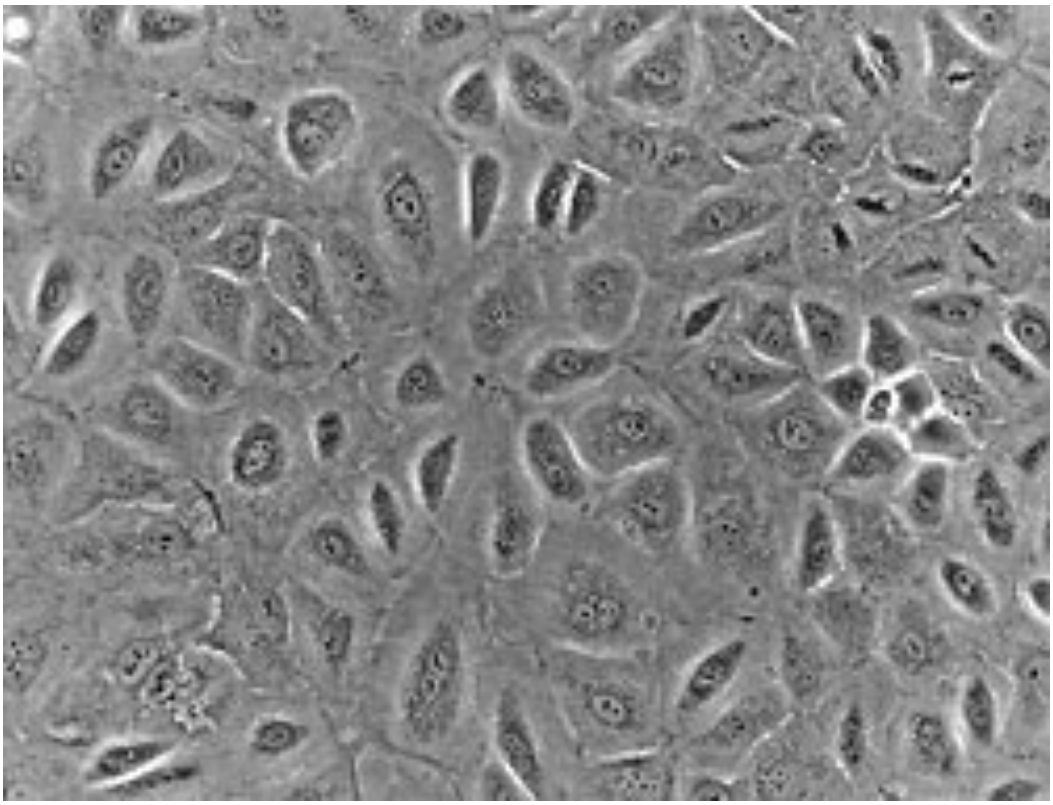


Figure 2.4.1: Confluent cell cultures of human peritoneal fibroblasts. HPFB are identified as spindle-shaped cells, growing in parallel, whorl-forming arrays. They demonstrate functional polarity, allowing effective regulation of the cellular traffic (Broche and Tellado, 2001). Phase contrast microscopy, magnification x 100 (Jörres *et al.,* 1996).

Ranvier in 1874 described ‘taiches laiteuse’ (milky spots), now known to be precursors of peritoneal macrophages. Containing typical omental capillary networks with surrounding macrophages (67%) and lymphocytes (10%) (Krist *et al.,* 1995, Hausmann *et al.,* 2000). Peritoneal macrophages produce early cytokines IL-1β and TNF-α and their antagonists, IL-1Ra and sTNF-R (Tellado *et al.,* 2000). Two embryologically distinct populations of peritoneal macrophages have been identified. Large peritoneal macrophages (LPM) originating from embryogenic precursors, regulated by specific transcription factors and tissue-derived signals. Small peritoneal macrophages (SPM) are bone-marrow-derived myeloid precursors, originating from circulating monocytes, Figure 2.4.2.

HPMCs express a MHC class II receptors initiated by the expression of IL-1, TNF-α, and IFN-γ by HPMC (Tellado *et al.*, 2000, Jayne *et al.,* 1998). SPM have high expression MHC-II, and LPM low expression. The LPM can proliferate within the peritoneum (Cassado *et al,* 2015). Immune stimulation alters the ratio of cells. The number of LPM decreases and those remaining migrate to the omentum. LPMs produce G-CSF, GM-CSF and killer cells and appear to be more specialised, this is shown in Figure 2.4.3. In contrast the SPMs and their precursors predominate within the peritoneum, due to recruitment from the circulation. SPM stimulation produces high level of pro-inflammatory mediators and greater levels of NO.

Activation of the cell population allows soluble antigen presentation to autologous T cells, and leucocyte recruitment in peritoneal cavity infection (Tollado *et al.,* 2000 Hausmann *et al.,* 2000). SPMs normally play a minor role in maintenance of the LPM numbers. Where LPMs are reduced in number, for example in sepsis, SPMs increase in importance, to try to maintain LPM numbers, which is important in the resolution of peritonitis (Cassado *et al.,* 2015).

The embryological origin of the macrophage subsets

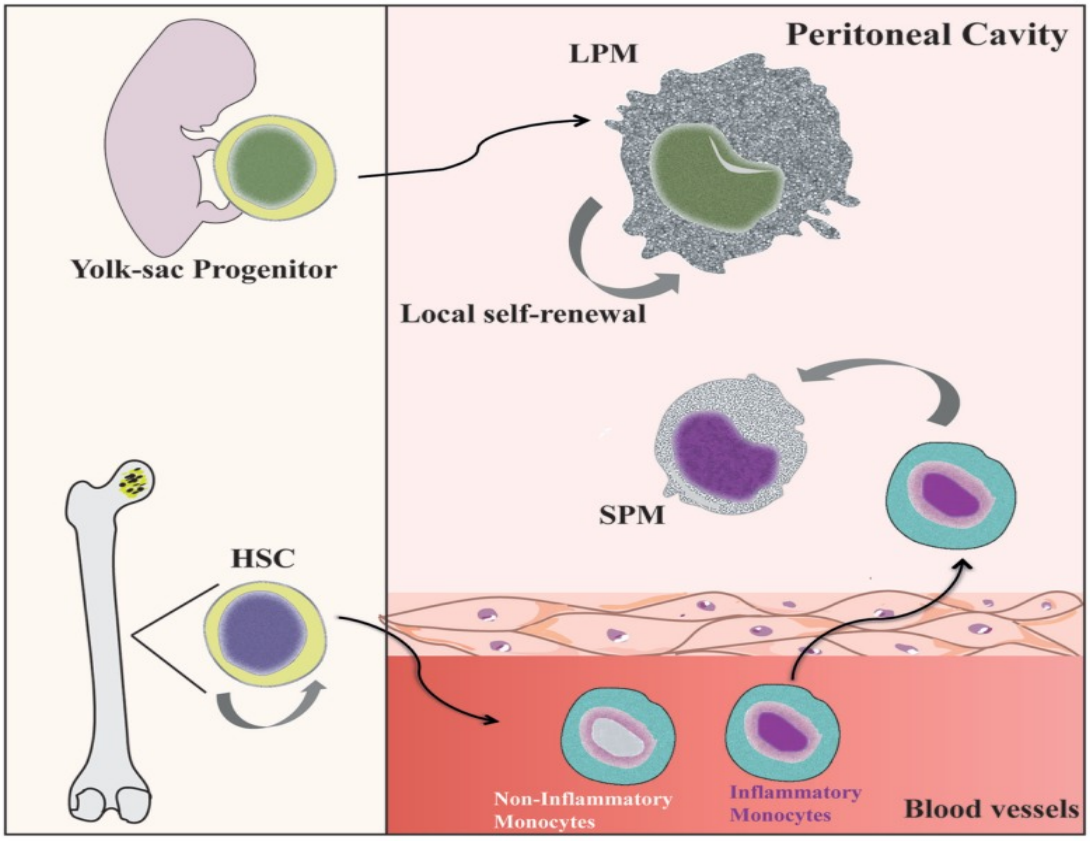


Figure 2.4.2: Demonstrates the different embryological origins of the peritoneal macrophage subsets. The small peritoneal macrophages (SPM) are generated from haematopoietic stem cells (HSC) in the bone marrow, by differentiation from blood monocytes. The large peritoneal macrophages (LPM) appear to be generated from the yolk sack and independent of haematopoietic progenitors. Local proliferation of LPMs ensures homeostatic maintenance by self-renewal. From Cassado *et al.,* (2015).

The response of the peritoneal macrophages to sepsis

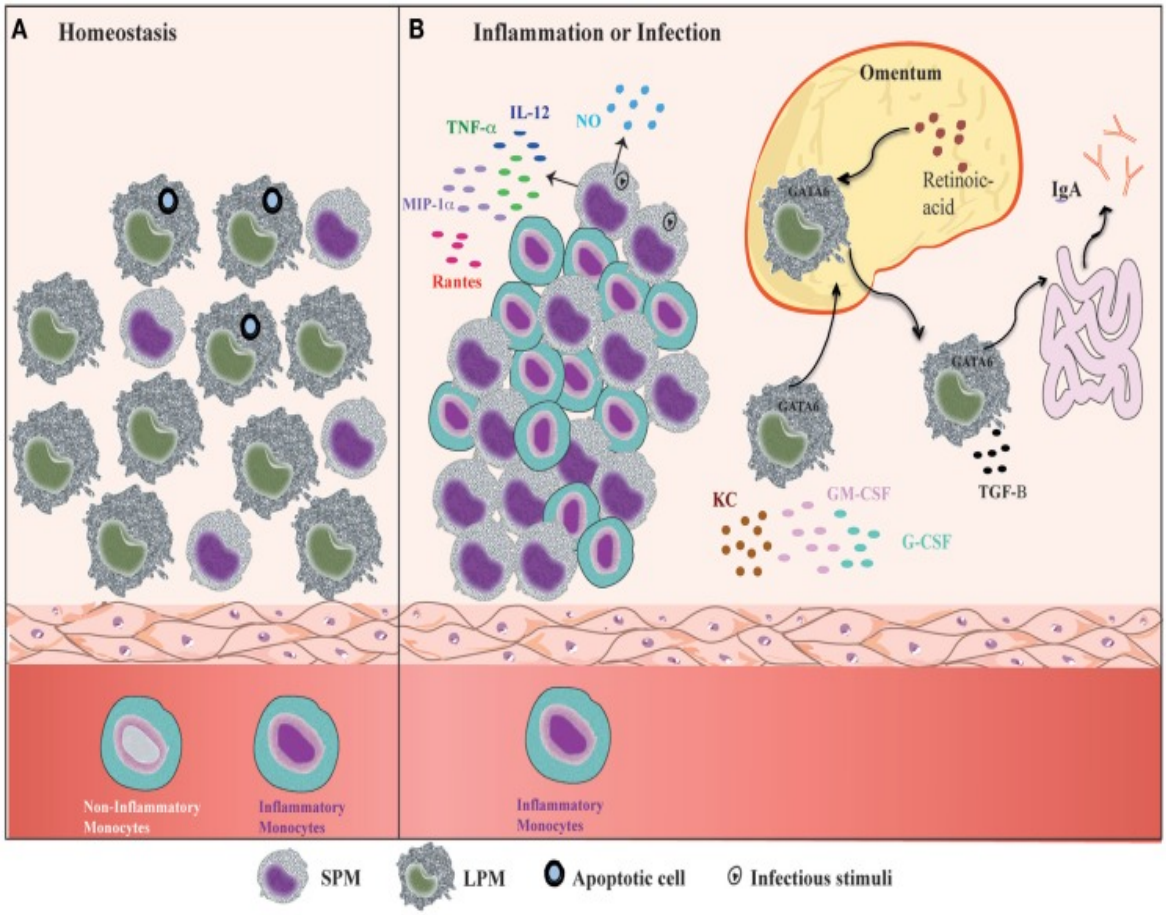


Figure 2.4.3: The left of the figure demonstrates homeostasis with LPM being the major peritoneal macrophage population, and are responsible for phagocytosis of apoptotic cells and tissue repair. In the presence of inflammation (right) LPM numbers decrease and the SPM numbers increase, supplemented by an influx of circulating monocytes. LPS, with NO and IL-12, are important in directing this change in cell population. This increases the production, by the SPM, of NO, IL-12, TNF-α, Rantes, MIP-1α. In contrast the LPM's are stimulated to produce G-CSF, GM-CSF and natural killer cells. LPS also stimulates the movement of LPM to the omentum in a pathway dependent upon retinoic acid, and the zinc finger transcription factor GATA-binding protein 6 (GATA-6). This stimulates the production IgA by the B cells in the intestine. The production of IgA is dependent upon TGF-β2. From Cassado *et al.,* (2015).

Immune function

Close contact in early peritonitis, between visceral, parietal and omental peritoneal membranes, increases the cell interactions (Kinnaert *et al.,* 1996). Up regulation and expression of adhesion molecules, chemo-attractants and other inflammatory products occurs. The peritoneal surface of the diaphragm has lacunae (large terminal lymphatics) draining via the thoracic duct to the venous system. Their patency and numbers are increased by raised intra-abdominal pressure (Walker and Condon, 1989). Respiration promotes lymphatic circulation and dissemination to the systemic circulation. Inflammation and fibrin obstruct lacunae drainage stimulating an immune response with associate hydro-thorax (Gürleyik *et al.,* 1996, Kumar *et al.,* 2014).

Inflammatory mediators and cytokines adversely affect diaphragmatic contractility (Wilcox *et al.,* 1992 and Labbe *et al.,* 2010). Fujimura's team (2000) and Labbe *et al.,* (2010) demonstrated diaphragmatic contractility was impaired early in sepsis, particularly slow twitch type I muscle fibres, and decreased as sepsis progressed. Ultimately type II fibres (fast twitch) being affected. Timing corresponds with an increase in local diphragmatic TNF-α production (type I) and the later cytokines (type II) (Callahan and Supinski, 2009). Specific cytokine up-regulation in respiratory muscle occurs, with enhanced proteolytic degradation, the later being more generalised (Callahan and Supinski, 2009).

Injecting intra-peritoneal zymosan antigen (from the yeast cell wall it is a potent activator of macrophages), and causes a dose dependent increase of TNF-α and IL-10 in mesenteric nodes, and corresponding response in distant organs (Sakahita *et al.,* 2000). Heidecke and colleagues (1999) found T cell anergy in peritonitis, with defective T cell proliferation and cytokine release correlated with mortality. Sepsis accelerates apoptotic cell death, anergy, and anti-inflammatory cytokine release from surviving cells (Green and Beere, 2000). Preventing this apoptosis improves the likelihood of survival (Hotchkiss and Karl, 2003). The more prolonged the sepsis, the more profoundly cellular immunity is affected (Hotchkiss and Karl, 2003). Macrophage migration and responsiveness is adversely affected by sepsis, possibly through invariant natural killer T-cells (Ayala *et al.,* 2014, Heffernan *et al.,* 2013). Cells migrate from the liver into the peritoneal cavity and blood with possible bi-directional stimulation between T-cells and macrophages (Heffernan *et al.,* 2013).

Pneumoperitoneum

Laparoscopic surgery causes less alteration in immune response after surgery than the open approach. Initially this was thought to be secondary to the reduction in abdominal wall trauma, which reduced the immune response. Principally studied in animal models, there are fewer human studies. Studies demonstrated there are a number of factors around generation of the pneumoperitoneum that modulate the immune response (Table 2.4.4). The majority of studies concluding there is a reduction or delay in the immune response following laparoscopic surgery, but not open surgery. At present it is unclear the importance of this reduced and delayed inflammatory response (Peters *et al.,* 2009, Goldfarb *et al.,* 2010, Han *et al.,* 2010).

Factors around laparoscopic surgery which are proposed to modulate the peritoneal environment

Laparoscopic factors modulating the peritoneal environment
Type of insufflation gas used.
Mechanical factors associated with abdominal wall and diaphragm distension.
Temperature alteration by pneumoperitoneum.
Pressure of the pneumoperitoneum.
Acidification and desiccation of the pneumperitoneum.
Preservation of cellular immunity with the pneumoperitoneum.
Unclear T-cell affect following open or laparoscopic surgery.

Table 2.4.4: Surgical factors modulating the peritoneal environment with laparoscopic surgery. Yahara *et al.,* (2002), Brokelman *et al.,* (2011) reviewed the factors.

Carbon dioxide is used to generate the pneumoperitoneum, as it is non-flammable and well dissolved in the blood. But it has been demonstrated to decrease the peritoneal macrophages cytokine production for up to three days after laparoscopic surgery compared with laparotomy (West *et al.* 1997). Predominantly the pro-inflammatory cytokine production was affected, whereas the IL-10 production was unaltered (Hanly *et al.,* 2007). Machado's team (2009), demonstrated a significant fall in TNF-α concentration in the peritoneum after carbon dioxide pneumoperitoneum, and TNF-α and IL-6 concentration in the serum, but not IL-10. Hajri *et al.,* (2000) noted a similar effect for IL-6, but not for IL-1. Machado and Coelho, (2012), proposed this is potentially mediated through the greater effect upon the small peritoneal macrophages (SPM) and their recruitment. SPMs normally play a small role in the maintenance of large peritoneal macrophage (LPM) numbers, but in sepsis LPM numbers drop and SPM numbers increase in importance and are central in the resolution of peritonitis (Cassado *et al.,* 2015).

The carbon dioxide acidification decreased TNF-α, and IL-1 cytokine production, macrophage recruitment and cellular immunity within the peritoneum and systemically (West *et al.,* 1997 and Kuntz *et al.,* 2000). Kawai's team (2014) demonstrated that carbon dioxide stimulates sensory neurons, but attenuates their inflammatory response. Cytokine expression is also reduced secondary to desiccation of the pneumoperitoneum and the open abdomen (Chekan *et al.,* (1999). Henry and Hofland (2005), demonstrated that hyperventilation during anaesthesia could ameliorate some of the hypercapnia and acidosis post-operatively.

The pressure of the gas generating the pneumoperitoneum is also important, with partial pressures above 12 mm Hg of carbon dioxide, being deleterious, though there is evidence of a negative effect above 8 mmHg (Matsuzaki *et al.,* 2014). Low pressures maintain mucosal blood flow to the gastrointestinal tract. There is decreased catecholamine release, decreasing haemodynamic fluctuation, and reduced cytokine and cellular immunity disturbance. Principally at lower pressures there is less suppression of the inflammatory and metabolic responses to injury by HPMCs, peritoneal polymorphonuclear leucocytes and peritoneal macrophages (Kopernik *et al.,* 1998 and Matsuzaki *et al.,* 2014). Neutrophil function does not return to normal until 4 ½ hours after the end of the pneumoperitoneum. The pressure of the pneumoperitoneum

negatively affects the lymph pumping, and bacterial clearance, and free radical scavenging (Gurtner *et al.,* 1995, Collet *et al.,* 1995, Taskin *et al.,* 1998).

Electron microscopy demonstrates mesothelial cells loose continuity, and fissures form allowing bacterial and macrophage migration (Liu *et al.,* 2006). These changes were seen within 30 minutes of the initiation of the pneumoperitoneum, with white cell migration occurring before 2 hours of surgery. In open surgery the opening up of intracellular spaces is only seen after 2 hours, and white cell migration occurring after longer procedures. (Liu *et al.,* 2006).

The temperature of the gas generating the pneumoperitoneum is important with Puttick and colleagues (1996) demonstrating increased TNF-α and IL-1 release with laparoscopy at room temperature compared to physiological temperature. IL-6 concentration was only marginally increased by laparoscopy at room temperature (Brockleman *et al.,* 2011). Comparisons of cellular immunity demonstrated decreased macrophage number and phagocytic activity, but an increase in macrophage cytokine production after laparotomy (Ure *et al.,* 2002). Following laparoscopy there is a decreased systemic inflammation and adhesion formation due to a decrease in cytokine concentration (Jacobi, 1998, 1999, 2001), but a preservation of neutrophil and monocyte cytotoxicity after carbon dioxide pneumoperitoneum (Alatamura *et al.* 2002).

Early on in the generation of the pneumoperitoneum venous return decreases, reducing cardiac output. Tolerated in healthy individuals, it is less well tolerated in those with cardiopulmonary disease. Research has examined lowering the pressure of the pneumoperitoneum (8-10 mmHg). This reduces general post-operative pain, analgesia requirements, and shoulder-tip pain (Sarli *et al.,* 2000,Barczynski and Herman, 2004, Sandhu *et al.,* 2009). But no study has evaluated surgeon satisfaction of low-pressure laparoscopy, particularly across a range of body habitus.

Clinically we are increasing the range of benign and malignant surgery, as well as surgery in the presence of sepsis, we are performing laparoscopically. There appears to be no harm to patients or adverse outcomes, but this is principally anecdotal (White *et al.,* 2010). Research evaluating the balance between the trauma of access, versus the magnitude of surgery, versus the physiology of the pneumoperitoneum, is yet to be

undertaken. Oncological procedures in patients who are potentially immunosuppressed from neo-adjuvant treatment, is a further area with limited research.

Introduction

Chapter 3 - Cytokines

3.1 Cytokines

Cytokine are small protein mediators with molecular weight less-than 40 kDa. Produced in a regulated fashion, to affect the activation and differentiation of the immune response. Active at low concentrations, their affinity being 10^{-9} to 10^{-12} M with a receptor occupancy of < 5% (Oberholzer *et al.,* 2000 a). Acting in a paracrine or autocrine fashion to stimulate cytokine and their receptor synthesis. Table 3.1.1 demonstrates pro-inflammatory cytokines action.

The actions of pro- and anti-inflammatory cytokines

Pro-inflammatory cytokine release initiates
Innate or adaptive immune response - including immune regulatory - effector cytokines
Initiates the release of anti-inflammatory cytokines IL4, 10 and transforming growth factor (TGF) - *β*
Anti-inflammatory cytokines release initiates
Inhibition of the production of pro-inflammatory cytokines

Table 3.1.1: The actions of the cytokine. Cytokines are released in a sequential fashion the so called 'cytokine cascade'. The pro-inflammatory cytokines promote and inflammatory response, in contrast the anti-inflammatory cytokines attempt to restore immunological equilibrium (Cohen, 2002).

Tang *et al.* (2010) reported that sepsis caused an immediate up regulation of pathogen recognition receptors, and activation of signal transduction cascades. Important inflammatory markers did not show any consistent gene expression patterns and were highly variable between individuals. Factors affecting cytokine concentration are highlighted in Table 3.1.2. Polymorphisms in genes may determine the concentrations of inflammatory and anti-inflammatory cytokines produced, determining whether there is a hyper or hypo-inflammatory response to infection (Freeman, and Buchman, 2000). The response being highly interactive and a dynamic process, reflecting heterogeneous genome-specific pathways (Schulte *et al.,* 2013). This requires tight regulation with anti-inflammatory cytokines, and soluble inhibitors of pro-inflammatory cytokines. Table 3.1.3 demonstrates the pro-inflammatory cytokines acute phase response.

Cytokine synthesis is tightly regulated self-limiting event. Not stored preformed, instead being synthesised from newly transcribed mRNA. Their mRNA has a short half-life due to an AU-rich region in their third untranslated sequence (Oberholzer *et al.,* 2000 b). Cytokine production and release is chiefly controlled at the level of gene transcription. NF-κB is one of the most important transcription factors in determining response (see Figure 3.1.4) (Panes and Granger, 1998).

Factors affecting cytokine production

Factors affecting cytokine concentration and their action
HLA haplotype particularly TNF-α and IL-1 – determines the action and concentration of the cytokine
Genetic polymorphisms both in loci for cytokine production and their receptors – determines the action and concentration of the cytokine
Age – Increase in IL-1 Ra, IL-6
Gender – Increased pro-inflammatory cytokines
Basal metabolic index – Increased IL-1, IL-6 and IL-18 in higher BMI
Oral contraceptive – Decreased TNF-α and IFN-γ
Oestrogen concentration - Increased IL-1 Ra, IL-17

Table 3.1.2: Demonstrates some of the important factors in cytokine production (Bone, 1996 a). The risk of death has been correlated with genetic polymorphisms at TNF-α and β loci (Freeman and Buchman, 2000). Genetic differences have also been identified in the TNF receptors, IL-1 receptors, Fc g receptors and toll-like receptors.

The acute phases effects of the rise in systemic inflammatory cytokines

Systemic effects of cytokine – acute phase response
Fever
Leucocytosis
Hypothalamic-pituitary-adrenal axis stimulation of catabolic hormones
Acute phase protein synthesis in the liver
Immune activation
Hypermetabolism
Anorexia
Protein catabolism, cachexia, and altered fat, glucose and trace mineral metabolism
TNF-α, IL-1 and IL-6 not only regulate the innate immune response, but also the acquired immune system in particular the T_H1 responses
IL-1, TNF-α, or lipopolysaccharide stimulate E-selectin to be displayed on the cell surface, the process involves nuclear factor Nκ-B, triggering cytokine production.

Table 3.1.3: The systemic effects of cytokine – acute phase response (Souba, 1994). These processes are accelerated if there is a second insult, such as infection, shock or ischaemia.

Schematic diagram of the network of intracellular signaling

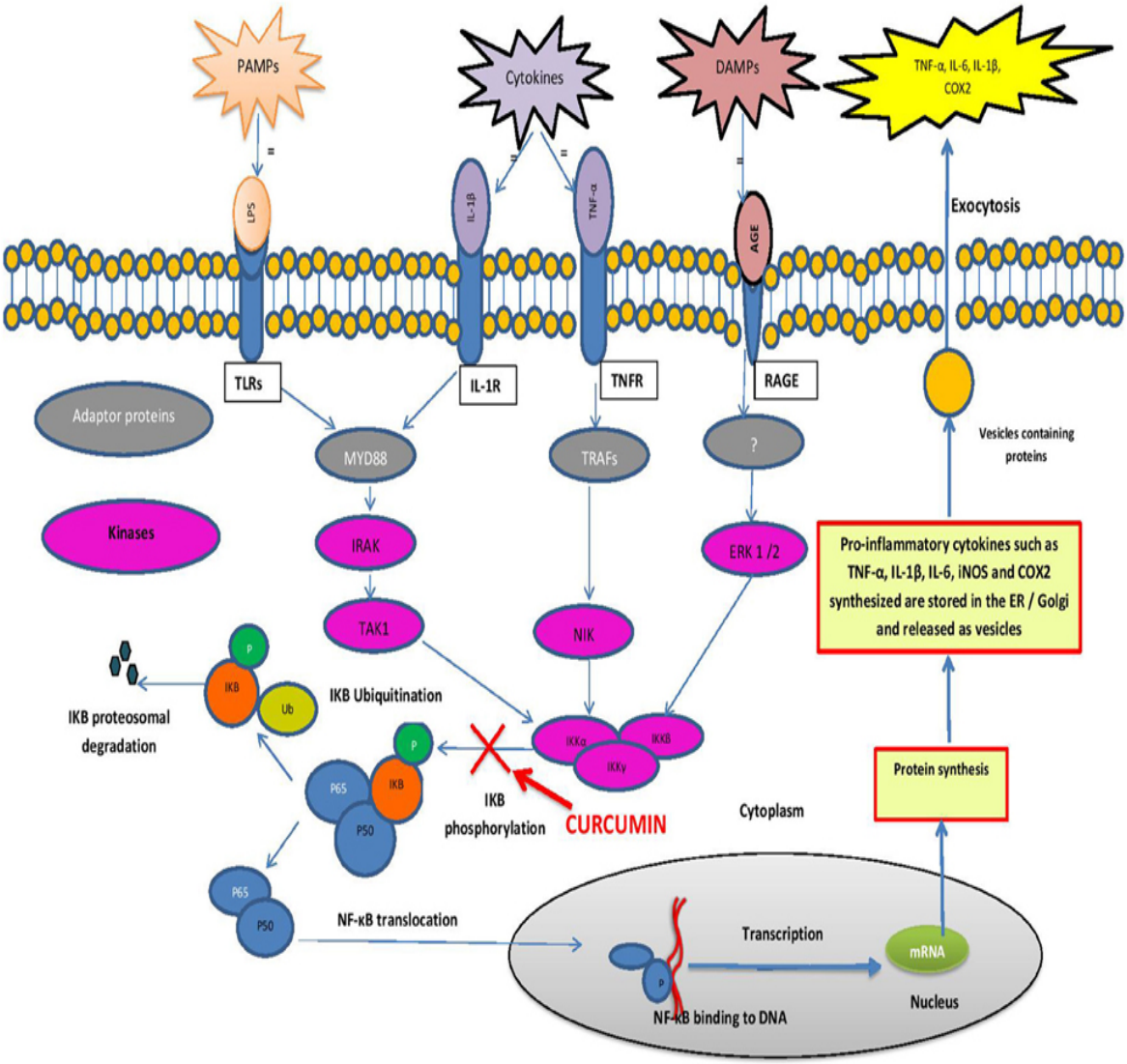


Figure 3.1.4: A schematic diagram of the network of intracellular signalling. Cells express membrane receptors such as toll-like receptors (TLRs), IL-1β receptors (IL-1R), TNF–receptors (TNFR) and receptors for advanced glycation end products (RAGE). These receptors recognise pro-inflammatory stimuli such as pathogen-associated molecular patterns (PAMPs), damage associated molecular patterns (DAMPs) and cytokines. Ligand bound PAMPs, DAMPs and cytokines activate downstream adapter proteins such as myeloid differentiationprimary response protein 88 (MyD88) and TNF associated factors (TRAF). MyD88 and TRAF activates specific protein kinases such as mitogen activated protein kinases (MAPK) such as IRAK, TAK1, NIK and ERK 1 / 2. These kinases activate IκB kinases (IKKα, IKKβ, IKKγ) that phosphorylate IkB-α. In stimulated cells, phosphorylation of IkB leads to its dissociation from the complex, and its proteasomal degredation, allowing NF-kB to translocate to the nucleus, where it binds to specific DNA sequnces present in the promoters of numerous target genes, encoding the pro-inflammatory cytokines (e.g., IL-1, Il-2, IL-6, TNF-α), chemokines (e.g., IL-8, MIP-1α, MCP-1, RANTES, eotaxin), adhesion molecules (e.g., ICAM, VCAM, E-selectin) as well as Cycloxygenase-2 (Cox-2) and inducible nitric oxide synthase (iNOS) (Losada *et al.,* 2014).

Cytokines are additionally controlled by post-transcriptional processing. For example IL-1 undergoes proteolytic cleavage from an inactive precursor, the TNF-α superfamily, are expressed as cell-associated proteins, cleaved from the cell membrane by matrix metalloproteinase or adamolysin (Black *et al.* 1997). The cytokines are retained in an inactive cytoplasmic complex regulating transcription of various proinflammatory and immunoregulatory cytokines. Shed extracellular domains of the cytokine receptors play a regulatory role. A class of non-coding RNAs modulate cytokine response, they comprise of microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). Important in innate immunity, mitochondrial functions, and apoptosis (Dey *et al.,* 2014). Both miRNAs and lncRNAs are important in pro- and anti-inflammatory response, circRNAs are less fully studied, but possibly regulate miRNA (Mao *et al.,* 2015). Non-coding RNA expression differs significantly dependent upon the microbial moieties encountered (Chen *et al.,* 2014). Study of miRNA in SIRS response reveal differential miRNA deregulation (Ma *et al.,* 2013).

miRNAs at the transcriptional and translational level regulate proinflammatory cytokine production, and the SIRS response (Zou *et al.,* 2016). miRNA expression maintaining the inflammation and immunosuppression, characteristic of ongoing sepsis. miRNA affects thrombocyte apoptosis, important in sepsis-induced coagulopathy (Larkin *et al.,* 2016). miRNA and lncRNA produce the lung, liver, kidney and skeletal muscle response to sepsis (Ho *et al.,* 2016). Organ-specific differentiation of regulatory non-coding RNAs, is a current focus of research, with the heterogeneity of patients with sepsis. The aim being to develop organ-specific delivery of non-coding RNA mediators or their antisense version, to down regulate the RNA enhancer. Their stability and ability to access sites like the CNS has lead to interest in them in pharmaceutical industry (Carpenter and Fitzgerald, 2019).

Patients with sepsis have features consistent with immunosuppression, when stimulated with lipopolysaccharide. Administering IFN-γ reverses this restoring macrophage TNF-α production and improving survival (Hotchkiss and Karl, 2003). Cytokine secretion by T-lymphocytes is suppressed after major surgery giving rise to an increased susceptibility to infection with intracellular pathogens.

3.2 Tumour Necrosis Factor - alpha

TNF-α is a central mediator of the immune activation, inducing the pathophysiological disturbances associated with bacteraemia and sepsis. TNF-α is a 157 amino acid polypeptide, 17 kDa in weight. With IL-1 it is a main player in infectious and noninfectious inflammatory diseases (Schulte *et al.,* 2013). TNF-α gene polymorphism affects transcription, translation and disease susceptibility (Feng *et al.,* 2015). TNF-α is synthesised by various cells of the reticular endothelial cell system, non-immune cells also synthesise TNF-α (Zhang *et al.,* 1997, Parameswaran and Partial, 2010). Production is tightly controlled at the transcriptional and translational level.

Release from macrophages begins within 30 minutes of the initiating event, as an early regulator of the immune response. Peak concentrations of TNF-*α* and IL-1 are detected 60 – 90 minutes after LPS administration (Dinarello, 2004). Circulating TNF-α half-life is short, 14 - 18 minutes, liver, skin, gastrointestinal tract and kidney removing it from the circulation. Trans-membrane TNF-α receptors, TNFR1, and TNFR2, activate immune cells to release downstream immune-regulatory mediators. Table 3.2.1 details the action of TNF-α.

TNF-*α* uniquely orchestrates the downstream cytokine cascade, with IL-1 support, it is considered to be a "master regulator" of inflammatory cytokine production (Parameswaran and Partial, 2010). Performed in an autocrine and paracrine manner by activating macrophages to secrete other pro-inflammatory cytokines, lipid mediators, and reactive oxygen and nitrogen species (Cohen, 2002), Figure 3.2.2.

The role of TNF-α in septic shock

Physiological changes produced by TNF-α in septic shock	
Hypotension	Fever
Tachycardia	Oliguria
Increased systemic capillary leakage	Decrease albumin synthesis
Pulmonary oedema	Haemorrhage
Decreased erythropoiesis	Enhanced microvascular thrombosis and inhibition of thrombomodulin on the cell surface
Alteration in consciousness	Chemotaxis to lymphoid tissue
Stimulation of accute phase proteins	Activation of complement and coagulation
Migration of neutrophils into the peripheral blood, but not monocytes	Release of neutrophils from the bone marrow
Activation and up-regulation of neutrophils, monocytes and microphage differentiation and activation.	Apoptosis in endothelial cells, hepatocytes and haemopoietic cells especially thymocytes (Papthanassoglou *et al.,* 2000)
Expression in endothelial cells of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, and chemokines (Shimaoka and Park, 2008)	Increases integrin adhesiveness in neutrophils and promotes their extravasation into tissues (Shimaoka and Park, 2008)

Table 3.2.1: The actions of TNF-α in septic shock (Beutler and Cerami, 1987, Zhang *et al.,* 1997, Newman *et al.,* 1996, Karima *et al.,* 1999, Baudo *et al.,* 1998, Oberholzer *et al.,* 2000 a, Papthanassoglou *et al.,* 2000, Dinarello, 2004, Conte *et al.,* 2006, Shimaoka and Park, 2008 Svedova, *et al.,* 2016).

Demonstration of the timing of the release of the inflammatory cytokines

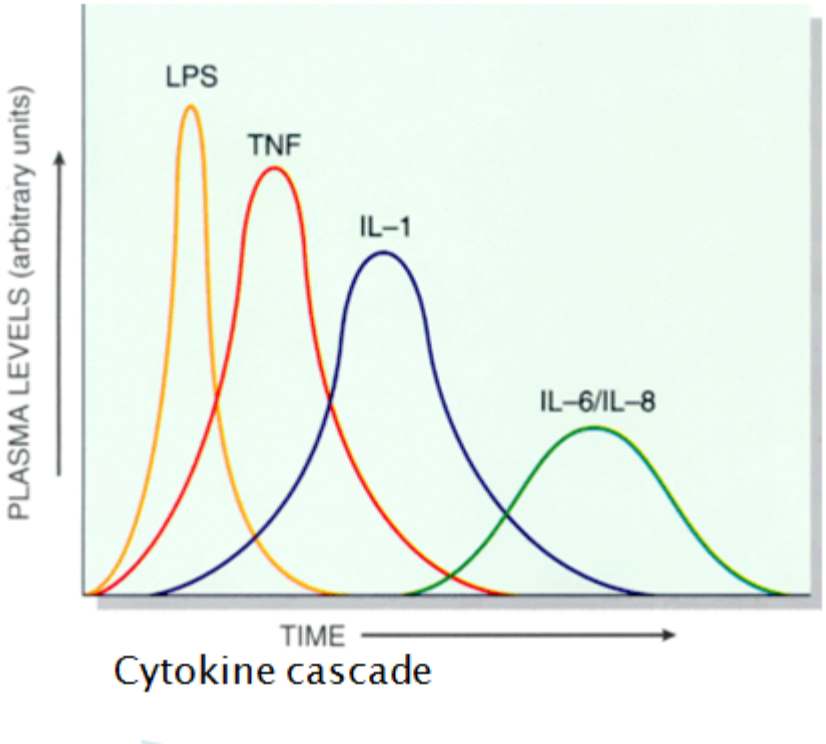


Figure 3.2.2: Demonstrates the casecade in the plasma levels of inflammatory cytokines in patients with sepsis (Boontham *et al.,* 2003).

Soluble cytokine receptors and receptor antagonists, modulate the action of the cytokines, sTNFRs, IL-1R2, and IL-1Ra, are important in the early stages of inflammation to dampen down the actions of TNF-α and IL-1. Elevated levels of sTNFRs and IL-1Ra were measured in septic patients, the plasma concentrations correlating with disease severity, and sTNFRs, with mortality (Gogos *et al.,* 2000). Particularly if levels are persistently elevated (Gogos *et al.,* 2000).

In murine models of septic shock IL-1Ra administration increased survival. The ratio between TNF-α and sTNFRs, rather than absolute plasma concentration alone, has prognostic value in septic patients (Modzelewski, 2003, Schulte *et al.,* 2013). Although the regulation pathway is currently unclear, tight regulation appears crucial for the positive outcome of sepsis. *Ex-vivo* studies demonstrated sepsis inhibits the ability to phosphorylate NF-kappa-light-chain-enhancer in activated B cells in lymphoid cells and monocytes, possibly contributing to the sepsis-induced immunosuppression (Hoogendijk *et al.,* 2017).

TNF has two soluble cell surface receptors sTNFR-p55 and sTNFR-p75. Produced by the proteolytic cleavage of the extracellular binding domain of the cell surface TNFRs. p55 expression is ubiquitous, p75 restricted to cells mainly of hematopoietic origin. Many cells express one or other receptors, few highly responsive cells express both (Stewart and Marsden, 1995).

3.3 Interleukin - 1

IL-1 has two structually related subsets, IL-1α and IL-1β, binding to the same receptor with equal affinity, synthesised from two separate genes (Schulte *et al.,* 2013). They have a half life of 6 - 10 mins. The genes encoding for IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra) are clustered together within the human major hitocompatability complex at q13-21 on chromosome 2 (Dinarello, 1996). As with TNF-α, gene polymorphism significantly predispose to sepsis (Zhang *et al.,* 2014).

IL-1Ra, is a 23kDa glycoprotein, competatively binding to the IL-1 receptors modulating cell signal transduction (Dinarello, 1998). Blockade of the receptor with IL-1Ra, has been shown to reduce mortality. Particular in those with severe sepsis with hepatobiliary dysfunction and disseminated intravascular coagulation, IL-1Ra has been seen to improve survival and have fewer safety concerns than activated protein C (Shakoory *et al.,* 2016). This requires larger randomised trials as there remain concerns about sepsis as secondary complication, and a poorer outcome from sepsis (Ali *et al.,*2015).

Rapidly after intravenous endotoxin infusion, into healthy volunters, circulating TNF-α and IL-6, but not IL-1, become detectable. Due to IL-1 principally being a membrane bound cytokine, involved in local paracrine and autocrine regulation (Davies and Hagen, 1997). The majority IL-1β remains in the cytosol in a precursor form, or as membrane associated in a biologically active form. In sepsis IL-1 is predominately in the beta form, it is converted to the active form by IL-1β converting enzyme, and secreted (Schulte *et al.,* 2013). Becoming detectable within two to three hours of sepsis (McAllister *et al.,* 1994). IL-1β acts to upregulate the expression of other cytokines and is degraded rapidly from its precursor by trypsin, plasmin and other proteases.

IL-1 is activated in parallel or in response to TNF-α. TNF-α acts on IL-1R1 and 2 to promoted the release of IL-1, which is released primarily from activated mactrophages. During infection it is also released from poly-morphonuclear leucocytes. IL-1 augments and acts in a similar fashion to TNF-α, activating both neutrophils and endothelial cell adhesion molecules (Dinarello, 1997).

There are two forms of the high affinity IL-1 receptor, and tissue distribution varies from 100 to 10,000 receptors per cell. Raised levels type II IL-1 receptor is seen in sepsis. IL-1 type II receptor is cleaved from the cell surface, and lacks an intracellular signaling domain to propagate the signal. The type II receptor can bind IL-1β inhibiting binding to type I IL-1 receptors, inhibiting amplification (Giri *et al.,* 1994). Circulating type II receptors increase in sepsis regulating IL-1 activity (Giri *et al.,* 1994). Monocytes from elective surgical patient have impaired LPS-stimulated IL-1 and IL-8 synthesis (onset of IL-6 synthesis is delayed) (Bone, 1996 b).

Studies support the central role of IL-1 in gram-negative bacterial sepsis (Pruitt *et al.,* 1995). As with TNF-α, IL-1β is a predictor of severity, but unlike TNF-α not of mortality (Bone, 1996 b). In contrast to TNF-α, IL-1 is not directly lethal, being equipotent in inducing cytokine synthesis, reproducing many of the acute haematological and metabolic phenomenon (Fong *et al.,* 1990). Table 3.3.1 details IL-1's action in sepsis.

IL-1's role after elective surgery remains unclear. Karayiannakis's team (1997) measured IL-1 receptor concentration finding no difference in concentration between the laparoscopic and open approach to cholecystectomy. Leung *et al.,* (2000) measured Il-1β, finding a peak in concentration at 2 hours after open and laparoscopic surgery.

The role of IL-1 in septic shock

Physiological changes produced by IL-1 in septic shock	
Fever	Anorexia
Malaise	Arthralgia
Headache	Haemodynamic abnormalities such as hypotension in shock
Production of granulocyte / macrophage CSF (GM-CSF)	Hepatic acute phase proteins
Cytokine production especially IL-6, IL-8 and TNF-α	Macrophages produce IL-1Ra higher concentration in more severe sepsis

Table 3.3.1: The action of IL-1 in sepsis, which is similar to TNF- α as seen in table 3.2.1 (Dinarello, 1997, Dinarello, 1998, Boontham *et al.,* 2003).

3.4 Interleukin - 6

IL-6 is a glycoprotein located on chromosome 7 at p21, with molecular weight between 21.5 and 28 kDa (May *et al.,* 1989). Produced by many cell types, as detailed in Table 3.4.1. Belonging to a family of at least six differently modified phosphoglycoproteins, released early in the acute phase response (Waage *et al.,* 1989). Gene polymorphism, post translational and post secretory modification, giving rise to differing isoforms. Levels are not detectable in the serum until four to eight hours after the TNF-α and IL-1β peak. Attenuation of the TNF-α and IL-1β peak concentration decreases the subsequent IL-6 response. Following elective surgery IL-6 peaks between 4 and 24 hours after surgery (Yuen *et al.,* 1998), with IL-6 being closely correlated to the magnitude of surgical stress (Schietroma *et al.,* 2016).

The cell types producing IL-6 and the stimulus for the cytokines release

Stimulus for IL-6 production	
LPS	TNF-α, IL-1
Cell types producing IL-6	
Macrophages	Dendritic cells
Lymphocytes	Endothelial cells
Fibroblasts	Smooth muscle cells
All nucleated cells *in-vitro*	

Table 3.4.1: Stimuli and cells producing IL-6 (Park and Pillinger, 2007, Scheller and Rose-John, 2006). Elevated IL-6 concentrations are measured in many acute conditions, such as burns, major surgery and sepsis.

Serum IL-6 levels are early and sensitive markers of tissue damage rising in proportion to the surgical trauma and associated injury. It is highly accurate in diagnosing sepsis (Hou *et al.,* 2015). Levels are not always detectable in patients under 60 years with uncomplicated surgery (Roumen *et al.,* 1992 and Ohzato *et al.* 1992). Injection of IL-6 by itself does not produce a sepsis-like state (Dinarello, 1997, Schute *et al.* 2013). Possibly IL-6 is a marker of the severity of the neuroendocrine and inflammatory response rather than the mediator (Preiser *et al.,* 1991).

Peak IL-6 concentrations follow TNF-*α* and IL-1 (Schute *et al.,*2013). IL-6 concentration correlating with indicators of disease severity scores; such as clinical scores, multi-organ failure and septic shock, and overall mortality. TNF-α, IL-1 and IL-6 activate procoagulation factors in the vascular endothelium, causing endothelial damage. This reduces its anticoagulant properties and fibrinolysis, leading to SIRS, shock, DIC and MODS (Nimah and Brilli, 2003). In animal studies, IL-6 plays a critical role in cardiac, liver and renal dysfunction in sepsis (Ding *et al.,* 2014). Both IL-6 and TNF-*α* correlate with APACHE II in ITU patients with pneumonia. Predicting the need for mechanical ventilation, early mortality and acute kidney injury (Bacci *et al.,* 2015).

Plasma IL-6 concentration in sepsis correlates closely with severity and outcome, being significantly higher in non-survivors (Boontham *et al.,* 2003, Hong *et al,* 2014). Severe sepsis and septic shock patients have a poor outcome and high circulating concentrations of IL-6. *In vivo* studies with IL-6 knockout mice, demonstrates deletion of the IL-6 gene decreases lung and peritoneal inflammation, and is protective against organ failure and mortality (Cuzzocrea *et al.,* 1999, Schulte *et al.,* 2013). IL-6 has a variety of biological effects demonstrated in Table 3.4.2.

The role of IL-6 in septic shock

Physiological changes produced by IL-6 in septic shock	
Fever	Mediation of the acute phase response
Lymphocyte proliferation	Coagulation system activation
Modulation of haematopoiesis	Myocardial depression
In combination with TNF-α augments T-cell proliferation and promotes PMN activation and accumulation	Synergistically with IL-1β to affect thymocyte proliferation

Table 3.4.2: The action of IL-6 in sepsis from the work of (Boontham *et al.,* 2003, Kopf *et al.* 1994, Dinarello, 1997, Vittimberga *et al.,* 1998, Pathan *et al.* 2004).

Exposure of endothelial cells to endotoxin results in increases in IL-6 concentration, possibly mediating a signaling cascade culminating in cell apoptosis (Papathanassoglou *et al.,* 2000). Failure of apoptosis in neutrophils in the presence of IL-6 potentiates tissue injury due to oxidative properties, proteolytic activities, and diminished numbers of activated neutrophils (Biffl, *et al,* 1996). T-helper 1 cells (Th1) predominate in microbial infection, activating Th2 to clear the pro-inflammatory response and stimulating the humoral response. In sepsis the Th2 response causes dysregulation of the cellular immune response, Th2 cytokines inhibit Th1 and vice versa (Shubin *et al.,* 2011).

IL-6 promotes an anti-inflammatory response, inhibiting the release of TNF-α and IL-1 and enhances the circulation levels of anti-inflammatory mediators, (Xiao *et al.,* 2005). IL-6 is protective in experimental endotoxemia (Schulte *et al.,* 2013). But genetic deletion of IL-6 did not alter the mortality in a model of polymicrobial sepsis by Remick *et al.,* (2005). Morphine, in animal models, has been demonstrated to decrease neutrophil recruitment into the peritoneal cavity in the early stages of sepsis, and increase Gram-positive bacterial dissemination from the gut lumen. This is proposed to be mediated through a pathway involving IL-17A (Meng *et al.,* 2015). Morphine has been demonstrated to be inhibitory to human macrophage function in sepsis (Liang *et al.,* 2016). This can induce peri-operative immunosuppression and impaired wound healing at sufficient doses (Ashcroft and Masterson, 1994).

IL-6 and CRP increase after both laparoscopic and open surgery, particularly the later (Karayiannakis *et al.,* 1997, Leung *et al.* 2000). Peak IL-6 concentration preceding CRP (Leung *et al.* 2000). Hill and colleagues (1995) and Akhtar *et al.,* (1998) failed to demonstrate an increase IL-6 concentration following open and laparoscopic inguinal hernia repair. Postulating a requirement for the surgical insult to be insufficient to increase IL-6 concentration. The later also demonstrated no increase in TNF-α concentration. Other researchers have found variable responses in various animal models (Johnson *et al.* 1994, Stage *et al.,* 1997).

3.5 Interleukin - 10

IL-10 is a 35-kDa synthesised as an 18-20 000 dalton monomer, which forms a non-covalently bound homo-dimer in solution. Gene-polymorphism alters the risk of developing sepsis after major trauma (Zeng *et al.,* 2009). Although anti-inflammatory, the IL-10 concentration has not been found to be predictive of survival (Hong *et al.,* 2014). Latifi *et al*. (2002) reported IL-10-deficient mice showed an earlier onset of lethality in sepsis and a reduced response to rescue surgery. Administration of recombinant IL-10 protein to IL-10 deficient mice increased survival and lengthened the therapeutic window for the rescue surgery. The timing of administration of IL-10 antibodies appears to be critical to survival; evidence is increasing of IL-10's role in transition between early reversible sepsis and late irreversible septic shock (Schulte *et al.,* 2013). Studies suggest IL-10 produces an early systemic anti-inflammatory response (Rivera-Chavez *et al.,* 2003 Taniguchi *et al.,* 2003). Table 3.5.1 lists the cells producing IL-10, and 3.5.2 the actions.

Cell types producing IL-10

Cell types producing IL-10	
Monocytes and macrophages	Dendritic cells
B and T lymphocytes	NK cells

Table 3.5.1: The main cell types producing IL-10, though many immune cells can produce the cytokine (Lyons *et al.,* 1997, Latifi *et al.,* 2002).

The role of IL-10 in septic shock

IL-10 action in septic shock
IL-10 acts to suppress the generation of Th1 T cells and their production of pro-inflammatory cytokines.
Strongly suppressive effect upon monocytes / macrophages, dendritic cells, neutrophils and T cells promoting anergy and apoptosis
Promoter of the Th2 cells and antibody production
IL-10 regulates IL-1α and β, TNF-α, IL-6, IL-8, IL-12 and IL-18, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, macrophage inflammatory protein-1a, normal T-cell expressed and secreted (RANTES), leukaemia-inhibiting factor and IL-10 itself are all suppressed
IL-10 stimulates the production of IL-1Ra and sTNFRs, thereby neutralizing the pro-inflammatory actions of IL-1 and TNF-α
Nitric oxide and synthesis of gelatinase and collagenase are also suppressed
No effect on the constitutive expression of TGF-β, a cytokine with anti-inflammatory properties
Interacts with the coagulation cascade, to inhibit the expression of tissue factor on monocytes

Table 3.5.2: The anti-inflammatory actions of IL-10 from the work of (Ertel *et al.,* 1995, Shubin *et al.,* 2011 and Boontham *et al*, 2003, Rivera-Chavez *et al.,* 2003, Oberholzer *et al.,* 2002). This wide range of biological properties has lead to a great deal of concern about its use in sepsis syndromes. Oberholzer's team believes this supports the hypothesis that during sub lethal endotoxaemia it is the inhibition of the pro-inflammatory cytokine release that is IL-10's predominant role (Oberholzer *et al.,* 2002).

Studies demonstrated the TNF-α and IL-1 response directly regulates IL-10 production, which in turn down regulates the concentration of TNF-α and IL-1 (Oberholzer *et al.,* 2002). Inhibition of TNF-α synthesis significantly attenuates IL-10 response. Inhibition of IL-10 causing an exaggerated TNF-α response to endotoxin, this interrelationship is shown in Figure 3.5.3 (Oberholzer *et al.,* 2002). IL-10 concentration increases after elective surgery and is postulated to limit the inflammatory response (Gilliand *et al.,* 1997). Neutrophils are essential for production of IL-10, which modulates the peritoneal monocytes phagocytosis, and expression of inflammatory cytokines (Ocuin *et al.,* 2011). Murine models have demonstrated neutrophil depletion alone did not alter survival, whereas depletion of neutrophils and inflammatory monocytes in peritoneal sepsis markedly reduced survival.

The role of IL-10 in stimulating B-cell and inhibiting macrophages and dendritic cells

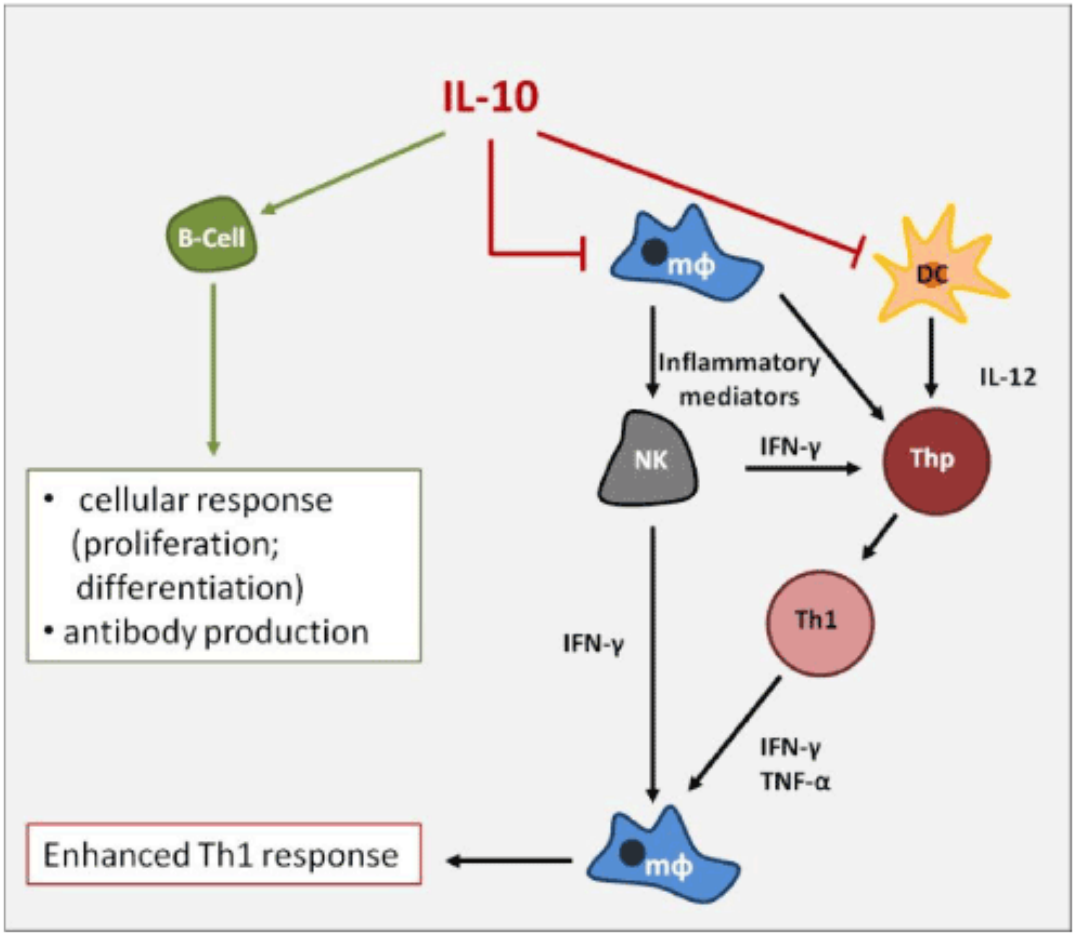


Figure 3.5.3: Demonstrating the role of IL-10. It is inhibitory to the macrophages and the dendritic cells, inhibiting the Th1 response. In contrast it promotes the cellular response via the B-cells (Oberholzer *et al.,* 2002).

3.6 Peritoneal cytokines

In animal studies there is a strong correlation between mortality and the intra-abdominal cytokine concentration of TNF-α, IL-6 and IL-10. Higher concentration of all three increase the risk of mortality, but the best correlation with APACHE-II score was with IL-10 (Spearman's rho 0.424, Hendricks *et al.,* 2010). In the first 72 hours of shock from generalised peritonitis, Riché and colleagues (2000) determined that IL-6 and TNF-α rose in the systemic circulation and then declined, whereas IL-1 barely increased. In the non-survivors IL-6 concentrations remained high, but TNF-α and IL-1 concentrations did not alter.

Martineau and Shek, (2000) using persistent bacterial peritonitis in a rat model found IL-6 concentration to be comparable, with significantly elevated TNF-α and IL-1β in the study group. No difference could be demonstrated for TNF-α concentrations between mono and poly-microbial peritonitis by Riché's team (2000). However it is postulated that there is synergistic effect in polymicrobial infections, leading to a worse outcome (Dupont *et al.,* 1998).

Animal i*n-vivo* experiments demonstrated significantly reduced TNF-α levels, in peritoneal macrophages, 24 hours after CO_2 laparoscopy compared to gasless laparoscopy and laparotomy, this effect lasted for up to three days (Mathew *et al.,* 1999). Systemic IL-6 was elevated, but depressed in peritoneal cells, there was no difference in IL-1 (Hajri *et al.,* 2000). T cell function and cell mediated immunity was maintained correlating with fewer postoperative septic complications following laparoscopic surgery compared to open.

Comparison of cytokine concentration in peritoneal drain fluid demonstrated no difference in pattern of response following laparoscopic and open colonic resection. The systemic and the drain concentration was significantly less in the laparoscopic resections (Wu *et al.,* 2003).

Measuring cytokines in the drain fluid following colorectal surgery has demonstrated elevation in cytokines after surgery. Falling by day 3 in un-complicated surgery. Those with an anastomotic leak or intra-abdominal complication, the drain concentration of TNF-α, IL-1 and 6 were an early diagnostic indicator (Yamamoto *et al.,* 2011).

Literature review has demonstrated IL-6 to be detectable first, possibly day 1, and TNF-α from day 2 in anastomotic leaks (Clini *et al.,* 2013). Similar results have been found by Sparreboom team (2016), in their meta-analysis, but TNF-α was only found to be significantly higher from day 3 onwards. In the later study the concentration in the drain rose before the systemic cytokines.

Introduction

Chapter 4 - Pain

4.1 Pain introduction

The most widely used definition of pain is the International Association for the study of pain (IASP) given in table 4.1.1. Pain is a subjective sensation, and measurement and analysis are difficult. Not only a sensory stimulus, it has a motivational and affective component, as in Table 4.1.2. Comparing studies is difficult due to the multiple measures adopted and environments measured in.

Kent (1985) demonstrated that anxious patients expected and remembered four times the amount of pain they actually had, whereas the low anxiety patient expected and remembered less than twice the amount of pain. The results of studies examining preoperative anxiety and postoperative pain are mixed, with many studies concluding that pain and anxiety are difficult to measure, however innate anxiety does not correlate with state anxiety (anxiety at a given time). There is closer correlation between pain and innate anxiety but it is still far from a perfect correlation (Chung *et al.,* 1997).

Definition of pain by the International Association for the study of pain

International Association for the study of pain (IASP)
'an unpleasant sensory and emotional experience associated with actual or potential tissue damage and described in terms of such damage' (Merskey and Bogduk, 1994).

Table 4.1.1: There are multiple definitions of pain in the literature. The most widely used is the International Association for the study of pain (IASP).

Factors affecting the experience of pain

Factors affecting the experience of pain	
Context of cultural learning	Previous experience
Anxiety and depression	Patient perception of their environment,
The patients, their relatives or the clinician's belief's about pain	Cultural background
Age	Gender
Placebo effect	Pre-existing pain

Table 4.1.2: Factors affecting patients' experience of pain (Katz and Melzak, 1999). Studies have found opposite findings for pain and increasing age (Lynch *et al.,* 1997, Chung *et al.,* 1997). Gender differences are widely studied and demonstrated environment plays a significant role (Lynch *et al.,* 1997, Chung *et al.,* 1997). Zatzick and Dimsdale (1990) demonstrated that there was no ethno-cultural difference between peoples discrimination of noxious stimuli, but there was significant cultural difference in reporting pain. It is this criterion for reporting pain, which may lead to ethnic bias. Vitale and colleagues (1991) demonstrated the importance of cultural factors in patient's duration of pain and their time to return to work following laparoscopic cholecystectomy. Postoperative pain has been reported to correlate with peri-operative pain levels (Ure *et al.,* 1994).

4.2 Pain assessment

In practice there is a marked disparity between how staff and patients rate their pain, even amongst specially trained staff. Research demonstrates uniform poor assessment and rating skills across all staff groups, particularly in comparison to other clinical signs (Grossman *et al.,* 1991). Guidelines for pain assessment include the timing of rating, the place, person and measure. Poor postoperative pain relief delays recovery, increases morbidity, reduces patients satisfaction and increases the risk of developing chronic pain and can increase mortality (American Society of Anesthesiologists, 2012, van der Voot *et al.,* 2015).

In a study of day case laparoscopic cholecystectomy Watt-Watson and team (2004), found all patients rated their worst pain, as moderate to severe at each 24 hour period to 7 days. Despite this only 50% took any analgesia after 72 hours. In 20% of cases this was due to the side effects of the analgesia. Surgical patients report more pain, but a systematic review of adult NHS patients reported over 50% of medical patients reported pain as a significant symptom (Greogory and McGowan, 2016). Several reasons have been proposed for patients' failure to report pain detailed in Table 4.1.3.

Reasons for patients not reporting their pain, and interventions to optimise pain management

Proposed reasons for patients failing to report pain	
Lack of knowledge regarding options for pain relief	Lack of knowledge of the risks of unrelieved pain
Experiencing less pain than expected	The patients experience of the side effects of analgesia
Belief that pain serves a purpose in recovery	Health care providers believes and responses
Multi-modal approach to pain is optimal including	
Multi-modal anaesthesia	Interactive patient counseling
Follow-up telephone advice and support	Pre-operative counseling

Table 4.1.3: Proposes reasons for patients failure to report pain, from Huang *et al.,* (2001) study of why patients fail to report pain and optimal measures to achieve post-operative pain relief.

Despite significant advance, focus and guidelines on postoperative pain, still 45% of patients are reporting being in extreme pain for a period of time after surgery. With pain being the reason for 38% of readmissions after day case surgery (White and Kehlet, 2010). Multi-modal analgesia, usually a NSAID and opiate aims to reduce side effects, by decreasing dose of each and gains from the synergistic action of the two drugs, this has improved postoperative pain management (Vadivelu, 2010). But of the patients experiencing extreme pain 30% report their pain not being fully addressed (Walker *et al.,* 2014). Despite this over 80% of patients expresses a high level of satisfaction with the care they received (Walker *et al.,* 2016).

4.3 Pain and the immune system.

Post-operative pain affects multiple organ systems, contributing to post-operative immunosuppression (Page *et al.,* 2001). Comparison of analgesia regimens demonstrated significantly improved immune function in the patient controlled analgesia group not containing morphine (Beilin *et al.,* 2003a). Preemptive analgesia was found to reduce post-operative pain, and cytokine production, and preserve cellular immunity (Beilin *et al.,* 2003b). Compared to morphine, tramadol pre and post operatively, fentanyls PCA and ropivacaine wound infiltration-based analgesia have all been demonstrated to preserve immune cell function and cell numbers better (Kim *et al.,* 2016).

The magnitude of neuropathic pain is directly proportional to the numbers of pro-inflammatory cytokine present at the site of neuronal injury. The strongest evidence is for TNF-α, but there is also evidence for IL-1, IL-6 and IL-17, and the anti-inflammatory cytokines IL-4, 10 and TGF-β (Hung *et al.,* 2017). Pro and anti inflammatory cytokines appear to have a significant role in neuropathic pain, although in small studies specific cytokines have been identified as treatment targets, the results of larger studies are mixed due to patients heterogeneity (Hung *et al.,* 2017). Exaggerated pain responses occur if healthy neurons are exposed to cytokines or gut contents, bacteria, fungi, or viruses (Maves *et al.,* 1993). El-Aleem and colleagues (2005) have studied both acute and chronic pain models finding anti-nociceptive and anti-inflammatory neuropeptides are regulated by the inflammatory process. Chronic pain and postoperative neuropathy is also being investigated (Cui *et al.,* 2000).

Injury increases levels of IL-6 and it's receptor and its trans-membrane signal transducer in peripheral nerves, dorsal root ganglia and the spinal cord (De Jongh *et al.,* 2003). IL-6 and receptor expression promote neuronal survival, enhancing the quality of neuronal repair (Their *et al.,* 1999 and Tancredi *et al.,* 2000). β-endorphin and enkephalin, release being enhanced by IL-6, and IL-6 administration having an analgesic effect reversed by naloxone (Bianchi *et al.,* 1999).

Inhibition of cytokine synthesis with pentoxifylline a phosphodiesterase inhibitor increases the nociceptive threshold. Pre-emptive administration for elective cholecystectomy decreased plasma IL-6 levels and reduced opioid requirement

(Wordliczek *et al.,* 2000). IL-1β acts peripherally on the primary afferent neurons to synthesize and release substance P, which contributes to neurogenic inflammation (Inoue *et al.,* 1999). Samad and colleagues (2001) note elevated IL-1β in the central nervous system (CNS) stimulating production of COX-2 increasing PGE_s production, and pain sensitivity.

Ren and Dubner, (2010), report a bi-directional interaction between the immune system and the nervous system. Injury to the Schwann cells triggering release of pro-inflammatory cytokines, and macrophage migration. Macrophages recruit PMNL's, releasing inflammatory cytokines triggering the nerve, with repetitive stimulation perceived as chronic pain. Inhibition of the immune system via the release of opioids, mainly β-endorphin, onto the nerve terminals improves chronic pain (Hua and Cabot, 2010). IL-6 in particular relaying peripheral immune signals to the CNS, inducing COX-2 and PGE2 release in vascular endothelial cells of the brain (Ren and Dubner, 2010). At present it is unclear, if the immune system initiates or maintains neuropathic pain in patients (Calvo *et al.,* 2012).

4.4 Surgery and pain

Patient studies report a wide range of pain following laparoscopic cholecystectomy, with 10% reporting no pain (Squirrell *et al.,* 1998). It is unclear if cholecystectomy in the presence of acute cholecystitis increases post-operative pain, with little published literature available.

Pain is most severe in the first 2-3 hours following surgery, and for the first twenty-four hours. Predictability allowing pre-emptive analgesia administration to reduce CNS hyper-excitability (Michaloliakou *et al.,* 1996, Wall, 1998). Ure and colleagues (1994) found pre-operative factors allowed relatively accurate post-operative pain levels. Despite staff and patient education, and advances in management, the incidence post-operative pain remains unaltered (Huang *et al.,* 2001). Studies comparing approaches to cholecystectomy demonstrate beyond the initial 1 - 2 weeks the advantage, in terms of pain, of the laparoscopic approach is lost, with equivalent pain scores at one month (Ros *et al.,* 2001).

Joris and colleagues (1995) demonstrated greater variability in pain following laparoscopic surgery. Pain is visceral after laparoscopic surgery, and parietal following the open approach. Following laparoscopic cholecystectomy pain characteristically originates in the right upper quadrant and around the port wounds. Pain diminishes after 24 hours and parietal pain being relatively minimal. Golder and Rhodes (1998) failed to demonstrate a significant benefit from reducing port size and an increase in difficult procedures secondary to localised inflammation. Cheah's team (2001) did demonstrate a benefit, but applied strict pre-operative selection.

A randomized prospective trial, by Singla's team (2014), demonstrated reducing intra-abdominal pressure to 7-8 mm Hg, and maintaining it at this reduced the frequency and intensity of post-operative pain. Similar results were found by Yasir's team (2012). Surwam and Yuwono, (2016), measured pain after open cholecystectomy with / without methylprednisolone administration pre-operatively. Methylprednisolone decreased IL-6, but not PGE2 post-operatively, pain decreased, but secondary to the concurrent reduction PGE2 with NSAID's. Table 4.4.1 lists potential sources of pain after laparoscopic surgery.

Potential sources of pain after laparoscopic surgery

Potential sources of pain after laparoscopic surgery	
Abdominal wall distension	Diaphragmatic, or sub-diaphragmatic fibres or stretch receptors
Loss of visceral surface tension	Weight on the diaphragmatic attachments of the liver
Length of the pneumoperitoneum	Volume of residual gas
Abdominal wall lift versus carbon dioxide pneumoperitoneum has demonstrated reduced shoulder tip pain when carbon dioxide is not used (Koivusalo *et al.,* 1996)	Type of gas generating the pneumoperitoneum
Temperature of gas, warming possibly reducing pain (Farley *et al.,* 2004)	Maximal pressure of the gas, maximal pain for 24 hours, but lasting a week, Wallace and team (1997)
Rate of insufflation important in relation to shoulder tip pain Berberoglu *et al.,* (1998)	Higher the insufflation pressure the higher the post-operative pain score Wallace *et al.,* (1997)
Splanchnic mucosal ischaemia caused by localized peritoneal acidosis	Transversus abdominis nerve block has also been demonstrated to reduce post-operative pain (Ra *et al.,* 2010)
Phrenic nerve neuropraxia is implicated, but the brevity of the pain suggests nerve is uninjured, but Matsui and colleagues, (1994) demonstrated a phrenic nerve block after anaesthetic induction significantly reduced shoulder tip pain	Lavage decreases postoperative pain possibly by displacing the sub-phrenic carbon dioxide or diluting local acid (Ure *et al.,* 1994), despite saline itself being acidic (Wills and Hunt, 2000)

Table 4.4.1: Potential sources of pain after laparoscopic surgery, (Wills and Hunt, 2000).

Shoulder-tip pain, increases in intensity from day two onwards, being more minor than the visceral pain, it is often ignored by patients. Quoted incidence being 30 -40% of cases (Cason *et al.,* 1996, Wills and Hunt 2000). Other common causes of postoperative pain include retained stones, usually asymptomatic, becoming nidus of inflammation or initiating other pathology, including fistula, abscess, or sinus tract formation (Binagi *et al.,* 2015, Jolobe, 2017). They present as non-specific right upper quadrant pain following cholecystectomy (Ramamurthy *et al.,* 2013), or ongoing inflammation and sepsis (Nayak *et al.,* 2013).

4.5 Measurement of pain

Pain assessment is a routine post-operative observation. Assessment requires an ability to communicate a pain description, and has lead to adoption of research tools into general clinical practice. Despite this Heikkila team found pain assessment was not documented in 35% of patients on day one and 46% on the second post-operative day (2016). Melzack and Casey, (1968) describe three distinct, measurable, dimensions listed in Table 4.5.1. Traditional models assess four parts Table 4.5.2. Pain measures are inherently subjective relying on self-reporting, Table 4.5.3 list the criteria for pain measures.

Measurable dimensions of the pain experience

Distinct measurable dimensions of the pain experience	
Sensory-discriminative	Sensory aspect of pain which defines the intensity, location and temporal aspects
Affective-motivational	Emotional and aversive aspects of pain and suffering
Cognitive evaluative	Patients interpretation of the meaning and consequences of the pain and injury, this includes impact on quality of life and death itself

Table 4.5.1: From the work of Melzack and Casey, (1968), the three distinct measurable dimensions of the pain experience. Patients experience of pain is comprised of all three parts, the proportion of each is unique to the individual and the cause of pain.

Four elements of the traditional models of pain

Traditional models of pain consist of four parts	
Nocieception	Lack of a pain stimulus makes the association between nocioception and pain response difficult to assess
Sensation	Easier to measure but are subjective depending on how patients report them
Suffering	Easier to measure but are subjective depending on how patients report them
Behaviour	Easier to measure but are subjective depending on how patients report them

Table 4.5.2: Describes the more traditional model of pain, and the ease of assessment. Sensation, suffering and behaviour are subjective, and depend on how the patient reports their pain, behaves while in pain, or the clinical parameters thought to be characteristic of the patient in pain.

Criteria pain measures should fulfill

Criteria a pain measure should fulfill	
Improve accuracy	Easily understood by the target population
Optimise reliability	Offer validity and reliability

Table 4.5.3: Demonstrates the criteria a pain measure should fulfill. No pain measure at present fulfills all these criteria adequately, but they are used as ideals. The visual analogue score comes closer than many.

Pain measures help in assessment of pain over a period of time and the effectiveness of interventions (Max *et al.,* 1990 Ong and Seymour, 2004). Currently used pain measures are unclear as to the minimum clinically important change in response, either to an intervention or surgery. The VAS is an incomplete representation of the pain experience and there is growing interest in using quality of life measures in conjunction with the VAS (Eaton *et al.,* 2013).

The timing of administration is also debated and not standardised. Lynch *et al.,* (1997), recommends assessment during movement or coughing and at rest, visceral pain is affected by coughing, but not mobilisation, whereas parietal pain is affected by coughing and mobilisation (Joris *et al.,* 1995). Movement depends upon the procedure and the mode of analgesia.

4.6 Specific measures of pain

Verbal Rating Score / Verbal category scale (VRS)

In the VRS, pain is described by a list of words graded in intensity, the patient choosing which word best matches their current pain, and the investigator scores their descriptors. The benefits and criticisms of the VRS are listed in Table 4.6.1.

Advantages and disadvantages with the Verbal rating score

Advantages of the VRS	
Good accuracy	Widely used and verified (Lara-Munoz *et al.,* 2004)
Good at assessing intensity	Good at demonstrating change following intervention
Disadvantages of the VRS	
There is a difference in the weighting between levels	Scoring system assume equal weighting between certain categories
Patients perceiving their pain as not falling into any category are forced to choose one that doesn't reflect their pain.	Data is ordinal data and should be analysed by non-parametric statistics but is not in all studies
The score also relies on a relatively good understanding of English, and the nuances of the language	Limited use in non-English speaking population
Limited use in the young	Limited use where there is a barrier to reading for example immediately post-operatively
Language barrier is a major limiter to use in practice	

Table 4.6.1: Benefits and criticisms of the verbal rating score, which is widely used in practice and in research.

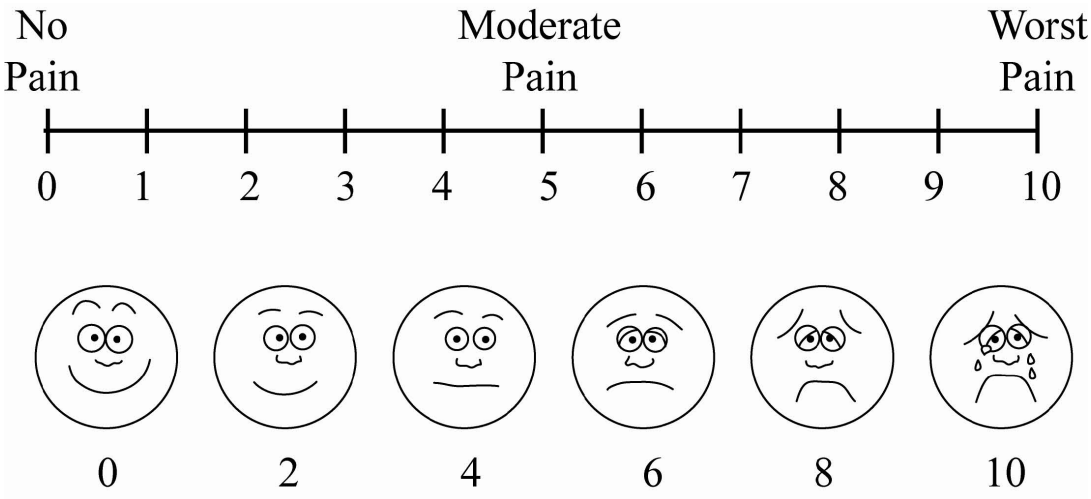
Numerical Rating Scores e.g. Visual Analogue Scale (VAS)

Numerical rating scores demonstrate good correlation to other pain measures and sensitivity to intervention (Chapman *et al.,* 1992). Clinically significant reduction in pain is seen as reduction as 10mm on the score (Myles *et al.,* 2017). Figure 4.6.2 demonstrates the VAS, and Table 4.7.3 the positives and negatives around the score. For post-operative use the simplest and most reproducible version of VAS was a 10mm line with annotation (B in Figure 4.6.2) (Kjeldsen and Klausen, 2016).

Melzack and Katz (1994) advocate the assessment of pain pre and post intervention. Accuracy can be improved by serial VAS measures and constructing a curve to give an integrated measure of the area under the curve of pain intensity (AUC) and pain relief (Matthews *et al.,* 1990).

Different representations of the VAS used in studies

A. Annotated Visual Analog Scale



B. Visual Analog Scale

Figure 4.6.2: Demonstrates different forms of Visual Analogue Scale (VAS). In (A) the scale is annotated to help patients where language is a problem. In (B) this variant is much simpler. In both examples the line is 10 centimeters long and the patient asked to mark the level they feel their pain is. In (A) the patient can see how the line is divided up, in (B) this is not as obvious. In this study we trialed all the version, (B) was chosen, because in the pilot it was found to be less confusing to post-operative patients, and it reduced numerical preference, and patients marking the whole numbers only. Numerical scales of 0-10 or 100 have also been used, these types of scale are called graphic-rating scales.

Advantages and disadvantages of the Visual analogue scale

Advantages of the VAS	
Simple to use	Independent of language
Easily understood by patients	Good correlation to other measures of pain
Quick to complete it is easy to repeat	Good compliance
Children as young as seven can reliably use it (Chambers and McGrath, 1998)	Good sensitivity to pharmacological and non-pharmacological interventions due to the number of response categories available to patients (Seymour 1982)
Disadvantages of the VAS	
Using the VAS with an attached rating scale, demonstrated clustering of responses around the descriptions with a consequent loss of sensitivity (Scott and Huskisson, 1976)	Using multiple choices on the scale rather than being more sensitive, the multiple scales have left patient's confused, and therefore there is a consequent loss of sensitivity (Jensen *et al.,* 1986).
Numerical scales have demonstrated digit preference	In the early post-operative period visual and motor coordination can be compromised and patients require additional instructions to complete the VAS (DeLoach *et al.,* 1998)

Table 4.6.3: Demonstrating the advantages and disadvantages of the VAS in both research and clinical practice.

Alternative measures

Due to variations in the severity of pain between studies, researchers have used alternatives, such as return to normal activities, or employment as an outcome measure. Variation being found between the self-employed and the employed, and discriminates against those not working (Gupta *et al.,* 2002).

Postoperative pain

Literature review established following cholecystectomy most authors use either the VAS or the Verbal rating scale (VRS). VAS and VRS scales demonstrate a high level of correlation (Jensen *et al.,* 1989). VAS is considered more sensitive in detecting small differences in pain levels, and changes after pharmacological intervention (Seymour, 1982). VRS has good reliability in assessing changes with analgesia intervention; finer changes in grade of pain are lost (Ong and Seymour, 2004).

4.7 Health related quality of life (HRQOL)

HRQOL encompasses physical, social, and emotional attitudes of the patient towards their present and previous health state. Policy makers use measures for pharmacoeconomic decisions, particularly in guiding clinical decision making. For patient's, quality of life as an outcome, is far more important than laboratory measures or clinical end points. Measures are frequently self-completed, aiming to assess physical and psychosocial attitudes and function. There are three groups of HRQOL measures, given in table 4.7.1.

The three main groups of Health related quality of life measures

	Different types of HRQOL measures
Global assessments	Provide basic information, measuring a single attribute on a visual analogue or a graded scale. For example the VAS measuring pain (Chapman *et al.,* 1992)
Generic questionnaires	Test more complex hypothesis clustering sub-scores into areas such as physical, emotional function, somatic sensation, and mental health. Generic scores can demonstrate unexpected relationships and are used to predict outcomes and to compare the study population with populations with other disease and/or the general population (Irvine, 1999). They may also contain sections that are not relevant to a particular disease, and omit important factors pertinent to the disease. For example the SF-36 (Ware and Sherbourne, 1992), Nottingham Health Profile (Hunt *et al.,* 1980)
Disease specific	Measures are used to chart the progress of a disease and measure the effect of treatment interventions. For example Gastrointestinal Quality of Life Index (Eypasch *et al.,* 1995), Diabetes Quality of Life measure (Burroughs *et al.,* 2004), Minnesota Living with Heart Failure (Bilbao *et al.,* 2016)

Table 4.7.1: Demonstrates the three main types of HRQOL scores used and their place in the assessment process. It is advocated that studies should include generic and disease specific measures to optimise the HRQOL information gained, at each stage of a disease and to avoid missing the unexpected (Guyatt *et al.,* 1993). All measures must be validated undergoing psychometric testing of validity, reliability and responsiveness (Guyatt *et al.,* 1993).

HRQOL and Surgery

HRQOL measures should assess all outcomes of surgery, including patient satisfaction, wellbeing, quality of life and functional outcome. Outcome of a procedure is important for, patient, clinician and funding organisation. It is vital advancements in surgery are validated and assessed fully before, and after gaining widespread adoption. This requires appropriate tools for assessment. It is perhaps surprising no standardised, validated quality of life instrument, exists for cholecystectomy (Carraro *et al.,* 2011). Many questionnaires trialed have had problems with reproducibility, restricted range of measures and language (Carraro *et al.,* 2011).

The European Association for Endoscopic surgery (Korolija *et al.,* 2004) performed a meta- analysis of published data to evaluate quality of life after laparoscopic surgery. Aiming to assess where the laparoscopic approach was beneficial and the optimal measures for future assessment. They found in the early period quality of life was improved by laparoscopic surgery, in the long term there was only minor benefit or equivalence between approaches (Nilsson *et al.,* 2004). Advocating the use of the SF-36 as the generic questionnaire and GIQLI (Appendix 1**)** for the disease specific instrument, suggest quality of life should be assessed at 1 and 6 months following surgery (Korolija *et al.,* 2004).

HRQOL markedly reduces while waiting for elective cholecystectomy (Somasekar *et al.,* 2002). The financial cost due to emergency admissions being sufficient to cover the cost of early surgery; particularly taking into account delayed cholecystectomy patients had technically more difficult procedures, and higher conversion rate. Identifying patients at risk of early readmission is difficult, but is more frequent after acute cholecystitis than biliary colic.

Vetrhus and colleagues (2004) randomised patients to cholecystectomy or observation. Observation group patients whose symptoms settled had no detectable difference in quality of life score, but had more recurrent episodes than the surgical group. The surgical group overall having higher quality of life scores, but patients with higher intensity and frequency of pain at randomisation, had further episodes of pain regardless of the group randomised to. Ozden and Dibaise, (2003) found no statistically significant difference between surgery and observation for patients with acalculous biliary pain.

Quintana and colleagues (2005) drew up criteria for appropriateness of cholecystectomy using the RAND appropriateness methodology Patients completed the Short-Form-36 (SF-36) and the Gastro-intestinal quality of life index (GIQLI)-before and 3 months after surgery. Their findings are demonstrated in Table 4.7.2.

Quintana *et al.,* (2003, 2005) Scale for the appropriateness of cholecystectomy

Patient group	Benefit
No symptoms of cholelithiasis	Less improvement in bodily pain, vitality, social function and physical impairment
Pain related to gallstones who were low surgical risk	Greatest improvements in, bodily pain, vitality, social function and physical impairment
Pain related to gallstones who were high surgical risk	Less improvement with a poor risk benefit ratio

Table 4.7.2: Quintana and colleagues drew up a scale for the appropriateness of cholecystectomy incorporating the assessment of HRQOL (Quintana *et al.,* 2003, 2005). Advocating identification of patients who are high-risk surgical candidates, or whose pain potentially had an alternative cause and carefully counseling them prior to making an informed decision about the modality of treatment.

4.8 Specific measures of quality of life

Gastrointestinal quality of life index (GIQLI)

Eypasch and colleagues (1995) developed the GIQLI with the aim to improve the quality of the data on patients' reported symptoms (Eypasch *et al.,* 1995). The structure of the measure is given in Table 4.8.1. The questionnaire in Appendix A.

Gastrointestinal Quality of Life index

Gastrointestinal quality of life index (GIQLI)	
Summary	Core section Additional modules can be added for specific diseases
Core section scoring	Five domains - 36 items graded responses scored 0 to 4 - Overall score 0 to 176 - Higher scores signifying better HRQOL
Domains	GI symptoms Emotions Physical Social function Medical treatment
Validity and reliability	Demonstrated to be high across a range of disease, with good discriminatory powers between severity of disease, distinguishing between mobile patients in the community, housebound and bed ridden patients
Responsiveness	High, for example mean scores (SD) following cholecystectomy showed an improvement from 87.3 (17.25) to 104.5 (17.52) two weeks following surgery (Eypasch and Williams, 1995)
Time to complete	10 – 15 minutes, most patients able to do with little or no help
Versions	Originally produced in German it has now been translated into English, French and Spanish (Slim *et al.,* 1999)

Table 4.8.1: The structure of the GIQLI (Eypasch *et al.,* 1995).

SF-36

Originally based upon concepts identified in the Medical Outcomes Study (MOS) (Ware and Sherbourne, 1992). The SF-36 is a generic questionnaire whose structure is demonstrated in Table 4.8.2, and Table 4.8.3 describes the summary scores. The questionnaire is given in Appendix A. In cholecystectomy patients no difference has been found in quality of life scores, with the SF-36 or other measures, between the open and laparoscopic approach after the first month. Carraro *et al.,* (2011), finding the quality of life was more closely related to factors around pre-surgery quality of life, than related to factors around surgery. Post surgical quality of life was also related to the accuracy of the pre-operative diagnosis, but this was secondary to pre-surgery quality of life (Carraro *et al.,* 2011).

The SF-36

SF-36	
Measure	Eight areas and a summary score of physical and mental health
Scoring	36 questions - 5 levels of responses - identical in ordering and layout - each item being included in only one of eight domains, which in turn become the two summary measures the physical and mental summary score
Domains	Vitality Physical functioning Bodily pain General health perceptions Physical role functioning Role emotional functioning Social role functioning Mental health
Validity and reliability	Reliability estimates for physical and mental summary scores usually exceeds 0.90 (Ware *et al.,* 1994). The SF-36 in studies of physical and mental health demonstrate an 80-90% empirical validity (McHorney *et al.,* 1993)
Responsiveness	For cholecystectomy patients it has been demonstrated to have limitations in discriminating between approaches to surgery (Carraro *et al.,* 2011).
Time to complete	10 – 15 minutes, most patients able to do with little or no help. Can be reliably completed by persons aged 14 years and upwards, being either self-administered, computerised-administration, or by a trained administrator.
Versions	Refined to be used worldwide and has been widely translated, approximately 50 languages, and takes into consideration cultural factors (Ware *et al.,* 1994)

Table 4.8.2: The structure of the SF-36.

The SF-36 component summary scores

	Predominant contributing domain and closest correlating domains to the component summary scores
Physical Component Summary Score (PCS)	- Physical role functioning - Role-physical - Bodily pain
Mental Component Summary (MCS)	- Mental Health - Role-emotional functioning
Contribution to both PCS and MCS.	- Vitality - General Health perceptions - Social role functioning

Table 4.8.3: Demonstrates how the eight individual domains contribute to the summary scores. Certain individual domains have a greater weighting to one or other summary score, others contribute more equally to both summary scores. Unsurprisingly the physical measures such as Physical functioning, Physical role functioning and Bodily pain, tend to better assess physical disorders. Mental health, Role-emotional, and Social functioning optimally assess mental health. There is only very weak correlation between mental health and the physical health measures. Skewing of scoring distributions occur in scales that have 20 or more levels, these include Physical functioning, General health, Vitality and Mental health (Ware, 2000).

GIQLI is not specific for gallbladder disease (Carraro *et al.,* 2011). Ibrahim's team (2016), and Quintana's team (2003, 2005), reviewed the literature for references to quality of life studies following cholecystectomy finding 38% of studies have used SF-36, and a further 38% the GOQLI. Only 21% used two measures, the commonest pairing being SF-36 and GIQLI. Concluding standardisation of instrument would permit easier comparison, suggesting GIQLI, in combination with either SF-36 or the EQ-5D5L, but the SF-36 has been more widely used to perform research with cholecystectomy patients.

Both the SF-36 and the GIQLI have also been the most widely used to compare approaches to cholecystectomy, and biliary disease in well recognised studies (Quintana *et al.,* 2003 – SF-36, Cararo *et al.,* 2011, Mentes *et al.,* 2001, Lien *et al.,* 2010 both questionnaires). Some studies have used EQ-5D5L (Wanjura and Sandblom, 2016) and other questionnaires, but there is more limited data for these questionnaires. The SF-36 and the GIQLI have been used together after cholecystectomy to determine the 'minimal clinically important difference' (MCID) to patients, or the minimal change that is of clinical relevance has been determined (Shi *et al.,* 2008, 2009). The wider use in the literature of the SF-36 and GIQLI was a significant determinant in using them in this study, to be able to compare this study's findings too.

Chapter 5 – Aims and Scope

Background

Gallstone disease is a significant western health problem. Nine million people in the UK population have gallstones and over 60 000 cholecystectomies are performed each year in the U.K. (Royal College of Surgeons, 2016). Fifty percent of biliary colic patients have further episodes of colic, and 1 to 2% suffers more serious complications (Wu *et al.,* 2015).

A pilot study was undertaken (**Chapter 6)** to test the anecdotal observation that patients undergoing laparoscopic cholecystectomy had a delay in presentation of sepsis, compared to open approach patients. Secondly to establish if pain was an early indicator of patients developing septic complications post procedure. The conclusion from this was that the laparoscopic approach patients did have a delayed presentation and pain was an early indicator of sepsis. The pilot identified a group of patients who experienced a lot of pain, at the level of the patients developing sepsis, but never developed sepsis. This group of patients rated their quality of life poorer than the main group of patients.

Changes in peritoneal cytokine concentration have been demonstrated to be early indicators of postoperative complications (Yamamoto's *et al.*, 2011 and Clini *et al.,* 2013). Peritoneal cytokine concentration changes preceding changes in systemic cytokines, and SIRS markers. The pilot study established that we could detect a change in cytokine concentration in systemic blood samples.

A literature review demonstrated IL-6 concentration correlated most closely with indicators of disease severity scores and overall mortality (Bacci *et al.,* 2015). From the literature TNF-α cytokine concentration was also important in early indication of complications and IL-1 had a role in mediating the pain response (Nicholson and Hall, 2011).

Various factors around laparoscopic surgery, including pressure, carbon dioxide and temperature, have been individually implicated, principally in animal models, to have a negative impact upon inflammatory cytokine concentration, and cell mediated immunity (Machado *et al.,* 2009, West *et al.,* 1997, Kuntz *et al.,* 2000 and Kawai *et al.,* 2014). In contrast Hanly *et al.,* demonstrated that the IL-10 concentration was unaffected, or increased, by the factors around laparoscopic surgery. Opiates particularly morphine, has been demonstrated to decrease neutrophil and macrophage recruitment and function, within the peritoneal cavity in the early stages of sepsis, and increase bacterial dissemination from the gut lumen. (Meng *et al.,* 2015, Liang *et al.,* 2016).

Cote *et al.,* (2015) and Concepción - Martin *et al.,* (2016), have demonstrated pain following ERCP as a good indicator of potential post procedural complications using it as an indicator for admission, but no one has examined this after cholecystectomy.

Aims

The aim of the main study was therefore to examine pain as early indicator of patients developing post procedural sepsis, allowing earlier initiation of treatment to reduce morbidity. Secondly the literature identified multiple factors around laparoscopic surgery that delayed the cytokine rise in patients undergoing laparoscopic surgery, but no one had examined multiple factors together. Thirdly to determine if we could use the quality of life (QoL) and Hospital anxiety and depression (HAD) scores to distinguish between the group developing sepsis and those who experienced a lot of pain but did not develop sepsis.

A secondary aim was to increase the rate of day case surgery by using the pain and quality of life scores to indicate who could be discharged home and was at low risk of sepsis, and postoperative pain problems.

Study design

The VAS identified an increase in pain score in those developing sepsis after surgery in the pilot study. But pain alone did not distinguish between those who had postoperative complications, from those who experienced significantly more pain only. This later group rated their quality of life poorer in the pilot. The SF-36 and the GIQLI, used together were able to identify this group. Ibrahim *et al.,* (2016) advocating the combined use of the SF-

36 and GIQLI, as they have been widely validated, and able detect minimal clinically important differences relevant to patients (Shi *et al.,* 2008, 2009).

Changes in the pain score pre-empted the rise in cytokine concentration and we wished to determine the factors that delayed the cytokine rise and identify a method for diagnosing sepsis earlier. I recorded the VAS, with the cytokines concentration, and routine clinical observations to identify those developing sepsis. The QoL questionnaires were completed to identify pre-operatively those who had significant problems with pain post operatively and determine the benefit they had from cholecystectomy. We included biliary emergency and ERCP patients, as I wanted to exclude factors around biliary disease affecting the cytokine or questionnaire response, as this had not previously been examined.

To decrease variation in clinical practice consecutive patients admitted under three consultants as either emergency admissions, or for cholecystectomy were approached to participate. For the ERCP arm one consultant performed all procedures. The ELISA plates included a standardisation curve on every plate. The study size, with measurements at multiple time-points, required multiple ELISA plates for each cytokine. Therefore I enroled 15 volunteer controls, their blood samples being plated on multiple plates permitting assessment of variation between plates to be assessed. They also give an indication of the size of a significant change in concentration.

Chapter 6 – Pilot study

6.1 The initiation of the study

The study proposal began as an observational discussion around patients developing complications after laparoscopic cholecystectomy appeared to have a delay in presentation of sepsis compared with patients with sepsis after open surgery.

This was an anecdotal observation, and I therefore undertook a notes review of two years of complications for three consultants, two performing laparoscopic surgery and one open surgeon. This demonstrated supporting evidence for the observation. The review highlighted pain at a level greater than expected, as a potential early indicator of postoperative complications.

As a surgical trainee I was aware of patients who had unexpected high levels of pain after surgery but did not develop postoperative sepsis. If pain was to be used an early indicator of complications, then this group of patients needed to be distinguished from the group of patients who potentially were developing postoperative complications.

The conclusions from the pilot would give the main study protocol.

6.2 Researching the pilot study

I realised I needed to understand the effect biliary disease had upon the cytokine concentration and pain measures. ERCP patients underwent manipulation of the biliary system but did not have surgical intervention; I recruited only elective ERCP's for benign disease in case malignant disease affected cytokine concentration or patient's perception of pain. I also recruited a group of biliary emergencies to understand the effect of gallstone disease on the measures recorded. Healthy volunteers gave a baseline cytokine concentration, and pain score, and gave feedback on the study, enabling changes to be made for the main study, as I was concerned patients' maybe inhibited from giving this information.

The pilot study also served as a feasibility assessment to performing the main study. For example in the biliary emergency and elective ERCP patients I could only assess systemic cytokine concentration, but it was unknown if the systemic cytokine concentration would change sufficiently. We also assessed the optimal pain assessment tool; the frequency of administration, and the acceptability to patients, particularly in the postoperative period was assessed. The clarity of written and verbal information was also assessed. In particular patients understanding this was a research trial, and not providing information on pain or sepsis to the clinical team.

If I collected the interventional data, this could potentially influence how investigations and prescribing was performed. Therefore the pilot was used to train independent observers. An anaesthetic nurse practitioner (ANP) and a first assistant nurse in theatre, and an endoscopy nurse for the ERCP's, and I observed all the cases, independently completing the pro forma. We assessed the ease of data gathering, completion of forms, standardised approaches to measuring each variable. We assessed diversity between assessments, and I invited their feedback. The ANP and first assistant were involved in camera holding for procedures, the endoscopy nurse did not scope patients. This potentially was a reason for good agreement in the surgical but not the endoscopy cases, particularly around the difficultness of the ERCP procedures.

I was proficient in the ELISA technique from previous research work, but used the pilot study to re-familiarise myself, and checked the kit was sensitive to demonstrate changes

in each of the cytokines. Finally I used the pilot to gather data to inform the power calculation for recruitment to the main study.

I presented the study plan to the surgical and anaesthetic department meeting to inform and receive feedback about feasibility. Agreement could not be reached on a standard analgesia and anaesthesia protocol, and clinicians did not want to place a drain in all surgical patients. No patient received prescribed premedication, and no adjunct analgesics were prescribed. Midazolam, morphine and buscopam were administered for the ERCP cases. For the open cholecystectomy patients antibiotics were given at induction to all patients, for the laparoscopic approach and the ERCP patients antibiotics were given at the discretion of the named consultant. Courses of antibiotics, after intervention and in the emergency patients were given at the senior doctors request.

Concluding from this only the patients of three surgical consultants to try and limit variation, as they shared a group of four anaesthetists and a junior team. The hospital had trainee surgeons and anaesthetists working under supervision of these consultants. Two higher surgical trainees performed cases, and two anaesthetic trainees.

6.3 Conducting the pilot study

For the pilot study I sought ethical approval from the hospital ethics committee and the city Research and Development Committee, this encompassed the ethical approval for the pilot and the main trial. The methods are as described for the main trial in Chapter 7; I highlight here the variation from the main study. Figure 6.3.1 demonstrates recruitment and Figure 6.3.2 the timeline for the pilot study. Two thirds of the way through the pilot I held a study group meeting. Following the meeting the suggested protocol refinements were implemented and feasibility assessed with the final group of patients recruited to the pilot. Table 6.3.3 details the changes made. This amended protocol became the main study protocol. The method for randomizing the pain questionnaires in given in Figure 6.3.4.

Recruitment for the pilot study

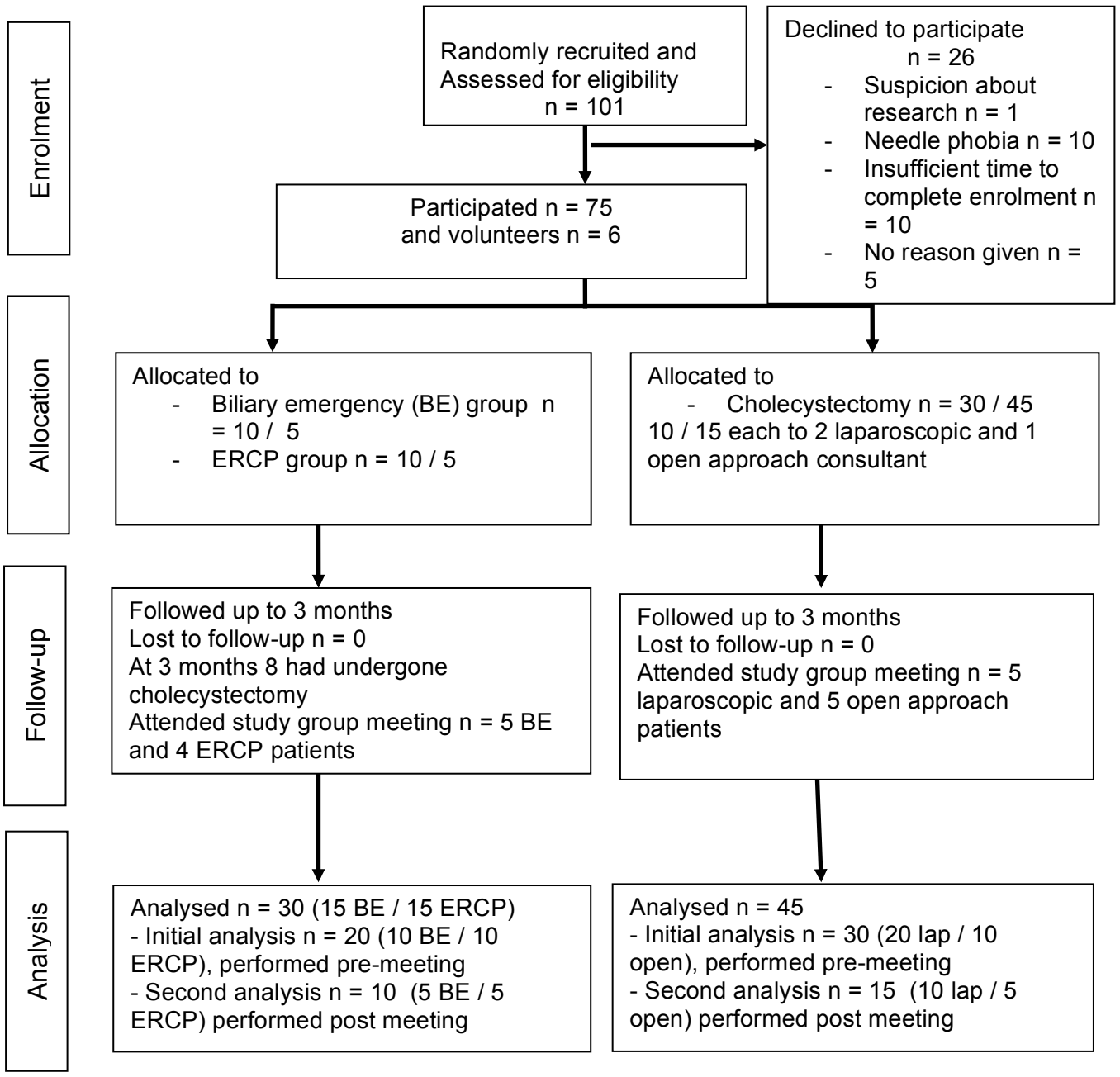


Figure 6.3.1: Demonstrates the recruitment to the pilot study. The first analysis was performed prior to the study group meeting; the second analysis being performed after the study group meeting, and was with adjusted timings of the samples (timings were as in the main study) and fewer blood tests. This second analysis demonstrated fewer patients declining to participate due to needle phobia, which had been one issue discussed at the study group meeting. Suspicion about research was patient believing the study was to cancel their surgery by measuring their pain.

Timeline for the pilot study

Time	Events
Enrolment	1. Patients recruited and informed about the study from SAU, Endoscopy, Surgical pre-assessment 2. Consented 3. VAS or VRS assessment of current, and least and worst pain expected. Current pain assessment repeated 4. Bloods taken for cytokines and WCC 5. Observations recorded from clinical notes 6. Quality of life and Hospital anxiety and depression forms completed 7. Clinical information about history of gallstone disease and analgesia recorded
Intervention	1. For the ERCP and the cholecystectomy arms the intervention was undertaken by the clinical team 2. Procedural data gathered by the independent observers
1 hours after enrolment / 1 hours after intervention	1. VAS or VRS assessment of current pain, and repeated after bloods 2. Blood, and if present drain fluid, samples taken for cytokines and WCC 3. Observations recorded from clinical notes 4. Data on analgesia and antibiotic requirements collected
3 hours after enrolment / intervention	1. VAS or VRS assessment of current pain, and repeated 2. Observations recorded from clinical notes 3. Data on analgesia requirements collected
5 hours after enrolment / intervention	1. VAS or VRS assessment of current pain, and repeated after bloods 2. Blood, and if present drain fluid, samples taken for cytokines 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected
7 hours after enrolment / intervention	1. VAS or VRS assessment of current pain, and repeated 2. Blood, and if present drain fluid, samples taken for cytokines 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected 5. Lead investigator asked nursing team to perform 11 and 17 hours VAS
11 hours after enrolment / intervention	1. VAS assessment of current pain administered by nurses on duty after briefing by lead investigator at the 7 hour assessment
17 hours after enrolment / intervention	1. VAS assessment of current pain administered by nurses on duty after briefing by lead investigator at the 7 hour assessment
24 hours after enrolment / intervention	1. VAS or VRS assessment of current and least and worst pain experienced. Current pain VAS repeated 2. Blood, and if present drain fluid, samples taken for cytokines and WCC 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected 5. Data on any episode of sepsis & interventions 6. Plan for discharge
Every 24 hours (or 48 hours after 1 week) if patient not discharged	1. VAS assessment of current pain, and repeated 2. Blood, and if present drain fluid, samples taken for cytokines and WCC 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected 5. Data on any episode of sepsis & interventions 6. Plan for discharge
3 months after intervention	1. VAS of current pain 2. Data on analgesia requirements and length of time in pain after discharge 3. Data on any episodes of sepsis after discharge 4. Timing of return to work 5. Quality of life and Hospital anxiety and depression forms completed

Table 6.3.2: Demonstrating the timeline for the pilot study for patient intervention.

The changes made from the pilot study to the main study

Changes made from the pilot to the main study	
Pilot	**Main study**
Consent was taken on the day of surgery	Consent was taken at pre-assessment
Bloods were taken at enrolment, 1, 5, 7 and 24 hours	Bloods were taken at enrolment, 2 and 24 hours
20 mls of blood were taken at each time point	5 mls of blood were taken at each time point. Reduced as was could collect enough to plate each sample on multiple ELISA plates
Bloods and VAS measured every 12 hours if not discharged at 24 hours	Bloods and VAS measured every 24 hours if not discharged at 24 hours
Drain, where present, systemic cytokines measured and the drain samples treated as the blood cytokines in Figure 7.7.1 page 194	Change could be seen in the systemic and drain. Blood cytokines measured as comparison across all arms was possible
I trialed the VAS and the VRS in a randomised approach as detailed in Figure 6.3.5	Choose the VAS as the results were more reproducible, and with less digit preference
The nursing staff administered the 11 and 17 hours VAS scores, which were frequently not completed	Pain was assessed at enrolment, 2, 4, 6 and 24 hours
Various versions of the VAS were trialed	Settled on B in Figure 4.6.2 page 109
Lead investigator and theatre nurses / endoscopy nurse observed each investigation to trial data collection format	Only nurses observed to reduce the risk of bias of the lead investigator being present
Reduction in the number of ELISA plates the volunteers were plated on as could reproduce all samples	All volunteers plated on at least two plates
Trialed lead investigator being called when a patient developed sepsis but this was unsuccessful	Adhered to the standardised blood and cytokine collection times
QoL measured at enrolment, 4, and 12 weeks	QoL measured at enrolment, 12, 26 and 52 weeks
Three controls completed QoL questionnaires verbally then on paper and three vice versa	No difference in response and standardised telephone completion of forms in main study

Table 6.3.3: Details changes made from the pilot study to the main study as a result of feedback from the study group meeting and findings from the pilot study.

The method for trialing the collection of pain data on the VAS and the VRS

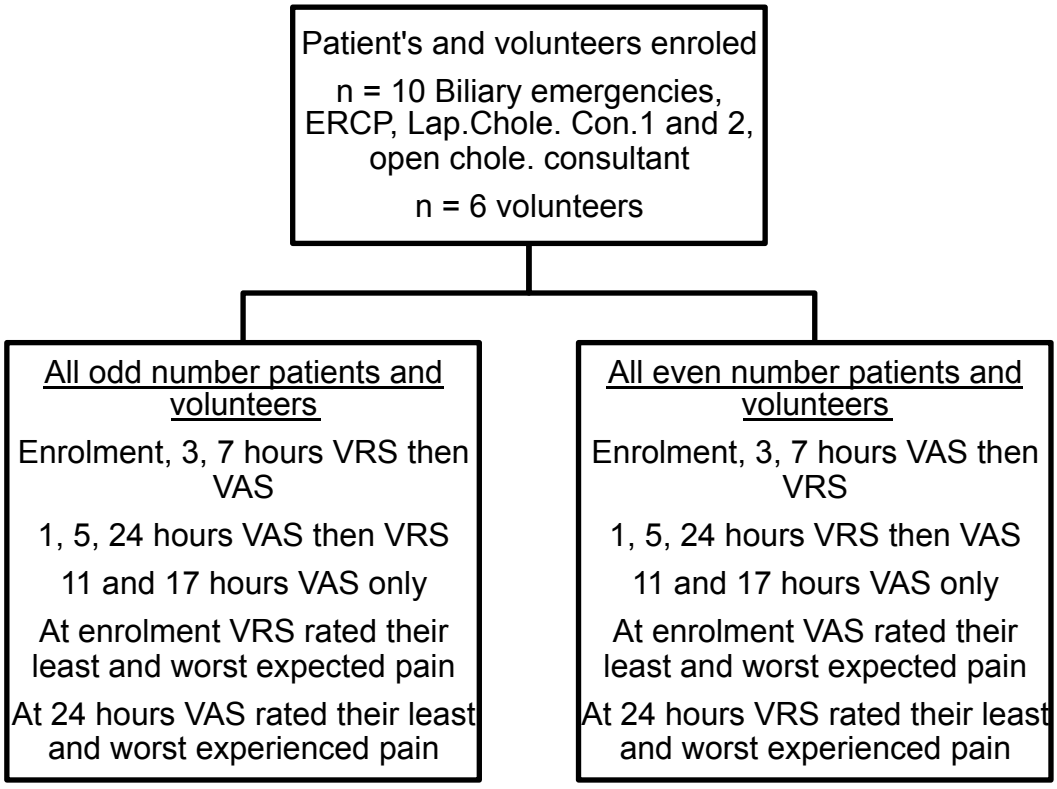


Figure 6.3.4: Demonstrates how the pain scores were administered for the first 10 patients enroled in the biliary emergency group, ERCP group, the two laparoscopic and the open approach consultant, and for the six, healthy volunteers, participating in the study. Participants were given a number at enrolment for their group, and patients were number consecutively and randomly recruited in each group. The pain scores were alternated to see if one was easier for patients, and for administration of the pain assessment in the study. Different styles of VAS were trialed to see if participants preferred one format. VAS – Visual analogue scale, VRS – Verbal rating scale, Con 1 / 2 – consultant one and two performing laparoscopic cholecystectomy (Lap. Chole.), the laparoscopic approach consultants were split, because the two consultants gave local anaesthetic by different routes at the end of the procedure. Open Chole. – Open cholecystectomy.

6.4 Outcome of the pilot study

Cytokines

The conclusions from the cytokine part of the study are demonstrated in Table 6.4.1, these Tables of conclusions were drawn up to be discussion points for the pilot study meeting. The details of the patients developing sepsis are given in Table 6.4.2, and the IL-6 concentration is plotted in Figure 6.4.3.

The conclusions from the cytokine part of the pilot study

Conclusions from the cytokine part of the pilot study
The systemic cytokine concentration did change in response to the intervention and we were able to distinguish those with sepsis from those who did not have sepsis
Although all cytokines demonstrated change, the significant change was seen in the IL-6 concentration, therefore the change of delta IL-6 was used to calculate an effect size, this was used to perform the power calculation for the sample size in the main study. Figure 6.4.3 demonstrates this change
My aim was to measure change in drain cytokine concentration, only four patients (2 laparoscopic and 2 open approach) had drains placed in the pilot. With systemic cytokines demonstrating a change, and the ability to compare all arms, we agreed to measure systemic cytokines
Three patients with drains developed sepsis their cytokine concentration demonstrated an increase from 7 hours in the drain fluid and 24 hours in the systemic blood in the open approach patient. In the 2 laparoscopic approach patients the drain cytokines increased from 18 hours and the systemic cytokines from 48 hours onwards. Drains were only placed in difficult cases in the pilot
IL-10 concentration systemically and in drain fluid did not rise significantly in any patients. But was highest in those developing sepsis and rose ahead of the other cytokines particularly in the laparoscopic approach patients
Five ERCP patients (50%) had a failed initial ERCP, requiring a second ERCP which was successfully completed the following day. Cytokine concentration rose none specifically after first ERCP decreasing only after the procedure was successfully completed, and therefore to continue following patients undergoing second ERCP
Two ERCP patients were admitted to HDU and demonstrated the greatest change in IL-6 concentration, in retrospect this potentially affected the main study power calculation
None of the biliary emergency group (7 biliary colic and 8 acute cholecystitis) underwent emergency ERCP and none of the ERCP group underwent emergency cholecystectomy. No pancreatitis or obstructive jaundice patients were admitted, therefore these were not planned for in the main study

Table 6.4.1: Demonstrates the conclusions from the cytokine part of the pilot study and the impact upon the main study.

The cause of sepsis in the patients in the pilot study

	Pain group on enrolment	Cause of sepsis	Co-morbidities	Level of care required
Laparoscopic patient 1	Severe	Chest infection	Asthmatic	Ward
Laparoscopic patient 2	Severe	Positive bile and blood cultures *E. coli*	Asthmatic	Ward
Open patient 1	Severe	Positive bile and blood cultures *E. coli*	-	Ward
ERCP 1st ERCP patient 1	Significant	Pancreatitis and chest infection	COPD	HDU
ERCP 2nd ERCP patient 2	Severe	Pancreatitis and chest infection	-	Ward
ERCP 2nd ERCP patient 3	Severe	Positive blood cultures for *E.coli*	Diabetic	HDU

Table 6.4.2: Demonstrates the cause of sepsis and the co-morbidities and level of care the patients required in the pilot study. Pain group is based on mild pain being < 4, significant being ≥ 4 - < 7 and severe being ≥ 7 on VAS or VRS. Patient 3 for ERCP had a sphincterotomy performed at their 2nd ERCP and were re-scoped to investigate bleeding after the procedure. All the ERCP's and cholecystectomy's' were rated as difficult. Laparoscopic or open patient – Laparoscopic or Open approach to surgery. ERCP 1st / 2nd / 3rd – ERCP completed at first attempt / second attempt / third attempt.

IL-6 concentration in participants in the pilot study.

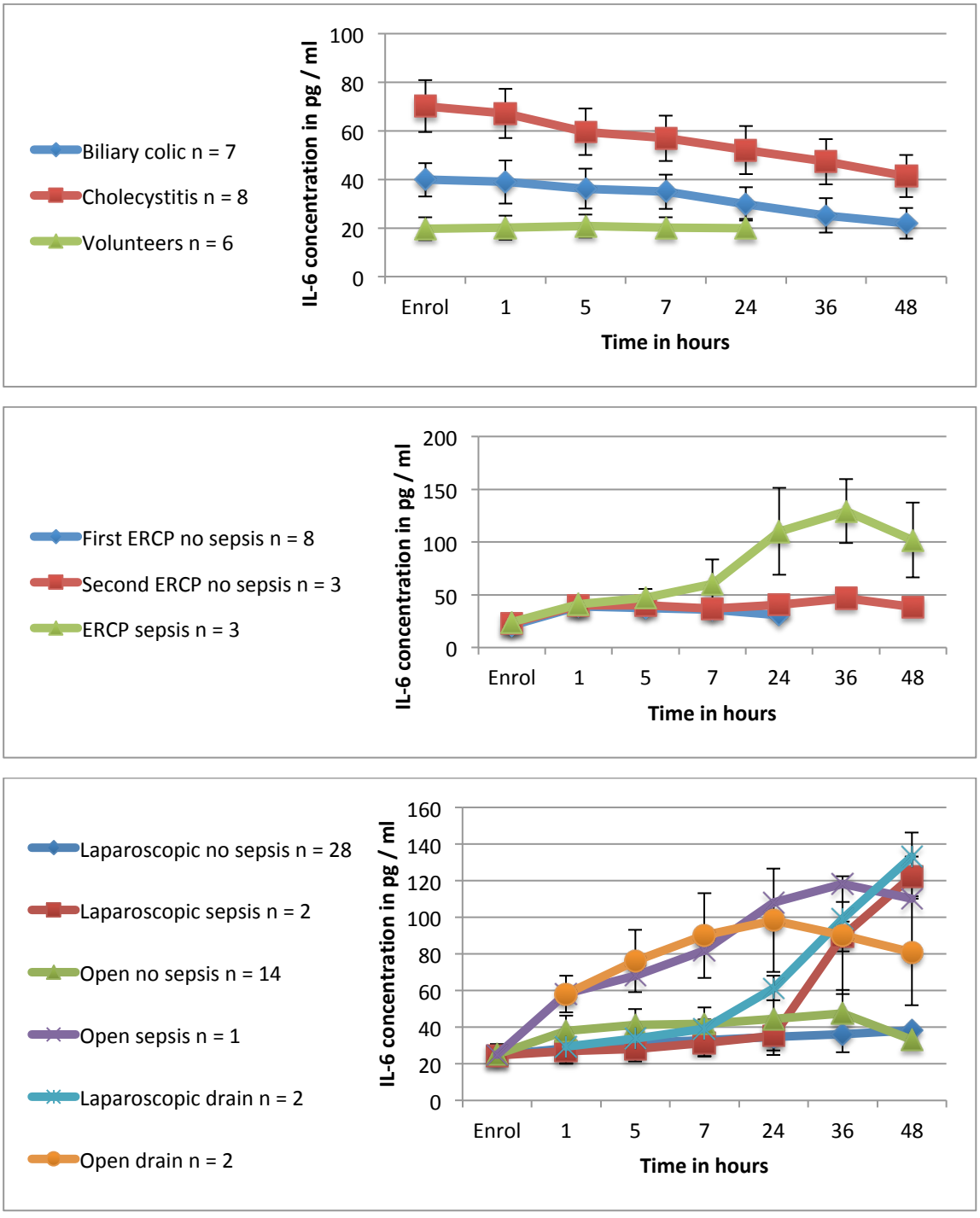


Figure 6.4.3: Demonstrates the systemic and the drain fluid (where available) IL-6 concentration in the biliary emergency, elective ERCP and cholecystectomy patients. Drain IL-6 concentration rising ahead of the systemic IL-6 concentration. The systemic cytokine concentration of the 14 open approach patients not developing sepsis is together but one of these patients had a drain and they are also included in the drain cytokine concentration.

Pain

As with the cytokine part of the pilot the conclusion from the pain part of the pilot are summarized in Table 6.4.4. Analgesia use was compared and converted to morphine equivalents, for ease of comparison. Table 6.4.5 demonstrates the conversion.

The conclusions from the pain assessment part of the pilot study

Conclusions from the pain part of the pilot study
The pain scores for the patients developing sepsis after procedures appeared to become significant prior to the cytokines, SIRS increasing significantly and the diagnosis of sepsis. Supporting the observation that pain was an early indicator of sepsis
Eight other patients pain scores were indistinguishable from those developing sepsis, but their cytokines did not change significantly and they were never diagnosed with sepsis
These patients scored their pain as 5 – 6 at enrolment, and 8 – 10 at the 5 hour assessment. Why did they not score their VAS within the severe pain category at enrolment, was it sample size?
These patients with significant pain following intervention but no sepsis had procedures rated as straightforward and not difficult and were as prevalent in the open cholecystectomy group as the open group, not opting for the perceived less painful procedure of laparoscopic surgery
NSAID's tended to be omitted and paracetamol and opiates prescribed, for comparison we converted all analgesia into morphine equivalents to aid comparison, Table 6.4.4, but recognised education events were required prior to undertaking the main study
Those developing sepsis and those who experienced more pain but did not develop sepsis, demonstrated a significantly greater use of opiate analgesia, and overall analgesia

Table 6.4.4: Demonstrates the conclusions from the pain part of the pilot study, and the questions that were raised and informed the main study protocol.

Morphine equivalent dose calculation

	Potency ratio with oral morphine	Equivalent dose to 10mg oral morphine
Codeine phosphate	0.1	100mg
Dihydrocodeine	0.1	100mg
Oral morphine	1	10mg
Tramadol	0.15	67mg
Intravenous morphine	3	3.3mg
Total Daily Morphine Equivalent Dose = (iv Morphine dose x 3) + (oral Codeine dose x 0.1) + (Tramadol dose x 0.15) + (oral morphine x 1)		

Table 6.4.5: Demonstrates the morphine equivalent dose for each of the opiates. Table from the Faculty of Pain Medicine (2019).

Quality of Life and Hospital Anxiety and Depression Scale

The principal questions from the QoL part of the study are highlighted in Table 6.4.6.

The conclusions from the quality of life part of the pilot study

Conclusions from the quality of life part of the pilot study
The level of anxiety was higher in the patients who completed their questionnaires on the day of the intervention, than those who completed it at pre-assessment (the surgical patients after the study group meeting).
The QoL scores were not significantly different pre-operatively for those developing and not developing sepsis, but they appeared clustered in the severe pain group and therefore slightly poorer
The QoL score was poorer in those developing sepsis at 4 weeks but had returned to the main group level at 12 weeks, but patients were not attending appointments at 4 weeks to complete the questionnaires and were therefore harder to complete
Patients who experienced significant amounts of pain scored their QoL lower at all time points at levels equivalent to those Quintana's group (2008), identified as not benefiting from cholecystectomy. This group also did not achieve the level Shi's group (2008, 2009), identified as the minimally clinically important difference in any of the domains. Their HAD score was also significantly higher and my supervisor and I were interested if the HAD questionnaire would provide shorter questionnaire but permit the same discrimination of groups
The patients experiencing a lot of pain but no sepsis scored particularly poorly in mental health and emotional domains, raising the possibility of using these domain scores to distinguish this group apart from the other patients. Potentially this could be performed pre-operatively, helping distinguish them from the patients developing sepsis post-operatively. But remembering patients with significant pain could develop post procedural sepsis
At 12 weeks the patients in the group experiencing a lot of pain during the study, still rated their pain higher & QoL lower, than the other patients, even those developing sepsis. This group had been back at work under a month at the 12 week time point. I wondered whether this had affected their quality of life scores and proposed measuring their scores further out to look for improvement. I proposed repeating the questionnaires at 6 months, Mr. Shehata proposed we also measure at 12 months to gain an understanding of whether this group gained benefits from undergoing surgery, being particularly interested in the SF-36 question "Compared to one year ago how would you rate your health in general now?"

Table 6.4.6: Demonstrates the principal conclusions from the quality of life part of the pilot study.

6.5 Conclusions from the review study group meeting

The review study group meeting was held at four to ten weeks after recruitment of two-thirds of the study group, to ascertain patients and health care providers feedback. Following the meeting changes could be made to the study design and the feasibility examined by recruiting the final third of pilot study group, and seek their views prior to recruiting for the main study, and I could optimise how I was going to gather the data. Figure 6.5.1 explains how the study group meeting was run. The people attending are given in Figure 6.5.2, and the problems and solutions encountered are given in Table 6.5.3. Tables 6.5.4 – 6.5.6 detail the main discussion points, Appendix 2 expands on the points.

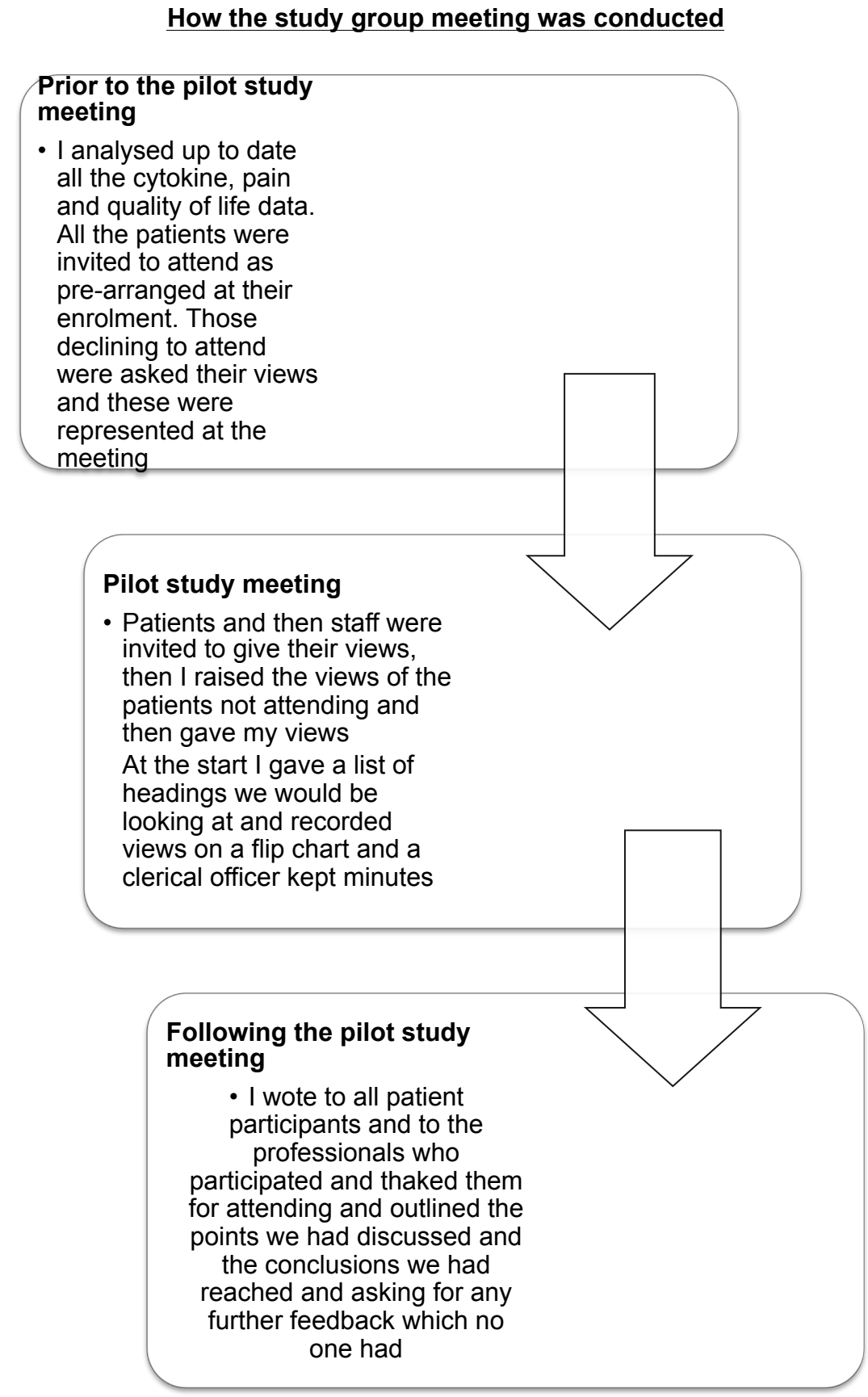


Figure 6.5.1: How the pilot study group meeting was conducted.

People attending the study group meeting

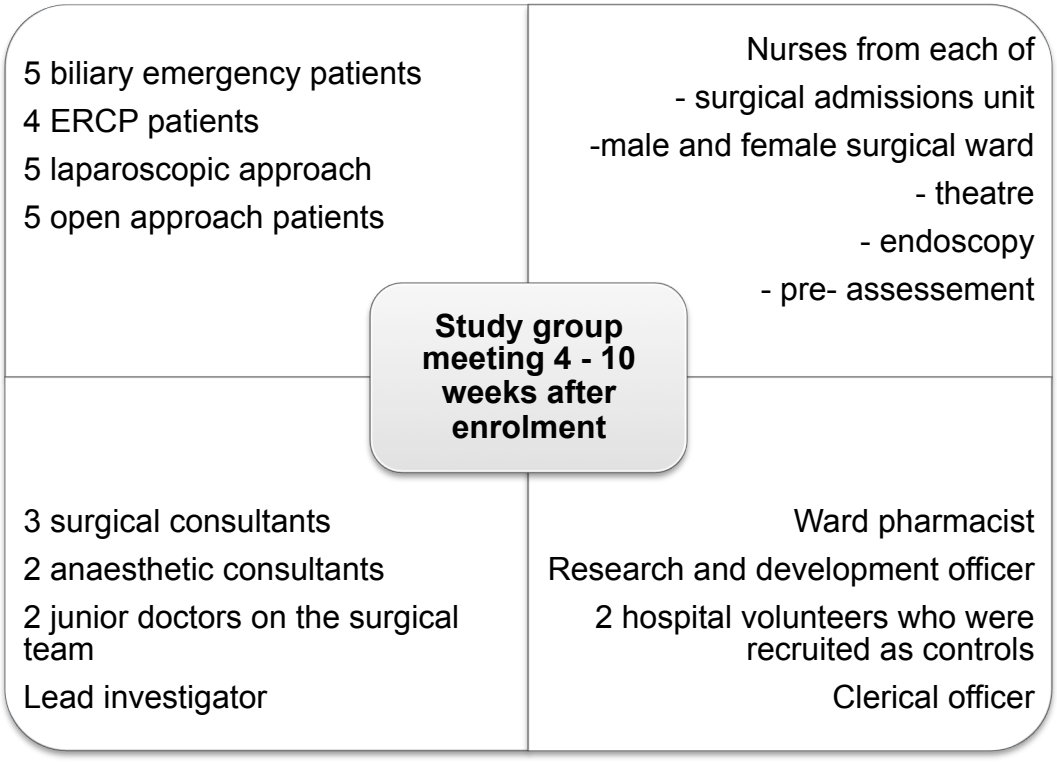


Figure 6.5.2: Demonstrates the people who attended the study group meeting. Two of the nurses and two of the doctors acted as controls; therefore all six volunteers were present. I invited all the patients, nurses and doctors involved. The clerical officer kept minutes for me giving me a full transcript of the meeting. The meeting was held after two thirds of the pilot patients had been recruited to allow changes to be made to the protocol and then the final patients to be recruited. This permitted changes to the protocol to be tested and their acceptability to the patients and team to be assessed, and influence the protocol of the main study. All the controls were recruited prior to the study group meeting to enable a wider range of opinion and feedback in case patients felt unable to raise issues.

Key problems and solutions conducting the pilot study meeting

Problems and solutions conducting the pilot study meeting
The meeting was held on an audit afternoon to optimise staff attendance, and parking / transport was paid for. For the patients, a nurse was also briefed prior to the meeting to be there if patients became distressed and patients were made aware of this. The meeting was held in the education centre and maps provided to the patients
A lot of discussion was generated from my prepared questions. Mindful of the consultants dominating the discussion I tried to avoid this, by bringing in information that patients not attending the meeting had given me in telephone discussion
The group experiencing more pain was over represented in those attending the meeting, and therefore potentially had more influence. I tried to counter this by seeking the views from the patients not attending the meeting and writing to everyone with the key points after the meeting
I broke the study into key steps in a timeline and sought opinions on each steps, this gave structure and it was recorded on a flip chart and by a clerical officer to give a meeting transcript for review after the meeting
We broke up for the patients to receive refreshments, while the staff discussed issues that were potentially sensitive for the patients, or not relevant such as the conducting the laboratory work or statistics
All the patients were invited back in to run through the conclusions of how the main study would be conducted, and everyone had the opportunity to raise further points

Table 6.5.3: The set up for the study group meeting to try and ensure that everybody views were taken into consideration, and the meeting could best inform the final study protocol.

Patients' concerns from the review study group meeting

Point	Decision
Patients' believed their reported pain was not addressed	**Decision** made about clearer written and repeated verbal information about the study group being separate from the clinical team at each clinical encounter
Too frequent blood tests during the study	**Decision** made to perform blood test at enrolment, one following the procedure and then every 24 hours. I was concerned we'd miss peaks in cytokine concentration but wanted a representative group to participate. Recruitment was an issue due to the blood test and this hoped to address this problem
Discussion with patients about increasing the information about analgesia and information in general about the reasons for performing the study	**Decision** made to educate staff with posters and presentations by myself and the pharmacist at ward and departmental meetings and staff induction. Information about the study included. Patients and staff were happy with the instructions provided for completing forms. We increased information to patients that participation was not going to exclude them from having surgery, as patients were concerned we were seeking to reduce the number of operations we were doing by performing the research. This was particularly prevalent amongst the patients who experienced a lot of pain
Confidentiality	**Decision** patients and staff were happy with the measures put in place to maintain the confidentiality of those participating

Table 6.5.4: Detailing the patients' principal concerns and the conclusions, which were reached, and adjustments made to the study protocol.

Procedural concerns from the review study group meeting

Point	Decision
Drain fluid cytokine concentration rose ahead of the systemic cytokine concentration	**Decision** made to measure systemic cytokines only as clinicians felt routine drain placement was inappropriate, and there was not a valid comparator in the ERCP or emergency group
To use the VAS or VRS for scoring pain	**Decision** made to use the VAS. Patient preference was to use the simpler VAS in part b of Figure 4.6.2 page 109 as the others confusing in the early period after anaesthesia. To also use this for least and worst pain
Timing and administration of the VAS	**Decision** made to complete VAS at pre-operatively and 2, 4, 6 and 24 hours, to optimise the completion of the VAS. Encourage the patients to be compliant with coughing prior to completing the VAS to measure visceral and parietal pain. To score the VAS twice to measure reproducibility
Poor adherence to the analgesia protocol	**Decision** made to educate staff with posters and presentations by myself and the pharmacist at ward, departmental meetings and staff induction. Information about the study included
Trainees performing the procedures	**Decision** made to perform the study in the second six months of the higher trainees attachment to the firm, which would also coincide with the junior doctors second six months of foundation year and therefore people should be more experienced and proficient
Standardisation the local anaesthesia approach and anaesthesia protocol	No decision could be reached **Decision** made to differ and to look if one route of local analgesia was optimal
Lack of space and time on the morning of surgery for all the people needing to review the patients	**Decision** made to complete consent, VAS, bloods and QoL at pre-assessment, but ERCP patients could be seen on the day of the procedure due to the list time
Taking cytokine concentration at the time of diagnosis of sepsis	This had not worked in the pilot study **Decision** made to record from the notes the time of diagnosis and SIRS and continue cytokine concentration measurements at the set times in the study
QoL forms	**Decision** patients found the QoL forms were repetitive; therefore we took the 4 week forms out and replaced them with measurements at enrolment, 12, 26 and 52 weeks. Surprisingly on discussion patients welcomed this as they felt we were following them up and checking they had recovered fully by longer term follow-up, this may have been the patients present at the meeting

Table 6.5.5: Details procedural concerns and decisions of protocol changes made.

Experimental concerns from the review study group meeting

Point	Decision
Financial concerns about number of ELISA kits due to number of blood and drain fluid measurements	**Decision** made to not sample drain fluid and reduce the number of blood tests, thereby reducing the number of kits, and not duplication of samples between plates, as this increased the accuracy of cytokine assessment
Variation between ELISA plates	**Decision** made to run the control patients samples on multiple plates to act as internal controls but this would require more blood to be taken from the controls
Taking one control sample or more and number of controls, gender and ethnic variation	**Decision** made to enrol 15 controls and commence one control in the study every hour from 8am to 10pm because of interest whether there was diurnal variation. We enroled 5 men and included 5 non-Caucasian controls to look for variation between groups. There was no diurnal variation, found therefore subsequent samples could have been standardised to the times of the main theatre list. But it allowed diurnal variation to be excluded from differences in the biliary emergency group.
Incomplete assessment forms	**Decision** the VAS and QoL forms were incomplete in particular in the surgical patients as they were called to theatre prior to completing them, by completing at pre-assessment we hoped to address this
Recruitment numbers	**Decision** using one surgeon would have reduced variation in surgical approach, but I was concerned about recruitment, therefore decision to use the patients of three consultants who shared a common junior team
Variation with independent observers	**Decision** Theatre observers achieved good reproducible observers, but the endoscopist struggled to rate the difficultness of procedure. Therefore we agreed to ask the opinion of the endoscopist after they had written the notes to rate the difficultness of the case, so it did not affect their judgments on their written conclusion if they knew the patient was participating in the study

Table 6.5.6: Detailing the concerns and the conclusions around the experimental work, and adjustments made to the study protocol.

6.6 Conclusions from the pilot study

In conclusion the pilot study demonstrated it was possible to measure a change in the systemic cytokine concentration and in the peritoneal cytokines in those developing sepsis following cholecystectomy or ERCP. The rise in peritoneal cytokines did pre-empt the rise in the systemic cytokines. But we had only been able to measure peritoneal cytokines in a small group of patients who had drains placed at the discretion of the operating surgeon. We included patients undergoing ERCP to examine the effect of instrumentation of the biliary tract without surgical intervention, and patients with biliary emergencies to increase understanding of biliary sepsis on the cytokine and pain response.

The post procedural VAS had confirmed the increase in pain score pre-empted the overt signs of sepsis, and the SIRS and cytokines It also increased prior to the rise in intra-peritoneal cytokines in the small number of patients it was measured in. There were a group of patients who experience significant amounts of pain postoperatively but did not develop sepsis. Their pain scores but not their cytokine concentration were similar to the group who developed postoperative sepsis. This group of patients with pain but not sepsis scored their quality of life poorly. This difference appeared to distinguish them from the other patients with sepsis, and the other patients not developing sepsis. Figure 6.6.1 demonstrates how the pilot study informed the main study.

How the pilot study and main study was designed

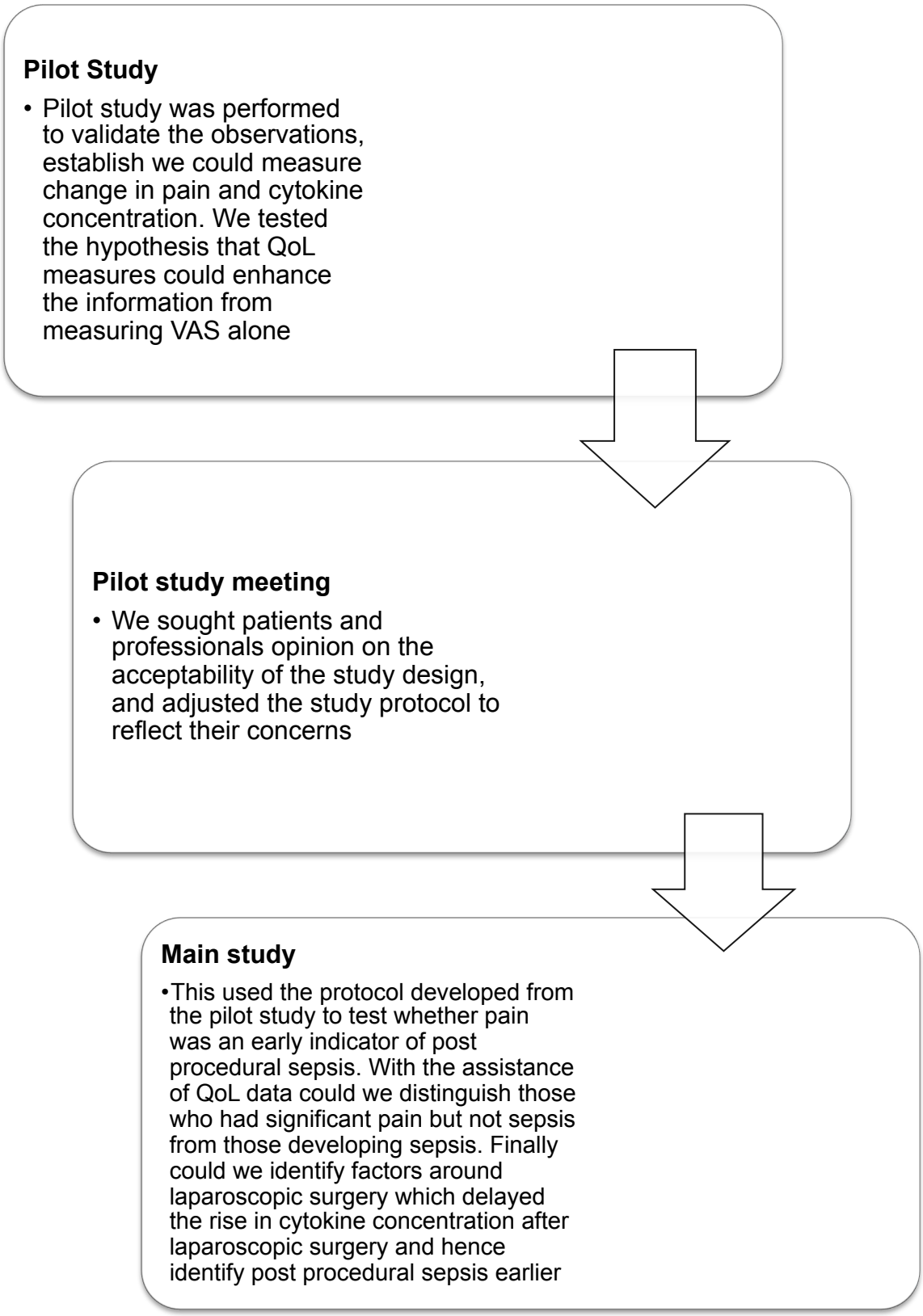


Figure 6.6.1: Demonstrates how the initial observation was tested and the protocol designed for the main study.

Chapter 7 – Material and Method

7.1 Recruitment

Table 7.1.1 details the patients recruited to each arm of the study. In each arm consecutive patients were approached to participate. The elective cholecystectomy patients were consecutively recruited from the pre-admission clinics at Nottingham City Hospital NHS Trust under the care of the same three consultants as in the biliary emergency arm. This was performed to try and minimise variation in management, as the same team was caring for the patients. Patients were recruited over a six month period.

The range of age and gender and ethnicity from the pilot study was used as a guide for recruiting a group of normal controls, which were recruited from medical staff, hospital volunteers and students. The control group did not have a history of biliary disease, and had not undergone cholecystectomy. Research and Ethical approval had been gained from the Trust's Research and Development Committee and Research and Ethics Committee (LREC reference number C1060303).

Groups' patients were recruited to, and the arm of the study the patients were in

Arm of study	Groups of patients were recruited
Biliary emergency arm	Consecutive patients attending surgical admissions unit for emergency admissions with gallstone related problems under the care of three surgical consultants
Planned ERCP arm	Consecutive patients attending endoscopy for planned ERCP for benign disease under one endoscopy consultant
Elective cholecystectomy arm	Consecutive patients attending for elective cholecystectomy only under the care of three surgical consultants
	Patients attending for elective cholecystectomy with an ERCP in the last year under the care of three surgical consultants
	Patients attending for elective cholecystectomy and requiring an on table cholangiogram (OTC) under the care of three surgical consultants
Control to all arms	Control group of volunteers

Table 7.1.1: Patients groups enroled in the study and the group of healthy controls also recruited to give a base line cytokine and VAS pain score.

7.2 Statistical analysis

The null hypothesis was that there was no difference between the patients admitted in each arm who developed sepsis and those who did not develop sepsis in terms of pain score. It was also proposed that there was no difference between the pain groups (as divided by the visual analogue score), in terms of cytokine concentration. The patients initial pain score and each cytokine concentration, for the two different approaches to cholecystectomy was subjected to an F-test to evaluate the equality of the population variance. This permitted evaluation of whether the two independent groups had been drawn from a normal population with the same variability, which was homogenous in nature. This was determined to be the case.

The data collected in the pilot study was used to perform the power calculation. The Nottingham University statistics department provided support in performing the power calculation. From the literature there was the most evidence for IL-6 being the most reliable cytokine for demonstrating a change in concentration in those develop sepsis after ERCP or cholecystectomy. Therefore the study was powered for a change in IL-6 concentration. The hospital morbidity and mortality data, and the individual consultant collected data, about their own morbidity and mortality for the previous twenty-four months was also used in the power calculation. An individual sample size was calculated for the biliary emergency, the ERCP and the cholecystectomy arms.

The power of the study was set as an 80% (or 0.8 $(1 - \beta)$) standard of detecting an effect, and the significance criterion used was 0.05 (α), with a two independent means, two-tailed T-test. From the pilot study the change in IL-6 varied between each arm, and the difference at 24 hours from enrolment was used to calculate the effect size for each group. The ERCP group had demonstrated a large effect size, calculated as 1.13, the biliary emergency group as 0.8 and the cholecystectomy group as 0.46. Reviewing this data, the change in IL-6 concentration, in the ERCP group was affected by the patients who were admitted to a higher level of care to treat their sepsis. The biliary emergency group in the pilot was only biliary colic and cholecystitis patients, and did not include pancreatitis and obstructive jaundice patients, and none of this group underwent an ERCP.

Table 7.2.1 demonstrates the sample size generated from the power calculation and the number of patients in each arm that were recruited to the study. It should be noted that we did not calculate the power for the group of patients who had undergone a recent ERCP, or the patients who underwent an OTC. These patients were going to be included with the patients undergoing cholecystectomy alone. We were able to recruit past the number of patients undergoing cholecystectomy alone recommended by the power calculation, therefore we removed the recent ERCP and OTC patients and analysed them separately. We did not perform a separate power calculation for them. No power calculation was undertaken for the healthy control group who acted as controls.

Sample size recommended from the power calculation and the number of patients in each arm recruited

	Sample size recommended	Sample size achieved
Biliary emergency arm	46 ± 8	78
- BC / AC		
- Panc. / OJ	-	60
	-	28
Elective ERCP arm	28 ± 5	52
- first ERCP	-	39
- second ERCP	-	8 with 5 emergency cholecystectomy
Elective cholecystectomy arm	153 ± 11	185
- laparoscopic cholecystectomy only	51 ± 7 each surgeon	67 and 58
- open cholecystectomy only	51 ± 4	60
Recent ERCP	-	34
OTC	-	32

Table 7.2.1: Demonstrates the number of patients recommended to be recruited from the power calculation and the number of patients recruited. There were no pancreatitis (Panc.) or obstructive jaundice (OJ) patients in the pilot study and a power calculation was not done for the sub groups within the biliary emergency group. The cholecystectomy arm over recruited and therefore the recent ERCP and the OTC group were analysed separately from the main cholecystectomy only group, and no sample size for the individual groups was calculated.

Data collected was continuous interval data, which was plotted to confirm that it had a Gaussian distribution, both for each of the cytokine concentration and the pain scores. This was performed for the biliary emergency, ERCP and cholecystectomy patients and the control group. This permitted the use of parametric statistics.

Parametric statistics were used with a mean and standard deviation for each variable. Comparison of the two approaches to surgery, or biliary emergency, required an unpaired Student's T-test. Occasionally data before and after intervention was analysed, or comparing how a subject responded in different parts of the study, in this case a paired Student's T-test was employed. Although it was proposed intervention would increase the cytokine concentration or pain score, this wasn't always the case in the pilot study especially for patients in the severe pain group. There was also variation in the IL-10 response. Therefore a two-tailed Student's T-test was employed to capture the potential variable response. The majority of groups were larger than ten, where smaller samples were present we were aware there may not be sufficient power to reject the null hypothesis.

When performing analysis of more than two groups, such as the different outcomes of ERCP then analysis of variance (ANOVA) was performed to examine the variance between the multiple means. This was performed to minimise the chance of a Type I error. This was particularly important when comparing the sepsis group to the other pain groups, because the group was smaller and the standard deviation wider. Where ANOVA demonstrated a significant difference then *post-hoc* tests were performed to determine where the difference was. Rarely was categorical data analysed, where it was a Chi square test was performed. Data was recorded on an Excel (Microsoft®) spreadsheet and statistics were calculated using the Excel functions.

7.3 Consent

The consent procedure is documented in the following flow diagram Figure 7.3.1.

Biliary emergency patients

The patients attending had been referred to surgical admissions unit from their general practitioner or the emergency department or out of hours clinics. They were admitted, clerked and analgesia prescribed by the clinical team. Once this was completed as lead investigator I spoke to them about the study and gave them the information sheet about the study (Appendix 3). After two hours I went back and asked them if they wished to participate and enroled them with the consent form in Appendix 4. All were made aware if their diagnosis changed from gallstones causing their underlying problem they were no longer eligible to participate in the study, and all their information would be removed.

Elective ERCP patients

The clinical team admitted the patients, and then the lead investigator informed them about the trial and gave them the information sheet about the study. After two hours if they wished to participate they were consented. This group was enroled on the day of their ERCP.

Elective cholecystectomy patients

The patients were enroled at pre-assessment up to 2 weeks before surgery. While waiting to be seen by the pre-assessment nurse they were told about the study and given the trial information sheet. After they had completed the standard hospital pre-assessment process (2 - 4 hours), they were asked if they wished to participate, if they did they were consented at pre-assessment, and consent confirmed on the day of surgery. The clinical team made the decision about undertaking OTC. Bloods were taken at pre-assessment and repeated on the day of surgery by the clinical team to make this decision. No patients approach to surgery was changed to retrieve a stone.

Protocol for consent

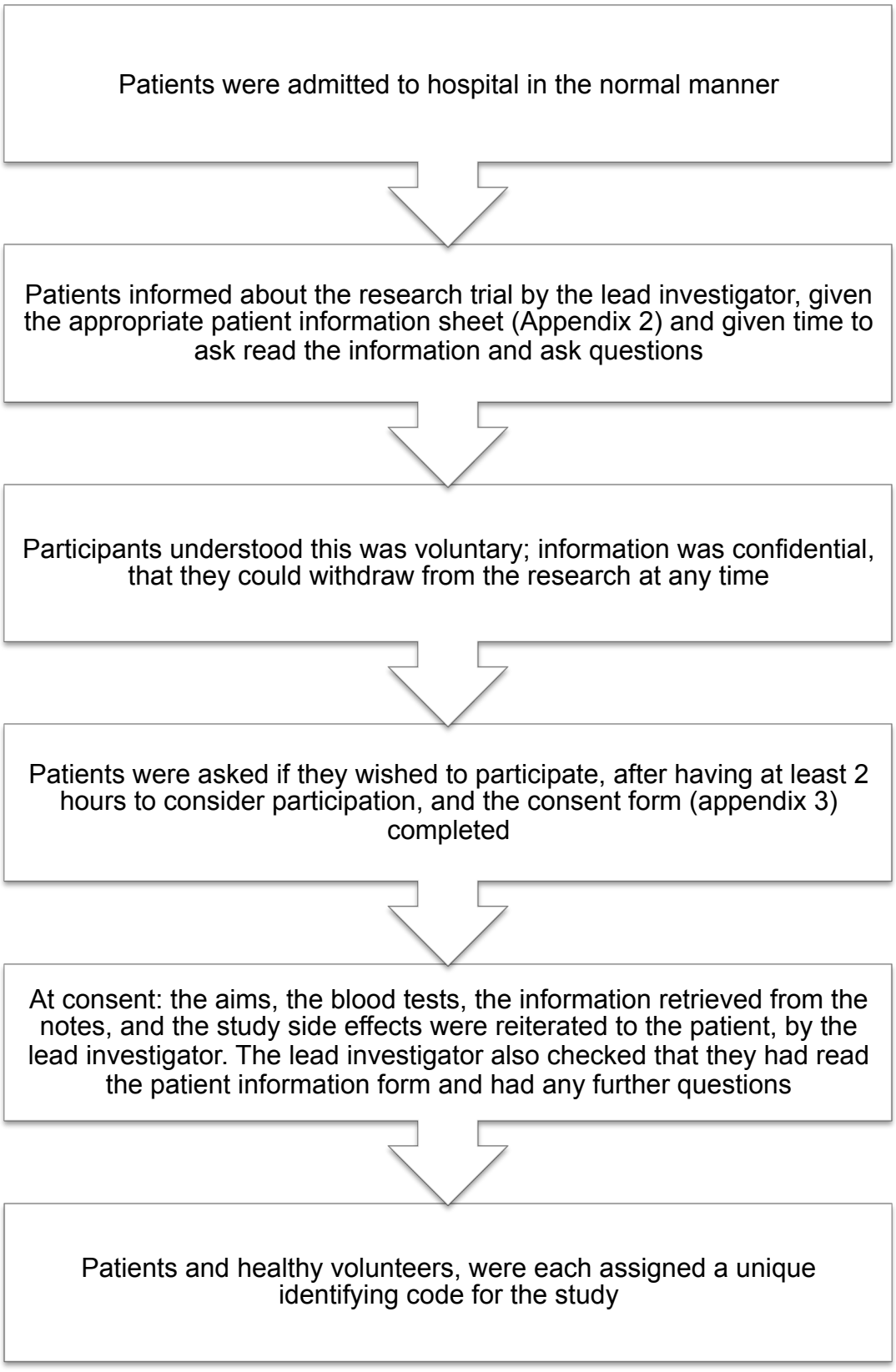


Figure 7.3.1: The consent procedure for the participants in the study.

Volunteers

Fifteen volunteers aged 18 - 70 years, male and female (ratio 1:2), and of diverse ethnicity were recruited. No financial inducement or reward was made or offered. They were given the information sheet after discussion about the study. Two hours later I asked them whether they wished to participate and we completed the consent form, and I asked for their permission to collect the clinical information we collected about the patients and their past medical history (Appendix 5). We wanted to ascertain if there was diurnal variation in the cytokine concentration, as the procedures were taking place in the daytime but the emergency admissions were occurring over 24 hours. Therefore we assigned them a time to commence the study between 8am and 10pm. One volunteer commenced the study at each hour, and the male volunteers were evenly spaced through the time points. The control patients consented to having a greater volume of blood taken to run their samples on multiple ELISA plates.

All participants

Everyone was aware that their participation was voluntary, with all their information kept confidentially under the unique identifier code. All knew they could withdraw from the research at any point without giving a reason for their decision, and had a telephone number and an email contact for the lead investigator to ask any questions. They understood the research group was separate from the team caring for them, and did not exchange information, therefore they would be asked to discuss their pain with the clinical team separately.

Where there were complications or the patient's were not discharged at 24 hours additional consent (Appendix 4), was taken in the same manner to continue scoring their pain and collecting bloods for cytokine concentration every 24 hours until 1 week. If still an inpatient at 1 week then every 48 hours until discharge.

7.4 Study timeline

Figure 7.4.1 gives the timeline for the study. This was the result of the discussion with the participants and clinical team in the pilot study.

Timeline for the patients recruited to the study

Time	Events
Enrolment	1. Patients recruited and informed about the study from SAU, Endoscopy, Surgical pre-assessment 2. Consented 3. VAS assessment of current, and least and worst pain expected. Current pain VAS repeated 4. Bloods samples taken for cytokines and WCC 5. Observations recorded from clinical notes 6. Quality of life and Hospital anxiety and depression forms completed 7. Clinical information about history of gallstone disease and analgesia recorded
Intervention	1. For the ERCP and the cholecystectomy arms the intervention was undertaken by the clinical team 2. Procedural data gathered by the independent observers
2 hours after enrolment / 2 hours after intervention	1. VAS assessment of current pain, and repeated 2. Bloods samples taken for cytokines and WCC 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected
4 hours after enrolment / intervention	1. VAS assessment of current pain, and repeated 2. Observations recorded from clinical notes 3. Data on analgesia requirements collected
6 hours after enrolment / intervention	1. VAS assessment of current pain, and repeated 2. Observations recorded from clinical notes 3. Data on analgesia requirements collected
24 hours after enrolment / intervention	1. VAS assessment of current and least and worst pain experienced. Current pain VAS repeated 2. Bloods samples taken for cytokines and WCC 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected 5. Data on any episode of sepsis & interventions 6. Plan for discharge
Every 24 hours (or 48 hours after 1 week) if patient not discharged	1. VAS assessment of current pain, and repeated 2. Bloods samples taken for cytokines and WCC 3. Observations recorded from clinical notes 4. Data on analgesia requirements collected 5. Data on any episode of sepsis & interventions 6. Plan for discharge
3 months after intervention	1. VAS of current pain 2. Data on analgesia requirements and length of time in pain after discharge 3. Data on any episodes of sepsis after discharge 4. Timing of return to work 5. Quality of life and Hospital anxiety and depression forms completed
6 months after intervention	1. VAS on current pain 2. Quality of life and Hospital anxiety and depression forms completed
12 months after intervention	1. VAS on current pain 2. Quality of life and Hospital anxiety and depression forms completed

Table 7.4.1: Timeline for the patient interventions taking place in the study.

7.5 Clinical information gathering

Consented patients were asked about their past medical history. Information was confirmed in their Nottingham City Hospital medical notes, and their admissions at Queen's Medical Centre Nottingham were checked on the hospital computer system and where appropriate these notes were requested. Patients' general practitioners were contacted for additional information where necessary. This information was recorded on the appropriate form shown in Appendix 5. Table 7.5.1 demonstrates the information gathered from their past medical history and current admission.

Data gathered was recorded on a Microsoft excel spreadsheet and analysed on a Microsoft excel programme (2000). The computer and spreadsheet were password protected. Data was stored under unique identifying number; this was used on all blood samples for cytokine analysis. Patients taking part in different arms of the study were given separate unique identifiers for each part, and the data paired up only after all the analysis had been completed.

Medical and nursing teams involved in the patients care were unaware of the patient's involvement in the research to try and reduce bias. The lead investigator did not take part in the medical care of these patients in anyway.

The information recorded from the patients' notes

Past medical history	
Gallstone disease	Admissions and GP treatment for - Biliary colic - Cholecystitis - Pancreatitis - Obstructive jaundice - ERCP - Known gallstones and readmissions
Past medical and surgical history	- Respiratory, cardiac, endocrine disease including diabetes, chronic pain conditions - Previous surgery particularly abdominal / pelvic surgery and whether open or laparoscopic
Medication	Current medications and allergies, recent analgesia use Previous steroids (oral and inhaled), immunosuppression and blood transfusion
Social history	Smoking
At admission, 2, 4, 6, 24 hours after admission or intervention	
Observation	- Pulse - Blood pressure - Respiratory rate - Oxygen saturations - Temperature - Basal metabolic index - WCC and CRP
Radiology	Details from ultrasound of biliary tree - Presence of single, multiple stones or sludge - Gall bladder wall thickness - Biliary tree dilatation and presence of stones
Medication	Analgesia – which, amount and time of dose Antibiotics

Table 7.5.1: The clinical information gathered from all the patients' notes.

7.6 Visual Analogue Pain score (VAS)

Prior to completing the VAS on each occasion patients were re-assured of confidentiality, reminded the research was independent of the medical team caring for them. Where patients complained to the lead investigator about pain or other problem it was suggested to them, and relatives if present, to discuss this with the medical team in charge of their care about the pain.

The protocol for completing the VAS is given in Figure 7.6.1. The enrolment VAS was taken for all patients at the time of consenting to participate in the study. The patients were given verbal information about completing the VAS at each occasion it was completed. The score was repeated at 2, 4, 6 and 24 hours after intervention or enrolment in the biliary emergency patients. For those not discharged it was completed every 24 hours until discharge, and every 48 hours if an inpatient over a week.

Patients not able to complete the VAS were asked to verbally score their pain on a scale of 0 – 10 by an independent person not involved in the patients care or the research team. Where this was not possible this data was omitted. The two VAS scores take pre and post the blood test were added together and divided in two to give the pain score, if scores were more than 20mm apart they were repeated a third time.

Protocol for completing the VAS

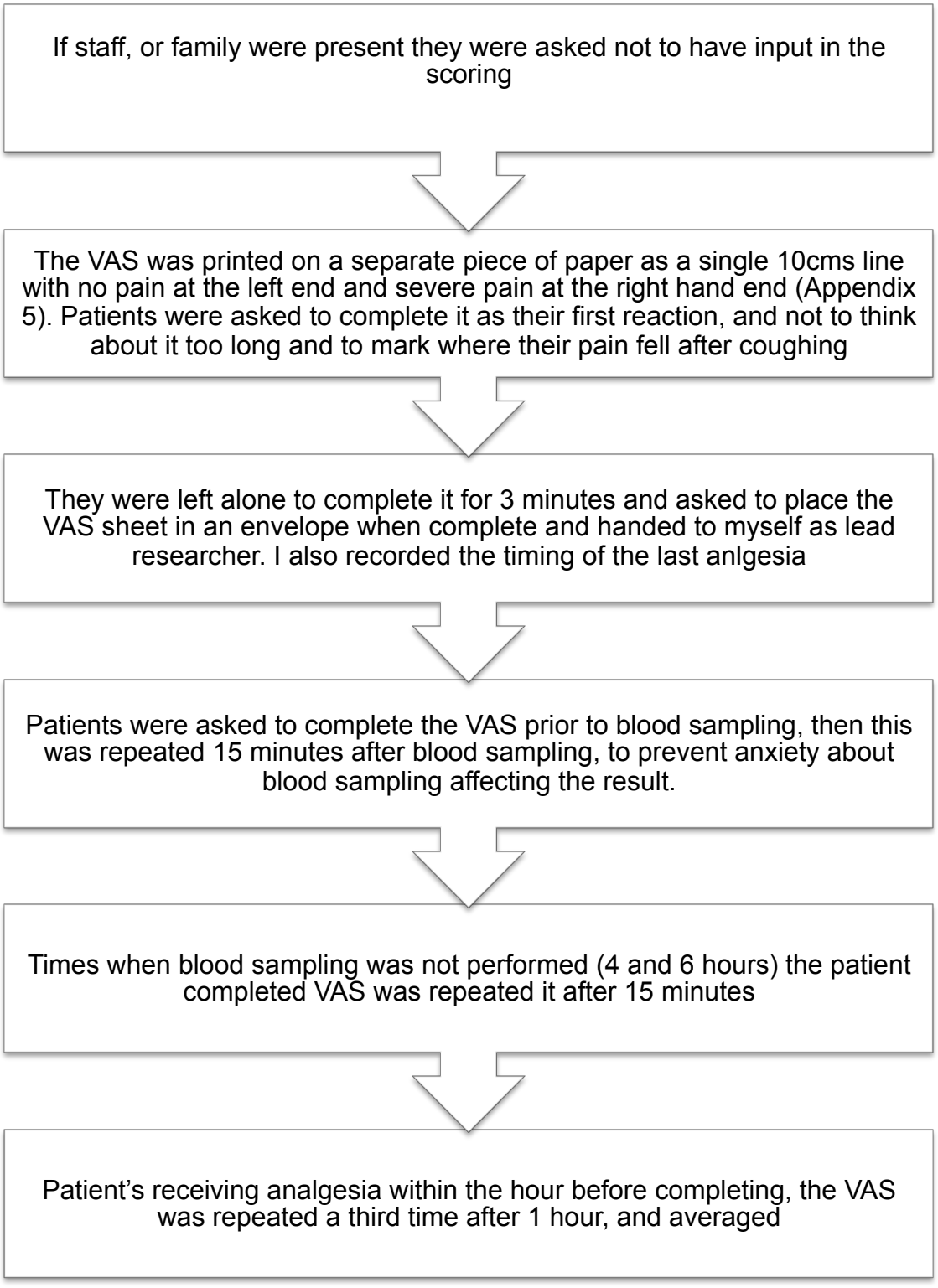


Figure 7.6.1: Demonstrates how the VAS was completed at each time point, the VAS was completed enrolment, 2, 4, 6, 24 hours after enrolment or after intervention, then every 24 hours for the first week, then 48 hours thereafter.

Least and most pain

VAS scores were collected for least and most pain expected at enrolment and least and most experienced over the preceding 24 hours at 24 hours after enrolment or intervention. The protocol for this is described in Figure 7.6.2.

12, 26 and 52 week data

Patients were all seen in outpatients at 10 – 14 weeks as per each consultant's protocol, and again at this point they were asked to score their pain using the VAS, and were asked if and when they had returned to employment or usual daily activities. At 12 weeks they were asked about the presence of shoulder pain after cholecystectomy. The Hospital Anxiety and Depression (HAD) and the quality of life (QoL) forms were completed during this appointment. At the 12 weeks appointment they were asked if we could send them a VAS form to rate their current pain, and the QoL and HAD scores, at 26 and 52 weeks. If they agreed they were asked to sign a consent form for this, and contact details were stored securely under their study identification on an Excel spreadsheet.

Protocol for completing the least and most pain assessment

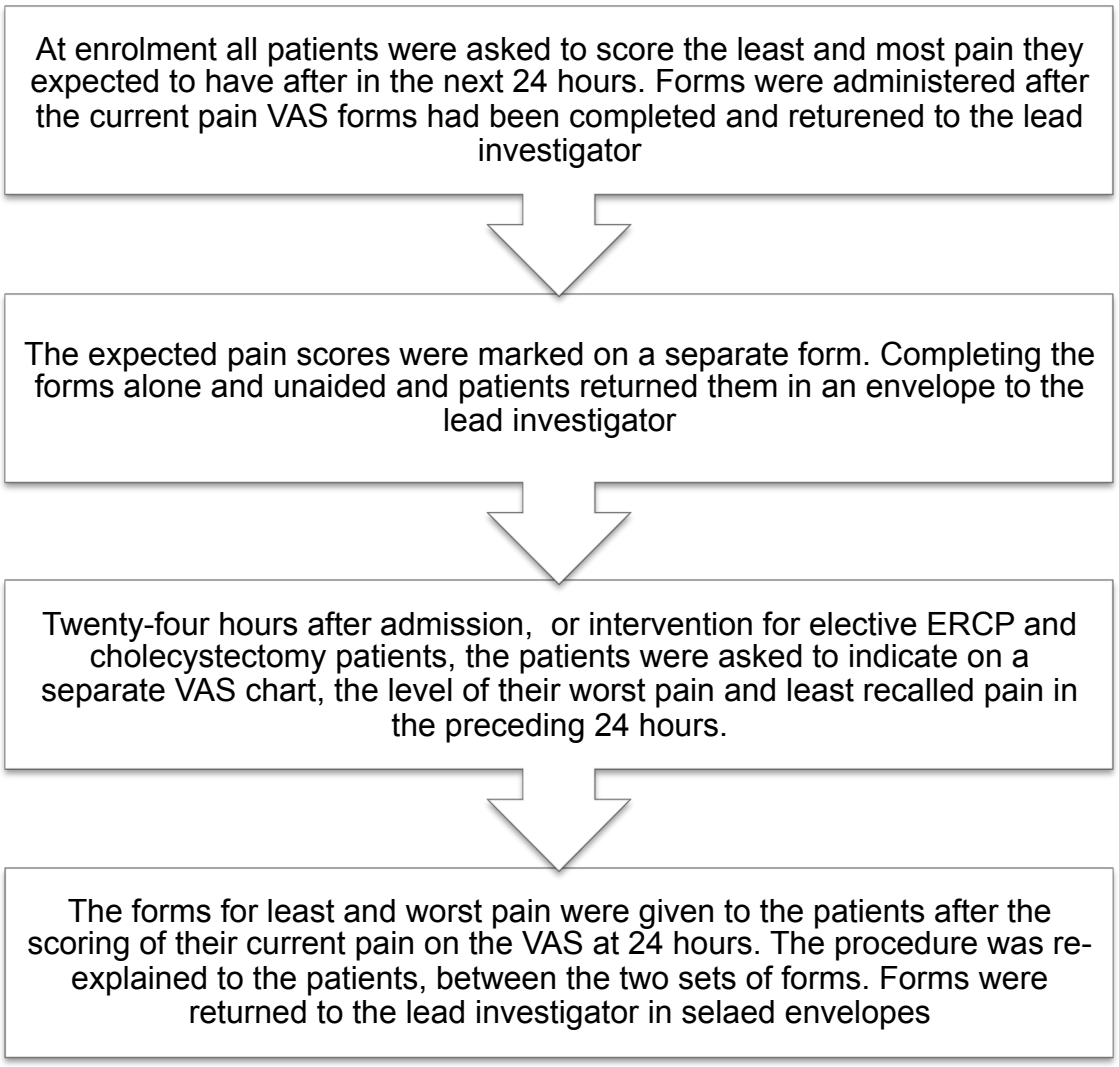


Figure 7.6.2: VAS data collection method for least and most pain recalled and experienced.

Semi-structured information gathering

At the time of completion of each VAS, patients were given time to talk about their pain. We also asked the patients about their past medical history of pain and gathered information from their notes Table 7.6.3 documents areas covered.

Questions asked directly to the patients about their pain at each time point

Semi-structured information gathering about pain	
All patients asked at each time point	Patients were asked if they had had problems with pain If pain control had been discussed with them, including side effects, and by whom They were asked if the information had been sufficient, and given in an appropriate way If they had had regular analgesia or refused analgesia had it been discussed with them
Patients who had had pain	Patients were asked if the issues had been addressed and who had addressed them If analgesia had been given, if it had been given in a timely manner and if they had had to re-request it
At 12 weeks out patient	Patients were asked how long they'd taken analgesia after discharge If they had seen a medical practitioner for advice, further prescription or treatment e.g. of infection or for analgesia They were asked whether they would consider further surgery by that approach again (laparoscopic or open)

Table 7.6.3: Information gathered in the semi-structured interviews from the patients about their pain at each time point and at twelve weeks following discharge. The notes, both medical and nursing, and the prescription chart were also reviewed for details of discussion about pain and the outcome.

7.7 Blood samples

Patients had 5 mls of blood taken at enrolment. This was at admission for the biliary emergency patients and just prior to their ERCP for this arm. But for the cholecystectomy patients it was at pre-assessment up to 2 weeks prior to surgery. Blood samples were collected as clotted samples in pyrogen-free tubes. As lead investigator I took all blood samples, being proficient in methods of venipuncture.

For the patients, their pre-procedural or admission blood investigations including full blood count, urea and electrolytes, liver function tests, amylase, C reactive protein and calcium were recorded from the hospital pathology computer system. White cell count at 2 hours and 24 hours were taken with the cytokine bloods but sent to the hospital laboratory in the routine manner. The protocol for handling of all blood samples is in Figure 7.7.1. Where patients were not discharged at 24 hours their bloods were repeated every 24 hours until discharge, up until 1 week. If not discharged then they were repeated every 48 hours onwards until discharge.

For the volunteers, bloods were taken at the same time intervals as the patients the time commencing from when they started the study between 8am and 10pm. Where the patients had 5 mls of blood taken at enrolment, 2 and 24 hours, the volunteers had 50 mls taken to allow each volunteer to have samples on multiple ELISA plates to ascertain variation between plates. The volunteers had a sample of routine bloods sent at the same as the patients. These were sent to the hospital laboratory, and the lead investigator consented each person to access their hospital records to obtain the results.

Protocol for the blood sampling

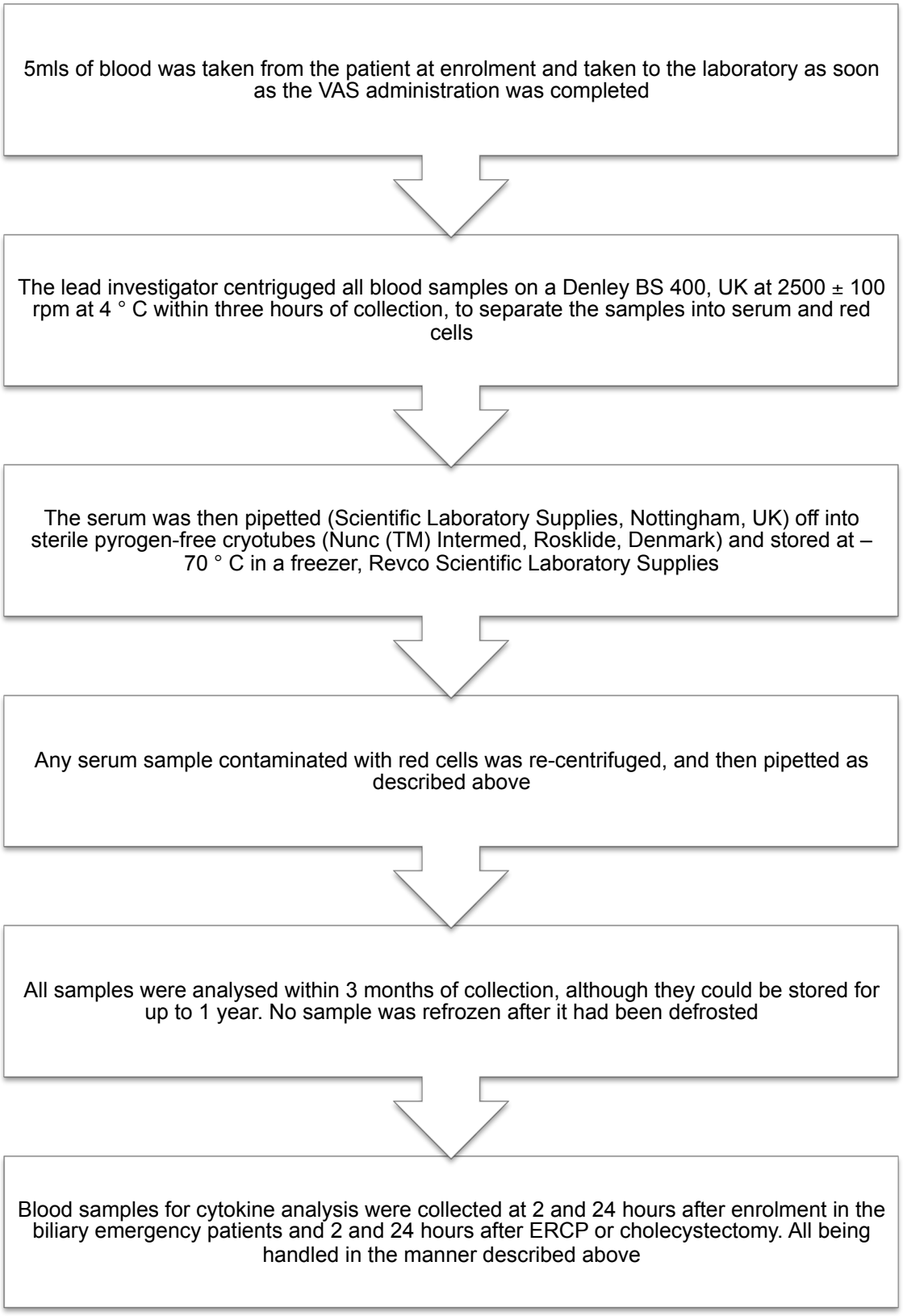


Figure 7.7.1: Blood samples from the patients for cytokines were handled and by the lead investigator as described above.

7.8 Operation and ERCP

The consultant, and where different, the operating surgeon, and anaesthetist were not aware a patient was participating in the study. The anaesthetic and operation were performed in the standard fashion of the practitioners undertaking the procedure and to clinical need. The study could not seek agreement on a standard protocol on performing surgery, anaesthesia or the administration of local anaesthetic. Surgical and anaesthetic consultants had been briefed about the study prior to its commencement and had given permission for operative and anaesthetic data to be recorded and included in the study, in line with the guidelines laid down by the ELREC for Nottingham City Hospital.

To limit the variation in practice, three surgical consultants patients participated in the biliary emergency, and cholecystectomy groups. One gastroenterology consultant undertook the ERCP procedures. Two surgical consultants were experienced laparoscopic surgeons, each having performed over one hundred laparoscopic cholecystectomy's' prior to the commencement of the study. The third consultant only performed open cholecystectomy and was equally experienced in the procedure. All the consultants had experience in performing open cholecystectomy, but no procedures were converted. Two trainees' (only higher surgical trainee's were involved) performed procedure and were closely supervised, and the consultant was a scrubbed assistant, performing both laparoscopic and open interventions. The research study was timed to run with their second six months with the firm so they had experience in the procedures.

Six anaesthetic trainees participated (only higher trainee's were involved), with the consultant present throughout the anaesthetic. The four anaesthetic consultants regularly performed the lists and were experienced in anaesthesia for laparoscopic and open procedures, and for emergency or elective procedures.

Operative data was collected by one of the theatre first assistants, or anaesthetic nurse practitioners and from the operative and anaesthetic records in the patient's notes. All data was collected on a standardised pro forma (Appendix 5) at the end of each procedure. The practitioners were regularly briefed by the lead investigator about the study, and pro forma completion, where possible data was verified from the patient's notes. Practitioners did not inform the theatre team that the patient was participating in the study; or take part in recruitment or data analysis. Data collected is demonstrated in

Table 7.8.1. The practitioners and the lead investigator had collected operative data together using the pro forma on the 45 pilot study patients (15 open and 30 laparoscopic approach patients - including patients 15 from each laparoscopic consultant), this standardised observation, and checked the method of data collection. The ERCP data was collected in a similar manor and was recorded by an independent assessor, who had observed 15 procedures with the lead investigator. The data on difficultness of the procedure was verified with the investigator after they had completed the patients ERCP report. Data collected is demonstrated in Table 7.8.2.

A standard protocol was discussed for local anaesthesia with the two surgical consultants performing laparoscopic surgery, but no agreement could be reached on the protocol to adopt. Therefore it was agreed to examine if one provided more benefit for patients by their post-operative pain scoring. The open patients did not receive local anaesthesia.

Operative data collected by the independent observers in theatre

Operative data
Volume of gas used and pressure of insufflating gas was collected from the standard theatre machines (Storz 264 305 20 electronic endoflator)
Volume of wash used was measured by subtracting the remaining volume at the end of the procedure (measured in a standard jug) from the initial volume
Density of adhesion, gall bladder wall thickness, and bile spillage, had previously been validated by the main investigator and theatre practitioners independently assessing 15 open and 30 laparoscopic procedures and scoring them on the study's pro forma. Operating surgeons observations were also recorded separately
The chief investigator recorded length of open incision at the 24 hour VAS recording. Wounds had clear glue dressing
One laparoscopic approach consultant infiltrated the peritoneum, right hemi-diaphragm and gall bladder bed with a standard 30mls of local anaesthetic of marcain 0.25 %. In four cases it was administered to the wounds as it had been forgotten The other laparoscopic approach consultant infiltrated 30mls of marcain 0.25% into the skin around the incisions No open patients received local anaesthetic

Table 7.8.1: The operative data that was collected by the practitioners, either alone or where possible the practitioners collected the data independently. Information on length and anaesthesia and surgical procedure was collected from the notes and by patient assessment.

ERCP data collected by the independent observer in endoscopy

ERCP data
Information about the sphincter and duct cannulation
Presence of a stone
Difficulty of the procedure

Table 7.8.2: The data about the ERCP was recorded by the endoscopy nurse and from the patients' notes. This had been validated as the operative data was, by observing 15 procedures with myself and recording them independently on the pro forma (Appendix 5). There was discrepancy between the observer and myself in the pilot on how difficult the procedure was. Therefore in the study at the end of the procedure the endoscopist was asked to rate the procedure and this was also recorded. The data being analysed as both observer data, and the endoscopist scoring of the difficultness of the case, this demonstrated no difference in the data but we used the endoscopists scoring in the final analysis. Other data was collected from the patient's notes.

7.9 Analgesia

The hospital adopted a standard post-operative and emergency admission analgesia protocol. The protocol was based on the World Health Organisation (WHO) analgesia protocol, shown in Figure 7.9.1, and was the protocol for analgesia used in this study. This was regardless as to whether the patient was admitted for an elective procedure or as an emergency.

Oral and rectal paracetamol were on the hospital formulary; diclofenac (NSAID's) was available orally or per rectum. Codeine phosphate and tramadol were available as tablets. Morphine was given sub-cutaneously or intra muscularly on the ward and intra-venously in theatre recovery. Standard patient controlled analgesia PCA with morphine was used as per the hospital protocol, with standard 5 minute lock out period. No pre-medication analgesia or anxiolytic was prescribed for any operative patient.

World Health Organisation analgesic ladder

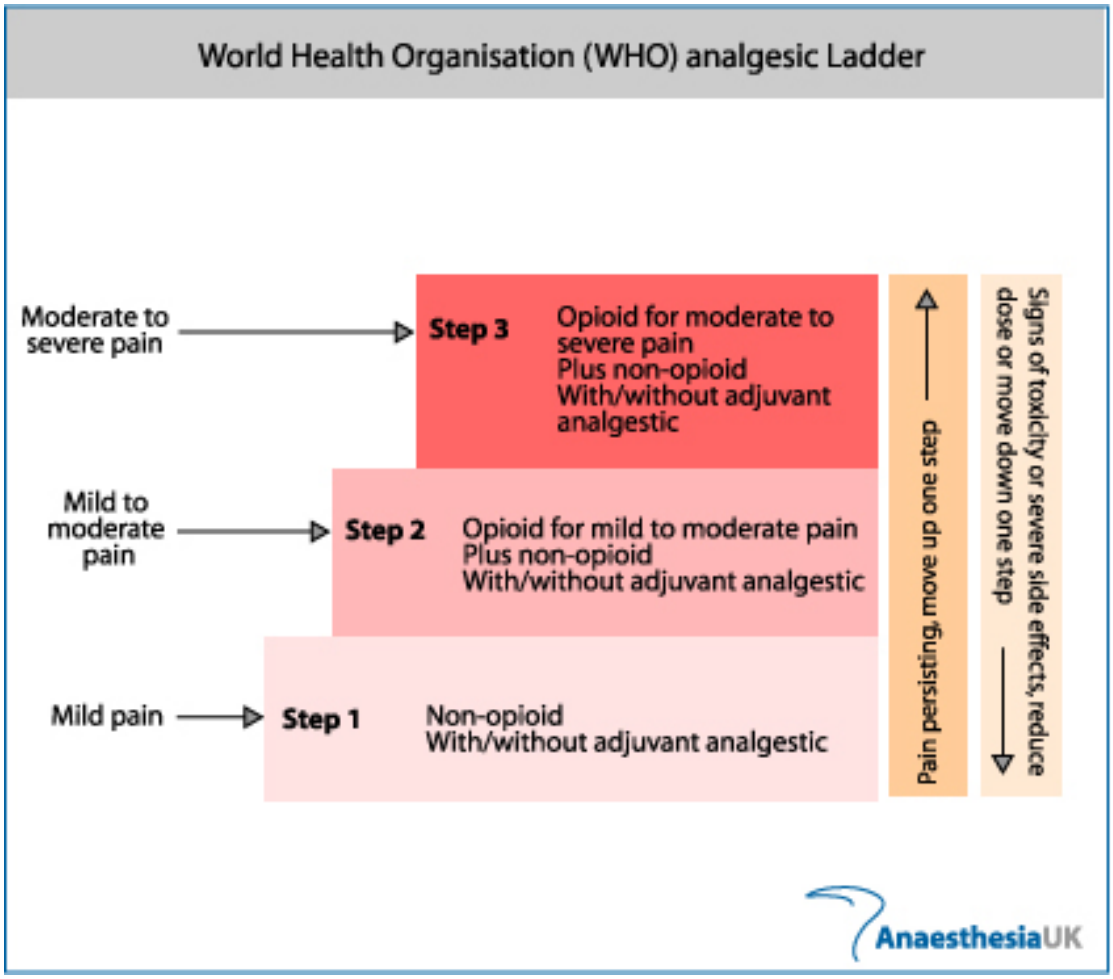


Figure 7.9.1: The World Health Organisations Analgesic Ladder. The patient should commence at the step appropriate to their pain. If pain is not controlled by analgesia at that level, then move up to the next step with the analgesia for that level. Reducing analgesia if pain adequately controlled or signs of toxicity or side effects. Diagram from Anaesthesia UK.

Patients reporting an allergic reaction to a form of analgesia were not enroled into the study. Patients with respiratory disease were enroled, but were not prescribed NSAID's. The admitting doctor or anaesthetist assessed and prescribed analgesia. Table 7.9.2 details the analgesia protocol. All patients were admitted to three surgical wards, and high dependency unit (HDU) or intensive care unit (ICU) if required. The admitting teams were unaware of which patients were participating in the study, and the VAS results were not available to them.

Pain problems in recovery were discussed with the list or duty anaesthetist. On the ward the surgical team assessed, referring to anaesthetics for advice, and changes with the patient controlled analgesia (PCA) prescription. Open approach patients, and patients returning to theatre were consented for PCA pre-operatively, which was re-explained in recovery when it was connected up. An acute pain team nurse reviewed patients on PCA once a day, referring if required to the acute pain doctor or duty anaesthetist. Change over from PCA to oral analgesia was by clinical assessment of the surgical team, in consultation with the pain team.

ERCP patients received midazolam, morphine and buscopan at the start of the procedure, titrated to need during the procedure. Post-procedure receiving analgesia as per the hospital protocol. Cholecystectomy patients' analgesia was prescribed in theatre. For administration in recovery patients had intravenous morphine prescribed, in 1mg increments up to 20 mg. Those still in pain were reviewed. Laparoscopic patients were blind to the route of local anaesthetic administration.

Recommended analgesia protocol

Analgesia protocol recommendations for all patients
On admission
Regular paracetamol and non-steroidal was prescribed, unless contra-indicated As required weak opiate prescribed If clinically judged necessary, morphine should be available intra-muscularly or sub-cutaneously on the as required prescription chart Nursing team to assess and administer analgesia on the ward, referring to the admitting team if assessment required
On discharge
Patients should receive 5 – 7 days of regular paracetamol and a NSAID If required 3 – 5 days of a weak opiate should be prescribed Patients' analgesia needs should be assessed and discussed with patients Patients' should receive information on pain management, and how to take analgesia and it's side-effects, alternative forms of analgesia, and where to seek advice

Table 7.9.2: The hospital protocol for the prescription of all patients admitted and discharged from the hospital. Prescriptions observing patients declared allergies.

Prior to commencement of the study, as lead investigator I presented to the medical staff, at the regular surgical and anaesthetic meeting. Presenting about the study and the hospital guidelines on pain management. The study was presented to the nursing staff at their ward meeting for each ward and to the theatre team. Written information was displayed prominently on the wards, in theatre, ERCP ward area, pre-assessment and outpatients. The Trust's analgesia protocol was also prominently available in these areas. The ward pharmacists and the pain team for surgery attended one of the presentations.

On medical staff change over the presentation was repeated at induction. New nursing staff were given written information and emailed the presentation. There was also a contact numbers of the lead investigator for questions on the protocol. Neither medical nor nursing staff was aware of which patients had consented to taking part in the research. Patients raising pain related concerns were given information about who to approach for advice. The research team did not prescribe analgesia or give advice about pain management. Staff questions on analgesia prescription were referred to the analgesia protocol or senior team member. The research team did not influence the management of patient's pain, and did not disclose the patients VAS response or response to any part of the research.

7.10 Antibiotics

The Trust's antibiotic protocol is described in Table 7.10.1. Where patients had a history of an allergic reaction to an antibiotic an alternative was substituted.

Antibiotic protocol – taken from Nottingham City Hospital antibiotic protocol

For biliary emergencies
For biliary colic no antibiotics are required Other biliary emergencies should be assessed and if not allergic prescribed a cephalosporin alone and or metronidazole or co-amoxiclav dependent on the admitting consultants preference Advice being adhered to in the study
For ERCP
Cephalosporin alone and or metronidazole or co-amoxiclav administered at the start of the procedure dependent on the admitting consultants preference and patient allergy status The need to continue antibiotics should be assessed at the end of the procedure Advice being adhered to in the study
For cholecystectomy
Antibiotics to be administered only if there is clinical requirement on the advice of the senior surgeon and anaesthetist Cephalosporin alone and or metronidazole or co-amoxiclav dependent on the admitting consultants preference, and patients allergy status Requirement for continuing antibiotics should be assessed at the end of the operation In this study all open cholecystectomy patients received a dose at induction, laparoscopic approach patients was at the surgeons discretion Antibiotics were not continued unless there is a clear clinical indication and instructions from the senior surgeon or anaethetist
Post-operative infections
Commence empirically on cephalosporin and metronidazole, or Co-amoxiclav by the appropriate route, refined when microbiological advice when available and patient allergy status Advice being adhered to in this study Trimethoprim was prescribed for urinary tract infections, and levo-floxacin for respiratory tract infections

Table 7.10.1: The Trusts antibiotic protocol, which was used for the study.

7.11 Assessment

Two hours

The time point of two hours was following the completion of surgery or ERCP was chosen, as this was a fixed time point where patients had begun to be sufficiently alert to complete the pain score, and the early cytokine profile could be assessed. If the time point had been from the start of the procedure then a proportion of patients would have had be excluded as their procedure was longer than two hours or they were not sufficiently alert. For the patients with emergency gallstone admissions, the 2 hours sample was taken 2 hours was after the enrolment. Volunteers' blood was taken 2 hours after they commenced the study. Figure 7.11.1 details the investigations completed at 2 hours. Figure 7.11.2 details the number of patients completing each time point. At 24 hours patients had been discharged, nine of the biliary emergency patients, five of the ERCP patients, and ninety-four laparoscopic cholecystectomy patients. Figure 7.11.3 details the pro forma completed and how they were completed.

Data collected at 2 hours after enrolment for the biliary emergency patients or 2 hours after ERCP or Cholecystectomy for those patients

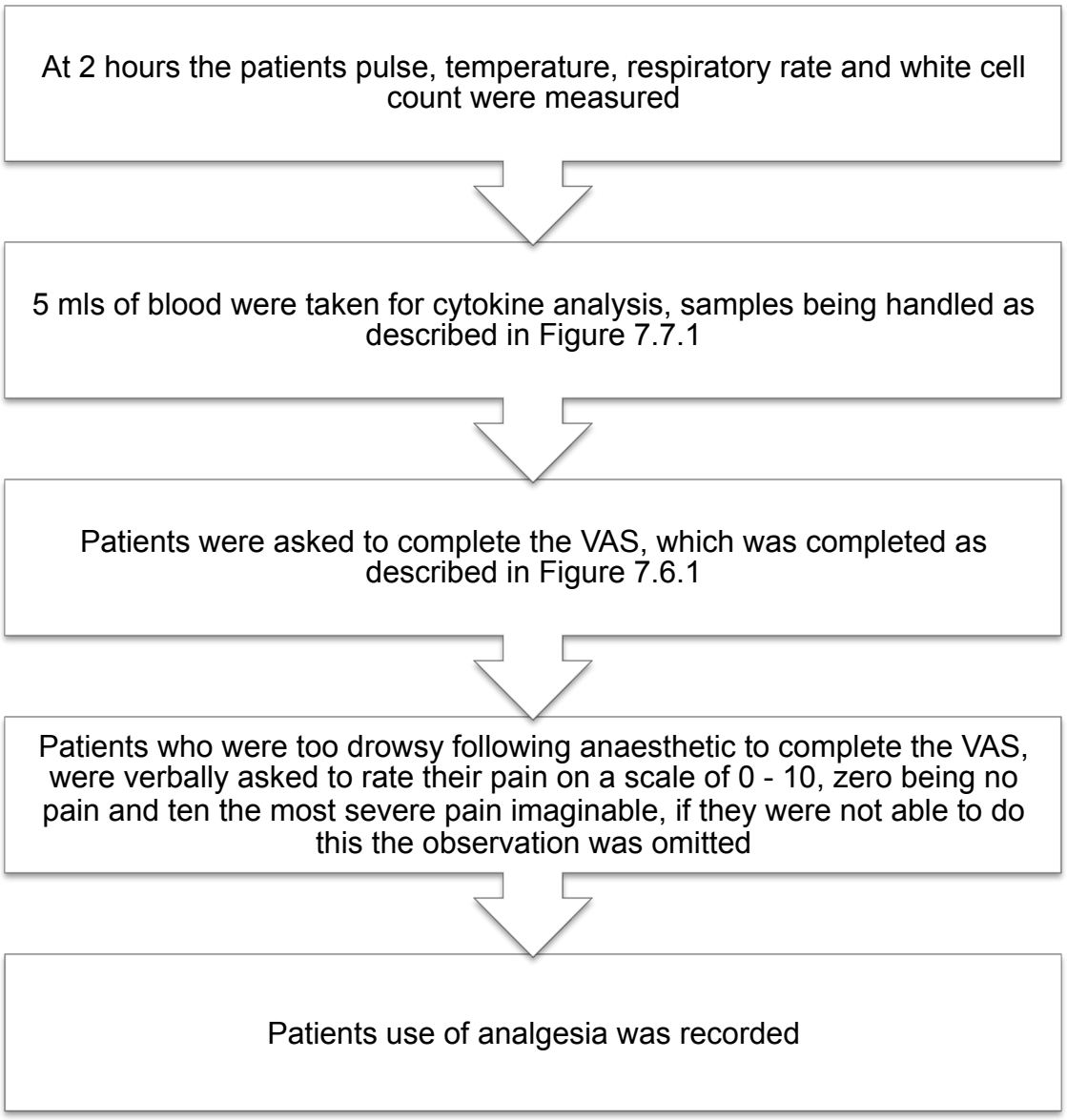


Figure 7.11.1: The data collected by the lead investigator at 2 hours. For surgery and ERCP patients the data was collected at 2 hours after the completion of ERCP or surgery, for biliary emergency patients data was collected at 2 hours after enrolment bloods were taken. Four surgical and three ERCP patients were not alert enough to complete the VAS; all were sufficiently alert to complete the numeric rating score.

Number of patients having bloods and VAS completed at each time point

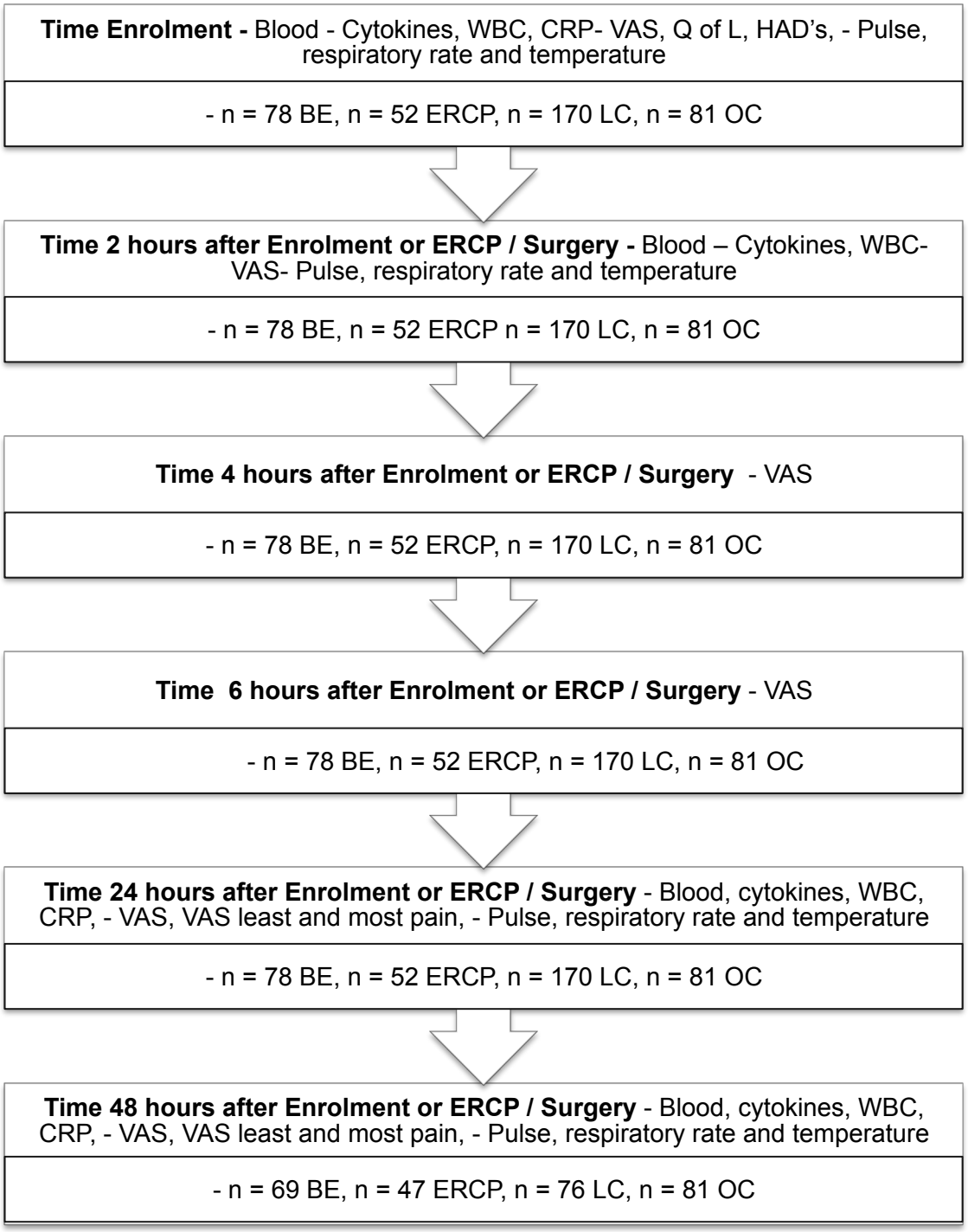


Figure 7.11.2: Demonstrating when bloods were taken specifically for the study, the VAS and QoL and HAD scores was completed, and also which observations were recorded. The number of patients in the study is recorded at each time point, which either remained an inpatient or came back to the hospital, or was telephoned. BE – biliary emergency, ERCP patients, LC – laparoscopic cholecystectomy, OC – open cholecystectomy.

Protocol for collecting data at 4 – 24 hours time points after enrolment or ERCP or Surgery

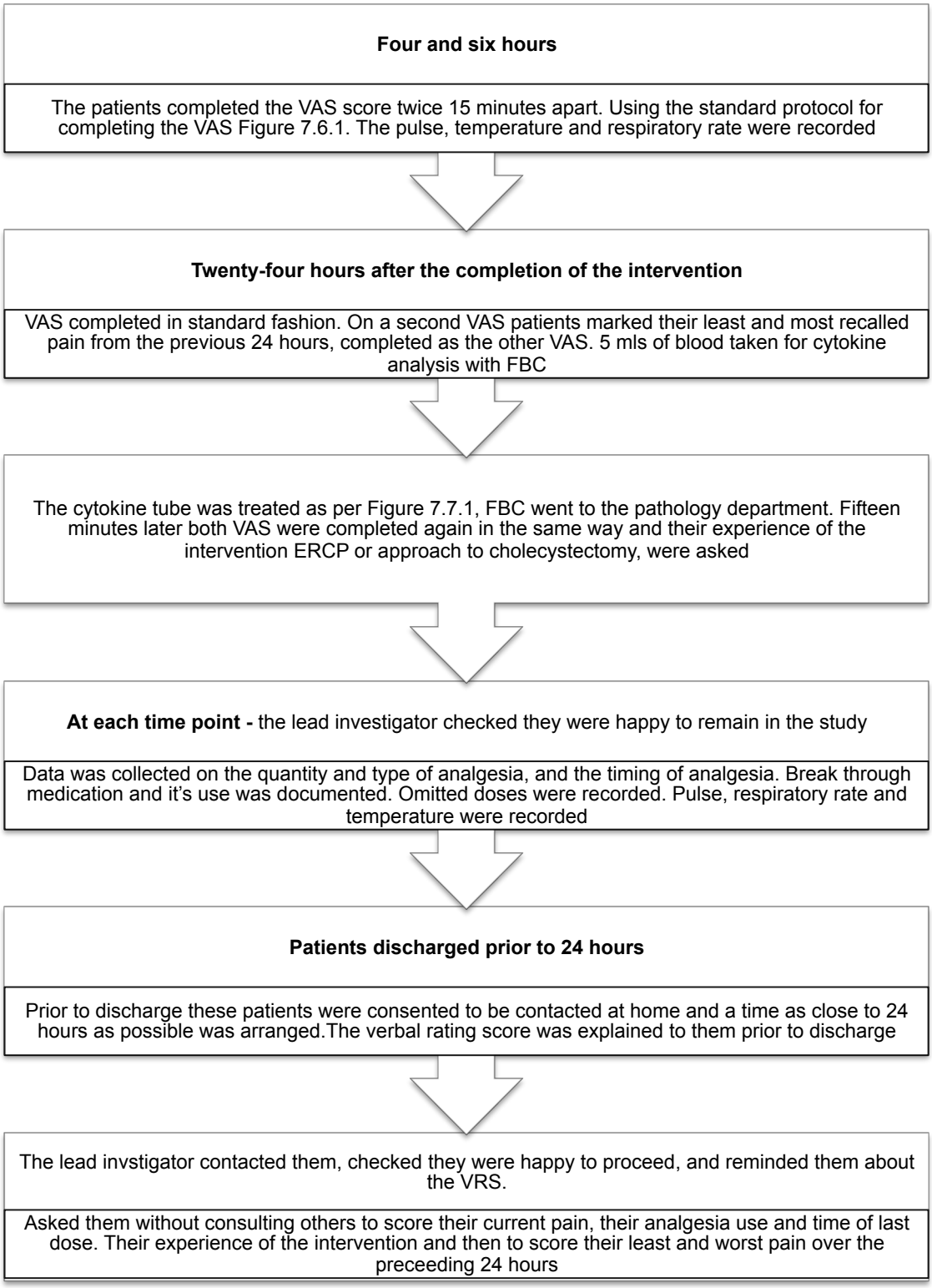


Figure 7.11.3: Administration of the visual analogue score for current and recalled pain and blood samples. VRS – verbal rating score, FBC – full blood count.

7.12 Quality of life data.

The patients who agreed to participate in the research were also asked if they would be willing to complete two QoL assessment forms and the HAD form at enrolment and 12, 26 and 52 weeks following admission with biliary emergency or for surgery or ERCP. The assessments used were the Short Form-36 (SF-36) and Gastrointestinal quality of life index and the HAD score (Appendix 1) (Zigmond and Snaith, 1983).

Figure 7.12.1 describes administration of questionnaires, and 7.12.2 describes the HAD scoring, the SF-36 and GIQLI forms being scored as per their scoring protocol. These were chosen as the European Association for Endoscopic Surgery recommended them as optimal measures of quality of life following cholecystectomy, allowing assessment of both general and disease specific quality of life. The timing of the assessment was also guided by the Associations recommendations (Korolija *et al.,* 2004).

We compared the results of completing the QoL and HAD forms by telephone and on paper in the pilot study. In this study half the group of healthy volunteers completed the measures on paper, and then completed them over the telephone with the lead investigator. The other half completed them the opposite way round to check for consistency in response. All of the volunteers were also asked to feed back on the forms, the advice given on completing the forms and finally on the telephone completion of the forms. They were also asked to comment on if they felt the lead investigator was leading the responses in any way. There was no difference in the responses to the questionnaires, therefore we were offered the patients the option of completing them by telephone or on paper.

The completion of the quality of life and Hospital anxiety and depression scale

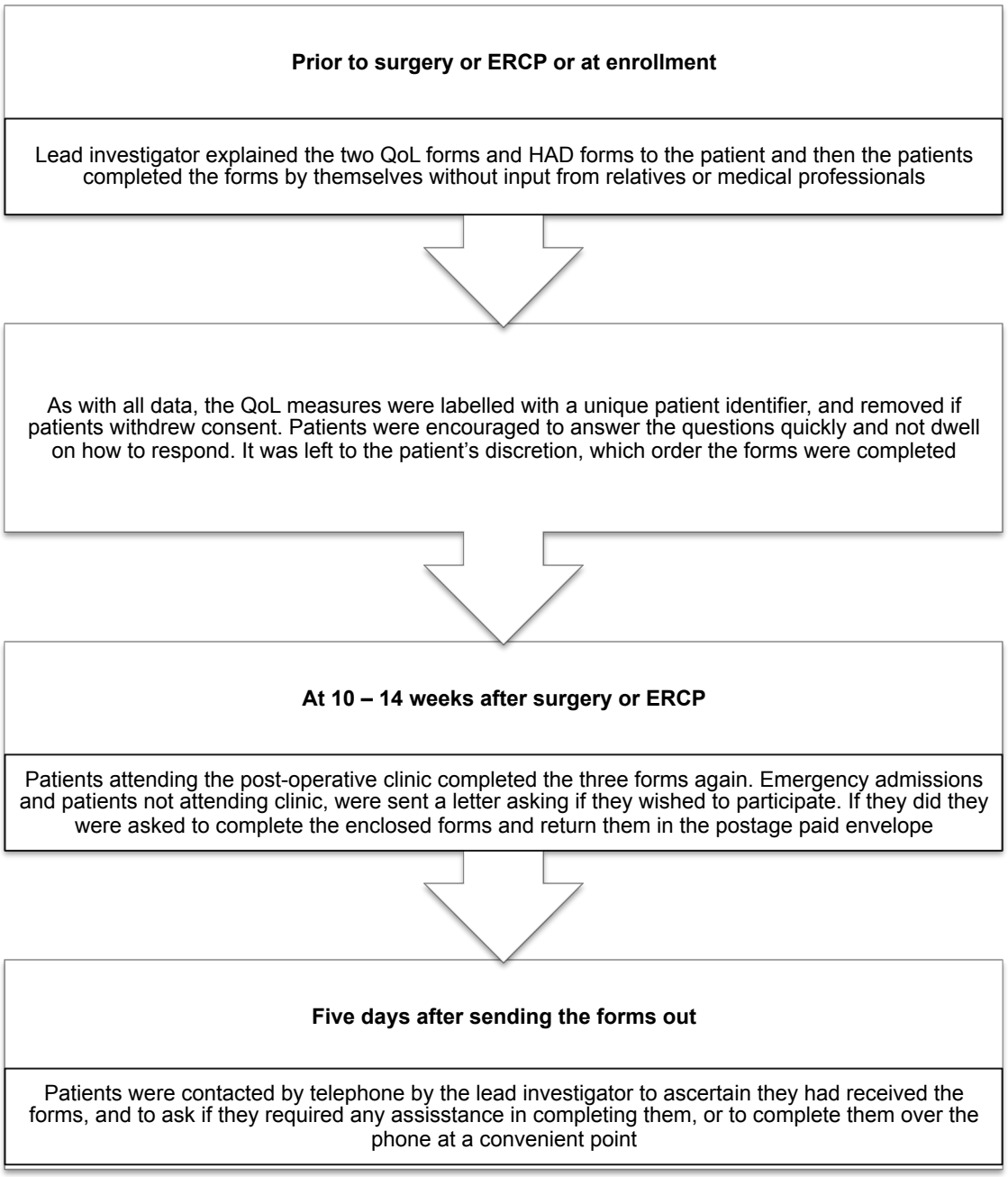


Figure 7.12.1: The collection of the QoL and HAD data, and the validation of the completion of the forms by telephone. The pilot volunteers were also split into two groups, for each intervention, half completing the forms by telephone and half on paper. Feedback was collected from the pilot volunteers and this was incorporated into the study.

The score ranges on the Hospital anxiety and depression scale

HAD score	Classification
0 – 7	Normal
8 – 10	Borderline
11 – 21	Abnormal

Table 7.12.2: Describes the interpretation of the Hospital Anxiety and Depression score. The score is composed of two domains the anxiety and the depression part and the two scores added together to give a maximal score of 21 (Zigmond and Snaith, 1983).

7.13 Enzyme Linked Immunosorbant Assay.

Enzyme linked Immunosorbant assay (ELISA) was used to quantify cytokine concentrations in serum samples. The kits' used were Biosource Enzyme Amplified Sensitivity Immunoassay (EASIA), Belgium.

The system uses an oligoclonal system where a blend of monoclonal antibodies directed against distinct epitopes on the specific cytokine. The Kohler and Milstein method of cell fusion is used to immortalise antibody-producing cells, to produce specific homogenous antibodies (detailed instructions for each cytokine are given in Appendix 7). The advantage of an oligoclonal system is the avoidance of hyper-specificity and increases assay sensitivity.

The EASIA uses a sandwich technique, shown in Figure 7.13.1. In the first step monoclonal antibodies (MAbs-1) coated onto the micro titer plate are used to capture the specific cytokine. The plates are then washed to remove unbound antigen. The plates are then incubated with a second monoclonal antibody which has horseradish peroxidase (HRP) attached (MAbs-2-HRP); this forms a sandwich of MAb-1-cytokine-MAbs-2-HRP. Excess unbound antibody is then removed in a second washing step. Bound labeled antibodies are detected by the addition of a chromogenic solution ($TMB+H_2O_2$). Following incubation a stop solution (H_2SO_4) is added and the plate read at the appropriate wavelength, the absorbance being proportional to the cytokine concentration.

A standard curve is constructed by reading the absorbance of the standards on the plate at 450nm and 490nm, within 15 minutes of applying the stop solution. The absorbances at 490nm were subtracted from those at 450nm. The 490nm wavelengths are used to subtract the non-specific emissions from all the other materials in the wells (e.g. polystyrene), this can be subtracted from the relevant emissions at 450nm. The result is then used to construct a standard curve, from which the values of the experimental wells can be extrapolated to determine the cytokine concentration. The procedure for all the cytokines is shown in Figure 7.13.2 to 7.13.4. The controls were plated on multiple plates to compare results across plates. There was not significant variation between plates but the plan was to re-run plates if there had been variation.

The ELISA sandwich technique

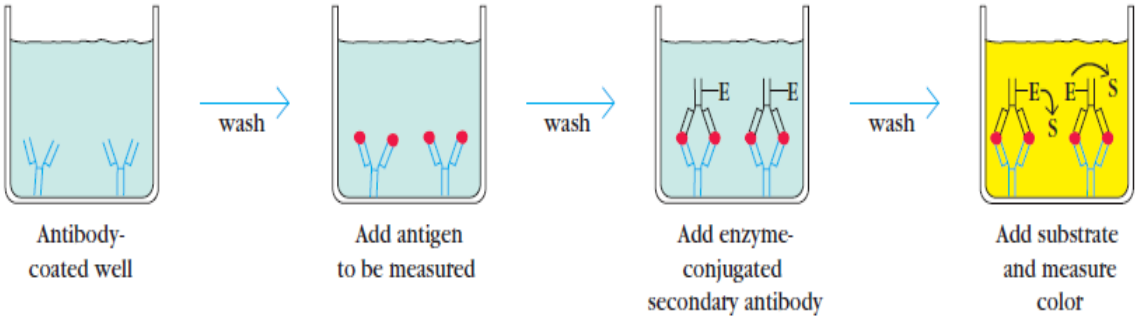


Figure 7.13.1: Diagram demonstrating the sandwich ELISA technique. It is used to identify a specific sample antigen. The wells of micro titer plate are coated with the antibodies. Non-specific binding sites are blocked using bovine serum albumin. The antigen-containing sample is applied to the wells. A specific primary antibody is then added after washing. This sandwiches the antigen. Enzyme linked secondary antibody is added that binds primary antibody. Unbound antibody-enzyme conjugates are washed off. The substrate for enzyme is introduced to quantify the antigens with a chromogen.

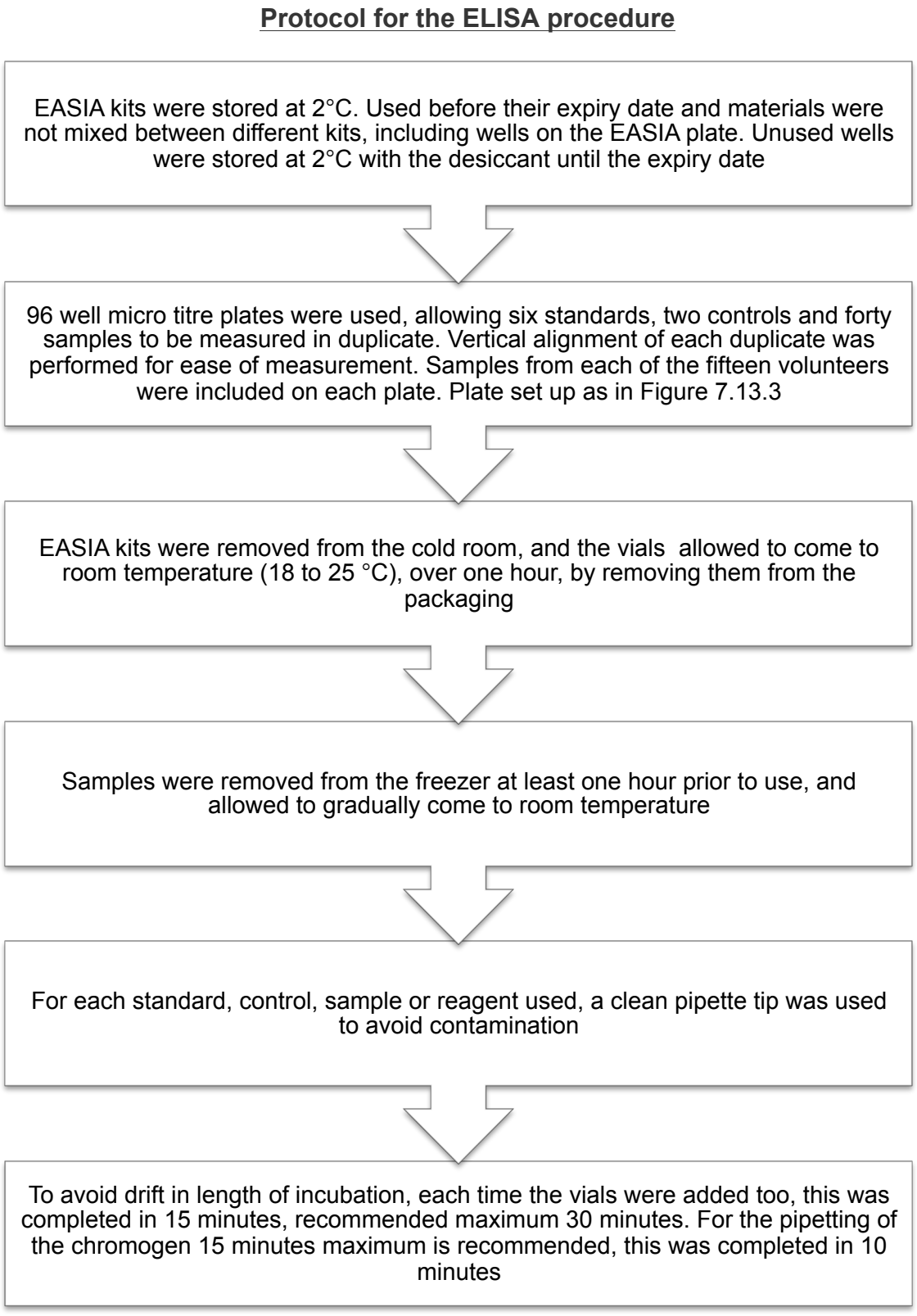


Figure 7.13.2: Preparation of EAISA plates, and handling of samples for cytokine analysis. The order for plating the samples on the EASIA is demonstrated in Figure 7.13.3. Appendix 7 details this procedure.

The layout of the ELISA plates

	1	2	3	4	5	6	7	8	9	10	11	12
A	S 0	S 0	P1 24	P1 24	P4 2	P4 2	P7 0	P7 0	P9 24	P9 24	V1 2	V1 2
B	S 15	S 15	P2 0	P2 0	P4 24	P4 24	P7 2	P7 2	P10 0	P10 0	V1 24	V1 24
C	S 50	S 50	P2 2	P2 2	P5 0	P5 0	P7 24	P7 24	P10 2	P10 2	V2 0	V2 0
D	S 150	S 150	P2 24	P2 24	P5 2	P5 2	P8 0	P8 0	P10 24	P10 24	V2 2	V2 2
E	S 500	S 500	P3 0	P3 0	P5 24	P5 24	P8 2	P8 2	P11 0	P11 0	V2 24	V2 24
F	S 1500	S 1500	P3 2	P3 2	P6 0	P6 0	P8 24	P8 24	P11 2	P11 2	V3 0	V3 0
G	P1 0	P1 0	P3 24	P3 24	P6 2	P6 2	P9 0	P9 0	P11 24	P11 24	V3 2	V3 2
H	P1 2	P1 2	P4 0	P4 0	P6 24	P6 24	P9 2	P9 2	V1 0	V1 0	V3 24	V3 24

Figure 7.13.3: Diagrammatic representation of the EASIA plate (96 wells, the blue label is not wells but the labeling of the grid). S is standard, with the standard concentration beneath in pg/mL (dark orange). V is for the volunteer with the volunteer number; the row below is time point 0, 2, and 24 hours (orange). P is for the patient samples with patient number; again the time is on the row below (yellow). All standards, patient and volunteer samples were plated twice. On the next plate patient (P) 12 was plated and volunteer (V) 4 was plated. Patients with more than three time point samples were all plated together. This was performed for each cytokine. One plate of only volunteer samples was performed for each cytokine (V1 – V14, with V15's three samples being plated on a separate plate and ensuring it was repeated). This meant every volunteer's cytokine concentration was measured at least twice for each time point.

Method for reading absorbances on the ELISA plate

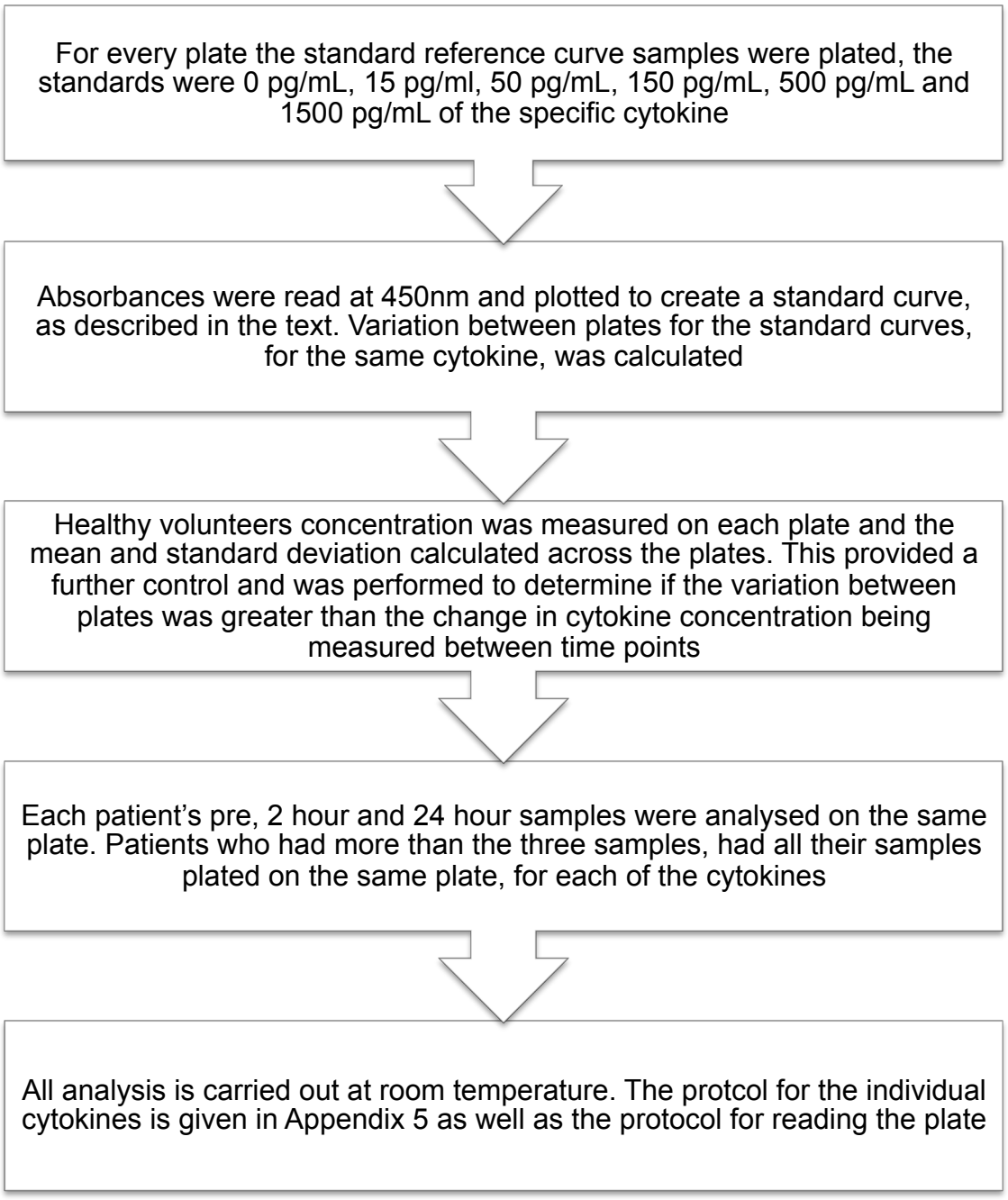


Figure 7.13.4: Method used for reading the absorbance's on the plate, the standard curve allowed comparison between plates. By plating the volunteers on each plate they acted as a second standard control to compare the plates. In addition if the variability for a volunteer between plates was greater than the change between time points, then we planned to re-run the plate as it would question the significance of the change in concentration observed, but this did not occur.

7.14 Disposal of samples.

A protocol was drawn up for where patients withdrew consent to participate in the research, and to dispose of samples where cytokine analysis had been completed demonstrated in Figure 7.14.1.

Protocol for disposal of samples and patient information

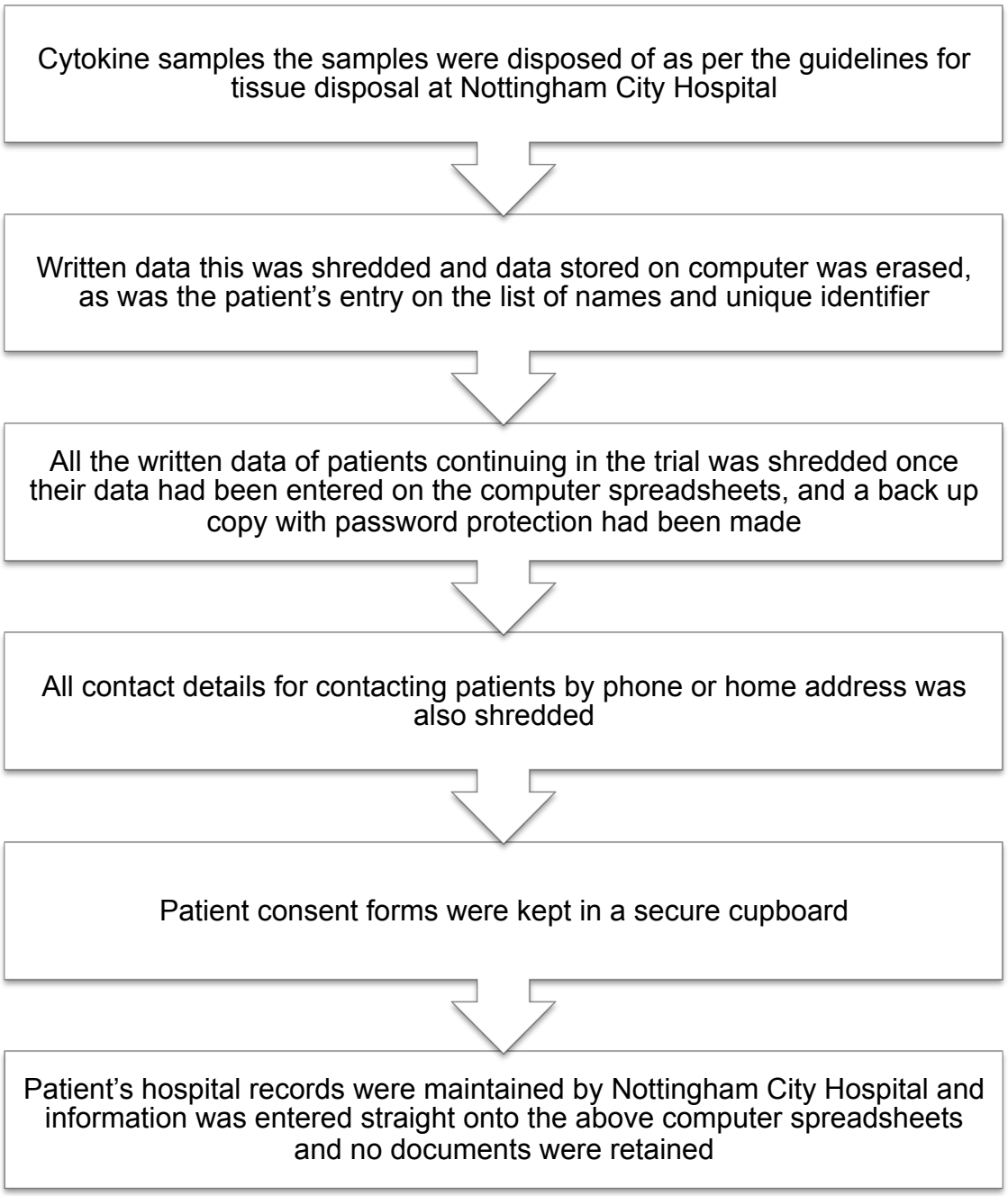


Figure 7.14.1: Protocol for the disposal of samples and patient information, for patients who withdrew from the study. The Figure also details the handling of the information collected to ensure patient confidentiality.

Results

Chapter 8 – Demographics and Pain groups

8.1 Patient recruitment

The aim of the study was to determine if pain was an early marker of sepsis following cholecystectomy. Patients were enroled who were undergoing elective cholecystectomy (n = 251). To determine the effect upon cytokines and pain of undergoing instrumentation of the biliary tree we enroled patients undergoing elective ERCP (n = 52), and the effect of biliary sepsis we included a group of patients with biliary emergencies (n = 78).

We over recruited in the elective cholecystectomy group; therefore we separated the group into those who underwent elective cholecystectomy alone, and those who had undergone ERCP within the previous year or those who underwent an on table cholangiogram. The groups were analysed separately as the recent instrumentation of the biliary tree affected the cytokine response, as will be discussed in Chapter 9.3.

Each of the three arms of the study, the biliary emergency, the ERCP and the cholecystectomy group contained patients who underwent ERCP. Patients developed sepsis in each arm of the study. Figure 8.1.1 is the consort diagram for the study.

Consort diagram for participation in the study

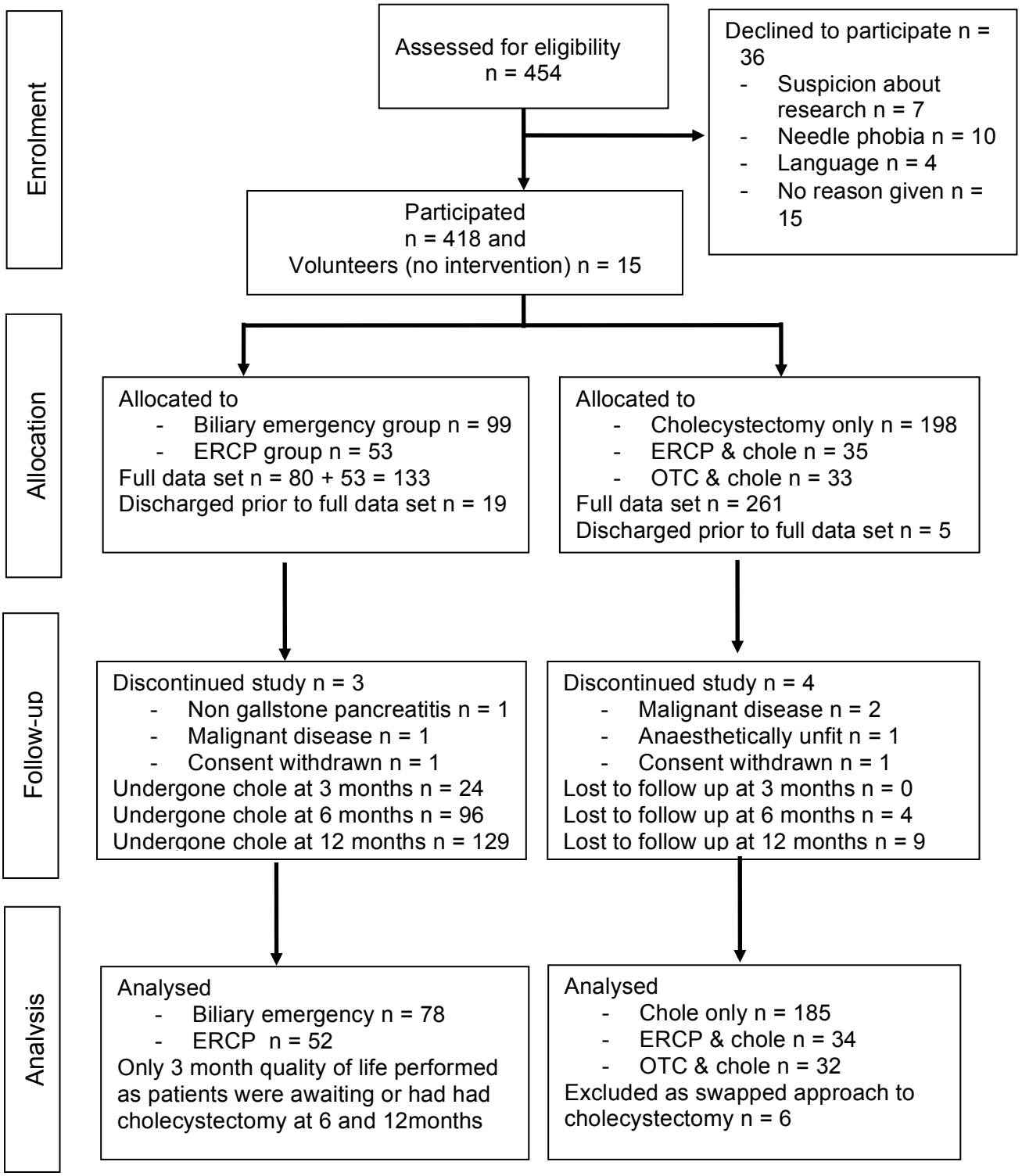


Figure 8.1.1: The consort diagram for the study. The biliary emergency patients and ERCP patients are together on the left of diagram, and the cholecystectomy patients are on the right, split into the cholecystectomy (chole) only group, recent ERCP and cholecystectomy patients and OTC and cholecystectomy patients. We also recruited 15 control patients from the staff, students and hospital volunteers.

Table 8.1.2 examines the number of patients approached to participate in the biliary emergency, ERCP and cholecystectomy part of the study. All groups are shown to aid comparison between arms and to demonstrate the equal uptake of the invitation to participate across the study. Five patients, all laparoscopic cholecystectomy patients went home on the day of surgery, and nineteen biliary colic patients were discharged on the day of admission. Their data was collected but due to exceeding the numbers required there data was not analysed with the main group, as they did not have a full set of cytokine data, and being at home they were potentially not representative of the main group of patients. Although it was not sought, patients approached did give reasons for not wishing to participate this data is demonstrated in Table 8.1.3.

Recruitment to the three arms of the study

	Number approache	Number participating	Full data se	Analysed
Biliary emergencies n = 78				
- Biliary colic	47	43 (91%)	24 (51%)	24 (51%)
- Acute cholecystitis	29	27 (93%)	27 (93%)	26 (90%)
- Pancreatitis	15	14 (93%)	14 (93%)	13 (87%)
- Obs. Jaundice	16	15 (94%)	15 (94%)	15 (94%)
ERCP n = 52				
ERCP	54	53 (98%)	53 (98%)	52 (96%)
Number who had participat previously	5	4 (80%)	4 (80%)	4 (80%)
Cholecystectomy laparoscopic n = 170 open n = 81 Total n = 251				
- Cholecystectomy only	219	198 (90%)	193 (88%)	185 (85%)
- ERCP & Cholecystectomy	38	35 (92%)	35 (92%) 33 (92%)	34 (90%)
- OTC Cholecystectomy	36	33 (92%)		32 (89%)
lumber participated previous reparticipating				
- Biliary emergency	25	22 (88%)	22 (88%)	22 (88%)
- ERCP	24	23 (96%)	23 (96%)	23 (96%)

Table 8.1.2: Demonstrates the number of patients approached and the numbers participating in the study. There were three surgical consultants admitting biliary emergencies and cholecystectomy patients, and one medical consultant undertaking the ERCP's. The lower numbers of biliary colic and cholecystectomy patients with full data sets is due to being discharged prior to 24 hours (laparoscopic group). There was no difference in numbers recruited to the biliary emergency and to the cholecystectomy only, cholecystectomy and recent ERCP and cholecystectomy and on table cholangiogram groups, for each of the surgical consultants.

Reason for not participating in the study

	Biliary emer	ERCP	Cholecystectomy					
			Only		Recent ERCP		With OTC	
			Lap	Open	Lap	Open	Lap	Open
Suspicion about any type of research	1	0	3	1	1	0	0	1
Needle phobia	2	0	4	3	1	0	0	0
Language problems	2	0	2	0	0	0	0	0
No reason stated	3	1	5	3	1	0	1	1
Discharged prior to 24 hours	19	0	5	0	0	0	0	0
Non gall stone pancreatitis	1	0	0	0	0	0	0	0
Malignant disease	0	1	1	0	0	1	0	0
Switched approach to chole.	0	0	0	5	0	0	1	0
Anaesthetically unfit	0	0	1	0	0	0	0	0
Consent withdrawn p way through study	1	0	0	1	0	0	0	0

Table 8.1.3: Demonstrates the reason why patients declined to participate in the study. The laparoscopic patients who were discharged prior to 24 hours were, with consent contacted at home and asked to score their pain at 24 hours. All their available data was excluded in the analysis, as they were potentially different to the in-patients, and the comparative cytokine data was not collected at 24 hours (four were operated on by consultant 1). Their pain scores were retrospectively compared to the patients not discharged, and no difference was detected. The malignant disease was a malignant biliary stricture found on ERCP, cholangiocarcinoma in a laparoscopic patient, and liver metastasis from a colonic malignancy in the open group. These patients' data was excluded. In the surgical patients there were patients who were anxious about completing particularly the pain scores and quality of life data in case it lead their operation being cancelled. Where consent was withdrawn part way through the study the patients were not included in the main analysis. Emerg. – emergency, lap. – laparoscopic, chole. – cholecystectomy.

8.2 Biliary emergency, ERCP and cholecystectomy patient demographics

Table 8.2.1 illustrates the patient demographics at enrolment for the biliary emergency group. Patients admitted multiple times with biliary emergencies were only enroled once in the study. Patients did participate in multiple arms of the study when they were readmitted for ERCP or cholecystectomy, but their data was kept separately and matched up at the end of the study.

Patients admitted in the biliary emergency group

	Biliary emergency n = 78			
	Biliary colic	**Acute cholecystitis**	**Pancreatitis**	**Obstructive jaundice**
Number	23	27	13	15
First episode of pain	6 (26%)	13 (48%)	4 (31%)	2 (13%)
Pain previously not admitted	9 (39%)	9 (33%)	8 (62%)	12 (80%)
Previous admissions	8 (35%)	5 (19%)	1 (8%)	1 (7%)
Length of symptoms (in hours ± SD)	9 ± 6	20 ± 8	28 ± 10	29 ± 8
Analgesia use (mg previous 24 hours)	23.5 ± 4.5	23.5 ± 4.5	23.5 ± 4.5	23.5 ± 4.5
ERCP this admission	0	0	10	15

Table 8.2.1: Demonstrates the patient history of the patients admitted with biliary emergencies who completed the study. The biliary colic group has had more previous admissions than the other groups. Analgesia use was calculated in morphine equivalent dose for the previous 24 hours to enrolment (Table 6.4.5 page 140). For the biliary colic group 70% had received paracetamol, whereas 85% of the other groups had received paracetamol.

Fifty-two patients having their first ERCP for benign biliary disease were included in the study, but patients' enroled in the study who had a second ERCP or cholecystectomy at the same admission were followed. Patients who had ERCP as part of their admission with biliary emergency were not included in this arm, only the biliary emergency arm. Failed ERCP patients were all admitted for at least one night, antibiotics continued and ERCP repeated within 48 hours of the first procedure. Five of the unsuccessful ERCP patients went for emergency cholecystectomy with on table cholangiogram.

As would be expected patients admitted for ERCP were older ($p = 0.044$) as the frequency of common bile duct stones increases with age. Significantly more males were admitted for both elective ERCP and underwent emergency ERCP for obstructive jaundice or pancreatitis ($p = 0.045$ and $p = 0.039$ respectfully).

Sphincter diverticulum or oedema was significantly more common in abandoned procedures that went on to have repeat ERCP or cholecystectomy. ($p = 0.048$). Sphincter canulation being unsuccessful in three cases all with sphincter oedema. Of the thirteen abandoned cases seven were performed by a non-consultant grade. The six failed ERCP performed by a consultant five went on to have cholecystectomy at this admission. All second attempt ERCP's were performed by a consultant. Table 8.2.2 demonstrates the patient flow in this arm of the study.

Of the 37 successfully completed first attempt ERCP's, two represented with biliary emergency symptoms. For the first a decision had been made not to perform a cholecystectomy due to her age (91 years) and co-morbidities. She represented with pancreatitis eight weeks later at another hospital and died, she was the only mortality within the study period. The second patient had a difficult but completed first attempt ERCP, the procedure was rated difficult as there was a significant amount of inflammation making the sphincterotomy more difficult. This patient represented with biliary obstruction, and had a repeat ERCP, which was rated as difficult and had a stent placed and early cholecystectomy.

Patients admitted in the ERCP group

	ERCP group n = 52	
	Successfully completed at first attempt	**Unsuccessfully completed at first attempt**
Number	39	13
Reason for ERCP **- Pancreatitis** **- Obstructive jaundice**	 13 (33%) 26 (67%)	 4 (31%) 9 (69%)
Difficultness & length of first ERCP (mins) **- straight forward** **- moderate** **- difficult completed** **- abandoned**	 20 – 37 ± 3 15 – 45 ± 4 4 – 58 ± 5 13 – 62 ± 6	 13 – 68 ± 6
Raised amylase after first ERCP	1 went onto develop sepsis	1 went onto have cholecystectomy and developed sepsis
Sepsis after first ERCP **- details**	2 1 raised amylase and chest infection 1 positive blood cultures	0
Cholecystectomy after first failed ERCP **- approach (LC / OC)** **- sepsis**	0	5 (all with OJ) 3 LC / 2 OC 1 LC / 2 OC +ve bile & blood culture
ERCP completed at second attempt	0	8
Difficultness & length of second ERCP (mins) **- difficult completed**	0	 8 – 67 ± 10
Raised amylase after second ERCP	0	2 both ERCP successfully completed
Sepsis after second ERCP **- details**	0	3 3 +ve blood cultures

Table 8.2.2: Demonstrates the outcome of intervention in the ERCP group. The positive blood cultures were all for *E.coli*. All the patients who went for emergency cholecystectomy had intra-operative bile cultures sent, four were positive (one laparoscopic cholecystectomy (LC) patients was negative). One laparoscopic cholecystectomy patient had positive bile cultures but was never diagnosed by the clinical team with sepsis; their cytokine concentration was seen to increase post procedure. LC – laparoscopic cholecystectomy, OC – open cholecystectomy, OJ – obstructive jaundice, mins – minutes ± SD, +ve - positive.

General practitioner referrals for cholecystectomy, to Nottingham City Hospital, were allocated from a pool to the general surgeons out patient clinics. Outpatient department clerks allocated patients to consultant's who performed cholecystectomy, on the basis of available slots. The second smaller source was patients brought back for review following their emergency admission; the consultant they had previously been admitted under saw these patients. Elective clinic slots were greater than four weeks after emergency admission. Emergency cholecystectomy procedures were excluded from this group.

Three consultants participated in this study as they shared theatre sessions, and anaesthetists, as well as junior staff. None had a specialist interest in hepatobiliary surgery, and all had at least 18 months experience as a consultant. Two performed laparoscopic surgery with conversion rates of 1% for elective procedures, and were proficient in converting to the open procedure. No laparoscopic cases were converted to open during the study. One surgeon only performed open cholecystectomy.

Based on a power calculation **(**Chapter 7.2 page 154**)**, we aimed to recruit 102 laparoscopic approach (51 each consultant) and 51 open cholecystectomy patients to demonstrate a difference between the two approaches. The complication rate was based upon the rate of complications in the previous two years for each of the consultant's, data gathered by Nottingham City Hospital at patient discharge.

In total I recruited 170 patients undergoing laparoscopic cholecystectomy and 81 open cholecystectomy patients, enrolment continued to study closure at six months, surgery was performed over an eight month period. When recruiting patients I tried to recruit consecutive patents, but in so doing I had a group of patients who had just cholecystectomy (n = 125 laparoscopic approach, and n = 60 open approach patients). There was also a group of patients who had had a recent ERCP (within a year) and a group who had an on-table cholangiogram (OTC) with their cholecystectomy (n = 45 laparoscopic approach and n = 21 open approach). Analysing the cytokines of the recent ERCP and OTC group, it appeared there was a different cytokine response to those just undergoing cholecystectomy. As I had recruited adequate numbers of patients just undergoing cholecystectomy to satisfy the power calculation, I decided to analyse the ERCP or OTC group separately and treat these patients as a separate group for analysis.

Analysis of demographics across the arms of the study demonstrated variation in both age and the gender ratio as demonstrated in Table 8.2.3. For the patients undergoing surgery they were listed from the consultant clinic they attended. The open approach consultant offered his patients referral to a consultant performing the procedure laparoscopically. Six patients asked to switch to laparoscopic cholecystectomy; this was in line with the consultant's own collected data of 10% switching. Five patients switching were female and the oldest was 42 years old. Although a full set of data was collected for theses patients, they were not included in the main analysis in case there preference in approach influenced their response in questionnaires. Their data is not presented unless otherwise stated.

The number of episodes of sepsis is also given for each group. In the group with pancreatitis, five had a raised white cell count and were treated for infection. But only three had a definite source of infection, and had two raised SIRS markers, and were diagnosed with sepsis the other two had no positive culture and one raised SIRS marker not fulfilling the definition of sepsis. For the ERCP group forty-seven patients just had an ERCP, and five developed sepsis. Five patients underwent an ERCP and emergency cholecystectomy, three of this group developed sepsis (Table 8.2.2).

Table 8.2.3 demonstrates the demographic data about each arm of the study and includes the data about the fifteen healthy controls that were recruited for comparison to the patients with biliary disease. This control group was a separate group of patients to the controls in the pilot study, as the timing of taking samples was changed from the pilot study.

Demographics of the patients participating in each arm of the study

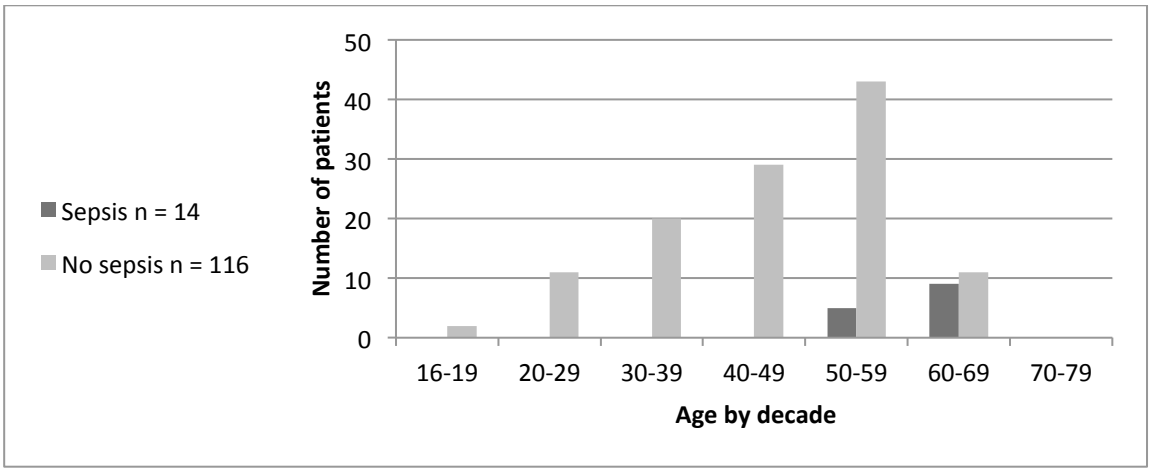
	Number	Gender M:F (% male)	Mean age (years ± SD)	Mean age M : F	Cases of sepsis (% of group with sepsis)
Biliary emerg.					
- BC	23	4 : 19 (17%)	34 ± 9	40 : 32	0 (0%)
- AC	27	6 :21 (22%)	41 ± 12	50 : 40	0 (0%)
- Panc.	13	5 : 8 (38%)	52 ± 7	53 : 51	3 / 2 * (23% / 15%)
- OJ	15	6 : 9 (40%)	54 ± 10	55 : 54	1 (7%)
ERCP - only	47	18 : 29 (38%)	57 ± 10	58 : 55	5 (11%)
- ERCP emerg. chole	5	3 : 2 (60%)	59 ± 3	61 : 60	3 (60%)
Lap. chole.	125	26 : 99 (21%)	49 ± 16	50 : 42	12 (10%)
Open chole.	60	23 : 37 (46%)	54 ± 12	53 : 60	8 (13%)
ERCP lap.	23	5 : 18 (28%)	56 ± 9	57 : 54	4 (17%)
ERCP open	11	5 : 6 (45%)	57 ± 8	57 : 60	2 (18%)
OTC lap.	22	5 : 17 (23%)	56 ± 8	57 : 55	5 (23%)
OTC open	10	6 : 4 (60%)	59 ± 9	59 : 61	3 (30%)
Healthy controls	15	5 : 10 (33%)	50 ± 15	54 : 49	0 (0%)

Table 8.2.3: Demonstrating the demographics of the patients participating in each arm of the study. The number of episodes of sepsis in each group is also shown. For the ERCP group forty-seven just under went an ERCP, and five of these develop post procedural sepsis. Another five of the ERCP patients under went emergency cholecystectomy, after a failed ERCP, and three of this group developed sepsis. M – male, F – female, SD – standard deviation, biliary emerg – biliary emergency, BC – biliary colic, AC – acute cholecystitis, Panc. – pancreatitis, OJ – obstructive jaundice, ERCP emerg chole – emergency cholecystectomy after failed ERCP, Lap. Chole. – Laparoscopic cholecystectomy, ERCP lap. – recent ERCP and laparoscopic cholecystectomy. * Five patients in the biliary emergency – pancreatitis group were treated for sepsis, but only three fulfilled the diagnosis of sepsis.

The mean age in those developing post-procedural sepsis was older than the patients not developing sepsis, for the elective ERCP arm and the cholecystectomy patients. For the cholecystectomy patients this was regardless as to whether they had had a recent ERCP or underwent an OTC. Figure 8.2.4 demonstrates the age of the patients diagnosed with sepsis in each of the arms of the study. The frequency of sepsis increased with age in all arms of the study. Kishimoto's team (2009) reported lower cytokine concentrations in their older patients. This was not seen in this study, but there was a tendency towards those older patients developing sepsis to require a higher level of care for their sepsis. Whether this masked the lower cytokine concentration is unclear, but the concentration at enrolment was not significantly lower.

Overall more males developed sepsis, but analysis by group, this only reached significance in the elective ERCP arm, laparoscopic ERCP patients, open ERCP patients, and laparoscopic on table cholangiogram patients. However the numbers are small and these were subgroups for which power calculations for recruitment was not performed ($p = 0.004$). The male patients did require a higher level of care. The demographics are demonstrated in Table 8.2.5.

Age of the patients diagnosed with sepsis. Biliary emergency and ERCP (upper diagram) and cholecystectomy patients (lower diagram)



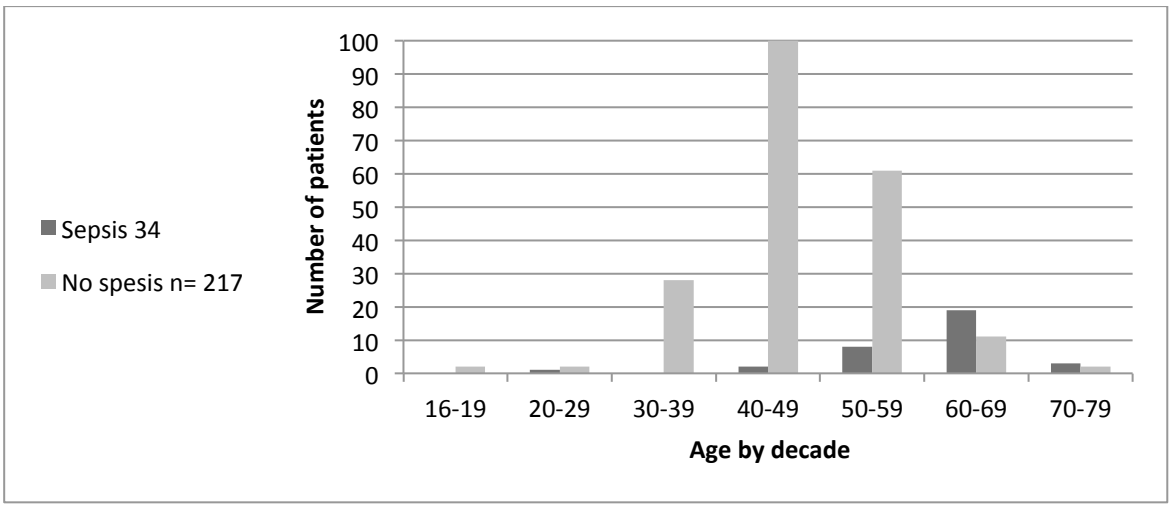


Figure 8.2.4: Demonstrates the age of the patients by decade and the number diagnosed with sepsis and those not diagnosed with sepsis. The biliary emergency and ERCP patients are the upper figure and the patients undergoing cholecystectomy are shown in the lower figure. This clearly shows the frequency of sepsis increases with age in all the arms of the study.

Demographics of the patients developing sepsis in each arm

	Cases of sepsis	Mean age of patient with sepsis (age ± SD)	Gender M : F (% of gender developing sepsis)	Procedure (Enrolment) to diagnosis (hours)	Level of care ITU : HDU : Ward
Biliary emerg.					
- BC (23)	0	-	-	-	-
- AC (27)	0	-	-	-	-
- Panc(13)	3 / 2 *		3 : 2 (60 : 25%)		0 : 1 : 4
HDU		66	1 : 0	9	
Ward		61 ± 4	2 : 2	6 ± 2	
- OJ (15)	1	69	1 : 0 (17 : 0%)	6	0 : 0 : 1
ERCP (52)					
- Only ERCP (47)	5	60 ± 4	4 : 1 (22 : 7%)	26 ± 6	0 : 0 : 5
- Chole OTC (5)			2 : 1 (60 : 30%)		0 : 1 : 2
HDU	3	69	1 : 0	57	
Ward		64 ± 5	1 : 1	50 ± 4	
Lap. chole. (125)	12		5 : 7 (19 : 7%)		4 : 3 : 5
ITU		63 ± 9	3 : 1	32 – 60	
HDU		56 ± 6	2 : 1	28 – 36	
Ward		50 ± 19	0 : 5	26 - 36	
Open chole. (60)	8		5 : 3 (21 : 8%)		2 : 3 : 3
ITU			2 : 0	2 – 33	
HDU		64 ± 6	2 : 1	20 – 25	
Ward		58 ± 14	1 : 2	20 - 28	
		54 ± 5			
ERCP lap. (23)	4		3 : 1 (60 : 6%)		0 : 1 : 3
HDU		65	1 : 0	32	
Ward		60 ± 3	2 : 1	29 – 36	
ERCP open (11)	2	60 ± 4	2 : 0 (40 ; 0%)	24 – 29	0 : 0 : 2
OTC lap. (22)	5		3 : 2 (50 : 12%)		0 : 1 : 4
HDU		64	1 : 0	31	
Ward		60 ± 3	2 : 2	28 - 38	
OTC open (10)	3	65 ± 3	3 : 0 (50 ; 0%)	26 - 29	0 : 0 : 3

Table 8.2.5: The table demonstrates the demographics of the patients diagnosed with sepsis, the time to diagnosis and the level of post-operative care they required. The ERCP patients who did not have a cholecystectomy had a wide time to diagnosis because two were diagnosed after the first ERCP and three were diagnosed after the repeat ERCP. The patients who had a cholecystectomy after ERCP were diagnosed after their cholecystectomy. As can be seen the men tend to require a higher level of care. M – male, F – female, SD – standard deviation, biliary emerg – biliary emergency, BC – biliary colic, AC – acute cholecystitis, Panc. – pancreatitis, OJ – obstructive jaundice, Lap. Chole. – Laparoscopic cholecystectomy, ERCP lap. – recent ERCP and laparoscopic cholecystectomy. ITU – intensive care unit, HDU – High dependency unit. * Five pancreatitis patients were treated for sepsis, three had positive cultures.

Table 8.2.6 demonstrates the co-existing co-morbidities and history of smoking of those developing sepsis to the rest of the patient group. The patients developing sepsis were more likely to have respiratory disease. These patients had been prescribed steroids for their respiratory disease, both inhaled and oral steroids, more frequently than the patients not developing sepsis who had respiratory disease. Respiratory disease and smoking was particularly prevalent amongst the laparoscopic approach patients developing sepsis ($p = 0.045$). Diabetes was also more prevalent amongst the laparoscopic approach patient ($p = 0.046$). The group developing sepsis had longer procedures, the combination of the co-morbidity, the prolonged pneumoperitoneum restricting ventilation and the majority of laparoscopic approach patients not receiving antibiotics, potentially predisposes to developing sepsis. In contrast all the open approach patients received antibiotics. This group of patients also received patient controlled analgesia, which potentially allowed them to be more comfortable and breath more deeply. The lower rate of cardiovascular disease amongst the laparoscopic approach patients, maybe due to the group being younger, than the other groups.

Co-existing co-morbidities in those developing sepsis

	Cases of sepsis	Co-existing co-morbidities						Smoker (number in group)	
		Resp. (number in group with problem)		Cardiac (number in group with problem)		Diabetes / endocrine (number in group with problem)			
		No. in grp	No. with sepsis	No. in grp	No. with sepsis	No. in grp	No. with sepsis	No. in grp	No. with sepsis
Biliary emerg.									
- BC (23)	0	1	0	0	0	0	0	9	0
- AC (27)	0	1	0	0	0	1	1	4	0
- Panc (13)	3 / 2 *	2	2	1	1	0	0	1	1
- OJ (15)	1	1	1	0	0	1	1	1	1
ERCP (52)									
- Only ERCP (47)	5	4	3	0	0	2	1	4	1
- Chole OTC (5)	3 / 4 **	2	2	1	0	0	0	1	1
Lap. chole. (125)	12	11	7	3	0	4	2	10	3
Open chole. (60)	8	6	2	2	1	3	1	5	1
ERCP lap. (23)	4	2	1	1	0	0	0	1	1
ERCP open (11)	2	1	1	0	0	0	0	0	0
OTC lap. (22)	5	4	3	0	1	1	1	1	1
OTC open (10)	3	0	0	0	0	0	0	0	1

Table 8.2.6: Demonstrates the co-morbidities of the group developing sepsis. The figure in brackets is the total number in the group with that co-morbidity. As can be seen many of the laparoscopic approach patients developing sepsis have respiratory problems or are smokers. Resp. – respiratory, biliary emerg – biliary emergency, BC – biliary colic, AC – acute cholecystitis, Panc. – pancreatitis, OJ – obstructive jaundice, Lap. Chole. – Laparoscopic cholecystectomy, ERCP lap. – recent ERCP and laparoscopic cholecystectomy.

* Five pancreatitis patients were treated for sepsis, three had positive cultures.

** Four emergency cholecystectomy had positive cultures, three had abnormal SIRS and were recognised by the medical team as having sepsis, all had received antibiotics at induction of anaesthesia.

The causes of sepsis are documented in Table 8.2.7. The timing of the diagnosis of sepsis was when the clinical team in the first documented it in the hospital notes. Time was taken from admission or the ERCP or surgical intervention. The cause of sepsis was taken from the clinical notes, microbiology results, imaging and findings at second procedures. There was no mortality from sepsis, or during the study, although one ERCP patient was admitted elsewhere, after the end of the study, with gallstone pancreatitis and died. This lady at 91 years had been deemed to frail for surgery. Secondary infections, documented as a second diagnosis, occurred in six patients, five cared for in ITU and one cared for in HDU all in the surgical group.

Two or more SIRS markers were elevated in all those developing sepsis at the time of diagnosis of sepsis, and in 18 of the acute cholecystitis patients at the time of admission. Ten patients had one SIRS marker elevated but never were diagnosed with infection, predominantly in the ERCP arm or the surgery and OTC group. These patients had no documented evidence of infection, except the one ERCP patient who underwent emergency cholecystectomy and had a positive bile culture but was not documented by the clinical team as developing sepsis. Two further patients with pancreatitis had an elevated white cell count, without overt signs of sepsis. All those with positive blood cultures had positive bile cultures.

Amongst the surgical patients sepsis was diagnosed in all the three groups of patients undergoing cholecystectomy, and for both approaches. Proportionally sepsis was more common after OTC (9.6%, 17% and 22% for the laparoscopic approach and 13.2%, 18.2% and 30% for the open approach), but the numbers are small.

As can be seen from Table 8.2.7 the causes of sepsis are varied but those developing respiratory sepsis had pre-existing respiratory disease. The majority of laparoscopic approach patients did not receive antibiotics and did not develop sepsis. Where there were pre-existing co-morbidities, particularly respiratory disease and diabetes, these patients potentially may benefit from targeted prophylactic antibiotics at surgery to reduce the rate of sepsis.

Causes of sepsis in each arm of the study

	Cases of sepsis	Positive bile cultures	Positive blood cultures	Chest infection	Other
Biliary emerg.	ITU - 1	-	-	-	1 (necrotizing pancreatitis)
- Panc(13)	Ward - 2	-	1	1 (RD)	-
- OJ (15)	1	-	1	-	
ERCP (52)	5	-	3 (1 RD, 1 D)	2 (2 RD)	-
- Chole OTC (5)	HDU - 1	1 (RD)	1	-	-
	Ward - 4	4	3	-	-
Lap. chole. (125)	ITU – 4	-	-	1 (clip dislodged cystic duct 2nd operation RD)	1 (Colonic perforation RD) 1 (Small bowel perforation) 1 (CBD injury)
	HDU – 3	-	-	3 (2 RD)	-
	Ward - 5	2 (2 D)	2	3 (3 RD)	-
Open chole. (60)	ITU – 2	-	-	1 aspiration pneumonia (RD)	1 clip dislodged cystic duct 2nd operation wound infection
	HDU – 3	3 (RD)	3	-	-
	Ward - 3	3 (D)	3	-	-
ERCP lap. (23)	ITU - 1	-	-	1 (RD)	-
	Ward - 3	1	1	2 (2 RD 1)	-
ERCP open (11)	2	-	-	2 (1 RD)	-
OTC lap. (22)	HDU - 1	-	-	1 (RD)	-
	Ward - 4	4 (2 RD)	4 (2 RD)	-	-
OTC open (10)	3	3 1 T- tube dislodged	3	0	0

Table 8.2.7: Demonstrates the cause of sepsis. The abbreviations are as previous tables for a full explanation of the table please see text. RD – respiratory disease, D – diabetes.

In the biliary emergency patients the acute cholecystitis patients were prescribed antibiotics at admission. The patients with pancreatitis and obstructive jaundice patients were prescribed antibiotics when clinical infection was documented. The ERCP patients received a prophylactic dose of antibiotics prior to the procedure, this was continued based on clinical assessment. This was predominantly the difficult but completed and failed first ERCP patients'.

The open cholecystectomy patients received a dose of antibiotics at induction, they were either continued if clinical concerns, or if the patient underwent OTC. Table 8.2.8 documents the patients receiving antibiotics. For those developing sepsis the six cholecystectomy and the three cholecystectomy with OTC patients who developed biliary and haematological sepsis all had a course of antibiotics prescribed from surgery. As did the patient who developed aspiration pneumonia following open cholecystectomy. Antibiotics had been stopped and were recommenced at the time of diagnosis of sepsis for the three patients developing a chest infection and one where the cystic duct clip was dislodged after open surgery.

The laparoscopic approach patients only received antibiotics at the time of surgery if there was clinical concern, this was usually for a distended thickened gall bladder where bile with aspirated. The antibiotics were continued based on the surgeon's request. As can be seen in Table 8.2.8 the laparoscopic approach patients who had had recent ERCP or had OTC were more likely to receive antibiotics. Only two of the laparoscopic approach patients developing sepsis had received antibiotics, even where bile had been sent for culture or OTC was performed. These two patients had received a course of antibiotics. This omission of antibiotics potentially increased the number of episodes of sepsis. No bile was sent for culture unless the patient underwent cholecystectomy.

The patients who received antibiotics at induction and a course of antibiotics in each arm

	Cases of sepsis	Received induction antibiotics	Patients with sepsis receiving induction antibiotics	Positive bile culture	% positive bile culture receiving course antibiotics
Biliary emerg.					
- BC (23)	0	0 (0%)	-	-	-
- AC (27)	0	27 (100%)	-	-	-
- Panc (13)	3	5 (39%)	3 (100%)	-	-
- OJ (15)	1	9 (60%)	1 (100%)	-	-
ERCP (52)					
- only ERCP (47)	5	16 (31%)	5 (100%)	-	-
- Chole OTC (5)	3 / 4	5 (100%)	4 (100%)	4	100%
Lap. chole. (125)	12	9 (7%)	1 (9%)	18	50%
Open chole. (60)	8	51 (98%)	8 (100%)	6	100%
ERCP lap. (23)	4	4 (17%)	0 (0%)	5	80%
ERCP open (11)	2	11 (100%)	2 (100%)	2	100%
OTC lap. (22)	5	18 (82%)	1 (20%)	5	20%
OTC open (10)	3	10 (100%)	3 (100%)	3	100%

Table 8.2.8: All of the acute cholecystitis, ERCP and open cholecystectomy patients received antibiotics at the commencement of the procedure or admission. The other patients only received doses based on clinical assessment. The table documents those who received a course of antibiotics; this was based predominantly on concern about positive bile cultures based on findings in theatre. Biliary emerg – biliary emergency, BC – biliary colic, AC – acute cholecystitis, Panc. – pancreatitis, OJ – obstructive jaundice, Lap. Chole. – Laparoscopic cholecystectomy, ERCP lap. – recent ERCP and laparoscopic cholecystectomy.

8.3 Significant pain experienced pain group

The visual analogue score is divided into three groups; mild, significant and severe pain. Table 8.3.1 demonstrates how the scores are divided between groups. Patients were placed in pain groups based upon their pain score at enrolment.

Prior to commencing the study a personal observation was that there was a group of patients who experienced more pain after surgery, but did not develop sepsis. In the pilot study, there was within the significant pain group, two distinct groups of patients, based upon their quality of life (QoL) scores throughout the study. The one group scored their quality of life similar to the mild pain group, and was denoted as 'Significant pain manageable'. The other group, within the significant pain group scored their quality of life as worse, for distinction they were termed 'Significant pain experienced' (Table 8.3.1).

The difference between the patients QoL scores in the significant pain group at enrolment will be discussed further in the pain chapter (Chapter 11). It is noted here to highlight the different demographics between the groups, in terms of the number of significant pain experienced patients in each group. Although these patients scored their pain similar to the group developing sepsis, only three of the group developed sepsis across the study. These three patients had cytokine concentrations similar to the other patients developing sepsis. The other patients in the significant pain experienced group had cytokine concentrations not significantly different to the significant pain manageable patients in the same arm of the study.

Identifying the separate group of the significant pain experienced group is important because the study aimed to identify sepsis early using patients pain score. The significant pain experienced group and the patients with sepsis scored their pain similarly, but the former did not develop sepsis. By using the VAS and quality of life scores at enrolment we can potentially identify the patients developing sepsis and initiate treatment earlier.

Pain groups based on the VAS and quality of life questionnaires

VAS pain score	Name of group	Quality of life score
< 4	Mild pain	Rate QoL high
≥ 4 - < 7	Significant pain manageable	Rate QoL as mild group
	Significant pain experienced	Rate QoL poor
≥ 7	Severe pain	Rate QoL intermediate between mild and significant pain experienced

Table 8.3.1: Demonstrates the different pain groups. The VAS is usually divided into three groups, mild, significant and severe pain. When we compared the groups, the significant pain group was composed of two distinct groups which could be separated based upon the patients quality of life scores, this will be discussed further in the next section and Chapter 11. There were significant pain experienced patients in each arm of the study. The severe pain group post operatively scored their quality of life similar to the mild and significant pain manageable group. QoL – quality of life.

In the pilot study I examined how patients pain scores changed in each arm of the study, initially performing this in the cholecystectomy patients. I realised that within the significant pain experienced group there was a group of patients whose pain score decreased post operatively and a group whose pain score remained unchanged or increased. Studying these patients further I realised they scored their QoL and Hospital anxiety and depression (HAD) score differently. The group whose pain score did not fall scored their quality of life and Hospital anxiety and depression score poorer at enrolment and throughout the follow up period. Whereas the group whose pain scores fell, scored their quality of life similar to the mild group. Like the severe pain group the mild and this group of significant pain group increased their quality of life following surgery. The same split in the significant pain group is seen in the biliary emergency patients and in the elective ERCP group.

From the pilot study this split was within the pain groups was only seen in the significant pain group. With the main study with bigger numbers of patients I wanted to know if the other pain groups displayed this dichotomy. In particular the severe pain group, because the significant pain experienced group appeared to experience a lot of pain. I therefore plotted out the change in pain score for every patient over the first 24 hours of the study. Figure 8.3.2 and 8.3.3 is for the patients who underwent cholecystectomy alone by both approaches. The other groups had similar plots, the larger number of patients in this group demonstrate the findings most easily. Patients in the same pain group tend to behave similarly for the mild and severe pain groups, but the smaller numbers in the severe pain group make the clustering appear more open.

For the significant pain group the distribution was bi-modal demonstrated in Figure 8.3.3. Comparison of the significant pain group patients' whose pain score fell post post-operatively, and the group whose pain score did not fall, demonstrated that the patients whose pain score fell, scored their quality of life significantly better than the group whose pain score increased.

The patients developing post procedural sepsis is excluded as their pain scores all increased following the procedure, as will be discussed in Chapter 10. Although the mild pain groups pain scores increased following cholecystectomy, like the

significant pain experienced group. The mild pain group then decreased after the early post operative period unlike the significant pain experienced group whose pain scores continued to increase to 24 - 48 hours. In the mild pain group this is likely to be post procedural pain, and their quality of life scores mirrored those of the significant pain manageable group.

There was no difference in cytokine concentration between the two groups in the significant pain group as will be discussed in Chapter 9. None of the other pain groups were seen to have these two groups of patients, not even the severe pain group, why this was unclear. The significant pain experienced group did receive more analgesia and whether this is the reason their pain was significant and not severe is a possibility.

Change in the pain score from enrolment to 24 hours in the mild pain group (upper) and severe pain group (lower)

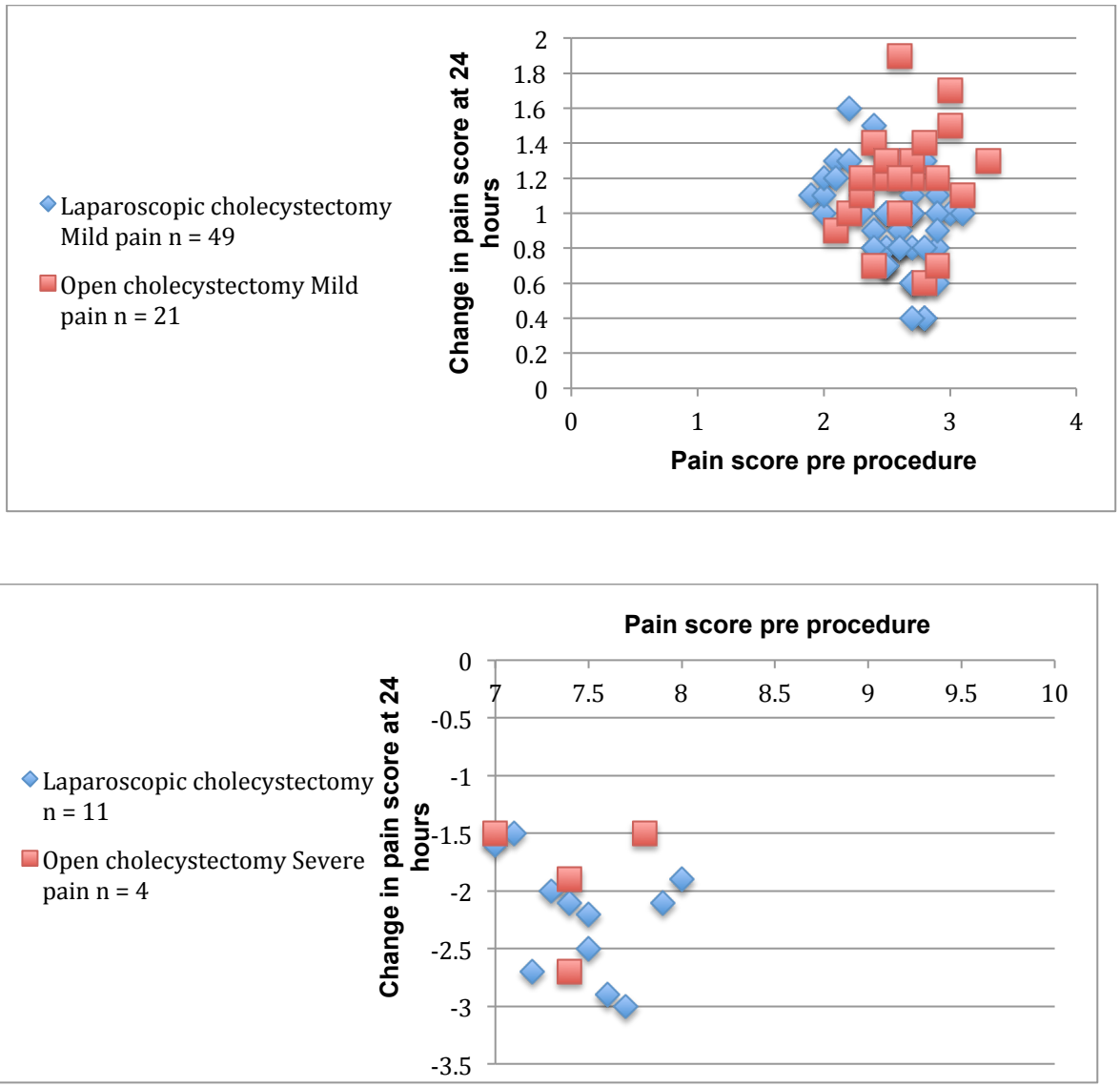


Figure 8.3.2: Demonstrates the change in pain score from enrolment to 24 hours after cholecystectomy. If the pain score at 24 hours decreased from the pre-operative score then it would be represented as a negative change and vice versa. The mild and the severe pain group are shown for comparison to Figure 8.3.3 the significant pain group. The patients developing sepsis are excluded for ease of demonstration.

Change in the pain score from enrolment to 24 hours in the significant pain group

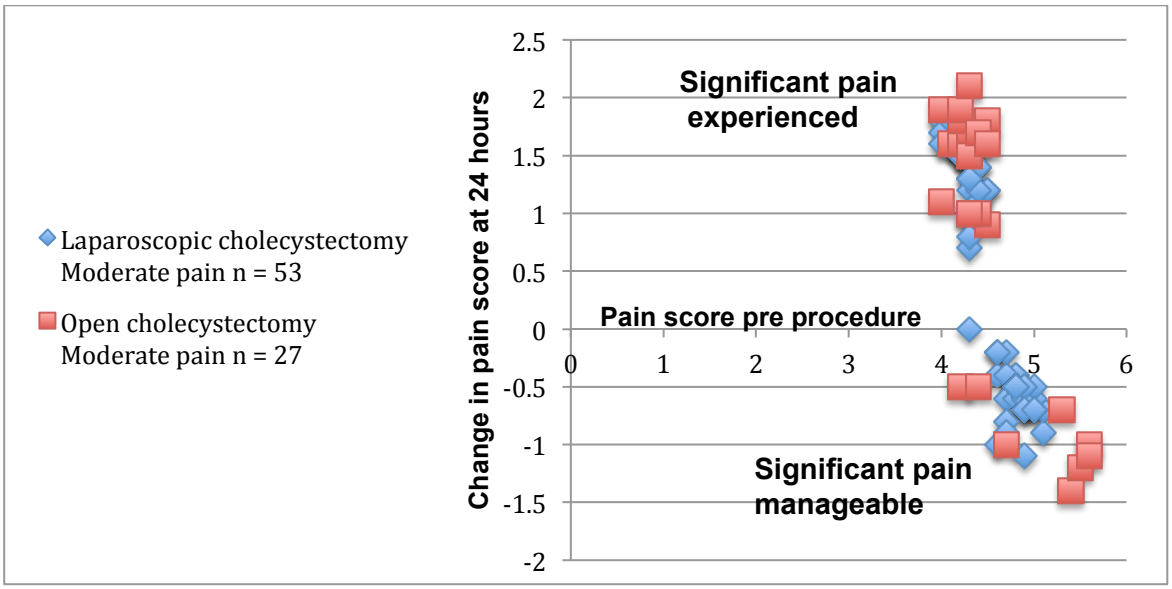


Figure 8.3.3: Demonstrates the change in pain score from pre-operatively to 24 hours after cholecystectomy, for the patients in the significant pain group. If the pain score at 24 hours decreased from the pre-operative score then it would be represented as a negative change and vice versa. The mild and severe pain group is shown for comparison in Figure 8.3.2. The patients developing sepsis are excluded for ease of demonstration. The patients in the significant pain group whose pain score decreased post-operatively were termed 'Significant pain manageable' and those whose pain score increased were termed 'Significant pain experienced'.

Biliary emergency group

The analgesia the patients received varied significantly different between the groups. Comparing pain scores in the biliary emergency group demonstrated at 24 hours those with pancreatitis and obstructive jaundice had received significantly higher morphine equivalent dose ($p = 0.003$) than the biliary colic and cholecystitis patients, if the significant pain experienced pain group were excluded. Including the significant pain experience group the difference in morphine equivalent doses at 24 hours was lost ($p = 0.31$), as the significant pain experienced group received equivalent doses of morphine to the pancreatitis and obstructive jaundice patients.

Patients admitted with a diagnosis of pancreatitis and obstructive jaundice all scored their pain in the severe pain group at enrolment. I was concerned that we were missing patients who would fit the description of the significant pain experienced group, and we were missing them because their pain at admission placed them in the severe pain group. As will be discussed later in the Chapter 11, the patients' quality of life and Hospital Anxiety and Depression scores were lower in the severe pain group, than the biliary colic and acute cholecystitis patients in the significant pain manageable and mild pain group; their scores were not as low as the patients who were in the significant pain experienced group. Neither was there the dichotomy in pain or quality of life scores.

A potential explanation for the absence of the significant pain experienced patients in the pancreatitis and obstructive jaundice group, is the patients in these groups have had their pain for significantly longer than the patients in the significant pain experienced group (Table 8.4.8). Although the length of symptoms does rely upon patients recall of the commencement of right upper quadrant pain, which can't be independently verified. Patients in the obstructive jaundice and pancreatitis groups also tend to be older, than the significant pain experienced group. Patients in the significant pain experienced group were significantly younger than all the patients having the same procedure or admitted with biliary emergencies ($p = 0.02$). There was a trend towards them being female but this was not significant. Table 8.3.4 demonstrates the spread of the pain groups across the arms of the study.

Number of patients in each pain group at enrolment into the study

	Cases of sepsis	Pain score			
		Number in mild pain VAS <4	Number in significant pain manageable VAS ≥ 4 - <7	Number in significant pain experienced VAS ≥ 4 - <7	Number in severe pain VAS ≥ 7
Biliary emerg. (78)					
- BC (23)	0	3 (13%)	3 (13%)	17 (74%)	0
- AC (27)	0	7 (26%)	10 (37%)	10 (37%)	0
- Panc(13)	3	0	0	0	13 (100%)
- OJ (15)	1	0	0	0	15 (100%)
ERCP (52)					
- First ERCP (39)	2	0	19 (49%)	4 (10%)	16 (41%)
- Second ERCP (8)	3	0	1 (12.5%)	1 (12.5%)	6 (75%)
- Chole OTC (5)	3	0	0	1 (20%)	4 (80%)
Lap. chole. (125)	12	53 (42%)	36 (29%)	22 (18%)	14 (11%)
Open chole. (60)	8	24 (40%)	13 (22%)	17 (28%)	6 (10%)
ERCP lap. (23)	4	0	16 (70%)	3 (13%)	4 (17%)
ERCP open (11)	2	0	6 (55%)	2 (18%)	3 (27%)
OTC lap. (22)	5	0	14 (64%)	3 (14%)	5 (23%)
OTC open (10)	3	0	6 (60%)	2 (20%)	2 (20%)

Table 8.3.4: Demonstrates the number of patients in each pain group in each arm of the study. Patients were placed in a pain group based upon their enrolment pain score, and their quality of life scores. Percentage in the group given in brackets. The ERCP patients are split into first and second ERCP to highlight how few patients in the significant pain experienced group had an ERCP because they tended to have surgery earlier in the course of their disease. The main number in each pain group is given at the top for of each column for the ERCP and biliary emergency patients. Biliary emerg – biliary emergency, BC – biliary colic, AC – acute cholecystitis, Panc. – pancreatitis, OJ – obstructive jaundice, Lap. Chole. – Laparoscopic cholecystectomy, ERCP lap. – recent ERCP and laparoscopic cholecystectomy.

For the biliary emergency patients it is very striking that the significant pain experienced group are predominantly represented in the biliary colic and the acute cholecystitis group. Caution has to be exercised particularly in interpreting the biliary colic data, as we enroled eighteen more patients into the biliary colic group, but they were all discharged prior to 24 hours (Figure 8.1.2). With the criteria used in this study all of the patients discharged were in the significant pain manageable group, and those who stayed in were the significant pain experienced patients. This is a significant potential bias.

Including the patients discharged in the demographics brought the biliary colic group closer in age and gender to the acute cholecystitis group. We did not include those discharged prior to 24 hours in the main analysis because we were not able to measure their cytokine concentration at 24 hours. We did contact them for their verbal rating score of their pain at 24 hours and they behaved like the other patients in the significant pain manageable group in other arms of the study. Despite this being the case we did not include their pain scores in the analysis, in case being discharged biased how they scored their pain.

The significant pain experienced group had been admitted previously with biliary colic, and reported more episodes of biliary colic, for which they had been prescribed regular analgesia ($p = 0.01$) (Table 8.2.1). The cholecystitis, pancreatitis and obstructive jaundice patients were more prevalent in the severe pain group and the significant pain manageable group ($p = 0.008$).

The significant pain experienced group had been admitted with non-specific abdominal pain under the general surgeons and gynaecologists, having diagnostic laparoscopies, looking for causes of pain and appendicectomy to exclude it as a cause ($p = 0.027$). Under the medical team they had been diagnosed with irritable bowel syndrome, asthma, reflux and depression ($p = 0.021$). The group was more likely to be current smokers or recently quit. Frequently they were in part time, or low paid employment or caring for another member of the family ($p = 0.009$). This difference between the significant pain experienced group and the other pain groups was seen in all arms of the study. There return to usual activities was significantly longer after surgery (26 ± 9 days compared to 46 ± 10 days $p = 0.008$).

ERCP patients

Four of the successfully completed first ERCP patients had quality of life scores, which placed them in the significant pain experienced group. One of the group who had a successfully completed second ERCP also was placed in the significant pain experienced group by their pain scores. This patient had a raised amylase and then a chest infection after their second ERCP. One of the patient's who had a failed ERCP and went onto have a cholecystectomy, also was identified as a significant pain experienced patient. This was the patient who did not develop sepsis after cholecystectomy and the bile culture was negative.

In total six patients (12%) undergoing ERCP were placed in the significant pain experienced group. This was a significantly smaller proportion of the group than the biliary emergency patients where 35% were in the significant pain experienced group, predominantly from the patients with biliary colic. The biliary colic patients in the emergency presentation group who were followed in the study were more likely to undergo cholecystectomy, than ERCP ($p = 0.005$). They were also more likely to have their surgery earlier (35 ± 10 versus 60 ± 11 days), perhaps reducing the risk of biliary obstruction because of the shorter time course of having biliary stones.

The severe pain group was over represented in the ERCP group, and the surgery patients who had had recent ERCP and OTC groups. This suggests a longer time course with symptoms prior to seeking intervention resulting in more complex biliary disease at the time of intervention.

Cholecystectomy patients

The significant pain experienced group was represented within the cholecystectomy arm. They were surprisingly maximally represented within the open cholecystectomy group. Speaking to the patients they felt their pain was such they opted for the shortest waiting time for surgery, which was open approach to cholecystectomy. They were less likely to have had ERCP or require OTC as they came to surgery earlier in the course of the disease and less likely to develop post-operative sepsis, possibly for the same reason. Table 8.3.5 highlights that the majority of the patients developing sepsis score their pain as severe at enrolment.

Numbers in each pain group developing sepsis

	Episodes of sepsis	Pain score (% proportion of patients participating scoring their pain in that group at enrolment)			
		Number in mild pain VAS <4	Number in significant pain manageable VAS ≥ 4 - <7	Number in significant pain experienced VAS ≥ 4 - <7	Number in severe pain VAS ≥ 7
Biliary emerg. (78)					
- BC (23)	0	0	0	0	0
- AC (27)	0	0	0	0	0
- Panc(13)	3	0	0	0	3 (23%)
- OJ (15)	1	0	0	0	1 (6.7%)
ERCP (52)					
- First ERCP (39)	2	0	0	0	2 (12%)
- Second ERCP (8)	3	0	0	1 (100%)	2 (25%)
- Chole OTC (5)	3	0	0	0	3 (75%)
Lap. chole. (125)	12	4 (7.5%)	3 (8.3%)	1 (4.5%)	4 (28.6%)
Open chole. (60)	8	3 (12.5%)	1 (7.7%)	1 (5.8%)	3 (50%)
ERCP lap. (23)	4	0	1 (6.3%)	0	3 (75%)
ERCP open (11)	2	0	1 (17%)	0	1 (33%)
OTC lap. (22)	5	0	2 (14.3%)	0	3 (60%)
OTC open (10)	3	0	1 (16.7%)	0	2 (100%)

Table 8.3.5: The table demonstrates the number of episodes of sepsis in each pain group in each arm of the study. The biliary emergency and the ERCP arm are separated into the sub groups. The percentage is the number of patients who scored their pain at that level in that subgroup who developed sepsis. The numbers in brackets are the number in that sub group developing sepsis the total is at the top of the cell. Biliary emerg – biliary emergency, BC – biliary colic, AC – acute cholecystitis, Panc. – pancreatitis, OJ – obstructive jaundice, Lap. Chole. – Laparoscopic cholecystectomy, ERCP lap. – recent ERCP and laparoscopic cholecystectomy.

8.4 Procedural data

The procedure information for the ERCP patients is given in 8.2.

For the cholecystectomy patients there was no difference in ASA grade between the elective cholecystectomy patients, who had cholecystectomy alone or had had recent ERCP or had on table cholangiogram. But the length of surgery was significantly longer in the significant pain manageable, severe pain and the group who developed sepsis ($p = 0.009$ Figure 8.4.1a). The volume of carbon dioxide, which is related to the length of the laparoscopic procedure, is also significantly greater for the same groups having laparoscopic procedure ($p = 0.020$). The volume of wash was also greater for the same groups ($p = 0.003$), it was used for dissection particularly in difficult cases (both open and laparoscopic). The volume is not a direct indication of the evacuation of carbon dioxide at the end of the laparoscopic procedures. We did not measure decompression of the abdomen at the end of the laparoscopic procedures and this is a limitation of the study. The % of time the pressure was over 12mm Hg was significantly longer in the severe pain and group developing sepsis.

Observers rated the severe pain group and the group developing sepsis as having more difficult procedures, both following surgery and elective ERCP (Figure 8.4.1c). The patient weight and BMI was greater in those developing sepsis, and those in severe pain who did not develop sepsis ($p = 0.030$). The same relationship was seen with the group who had had a recent ERCP and those who had an on table cholangiogram. The elective ERCP patients are also shown for comparison, and demonstrate the same pattern (Figure 8.4.2).

Operative data for laparoscopic and open cholecystectomy patients (part a)

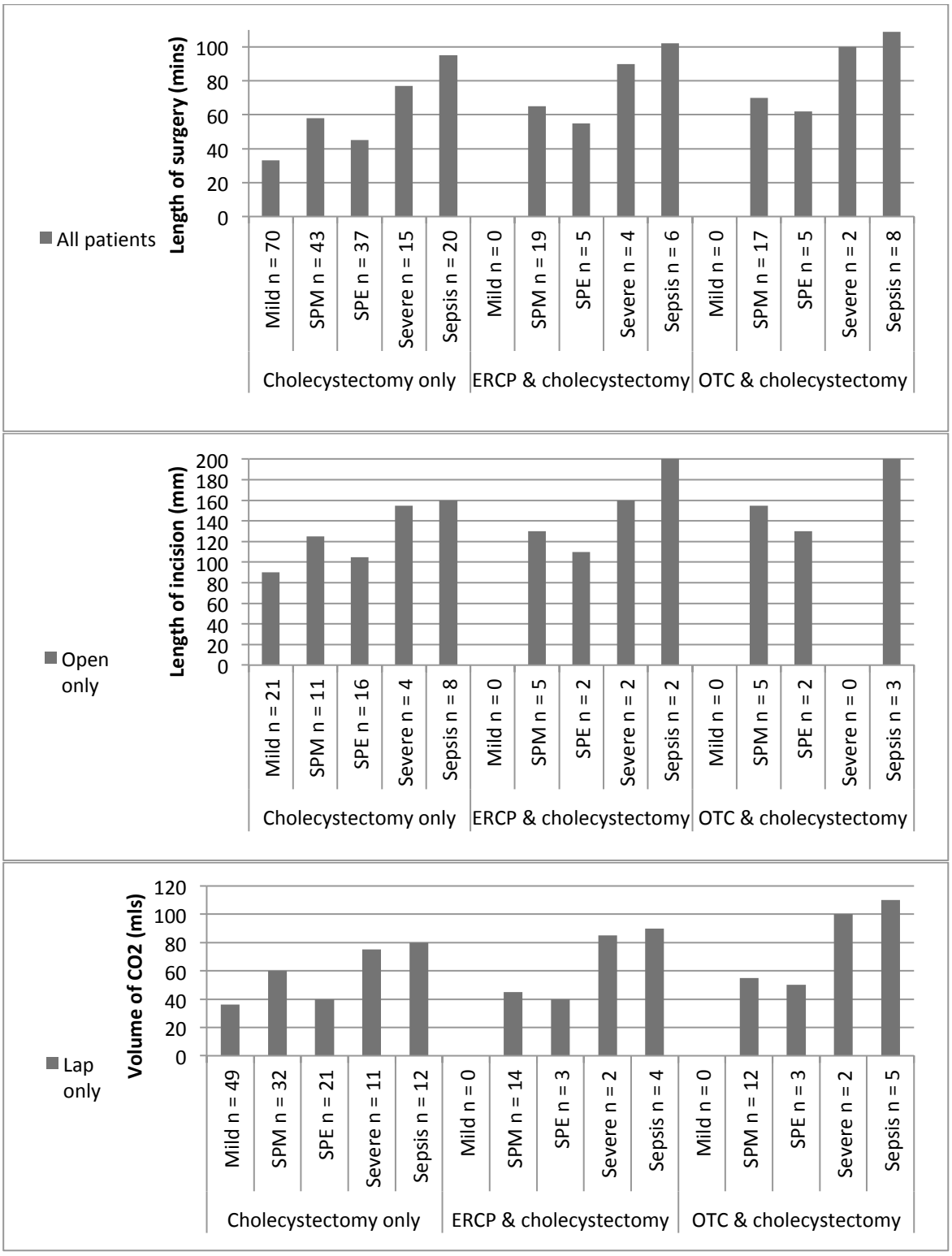


Figure 8.4.1 a: The first part of the operative data, the length of surgery, length of incision, open approach patients only, volume of carbon dioxide, laparoscopic approach patients only.

Operative data for laparoscopic and open cholecystectomy patients part b

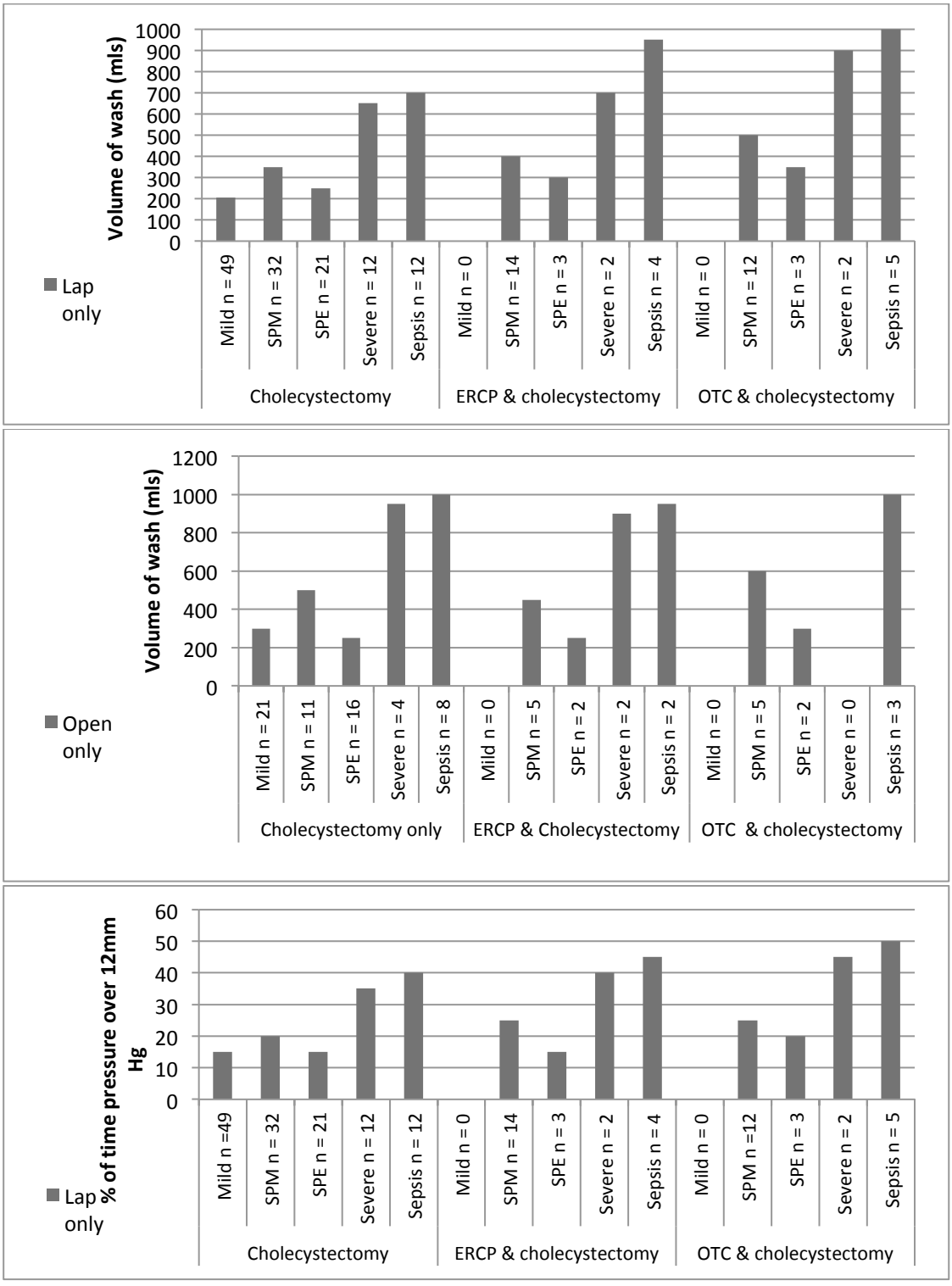


Figure 8.4.1 b: The second part of the operative data, the volume of wash for the laparoscopic approach patients above and the open approach patients middle, and % of time of the pneumoperitoneum that the pressure was greater than 12mm Hg.

Procedural data for cholecystectomy and ERCP patients (part c)

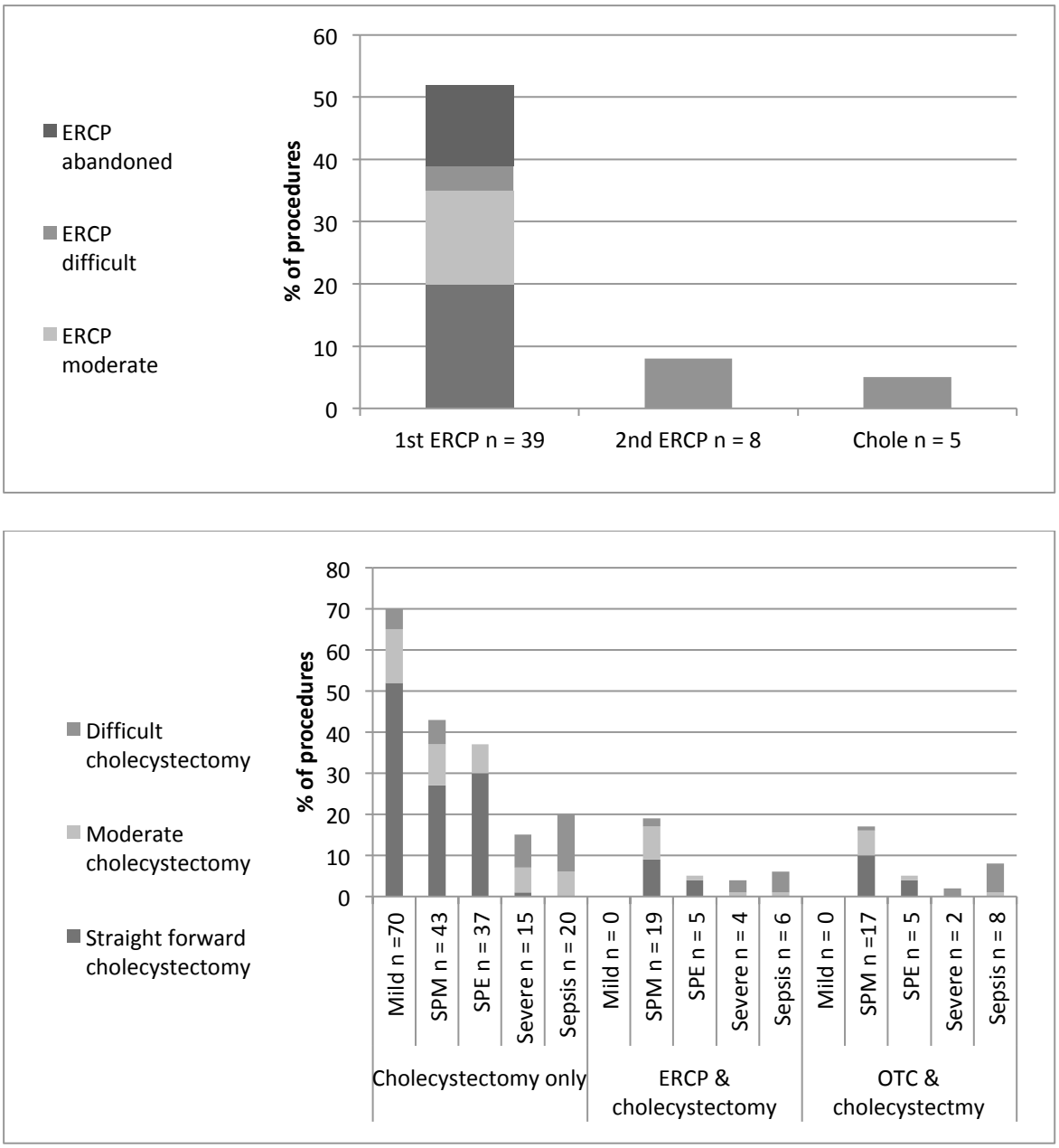


Figure 8.4.1 c: The Figures demonstrate the rating of the procedures, as to how challenging they were. The elective ERCP patients are upper, the surgical patients lower. Both the ERCP's and cholecystectomy's were rated by the independent observer, and the clinician undertaking the procedure. There was good agreement (greater than 90%) with the operative procedures and 70% with the ERCP's, for the later the clinician's rating was used. SPM – significant pain manageable and SPE - significant pain experienced.

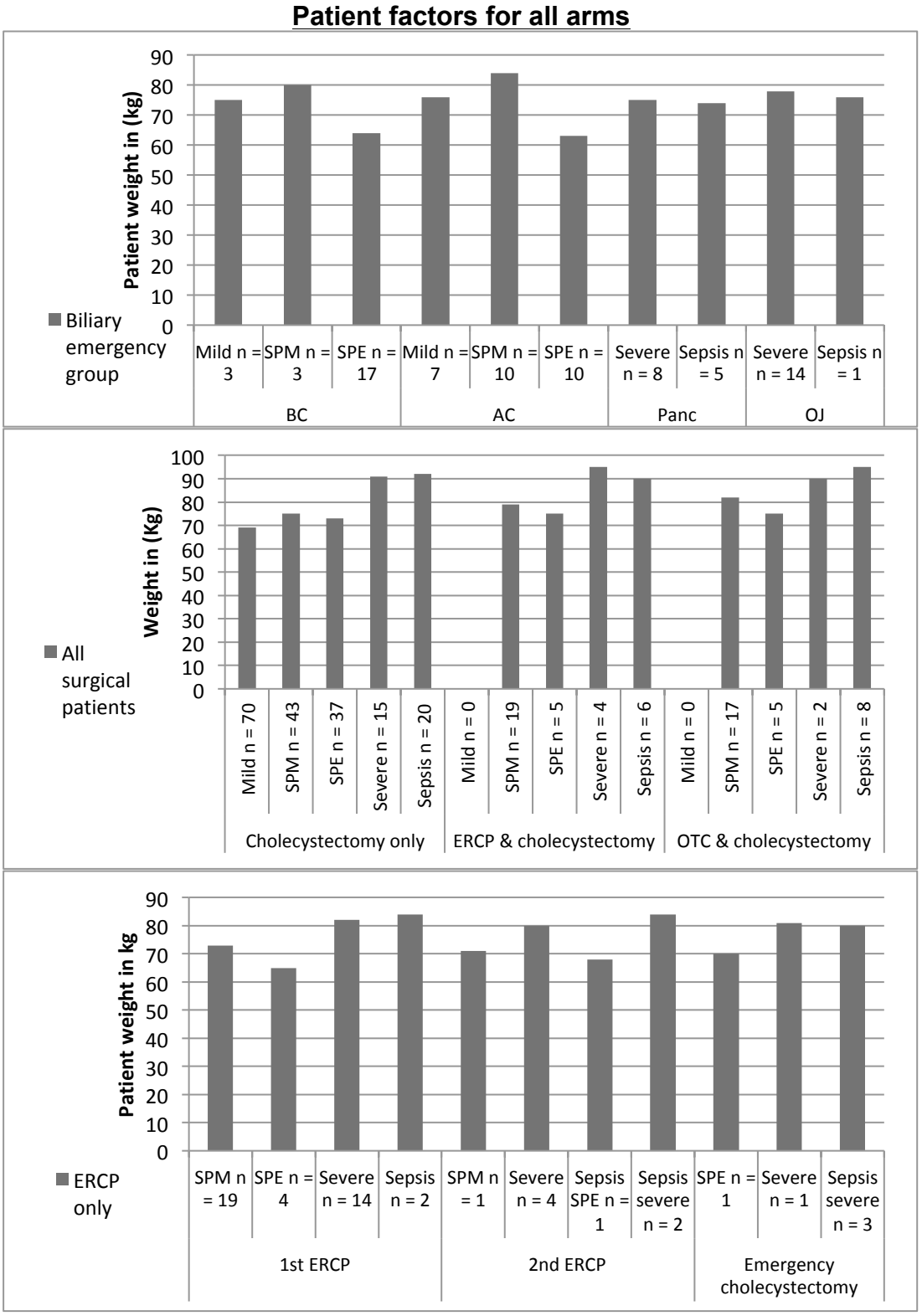


Figure 8.4.2: Demonstrates the patients weight by enrolment pain group. The biliary emergency group are shown for comparison (upper figure), the middle figure is the elective ERCP patients, the lower the surgical patients. The two pancreatitis patients without a firm diagnosis of sepsis are included in the sepsis group, excluding them did not significantly alter the weight in the sepsis group. SPM – Significant pain manageable, SPE – Significant pain experienced, 1st and 2nd refer to first or 2 ERCP.

Analysis of analgesia use demonstrated the significant pain experienced group required more analgesia, and were receiving more analgesia prior to admission. This is displayed in Table 8.4.3. All the patients who developed post procedural sepsis had more pain than the patients who did not develop sepsis excluding the significant pain experienced group. Of the group not developing sepsis the severe pain group had more pain than the mild and significant manageable but this did not reach significance, Table 8.4.4.

The numbers are small, but the patients developing sepsis who required care above ward care (n = 15), received the highest doses of opiate based analgesia, this did not reach significance until the second 24 hours following enrolment or intervention.

Analgesia received prior to enrolment and in the first 24 hours after enrolment

	Episodes of sepsis	Previous 24 hours		First 24 hours	
		None Sig. pain exp. group Morphine equivalent analgesia use (mg in previous 24 hours to admission)	Sig pain exp. group Morphine equivalent analgesia use (mg) in previous 24 hours to admission	None Sig. pain exp. group Morphine equivalent analgesia use (mg in first 24 hours of admission)	Sig pain exp. group Morphine equivalent analgesia use (mg) in first 24 hours of admission
Biliary emerg. (78)					
- BC (23)	0	20 ± 10	40 ± 10	40 ± 10	90 ± 10
- AC (27)	0	20 ± 10	40 ± 10	50 ± 10	90 ± 10
- Panc (13)	3	40 ± 10	0	80 ± 20	
- OJ (15)	1	40 ± 10	0	80 ± 20	
ERCP (52)	5	10 ± 10	30 ± 10	40 ± 10	90 ± 10
- Chole OTC (5)	3	30 ± 10	60	80 ± 20	90
Lap. chole. (125)	12	5 ± 5	20 ±10	40 ± 10	100 ± 10
Open chole. (60)	8	5 ± 5	20 ± 10	55 ± 10	90 ± 10
ERCP lap. (23)	4	10 ± 10	20 ± 10	70 ± 15	100 ± 10
ERCP open (11)	2	10 ± 10	20 ± 10	90 ± 20	110 ± 20
OTC lap. (22)	5	10 ± 10	20 ± 10	60 ± 15	100 ± 10
OTC open (10)	3	10 ± 10	20 ± 10	90 ± 20	110 ± 20

Table 8.4.3: Demonstrates the analgesia requirements for the patients in the significant pain experienced group compared to all the other patients. This is given for the 24 hours prior to admission, and the first 24 hours of the admission. The severe pain group received the majority of the analgesia prior to admission. The analgesia is given in morphine equivalent doses to aid comparison between groups. The biliary emergency group had received analgesia from their GP and emergency care and this is included in the analgesia prior to enrolment.

Analgesia use in the first 24 hours by pain group for those developing and not developing sepsis

	Episodes of sepsis	No Sepsis			Sepsis
		Mild & Sig. pain manag. group Morphine equivalent analgesia use (mg) in first 24 hours of admission	Sig. pain exp. group Morphine equivalent analgesia use (mg) in first 24 hours of admission	Severe pain group Morphine equivalent analgesia use (mg) in the first 24 hours of the admission	Sepsis group Morphine equivalent analgesia use (mg in first 24 hours to admission)
Biliary emerg.					
- BC (23)	0	40 ± 10	90 ± 10	-	-
- AC (27)	0	50 ± 10	90 ± 10	-	-
- Panc (13)	3	-	-	80 ± 20	90 ± 10
- OJ (15)	1	-	-	80 ± 20	90 ± 10
ERCP (52)	5	20 ± 10	90 ± 10	40 ± 10	80 ± 20
- Chole OTC (5)	3	-	90	70 ± 20	90 ±10
Lap. chole. (125)	12	40 ± 10	100 ± 10	50 ± 10	70 ± 10
Open chole. (60)	8	45 ± 10	90 ± 10	60 ± 10	75 ± 10
ERCP lap. (23)	4	50 ± 20	100 ± 10	80	90 ± 10
ERCP open (11)	2	70 ± 10	110 - 120	90 - 95	100 ± 10
OTC lap. (22)	5	60 ± 15	100 ± 10	75 - 85	90 ± 10
OTC open (10)	3	90 ± 20	110 - 120	-	100 ± 10

Table 8.4.4: Demonstrates the analgesia use for the pain groups. The mild and the significant pain manageable (Sig. pain manag.) are grouped together because their analgesia use was equivalent. Where there were only two patients in the group the two doses of analgesia is given without standard deviation. Sig. pain exp. – significant pain experienced.

The male patients move from the milder pain group to the more severe pain group at 24 hours as demonstrated by Figure 8.4.5. There is a tendency towards more males developing post procedural sepsis, which is significant in the emergency, elective ERCP, laparoscopic cholecystectomy only, recent ERCP and laparoscopic patients, and laparoscopic on table cholangiogram patients, but the numbers are small ($p = 0.021$, 0.028, 0.045, 0.006, 0.020 respectfully).

The gender ratio by pain score of the patients by enrolment pain group

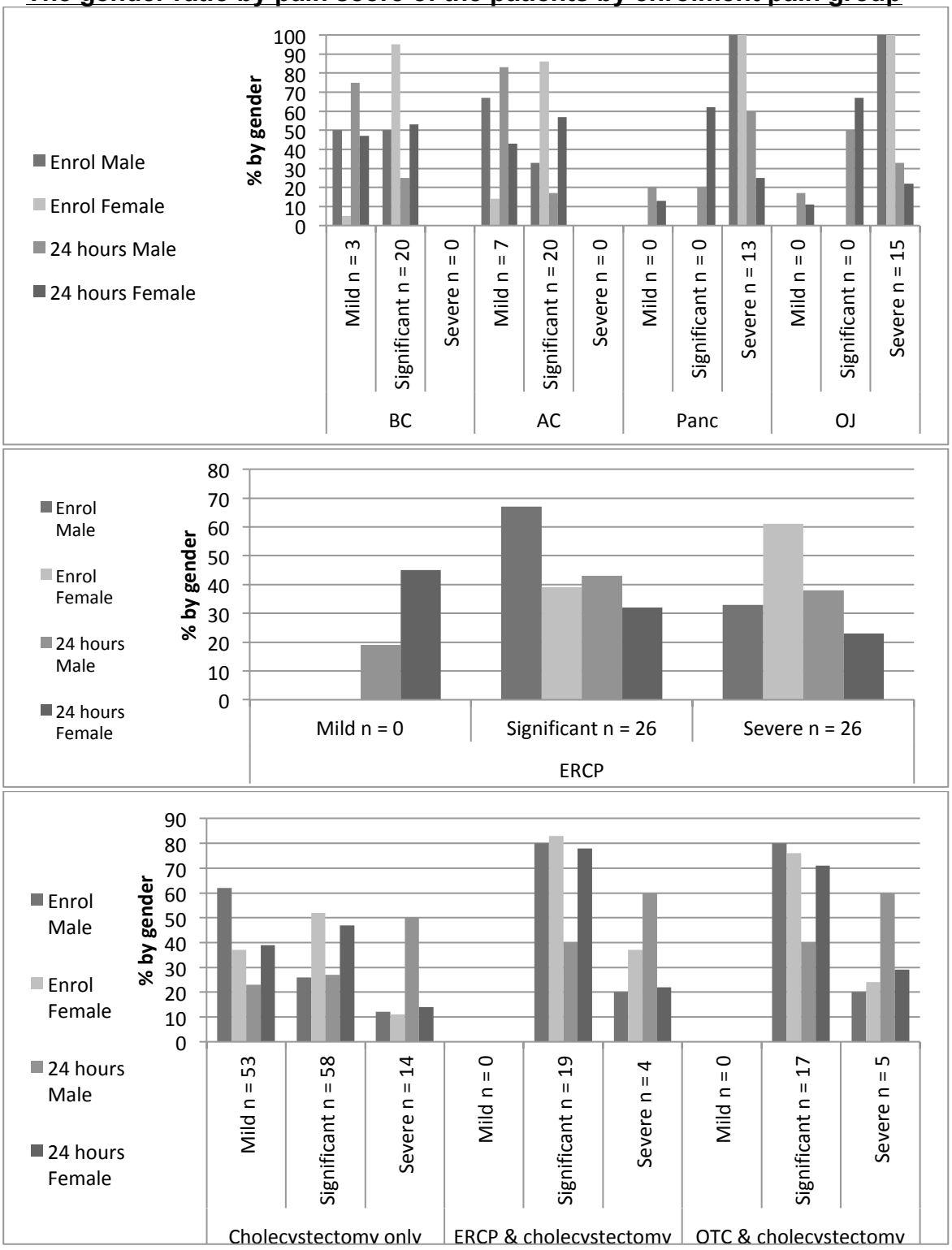


Figure 8.4.5: Demonstrates the percentage of each gender who score their pain in each of the pain groups, at enrolments and at 24 hours, the percentage is plotted against pain score at enrolment. The upper is the biliary emergency, middle ERCP arm, the lower is the surgical arm, the laparoscopic and open approach been added together. As can be seen the male patients move from the mild pain to the severe pain group, partly because they are more likely to develop post operative sepsis.

At enrolment and at 24 hours after admission, as well as rating their current pain patients were asked to rate their recalled least and worst pain in the preceding 24 hours. The results are displayed in Figure 8.4.6. In the laparoscopic approach group nursing staff administered analgesia and subjectively assessed pain when patients required analgesia. No objective measure was used to assess pain. Administration was delayed waiting for prescribing or due to busy wards. This could contribute to the greater pain experienced than expected in the laparoscopic approach patients. Open approach patients had patient controlled analgesia, excluding the significant pain experienced group there was no difference between the pain groups analgesia due to being in the lock out period. The significant pain experienced group had significantly more unsuccessful attempts, even than the group developing sepsis ($p = 0.009$).

The figure demonstrates the subjective nature of patient's assessment of their pain. With the significant pain experienced group rating their recalled pain as greater than the scores they gave their pain over the first 24 hours. The group also significantly more frequently believed that their pain was not recognised by the clinical team caring for them Table 8.4.7. They believed others had received analgesia, but they had not. This is despite them receiving significantly more morphine equivalent dose analgesia than the other patients in the same arm (Table 8.4.4). They also felt that the clinical team under-estimated the level of pain that they were experiencing and underplayed it; by explaining to them it was normal. But it does appear they did not benefit from the analgesia they did receive. The significant pain experienced patients had had more experience of surgery than the other patients, and this may aid their expectations of post procedural pain, as they accurately predicted their level of pain.

Reviewing the significant pain experienced patients' prediction of and recalled pain scores, did demonstrate a potential bias in scoring. They tended to score their least pain in the centre of the line, and their most pain at the right hand end of the VAS line (worst possible pain Figure 4.6.2 page 109). Their scoring at the individual time points was not as high and did not demonstrate this bias. The significant pain experienced group's belief that they would experience a lot of pain, and their recalled memory of being in a lot of pain was seen across each of the arms of the study.

Even for the mild, significant pain manageable, and severe pain patients the satisfaction level that pain had been recognised and addressed was only 65 – 70%. When asked about this, these patients responded that they believed having pain post-operatively was normal, no analgesia would take all pain away, and that the nursing team was very busy and they could cope with their pain. This group also sought to try and avoid medication due to the side effects, whereas the significant pain experienced patients would put up with the side effects to have the medication.

The least and most pain expected and recalled by the patients in each arm

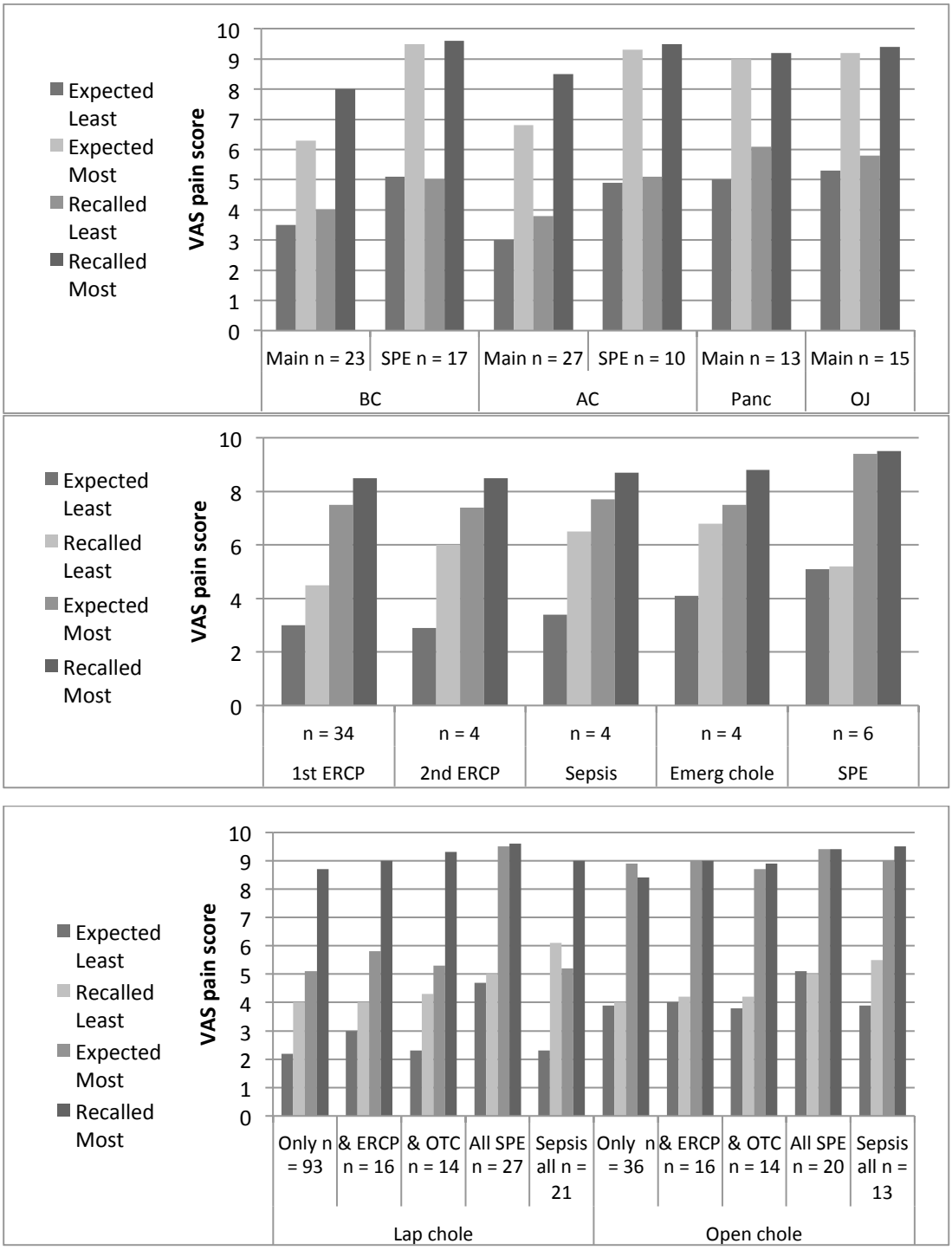


Figure 8.4.6: Demonstrates the least and worst pain expected and recalled pain by the biliary emergency patients (top), ERCP (middle) and cholecystectomy (bottom). From the lower figure it can be seen the laparoscopic approach patients did not expect to be in as much pain (green line) as the open patients. Both recalled the same level of pain (purple). BC main – biliary colic non significant pain experienced group, BC SPE – significant pain experienced, AC – acute cholecystitis, Panc – pancreatitis , OJ – obstructive jaundice, chole – cholecystectomy, and OTC – on table cholangiogram.

Least and most recalled pain in the first 24 hours and patient's satisfaction

	Total patient numbers in group	Significant pain experienced	Non significant pain experienced % who felt pain was recog. and treat.	Significant pain experienced % who felt pain was recog. and treat.
Biliary emerg.				
BC	23	17	68%	34%
AC	27	10	69%	31%
Panc	13	0	73%	0%
- Sepsis	3	0	66%	0%
OJ	15	0	70%	0%
- Sepsis	1	0	100%	0%
ERCP				
Completed first	39	4	70%	33%
- Sepsis	2	0	100%	0%
Completed 2nd	8	1	72%	0%
- Sepsis	3	0	66%	0%
Chole OTC	5	1	70%	0%
- Sepsis	3	0	66%	0%
Lap. chole.	125	22	74%	35%
- Sepsis	12	0	70%	0%
Open chole.	60	17	72%	36%
- Sepsis	8	0	69%	0%
ERCP lap.	23	3	69%	39%
- Sepsis	4	0	67%	0%
ERCP open	11	2	71%	37%
- Sepsis	2	0	70%	0%
OTC lap.	22	3	72%	35%
- Sepsis	5	0	69%	0%
OTC open	10	2	71%	36%
- Sepsis	3	0	68%	0%

Table 8.4.7: The patients were also asked 'Do you believe the doctors and nurses looking after you recognised the amount of pain you have been in?, and has this pain been treated with appropriate pain relief?'. The significant pain experienced patients were very different to the other patients. Where there was just one patient their response is given. The patients with sepsis scored their satisfaction similar and their recalled worst pain to the main group. N – no, BC main – biliary colic non significant pain experienced group, BC SPE – significant pain experienced, AC – acute cholecystitis, Panc – pancreatitis , OJ – obstructive jaundice, chole – cholecystectomy, and OTC – on table cholangiogram.

The patients who underwent laparoscopic surgery, but were not in the significant pain experienced group, expected to have significantly less pain than they had after surgery. It was evident the laparoscopic patients expected it to be a very minor procedure. Whereas the open approach patients were more accurate in predicting the amount of pain they would have following surgery. This held true for those developing sepsis and those who did not develop sepsis, and is seen in Figure 8.4.6. Potentially this could be because the anaesthetist discussed patient controlled analgesia with the open approach patients as this was used for all the open cholecystectomy patients. But this discussion took place after the scoring of expected pain, although written information about the surgery, discussed patient controlled analgesia, and this was given to the patients when they were listed for surgery.

All the patients believed that the setting of expectations of pain levels and controls was poorly managed. Likewise there was very minimal discussion about pain control and patients managing their own pain, and the differences between analgesia. Very few patients recalled being asked their level of pain outside the study, and did not know if a nurse had assessed it without discussion. It was also a frustration to patients that the pain scores they were filling out for the study were not fed back to the clinical team.

Reviewing the prescriptions it was evident that although we had performed education sessions about the WHO analgesia ladder and about the use of NSAID's, NSAID's were under prescribed and under used. Discussion groups were held following the study, with medical and nursing staff, to discuss this as there was resistance on both sides to prescribing and administering NSAID's. The principal reason being the side effects of the drugs, and a belief the patients required stronger analgesia, though this was not measured or discussed with the patients.

At 2 and 4 hours after surgery there was not a significant difference in analgesia use between the groups based on VAS score at enrolment. The intra-operative analgesia received was included in the analgesia use at 2 hours. The difference was based on the length of the procedure and the difficultness of the procedure, which were interlinked. The laparoscopic approach patients received local anaesthesia at the end of the procedure, and received less opiate analgesia but this did not reach significance. From 6

hours onwards the significant pain experienced and the group developing sepsis received more analgesia.

Table 8.4.8 demonstrates the difference in the length of symptoms prior to the admission for the arm they were in and their length of stay. For all arms those developing sepsis and the significant pain experienced group stayed longer. The level of care required for those developing sepsis is given. The significant pain experienced had had their symptoms for a significantly shorter period prior to admission compared to all the other groups ($p = 0.001$).

Patient's length of symptoms prior to enrolment & their length of stay

Number (Total / Significant pain experience)	Main group excluding significant pain experienced group			Significant pain experienced group		
	Mean length of sympt. prior to enrol. (days) ± SD	Mean length of stay no sepsis (days) ± SD	Mean length of stay sepsis (days) ±SD	Mean length sympt. prior to enrol. (days) ± SD	Mean length of stay no sepsis (days) ± SD	Mean length of stay sepsis (days) ± SD
Biliary emerg.						
BC (23 / 17)	120 ± 30	1 ± 1	-	30 ± 30	3 ± 0.5	-
AC (27 / 10)	90 ± 60	3 ± 0.5	-	30 ± 30	3.5 ±0.5	-
Panc(13 / 0)	90 ± 90	4.5 ± 0.5		-	-	
- Sep HDU (1)			16			-
- Sep ward			9 ± 2			-
OJ (15 / 0)	90 ± 90	4.5 ± 0.5		-	-	
- Sep ward			8			-
ERCP						
First (39 / 4)	90 ± 30	0.5 ± 0.5		45 ±15	2.0 ± 0.5	
- Sep ward			3 ± 0			-
Second (8 / 1)	120 ± 30	2 ± 0.5		45	-	
- Sep ward			4.5 ± 0.5			7
Emerg Chole (5)	120 ± 30	6 ± 1		45	8	
- Sep HDU			9			-
- Sep ward			7 ± 2			-
Lap. chole. (125)	90 ± 45	1.25 ± 0.5		30 ± 15	2.5 ± 0.5	
- Sep ITU			14 – 90			
- Sep HDU			7 – 11			
- Sep ward			3 - 7			9
Open chole. (60)	100 ± 30	5 ± 2		35 ± 20	7 ± 1	
- Sep ITU			7 – 11			
- Sep HDU			6 – 10			
- Sep ward			4 - 5			10
ERCP lap. (23)	100 ± 30	3 ± 1.5		40 ± 20	5 ± 1	
- Sep HDU			9			-
- Sep ward			7 ± 2			-
ERCP open (11)	90 ± 30	5 ± 1		30 - 40	7 - 8	
- Sep ward			8 ± 2			-
OTC lap. (22)	115 ± 30	4 ± 1		45 ± 20	6 ± 2	
- Sep HDU			11			-
- Sep ward			7 ± 2			-
OTC open (10)	110 ± 30	6 ± 1		30 - 40	7 - 8	
- Sep ward			8 ± 2			-

Table 8.4.8: Demonstrates the mean length of symptoms and the length of stay for the significant pain experienced patients, compared to the other patients. In those developing sepsis the length of stay is broken down into the level of care they required. All the significant pain experienced patients developing sepsis were cared for on the ward.

At the 12 weeks out patient appointment patients scored their pain at that time Figure 8.4.9. A number in the sepsis group had not long been discharged, particularly the ITU patients. Despite this the significant pain experienced patients pain group scored their pain significantly higher at every time point ($p = 0.0009$). At 26 weeks the significant pain experienced patients pain had decreased, but it remained higher than the other groups. By 12 weeks there was no significant difference between the laparoscopic and open approaches to surgery. There was also no difference between the two approaches for the significant pain experienced groups. At 6 and 12 months $n = 4$ and 9 patients were lost to follow-up, these patients were in the mild and the significant pain manageable group, 3 and 6 respectfully from the laparoscopic approach group.

The biliary emergency and ERCP patients are not shown as by six the majority, and 12 months after admission all had undergone surgery ($n = 96$ and $n = 129$ respectfully).

Pain score from enrolment to a year after cholecystectomy

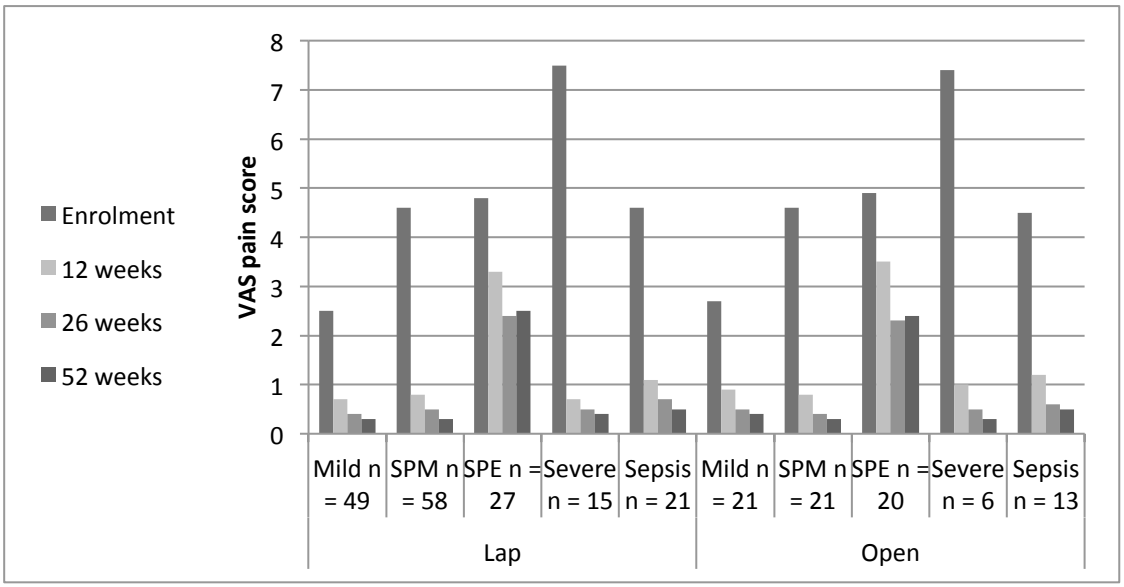


Figure 8.4.9: Figure demonstrates the change in pain score from enrolment to a year after surgery. The numbers in each pain group include those just undergoing cholecystectomy, those who had had recent ERCP prior to their cholecystectomy and those who had cholecystectomy and OTC. They are arranged by the pain group they were placed in at enrolment. At 26 weeks the Mild group was 48 / 20 and Significant pain manageable (SPM) 56 / 21, and at 52 weeks 46 / 19 and 55 / 20 respectfully. This was due to 4 patients being lost to follow up at 6 months and 9 at 12 months. The other groups remained unchanged and no one was lost from follow up at 3 months. The ERCP and biliary emergency patients are not included as the majority had undergone cholecystectomy. SPE – significant pain experienced.

Results

Chapter 9 – Cytokines

9.1 Biliary emergency - Cytokines

The biliary emergency patients were all enroled into the study within the first four hours of their admission to the surgical admission unit. Admissions were from general practitioners and the emergency department. Those with the highest cytokine concentration had the most elevated SIRS markers.

The cytokine concentration was not different for the patients who were in significant pain experienced group. They are not plotted separately in the biliary emergency cytokine concentration figures in Figure 9.1.1, but 74% of the biliary colic and 37% of the acute choloecystitis group were in the significant pain experienced group (Table 8.3.4). None of the obstructive jaundice and pancreatitis patients were placed in the significant pain experienced group.

Both early and late cytokines were elevated in the biliary emergency patients and inflammatory cytokines concentration decreased from admission onwards. The patients with obstructive jaundice and pancreatitis had the highest cytokine concentration on admission. The healthy control group's mean concentration is shown for comparison for this group and were used as controls throughout the study.

Cytokine concentration in the biliary emergency patients

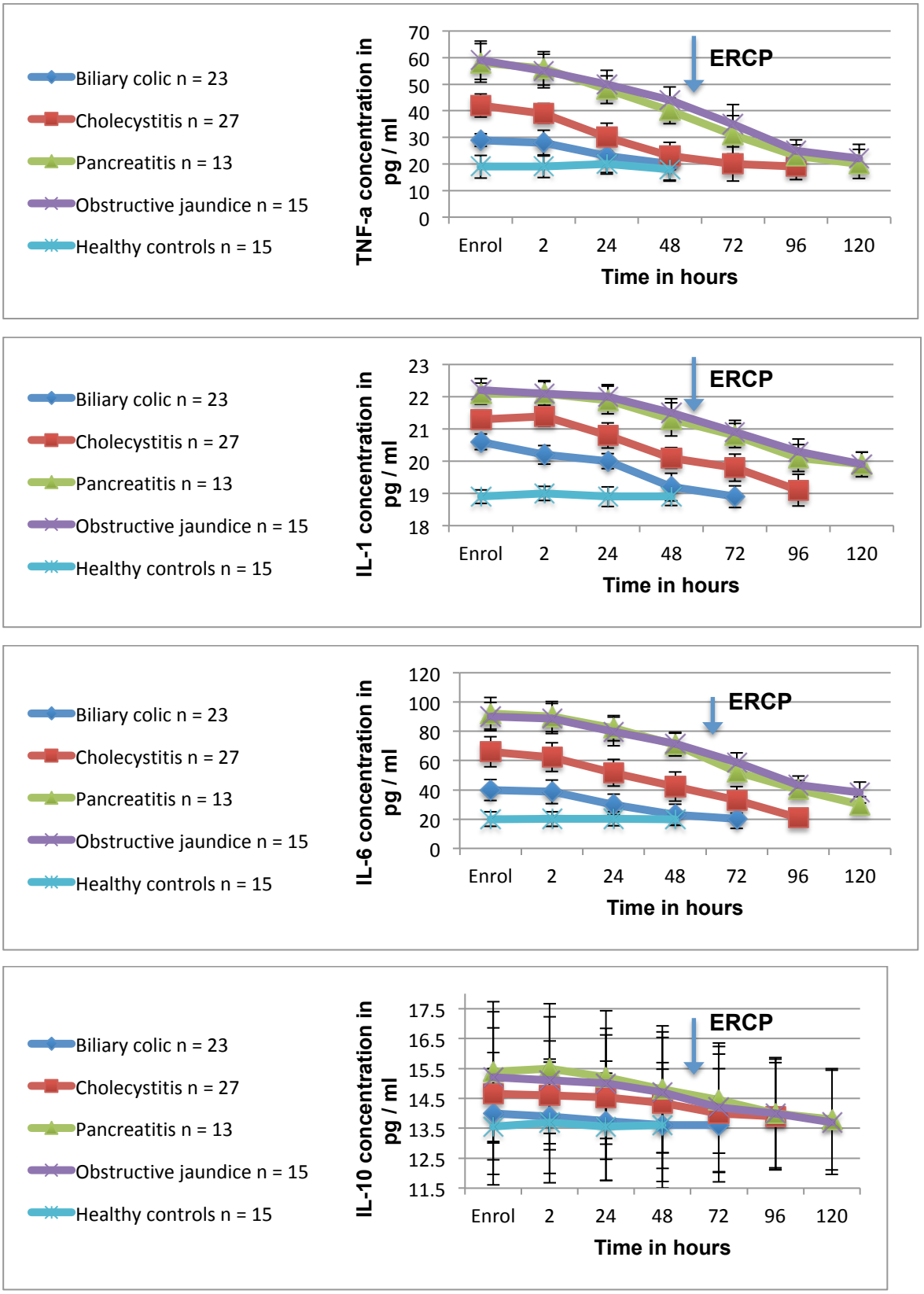


Figure 9.1.1: Demonstrates cytokine concentration (± SD) (pg/ ml) in biliary emergency patients. ERCP was undertaken in 25 of the obstructive jaundice and pancreatitis patients. Enrol – Enrolment.

By 48 hours for the biliary colic patients the cytokine concentration had returned to the level of the healthy controls. In the acute cholecystitis patients the cytokine concentration levels had returned to the healthy controls concentration by 48 hours for TNF-α, and 72 – 96 hours for IL-1 and IL-6. In the obstructed patients, undergoing ERCP to relieve the obstruction increased the decline in the cytokine concentration. TNF-α concentration declined ahead of IL-1 and IL-6 (Figure 9.1.2).

In contrast to the inflammatory cytokines, the IL-10 concentration did not change significantly during the study, particularly in the patients with biliary colic and acute cholecystitis. For the pancreatitis and obstructive jaundice patients the concentration was elevated at admission, but the change in concentration did not reach significance. All the biliary colic patients had had pain for at least 9 hours, and nearer to 24 hours in the other biliary emergency patients at admission (Table 8.1.2) therefore the peak in IL-10 concentration may have been missed.

Twenty-five of the twenty-eight obstructive jaundice and patients with pancreatitis underwent ERCP at this admission. This was undertaken between 48 and 72 hours. The cytokine concentration for these patients was measured pre ERCP, 2, and 24 hours after the procedure and is given in Figure 9.1.2. The inflammatory cytokine concentration increases two hours after ERCP, for both the early and late cytokine concentration and then continues the decline as the system drains. IL-10 concentration was unchanged after ERCP.

Ranson's criterion is a clinical prediction tool predicting prognosis and mortality risk of acute pancreatitis, with scores greater than or equal to 3 indicating severe pancreatitis. Patient's scoring 3 to 4 have a predicted mortality of 15%. The scoring is repeated at 48 hours. The TNF-α and IL-6 concentration most closely matched the Ranson criteria score and the SIRS markers.

Five patients with pancreatitis and the one patient with obstructive jaundice had a raised white cell count, and were commenced on antibiotics. These patients had the highest IL-10 concentration. Four were diagnosed with sepsis (3 pancreatitis and 1 obstructive jaundice) and had the highest IL-10 concentration, the patient with necrotizing pancreatitis, was admitted to HDU, had the highest IL-10 concentration (Figure 9.1.3).

This patient scored 4 on the Ranson's criteria at admission and 3 at 48 hours. Two other pancreatitis patients developed overt sepsis, From Table 8.2.7 there was one each of, positive blood cultures for *E. coli*, culture positive chest infection, scoring 3 on the Ranson's criteria at admission and 2 at 48 hours. The obstructive jaundice patient who developed sepsis was cared for on the surgical dependency unit, a step up from the ward. They had the second highest IL-10 concentration. The patients with a definite diagnosis of sepsis, all had the highest inflammatory cytokine concentration, and SIRS markers.

The two other pancreatitis patients with a raised white cell count only had one raised SIRS marker, and did not have positive cultures, but were commenced on antibiotics for the possibility of a chest infection, and the other for potential line sepsis. They scored 2 on the Ranson's criteria at admission and 1 at 48 hours

These five were the sickest patients in the group according to SIRS, and cytokine concentration. They were also the oldest in the group. The episodes of sepsis were diagnosed from day 4 of the admission onwards. All the patients underwent an ERCP on the third day of their admission. Their cytokine concentration is demonstrated in Figure 9.1.3.

Cytokine concentration in emergency patients undergoing ERCP

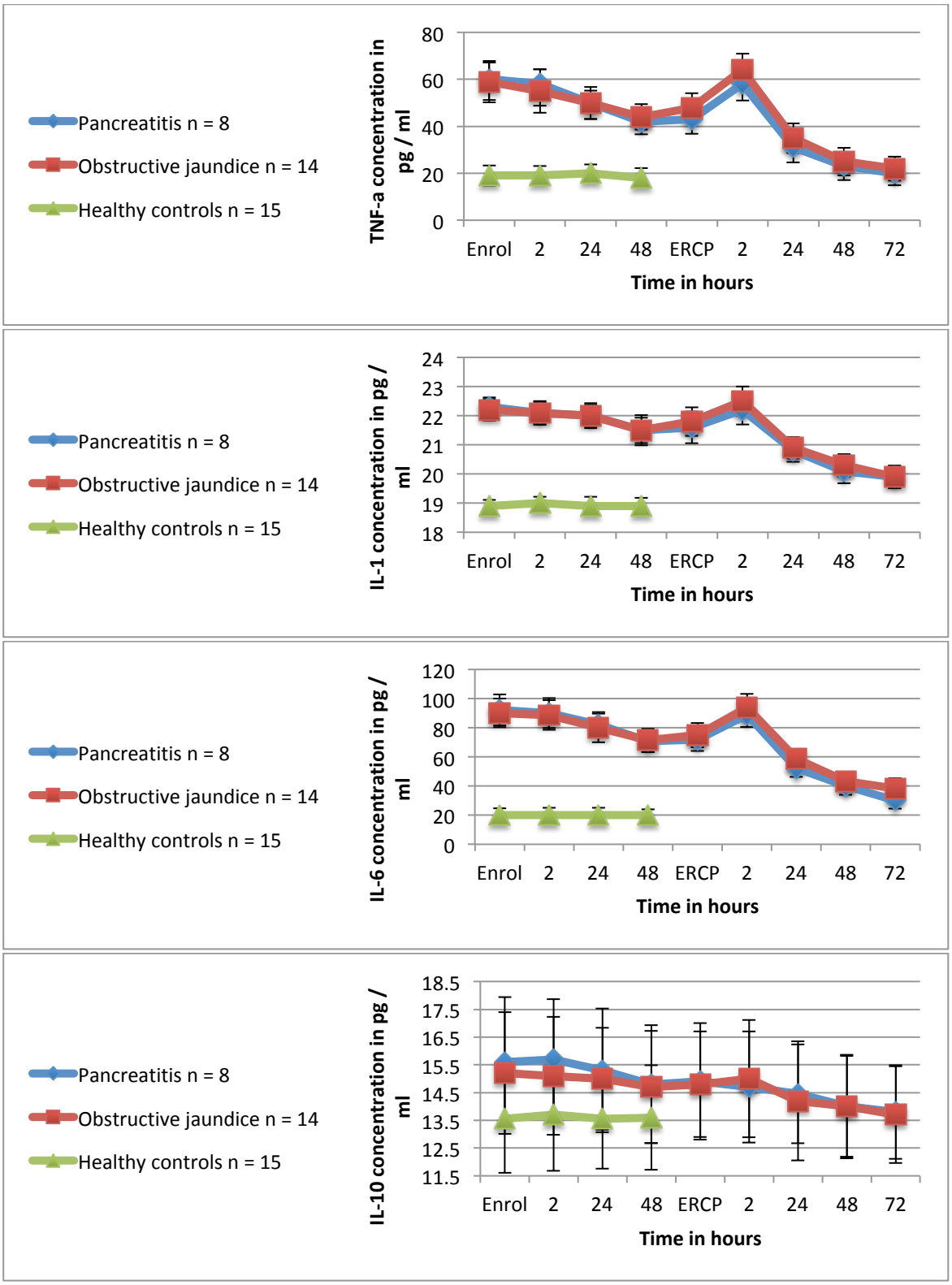


Figure 9.1.2: Demonstrates cytokine concentration (± SD) (pg/ ml) in the biliary emergency patients undergoing ERCP. Only the pancreatitis and obstructive jaundice patients underwent ERCP, control patients are shown for comparison. All ERCP's were successful on the first attempt Enrol – enrolment.

Cytokine concentration in emergency patients developing sepsis

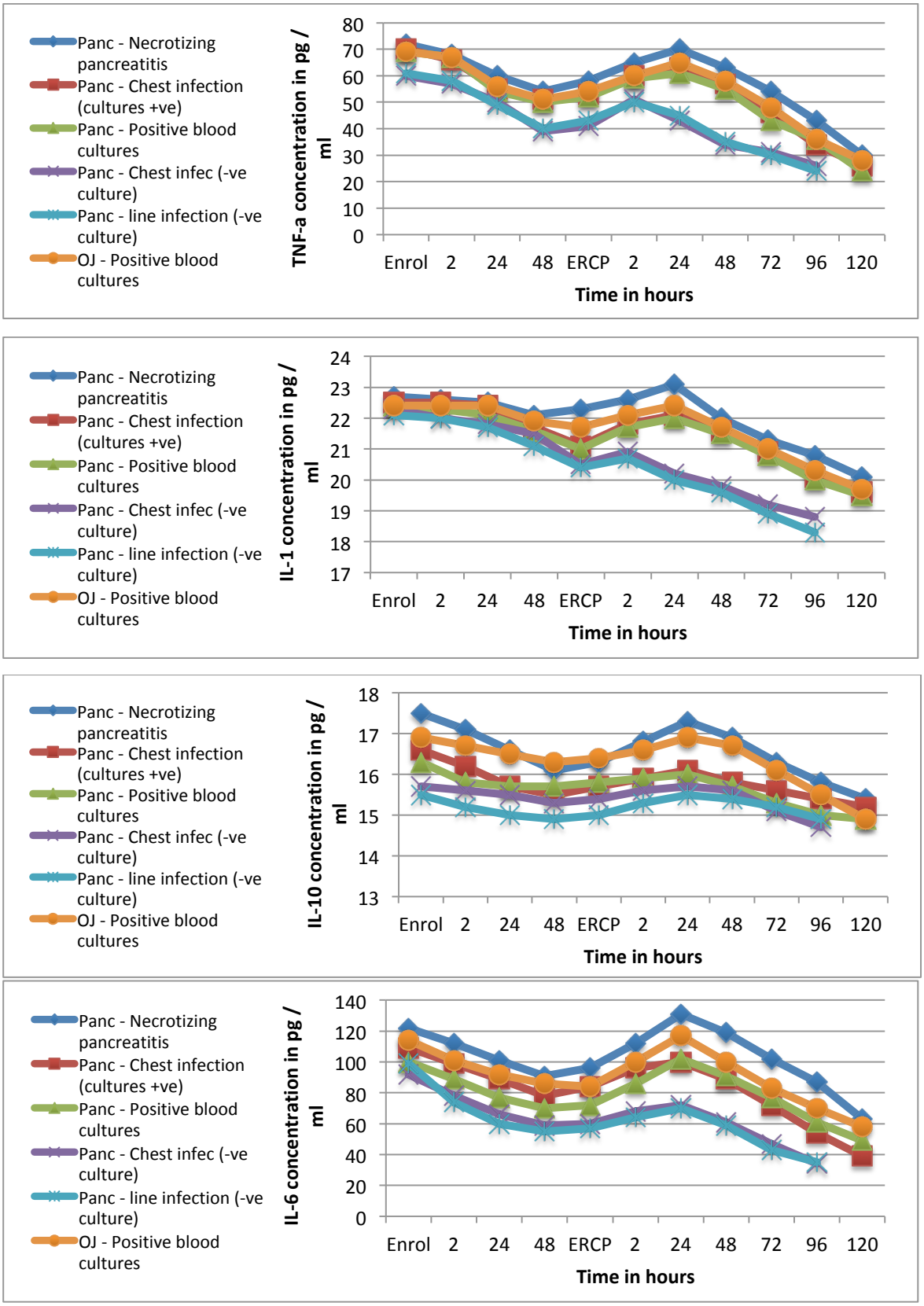


Figure 9.1.3: Cytokine concentration in the four biliary emergency patients developing sepsis, and the two who were thought to possibly have sepsis. 72 hours from admission and the pre-ERCP value were equivalent and so plotted together.

9.2 ERCP patients - Cytokines

The ERCP patients were sent information about the study, and enroled if they wished to participate on the day of their ERCP, in contrast to the cholecystectomy patients who were enroled a few days before the procedure.

Figure 9.2.1 demonstrates the change in cytokine concentration following ERCP. Prior to ERCP the TNF-α, IL-1 concentration was elevated above the healthy controls. The systemic TNF-α, IL-1 and IL-6 concentration was highest in the group that ended up undergoing an emergency cholecystectomy, ($p = 0.008$ for IL-6). This possibly indicates a greater level of intra-peritoneal inflammation, which distorts the anatomy making it more difficult to perform the ERCP. But there was not a significant difference in the cytokine concentration between those who had a successful first ERCP and those who required a second ERCP to achieve drainage of the system.

Following ERCP the pro-inflammatory cytokine concentration increased when measured at two hours after the procedure. The concentration at 24 hours was dependent upon the outcome of the procedure. In those where the first ERCP had been successfully completed the cytokine concentration returned towards the level of the healthy controls. In those having second procedures the cytokine concentration did not fall to the level of the healthy controls until after the second successfully completed ERCP. This pattern of the inflammatory cytokines increasing at 2 hours after the procedure was also seen in the biliary emergency patients who underwent emergency ERCP, Figure 9.1.2. This entire group had successful ERCP's on the first occasion.

The peak in cytokine concentration at 2 hours could represent the response to the procedure, with the cytokine concentration not falling until the obstruction was relieved. Similar pattern in cytokine response after ERCP have been found by Adas *et al.,* (2013), and Wozniak *et al.,* (2001), they found a peak in inflammatory cytokine one hour after ERCP and then a fall in concentration after successfully completed procedures. In those developing sepsis after the first or second ERCP in this study, the cytokine concentration increased further at 24 hours after the successfully completed ERCP, and then decreased slowly back to the level of the healthy controls. Sepsis only being diagnosed after successfully completed ERCP's.

The IL-10 concentration prior to ERCP was elevated above the healthy controls but not significantly. Following successful ERCP and in those developing sepsis the IL-10 concentration decreased. The biliary emergency patients undergoing ERCP, their IL-10 concentration mirrored the elective ERCP patients, Figure 9.1.2. Those undergoing a second ERCP, in the elective ERCP group, their IL-10 did not decrease until after the ERCP was successfully completed. For those undergoing emergency cholecystectomy the IL-10 concentration rose following the surgery, the IL-10 concentration did not fall until after the cholecystectomy was performed, and if present, the sepsis had resolved Figure 9.2.2.

Cytokine concentration in the ERCP patients

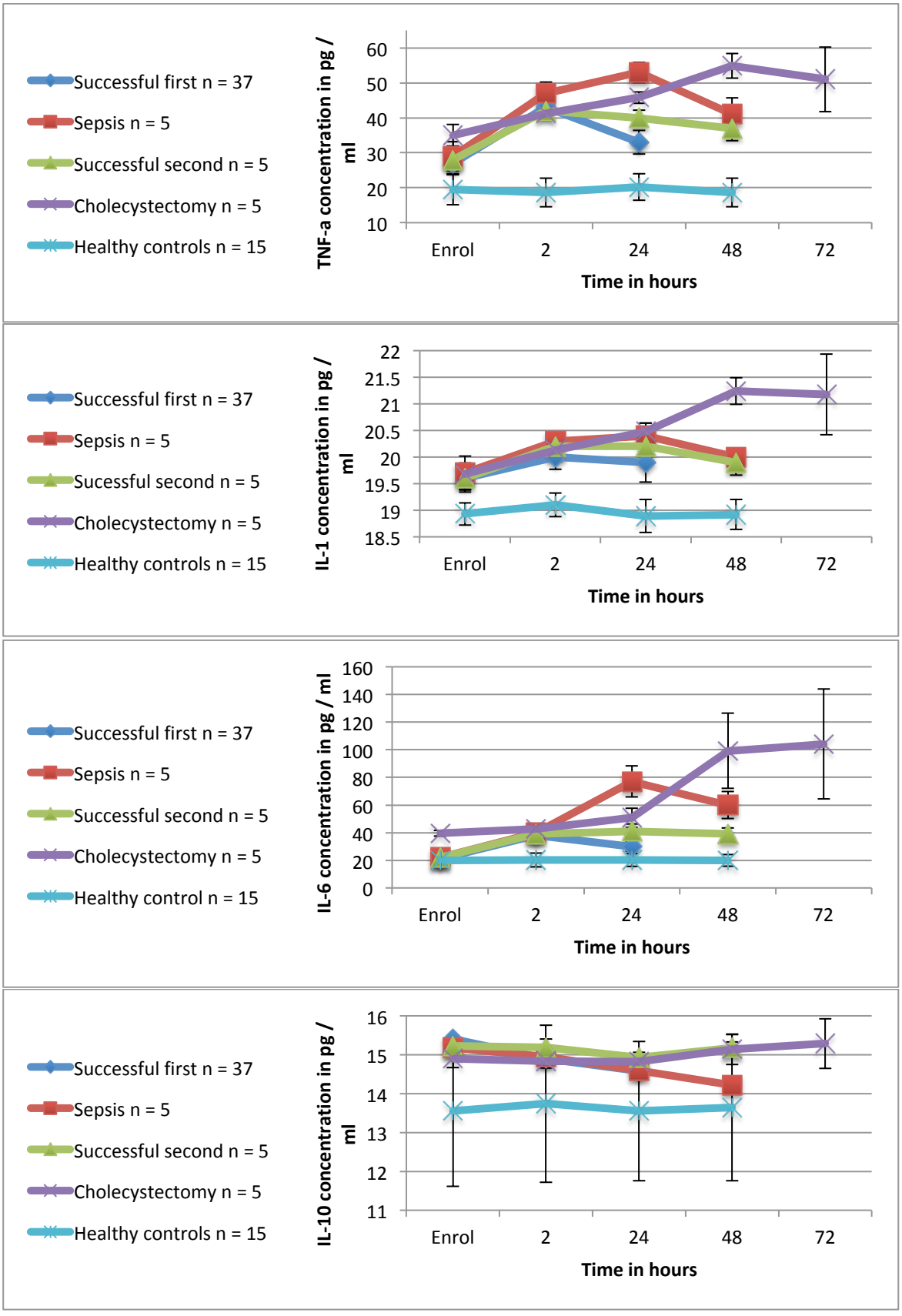


Figure 9.2.1: Cytokine concentration (± SD) (pg/ ml) in ERCP patients. For appropriate groups arrow demonstrates 2nd ERCP or cholecystectomy timing. Enrol – Enrolment.

Five patients underwent cholecystectomy and on table cholangiogram (OTC) following a failed first ERCP (Figure 9.2.2 patient A – E). As they had had an ERCP prior to emergency surgery, their results were not analysed with the elective cholecystectomy patients. Three procedures were performed laparoscopically (A – C), and two open procedures (D – E). All had bile cultures sent, four were positive for *E.coli* in four (B - E). Three developed overt signs of sepsis, all had positive bile and blood cultures for *E.coli,* and were diagnosed with sepsis by the clinical team (C – E). Those undergoing cholecystectomy, their pro-inflammatory cytokine concentration increased at 24 hours after ERCP, remaining elevated at 48 hours and then falling more slowly after the cholecystectomy, which took place between 24 and 30 hours after the ERCP.

Two laparoscopic approach patients did not develop sepsis, one had negative bile cultures (A), and one had positive bile cultures (B). Although not developing overt signs of sepsis, both had a greater cytokine rise, and earlier than the elective laparoscopic cholecystectomy patients who did not develop sepsis.

The open patients (D and E) and the culture negative laparoscopic approach patient (A) displayed a cytokine concentration peak at 24 hours following cholecystectomy. This mirrored the open elective cholecystectomy patients. Patient A's IL-10 concentration fell rapidly following surgery (negative culture). Patient B, who had positive bile culture but no other signs sepsis, their cytokine concentration mirrored the laparoscopic approach and the two open approach patients developing overt sepsis (C - E) peaking at 48 hours after surgery.

The two patients not developing overt sepsis had 2 SIRS markers elevated at fours hours after surgery, otherwise only one SIRS marker was elevated until 24 hours after surgery, and then all returned to normal.

As will be discussed later in this chapter, the elective laparoscopic cholecystectomy only patients developing sepsis after surgery, their cytokine concentration didn't increase until 48 hours after the procedure. Patients having elective laparoscopic or open cholecystectomy after ERCP, the rise in pro-inflammatory cytokine was delayed to between 48 to 72 hours. In contrast the three patients undergoing emergency laparoscopic cholecystectomy after ERCP demonstrated a rise in in pro-inflammatory

cytokine concentration from 2 hours onwards. In the laparoscopic approach patients with positive bile cultures (B – C) the cytokine concentration carried on increasing until 48hours and then declined. The reason for the difference in cytokine response is difficult to determine with the small patients numbers. One theory to test would be the ERCP being only the day before and the obstruction not being relieved, initiated an increase in cytokine concentration prior to surgery. These patients demonstrated a significantly higher systemic pro-inflammatory and IL-10 concentration prior to the procedure, except for IL-1 ($p = 0.031, 0.391, 0.007, 0.011$ for TNF-α, IL-1, IL-6 and IL-10).

Cytokine concentration in ERCP patients who underwent cholecystectomy

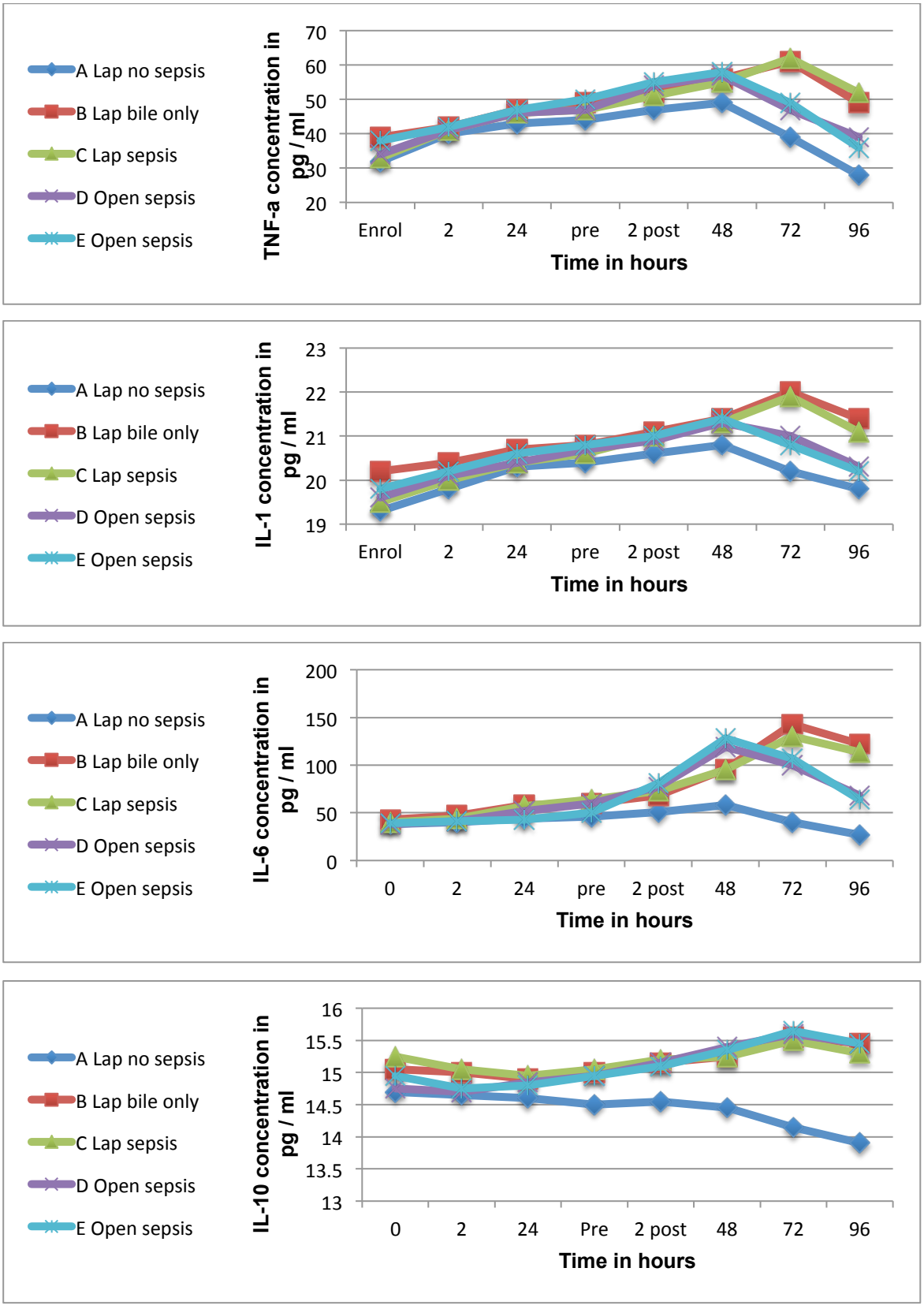


Figure 9.2.2: Cytokine change in the five patients undergoing emergency cholecystectomy after elective ERCP. 48 hours after ERCP, the concentration corresponds to 24 hours after emergency cholecystectomy.

9.3 Elective cholecystectomy – Cytokines

Surgical intervention evoked a cytokine response, this was compared with a two-tailed T-test to ensure that increases and decreases in concentration were captured. Within the group having cholecystectomy we recruited past the number of patients the power calculation indicated were required. Therefore the group was split into patients who had only cholecystectomy (125 laparoscopic approach and 60 open approach). A second group who had had an ERCP prior to cholecystectomy; all ERCP's had been in the preceding year (4 – 28 weeks before surgery), (23 laparoscopic approach and 11 open approach to cholecystectomy). Finally a third group who had abnormal liver function tests at pre-assessment, which remained abnormal on the blood test performed on the day of surgery, and therefore underwent cholecystectomy and on table cholangiogram (OTC) (22 laparoscopic approach and 10 open approach).

Within the cholecystectomy patients there were patients whose pain and quality of life scores placed them in the significant pain experienced group (Chapter 8.3). This group's IL-10 and inflammatory cytokine concentration was no different to other patients who had the same procedure and did not develop sepsis.

The results of the cytokine analysis are demonstrated in Figure 9.3.1, this Figure demonstrates all the patients together. To make interpretation easier, Figure 9.3.2 is the laparoscopic approach patients and 9.3.3 the open approach patients. For ease of interpretation the standard deviation is omitted from Figure 9.3.1, but is included with the mean in the later two Figures.

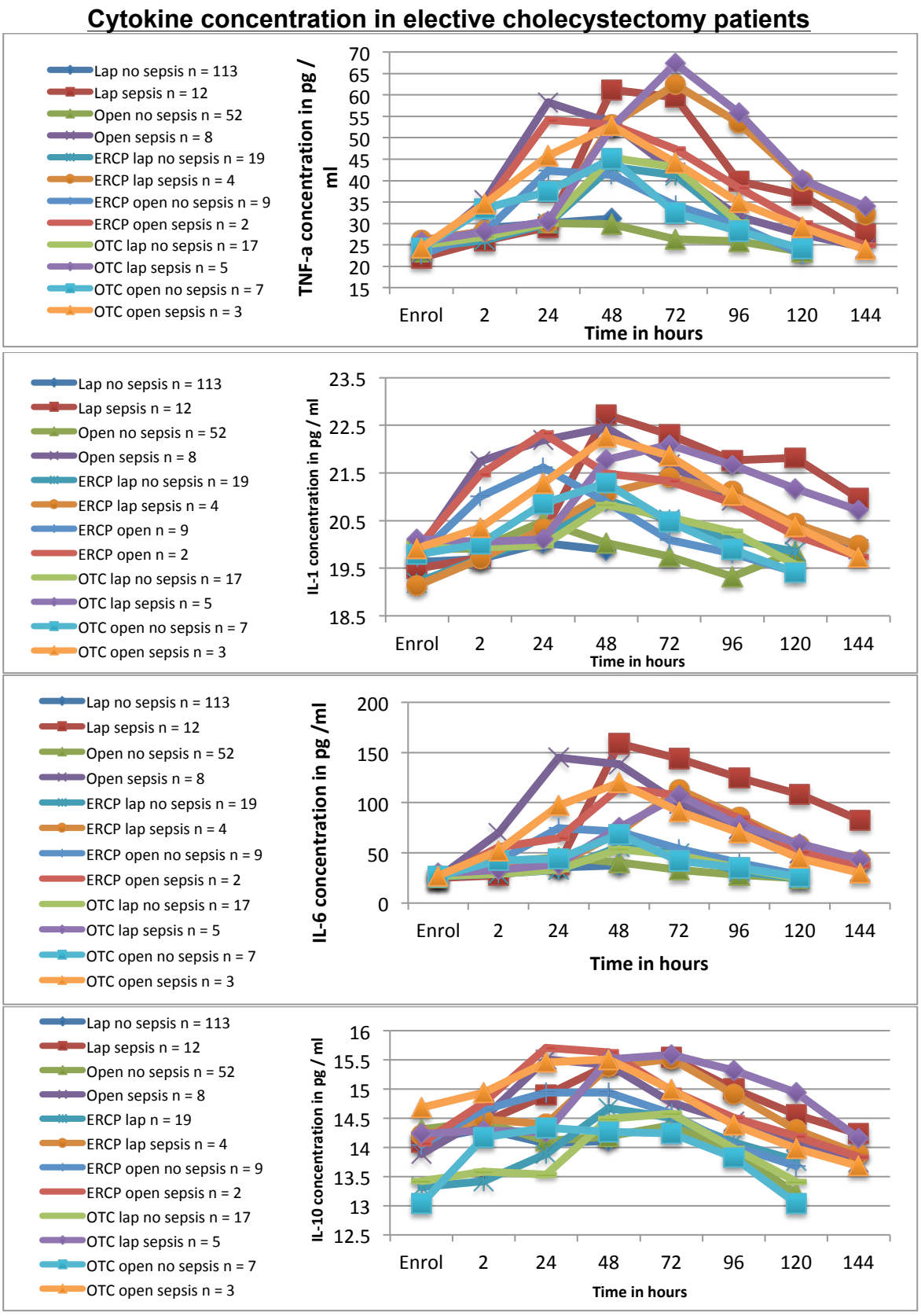


Figure 9.3.1: Demonstrates the cytokine concentration of all the patients undergoing elective cholecystectomy. Lap – laparoscopic, Enrol – enrolment.

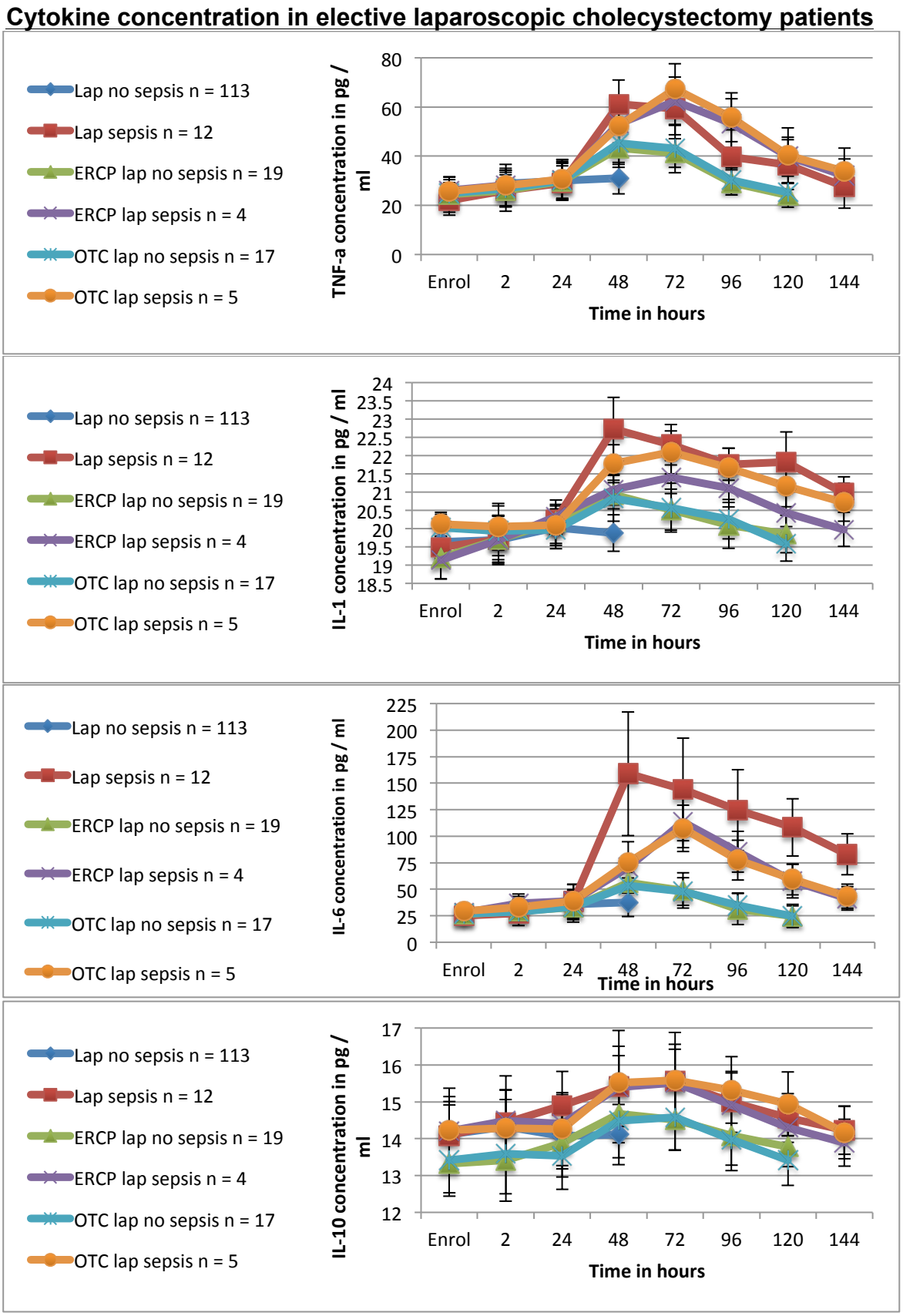


Figure 9.3.2: Demonstrates the change in cytokine concentration (pg / ml ± SD) for the laparoscopic (Lap) approach to cholecystectomy. Enrol – enrolment.

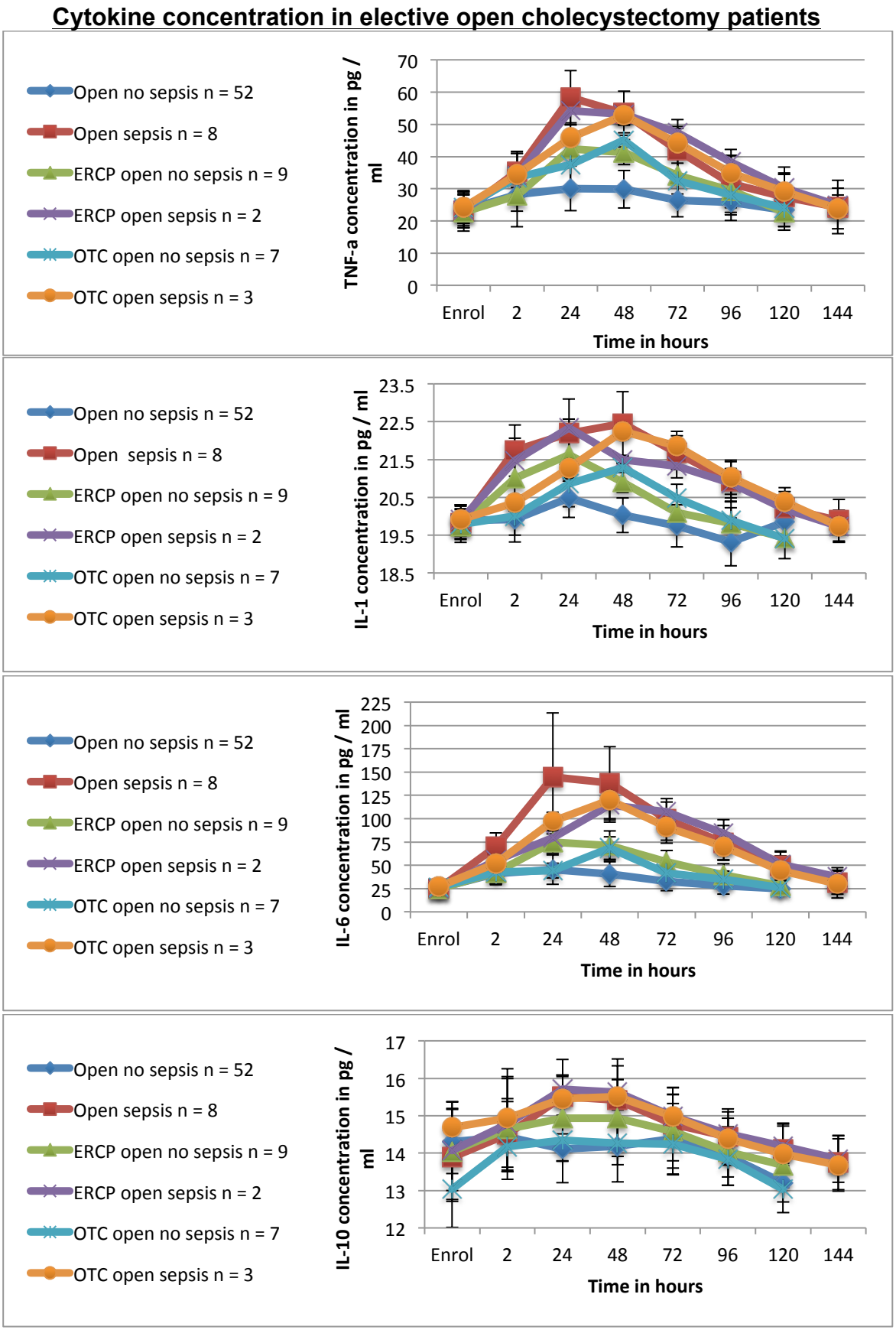


Figure 9.3.3: Demonstrates the change in cytokine concentration (pg / ml ± SD) after elective cholecystectomy. Enrol – enrolment.

Initially considering the inflammatory cytokines it can be seen that there is a difference between the open and laparoscopic approach patients cytokine response. For all the laparoscopic cholecystectomy patients the TNF-α, IL-1 and IL-6 concentration remained stable, or increase slightly up to the assessment at 48 hours. In contrast the cytokine concentration in the open approach patients begins to increase from 2 hours onwards. As would be expected the patients who did not develop sepsis, for all groups, had the smallest rise in cytokine concentration. Further interpretation does have to consider there are small numbers of patients in some of the groups, as it was not planned to analyse the recent ERCP and OTC patients separately.

Patients not developing sepsis

For the open cholecystectomy only patients, the peak cytokine concentration is reached between 24 and 48 hours. In contrast all the laparoscopic approach patients cytokine does not increase until 48 hours at the earliest. The open and laparoscopic cholecystectomy only patients, not developing sepsis, have the smallest rise in cytokine concentration. The patients who have had recent ERCP or OTC, and do not develop sepsis, their peak cytokine concentration is higher than the patients who undergo cholecystectomy only. All the patients developing sepsis increased their cytokine concentration significantly above those not developing sepsis ($p = 0.019, 0.030, 0.001, 0.025$ for TNF-α, IL-1, IL-6 and IL-10). This is seen with both approaches. IL-6 demonstrated this most clearly, particularly in the laparoscopic approach patients. The IL-6 concentration remaining significantly higher, for longer, in the group developing sepsis, reflecting its role as a late cytokine.

The patients who have had a recent ERCP and then open cholecystectomy, without sepsis, their cytokine concentration peaks at 24 - 48 hours, the same as the open cholecystectomy alone patients. The OTC with open cholecystectomy patients, their peak cytokine concentration was at 48 hours. In the laparoscopic approach patients, the recent ERCP and on table cholangiogram patients, not developing sepsis, have a peak cytokine concentration at 48 hours, the same as the laparoscopic cholecystectomy patients alone.

Patients developing sepsis

For the patients developing sepsis, the cytokine concentration is greatest in the patients who have cholecystectomy only. This group includes patients who were admitted to ITU, and particularly in the laparoscopic group, had bowel injuries and not just respiratory or

positive blood and bile cultures. This is reflected in the wide standard deviation due to the multiple causes of sepsis (particularly seen with IL-6). With the small number of patients and different causes of sepsis, it is difficult to separate procedure and sepsis related changes in cytokine concentration.

For all the patients developing sepsis, the open approach patient's cytokine concentration peaks earlier. The patients having open cholecystectomy alone their cytokine concentration peaks at 24 hours and remains elevated at 48 hours then declines. The laparoscopic cholecystectomy only group, developing sepsis, peak cytokine concentration is at 48 to 72 hours and then begins to decrease. For both laparoscopic and open approach patients the biggest increase in cytokine concentration is seen with IL-6.

The recent ERCP patients undergoing open cholecystectomy, have a peak cytokine concentration at 24 hours like the open cholecystectomy alone patients, for TNF-α and IL-1. In contrast the IL-6 concentration peaked at 48 hours, 24 hours later than the early cytokines and the open cholecystectomy only patients developing sepsis. For the laparoscopic approach patients having a recent ERCP, the cytokine concentration has increased at 48 hours like the cholecystectomy alone patients, but the peak is at 72 hours for TNF-α, IL-1, and IL-6.

For the OTC patients having an open cholecystectomy all three inflammatory cytokines have risen at 24 hours, but their peak cytokine concentration is at 48 hours. For the laparoscopic route again the cytokine is increasing at forty-eight hours but peaks at 72 hours.

IL-10 concentration does not significantly increase in those developing sepsis, above those not developing sepsis, but the concentration is higher in those developing sepsis for both approaches. In the laparoscopic approach patients the IL-10 concentration begins to increase earlier (24 hours) than the inflammatory cytokines but peaks at the same time point 48 to 72 hours for all approaches. The peak IL-10 concentration in the open approach patients occurs at 24 to 48 hours regardless of whether they have cholecystectomy alone, recent ERCP or OTC. This occurs with the inflammatory cytokines in the cholecystectomy only group and ahead of the recent ERCP and OTC patients.

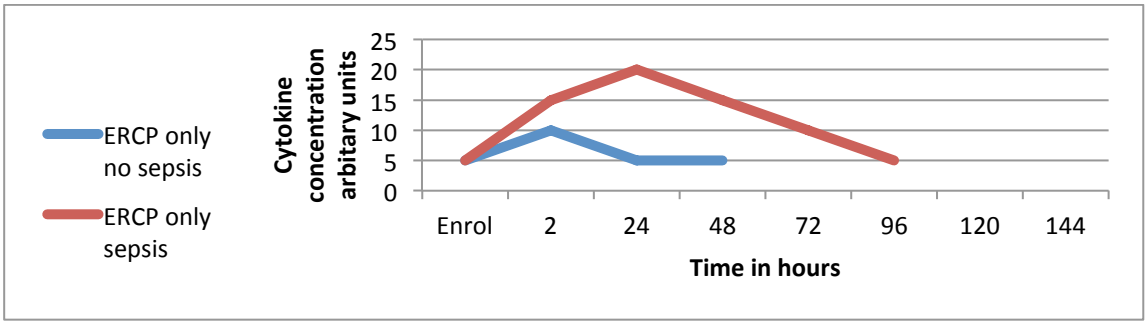
The IL-10 concentration is regulated by the TNF-α and IL-1 concentration but the rise in IL-10 precedes the rise in inflammatory cytokines. Whether this is because we are measuring systemic and not peritoneal cytokines is unclear. Hanly's team (2007) found peritoneal acidification with carbon dioxide, correlated with a fall in the peritoneal inflammatory cytokines. But even in the absence of lipo-polysaccharide, peritoneal acidification stimulated a rise in IL-10 concentration. Conclusions from this study are difficult due to measuring systemic cytokines, but we certainly do see a rise in IL-10 concentration in all patients, and the IL-10 concentration rise precedes the inflammatory cytokine rise and in the open and laparoscopic groups, including those who have had a recent ERCP or OTC.

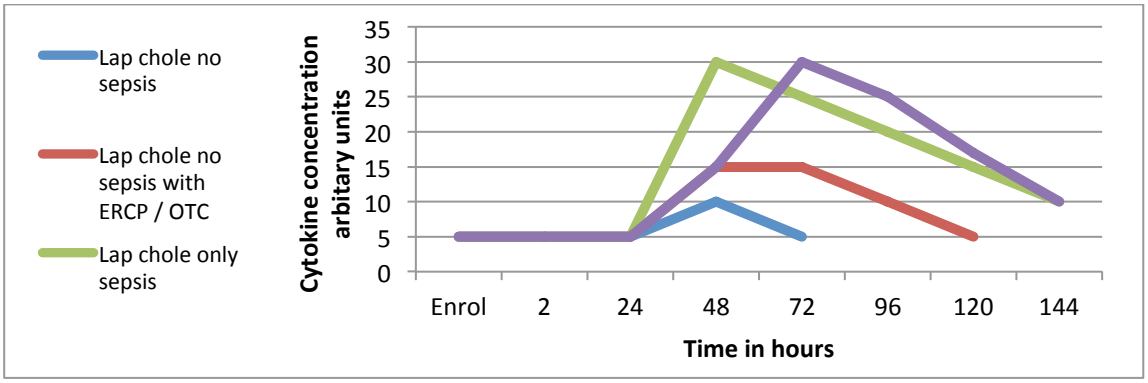
Reviewing the cytokine concentration by the level of care the patients required demonstrated that the ITU then the HDU patients had the greatest increase in cytokine concentration, the ward patients had the smallest increase. This was seen in all the arms of the study. The length of the procedure was longer for those requiring the highest level of care. Longer laparoscopic procedures are a longer pneumoperitoneum, with greater acidification of the pneumperitoneum, inhibiting the increase in inflammatory cytokines, but not affecting the IL-10 concentration.

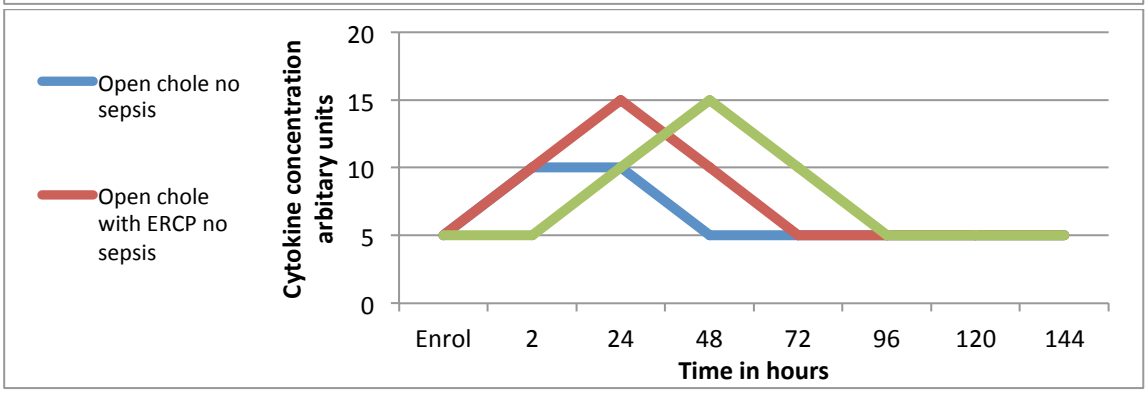
The multiple different groups can make this hard to visualize the change in inflammatory cytokines, therefore Figure 9.3.4 demonstrates the findings as a model with arbitrary units. Figure 9.3.5 demonstrates a model of the change in IL-10 concentration, with IL-6 representing the inflammatory cytokines.

We only measured the cytokine concentration every 24 hours, except for 2 hours time point, after the procedure. Therefore we do not know if the cytokines peaked earlier or increased higher between the times we measured. Also we measured the systemic cytokines and not the peritoneal cytokines and therefore we are inferring that the pattern is the same. From the pilot, where drain fluid was collected to measure peritoneal cytokines we established that this was an appropriate assumption to make, but there was only three patients with drains.

Model for inflammatory cytokine concentration change after intervention







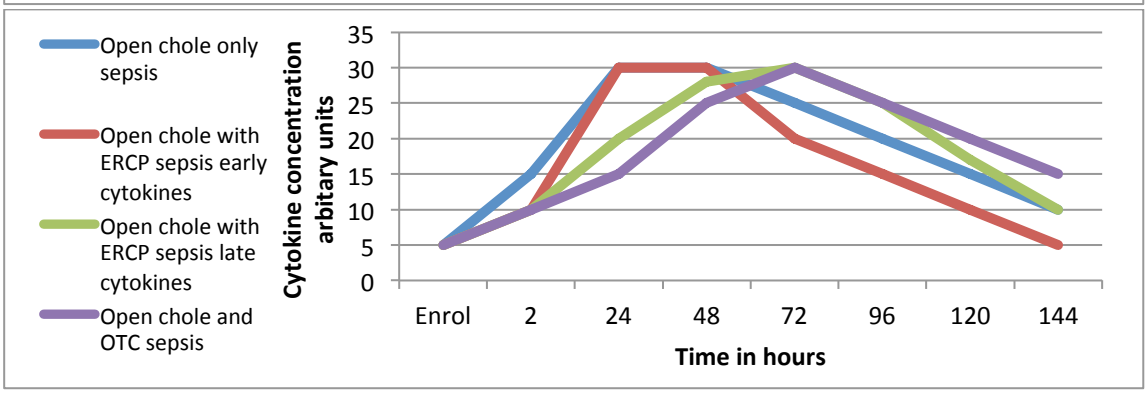


Figure 9.3.4: Model of the cytokine concentration changes found in the study. The three inflammatory cytokines measured followed this pattern. The ERCP only (above), laparoscopic (second) and open (lowest two) approach is separated for ease of interpretation. The Figures highlight the delay in the laparoscopic approach, and the open approach with ERCP late cytokines or OTC in those developing and not developing sepsis. Enrol – enrolment, Early – early cytokines TNF-α and IL-1, Late – IL-6. Lap or Open no sepsis is cholecystectomy only group.

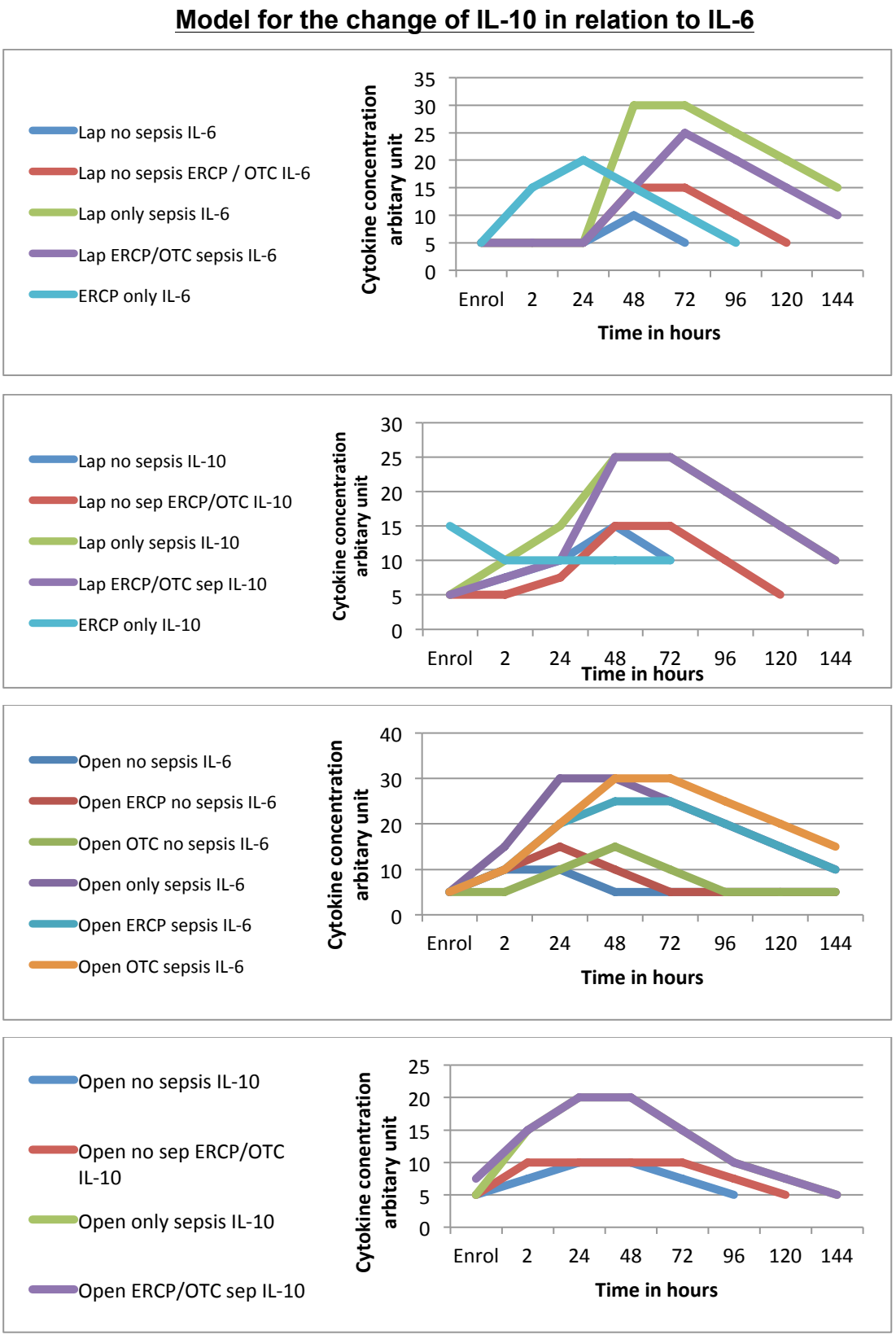


Figure 9.3.5: Demonstrates the changes seen in IL-10 concentration compared to IL-6 as an example of an inflammatory cytokine. IL-6 and IL-10 laparoscopic approach (upper), IL-6 and IL-10 open approach (lower). The IL-10 concentration is less inhibited by the laparoscopic approach or recent ERCP or OTC. Lap / Open no sepsis is the cholecystectomy only patients.

Across a number of different laparoscopic procedures, such as colorectal resections, splenectomy and cholecystectomy it does appear that there are factors within laparoscopic surgery that attenuate the rise in cytokine concentration in the early post operative period (Sammour *et al.,* 2010, Kvanstom *et al.,* 2013, Wu *et al.,* 2012). Sammour team (2010), reached the conclusion that the rise in post-operative cytokine concentration is proportional to the magnitude of the operation, which is important. This out weighs the approach to surgery affect upon the cytokine concentration. The literature demonstrates the larger laparoscopic colorectal resections demonstrating a change in cytokine concentrations, whereas smaller operations such as hernia repair did not demonstrate a change in cytokine concentration (Sammour *et al.,* 2010, Kvanstom *et al.,* 2013, Wu *et al.,* 2012). Certainly in this study the cytokine concentration is not significantly different between the two approaches to surgery. But increased intervention, such as OTC, or increased dissection due to recent ERCP or OTC does increase the cytokine concentration in the patients not developing sepsis.

Biliary obstruction and delayed cytokine increase

The clinical data illustrated the inflammatory cytokine concentration rising at the same time point in those with and without sepsis having the same procedure. The difference being the peak concentrations was greater and sustained for longer in the group developing sepsis. Being an observational study with set timings of systemic blood tests we do not know the exact timing of the onset of sepsis, but the cytokine concentration increase mirrored the SIRS markers and the clinical diagnosis of sepsis.

The findings indicate that factors around laparoscopic surgery delay the rise in cytokine concentration. But the findings cannot solely be explained by this, as those with instrumentation of the biliary tree, either as a recent ERCP or OTC also have a further delay on the rise in cytokine concentration. This could be because the system is obstructed (OTC patients), or has recently been obstructed, potentially with or without remaining oedema due to the recent ERCP. In these patients theoretically there is localised inflammatory markers that have not fully drained. Cholecystectomy, particularly in the presence of biliary inflammation, facilitates drainage of these localised inflammatory mediators into the systemic circulation. Potentially factors around surgery, and the biliary intervention, affect when this is detectable systemically.

Supporting this is the finding of the mean time from ERCP to surgery, for those developing sepsis was 40 ± 7 days. In contrast those not developing sepsis the mean time between ERCP to surgery was 103 ± 23 days (p = 0.002). This additional time theoretically giving time for the system to drain, and potentially bacterial overgrowth, due to biliary stasis, to be resolved and oedema settle. There was no difference between the patients who had had sphinceterotomy, versus those who had a stent *in-situ*. From research, biliary stasis is known to be inhibitory to the inflammatory cytokines but not IL-10 (Nesseler *et al.,* 2012). Factors around the pneumoperitoneum in the laparoscopic approach patients may magnify the delay in cytokine response. The OTC group where the system was obstructed had a trend towards more episodes of sepsis particularly in the laparoscopic approach patients, but this did not reach significance. These operations were longer and noted to be more complex. As was those who had had a recent ERCP, the operations were difficult due to adhesions, difficult to delineate anatomy, bile spillage and oedematous gall bladder.

Source of sepsis

Thirty four patients who underwent cholecystectomy developed sepsis post-operatively. Eight of the elective ERCP patients, five ERCP only patients and three after emergency cholecystectomy after ERCP, were diagnosed with sepsis. The proportion of each type of post-operative sepsis did vary between the groups.

There was no evidence of a septic nidus from the enrolment cytokine bloods in any of the elective cholecystectomy patients, but this may not be evident at the systemic level only at the peritoneal cytokine level. We know from culture results in this study between 10 – 20% of surgical patients had positive bile cultures (Table 8.2.8). The presence of a septic nidus within the gall bladder, with the delayed cytokine response in the laparoscopic approach patients, and in the patients where the biliary system is obstructed or has recently been obstructed would be expected to predispose these patients to post-operative sepsis.

The open approach patients, receiving prophylactic antibiotics, had more episodes of biliary sepsis with positive peripheral blood cultures (p = 0.041), predominantly in the OTC group. This was also seen in the laparoscopic group undergoing OTC. These

patients were more likely to have had a significant bile spillage, defined as more than a small leak on cannulation of the duct.

Respiratory complications were significantly more frequently seen in the laparoscopic approach patients (Table 8.2.7). Smoking was also more common in those developing sepsis, and is a potential confounder. The laparoscopic group, also had a higher rate of sepsis amongst those who were diabetic (Table 8.2.6) ($p = 0.045$). There was a trend towards this in the open patients but it was not significant. Confounding conclusions about sepsis was the difference between the approaches in terms of the administration of prophylactic antibiotics. All of the open cholecystectomy patients received antibiotics, but for the laparoscopic approach patient, even with OTC or recent ERCP it was at the surgeon's discretion. The majority of the laparoscopic cholecystectomy patients who developed sepsis had not received antibiotics (90%) Table 8.2.8.

In conclusion we have demonstrated that the laparoscopic approach patients have a delay in their cytokine response following surgery. Instrumentation of the biliary tree also contributes to the delay in the increase in cytokine concentration after surgery. Patients who rate their pain as severe pre-operatively, those with pre-exiting respiratory disease and diabetes are at increased risk of developing post-operative sepsis. Therefore we should consider prophylactic antibiotics in these patients and patients having OTC or who have had a recent ERCP.

Results

Chapter 10 – Pain

10.1 Biliary emergency - Pain

The biliary emergency patients were enroled into the study on surgical assessment unit. They had all been reviewed and received analgesia prior to admission, from either their general practitioners or the team assessing them in the emergency department. On admission the admitting team also administered analgesia. When the patients were enroled they had been on surgical admissions unit on average for four hours, having been admitted and given time to review the information about the study.

Analysis of the mean pain score for those with biliary disease demonstrated that those with obstructive jaundice and pancreatitis had significantly more pain initially ($p = 0.002$). At two hours this difference was lost ($p = 0.032$ and $p = 0.022$ pre and $p = 0.484$ and $p = 0.521$ at 2 hours for pancreatitis and obstructive jaundice respectfully), due to administration of analgesia.

Analysis based on quality of life data demonstrated those in VAS significant pain group at enrolment into the study (VAS greater than or equal to 4 to less than 7), could be split into two groups; those who scored their quality of life similar to the mild pain group, denoted as 'Significant pain manageable'. The second group denoted as 'Significant pain experienced', scored their quality of life as significantly poorer than those in the mild or significant pain manageable group. Discussed Chapter 8.3.

Figure 10.1.1 demonstrates changes in the pain score for each group. The second part of the figure highlights the patients in the significant pain experienced group experienced more pain than the other patients with the same admission diagnosis. The significant pain experienced group had a longer admission than the other patients with identical diagnosis (Table 8.4.8).

The doctors' emergency admission pro forma had a question to verbally rate patient's pain, no other arm of the study was independently asked their pain score by the clinical

team treating the patients. This is included in the second part of Figure 10.1.1 to demonstrate the significant pain experience patients had responded to analgesia received prior to enrolment, and their pain had been acted upon. In Table 8.4.7 we highlighted the significant pain experienced group's dissatisfaction with the response and treatment of their pain throughout their admission, but these patients have received analgesia and were admitted.

The pain score on the admission pro forma places the significant pain experienced in the severe pain, and not the significant pain group. We reflected whether patients, particularly in this group scored their pain differently verbally when asked by a doctor who would be prescribing analgesia, this was not seen with the VRS in the pilot. The pancreatitis and obstructive jaundice patients' pain score had not decreased from their admission scoring and they score their pain as severe on the VAS at enrolment.

VAS at each time point for each of the biliary emergency patients, and the VAS score in the biliary colic and acute cholecystitis group

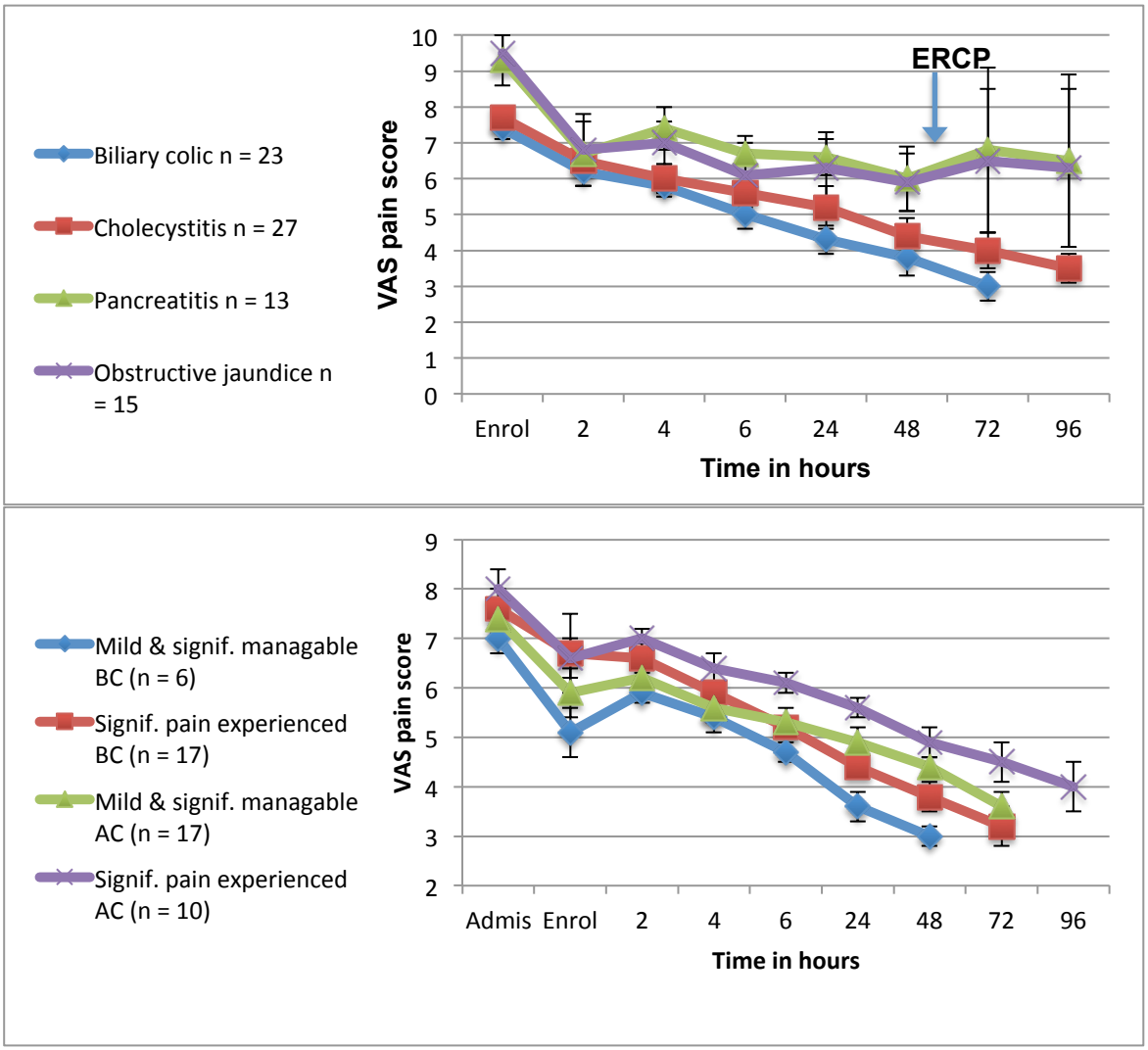


Figure 10.1.1: Demonstrates the visual analogue pain score (±SD) at each time point for the biliary emergency admissions. The timing of the ERCP's is marked in the top figure, only the pancreatitis and obstructive jaundice patients underwent ERCP, all these two groups scored their pain as severe. The lower figure demonstrates the biliary colic (BC) and acute cholecystitis (AC) patients split into the significant (Signif.) pain manageable and mild pain group and significant (Signif.) pain experienced group. The doctors admission clerking pro forma asked a verbally rating of pain, which is given in the lower Figure, this had dropped by enrolment, hence patients were in the significant and not severe pain group. Admis – admission, Enrol – enrolment.

All the patients with an elevated white cell count were commenced on antibiotics, none of the biliary colic patients received antibiotics. All the acute cholecystitis patients received antibiotics, but no further sources of sepsis were documented besides cholecystitis. Five pancreatitis patients received antibiotics, one with positive blood cultures for *E.coli*, one with a chest infection, and one with necrotizing pancreatitis, diagnosed on a CT scan. Two further pancreatitis patients had raised white cell count and were commenced on antibiotics. One for a possible chest infection, and line sepsis as a potential source was raised with the other. These did not fulfill the definition of sepsis. One of obstructive jaundice patient developed sepsis

The majority of patients with obstructive jaundice and pancreatitis were discharged on day five. Seven pancreatitis patients and eight with obstructive jaundice were not discharged due to being in pain. The patients who developed sepsis, and the two with raised white cell count, had an increase in their pain score on day five, whereas the other patients pain score continued to decrease. This rise in pain was not mirrored by a rise in SIRS markers, which did not rise until at least 18 ± 8 hours later, from the observation chart. The cytokines did not increase till 24 hours afterwards, but the cytokines were only being measured every 24 hours. The pain score remained elevated on day six only falling from day seven onwards. Figure 10.1.2 demonstrates the change in VAS pain score. The biliary colic and acute cholecystitis patients were omitted as none of this group developed sepsis.

The highest VAS scores was in the patient with necrotizing pancreatitis (admitted to HDU) and the obstructive jaundice patient (admitted to surgical dependency unit a step up from normal ward care). The lowest VAS scores were those where the source of sepsis was not fully determined. The numbers of patients are too small to draw conclusions, but throughout the study patients requiring higher levels of care had higher pain scores. The six patients with sepsis, their individual pain score are plotted in the second part of Figure 10.1.2, to illustrate the difference in pain scores between patients. As with other arms of the trial, the patients who developed sepsis, their pain score began to fall ahead of the decline in SIRS markers and pre-empted a significant fall in the systemic cytokine concentration.

VAS in the pancreatitis and obstructive jaundice patients, highlighting those developing sepsis

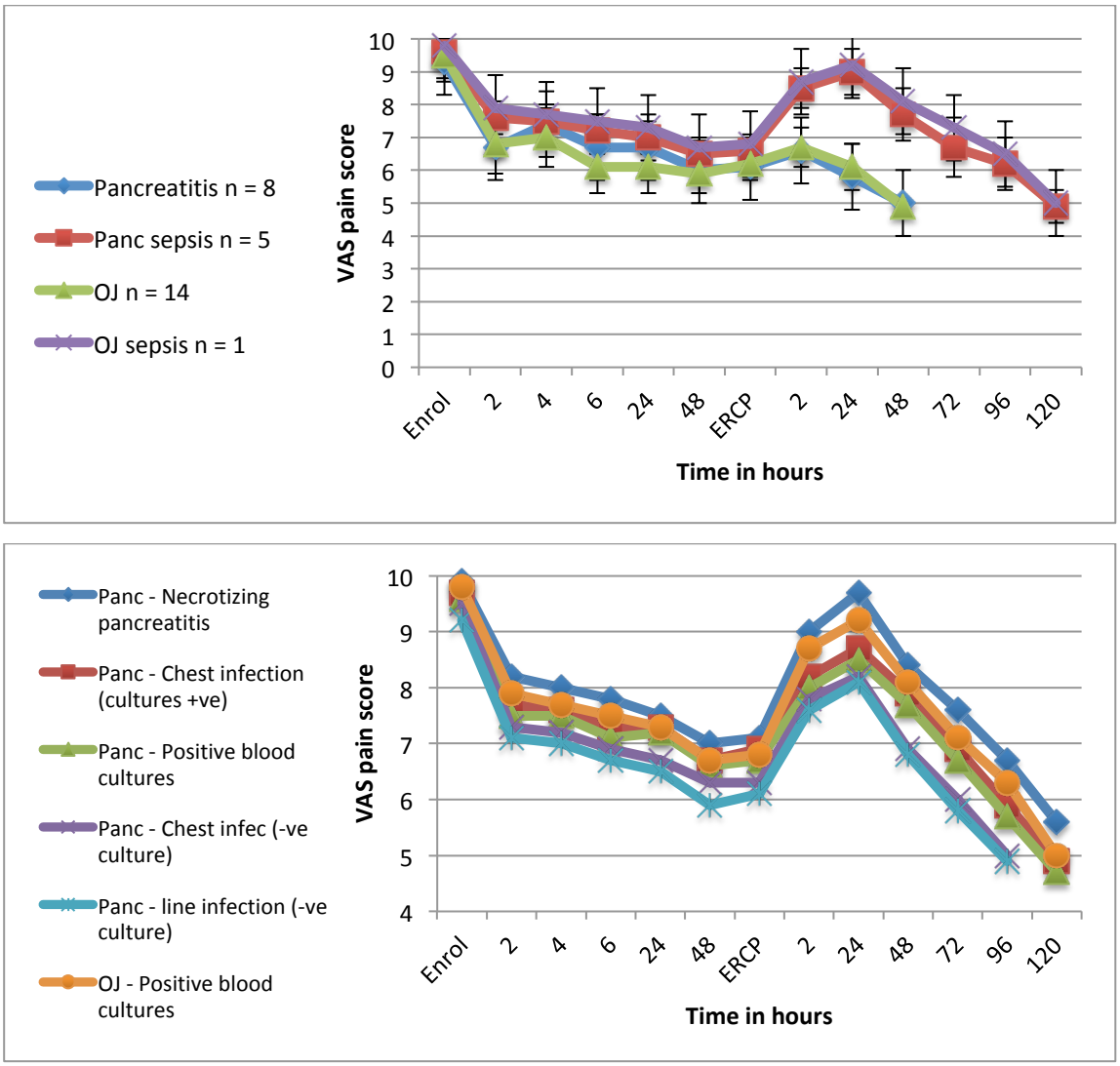


Figure 10.1.2: The change in pain score in those patients developing and not developing sepsis who were admitted with pancreatitis or obstructive jaundice. The biliary colic and acute cholecystits patients have been omitted for ease of illustration, they also did not undergo ERCP, and none of these patients developed sepsis. Twenty-five of the twenty-eight obstructive jaundice and pancreatitis patients underwent an ERCP, between 48 and 72 hours after admission. The second half of the figure illustrates the individual pain scores in the four developing sepsis, and 2 with proposed sepsis but negative cultures. The patient with necrotizing pancreatitis and a chest infection required the highest level of support in the group of patients. Panc – pancreatitis, OJ – obstructive jaundice, Enrol - enrolment.

10.2 ERCP patients – Pain

The majority of patients had their ERCP completed at first attempt and were discharged on the day of the procedure Table 8.4.8. Two of this group developed sepsis. Eight patients had a second ERCP after an unsuccessful first procedure, three of this group developed sepsis. Five additional patients had an unsuccessful first ERCP and underwent an emergency cholecystectomy rather than a repeat ERCP at the clinician's discretion. This was made because the patients had two raised SIRS marker and concern about sepsis, but without substantive diagnosis. This was reflected in the raised cytokine concentration, and had very difficult unsuccessful ERCP's. They were perceived by the clinical team to be more unwell than the patients who underwent a second ERCP; none of this group had two raised SIRS markers elevated.

The patients who were discharged on the day of the procedure (n = 32) were asked if they would return for bloods and pain score for the study, reimbursement for travel was made. Twenty-three patients returned and nine were contacted by telephone. By 48 hours 37 had been discharged and 35 were contacted by telephone for their pain score. None of the patients developing sepsis had been discharged. Three patients undergoing second ERCP were discharged on the day of the second procedure, the rest, except for those developing sepsis, were discharged the following day. Having been discharged the patients pain scores may not be equivalent to the inpatients.

Of the seven patients not discharged on the day of ERCP who had had a successfully completed ERCP, six stayed due to pain, one because of social circumstances. Four patients (8%) were identified as fulfilling the significant pain experienced criteria; none were discharged on the day of the procedure, remaining in up to 72 hours after the procedure. Two remaining in due to pain developed sepsis, a chest infection and positive blood cultures. The significant pain experienced group was under represented in this arm, possibly reflecting the fact they underwent surgery earlier in the course of their disease Table 8.4.8.

Patients in the severe pain group at enrolment were significantly less likely to have a successfully completed ERCP at the first attempt ($p = 0.025$). This was principally due to having difficult anatomy, and difficult procedures. The severe pain group patients were more likely to develop post procedural sepsis ($p = 0.021$). Whether the increased

pain score at enrolment indicated localised intra-peritoneal sepsis, which became apparent post procedure is not possible to comment on from this study.

Figure 10.2.1 demonstrates the pain score for the patients undergoing ERCP. The top figure demonstrates the pain score after the first ERCP. The lower figure demonstrates those who underwent a second ERCP or cholecystectomy, denoted as abandoned in the upper figure. The two developing sepsis after successful first ERCP are shown in both figures Figure 10.2.1 demonstrates the higher pain score in the significant pain experienced group and how they are more difficult to separate from the group developing sepsis following ERCP.

The VAS pain score after first and second ERCP

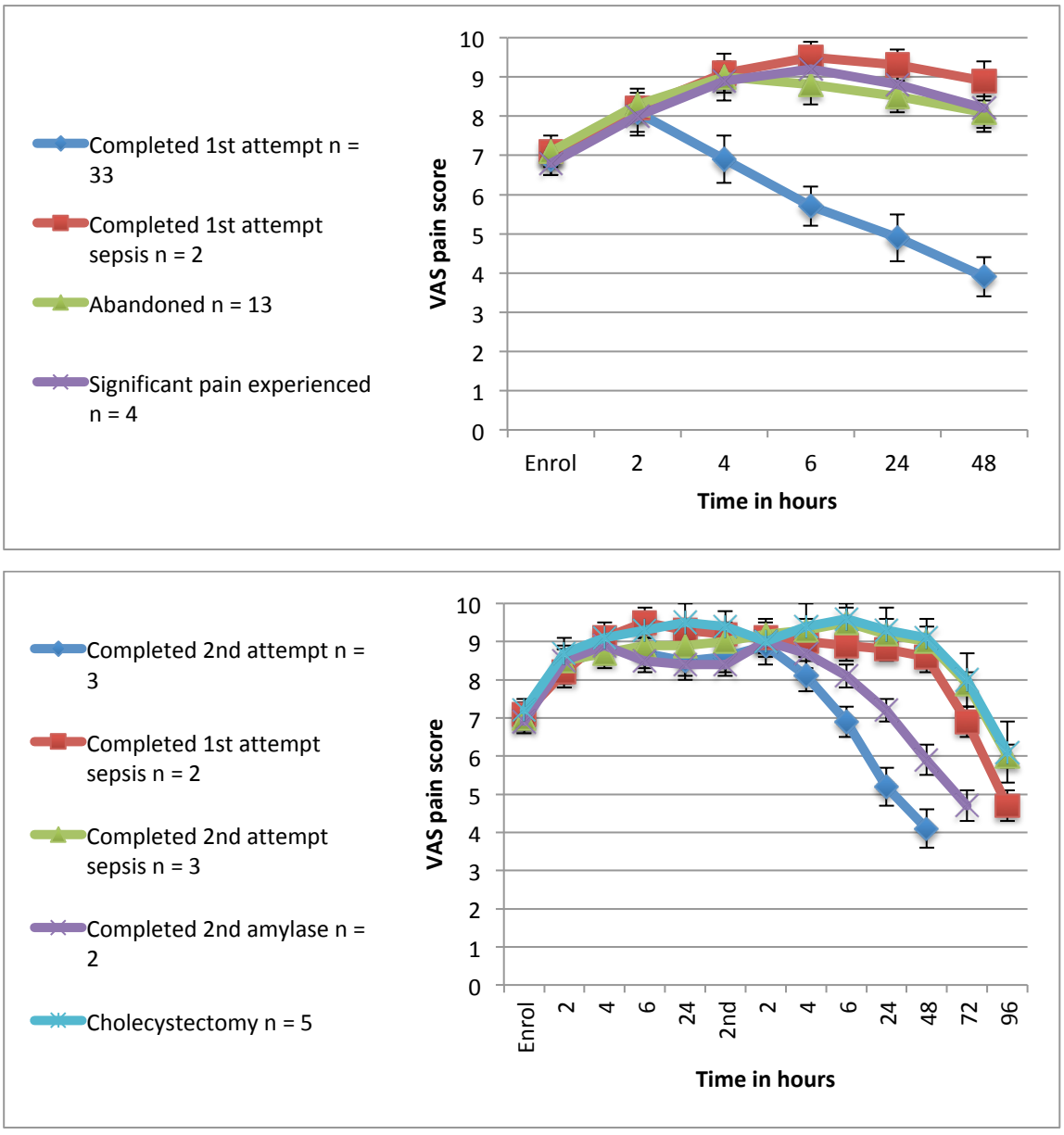


Figure 10.2.1: demonstrates the pain score for the patients undergoing ERCP. The top figure demonstrates the pain score after the first ERCP. Those where it was abandoned were the people who went on to have a second ERCP, or cholecystectomy shown in the lower figure. The patients who had a raised amylase after the second ERCP there pain score was slower to fall, than where there was no problem post ERCP. The patients with sepsis had the highest pain score. Enrol – enrolment, 2nd – second ERCP.

As can be seen from the Figure 10.2.1 the pain the pain scores have increased two hours after the procedure in all groups of patients. From four hours onwards the pain scores fall in the patients where the ERCP has been successfully completed, but not in those where the system is still obstructed or who develop sepsis following the procedure. The SIRS and cytokines rising in the 2 developing sepsis only in the 24 hours bloods, but the pain score being higher from four hours onwards. Of the eight patients who had a second ERCP, three developed sepsis, these patients also had a higher pain score from 4 hours after the second procedure but only developed clinical signs of sepsis 24 hours after the successfully completed ERCP.

Of the five not developing sepsis after the second ERCP, two had a raised amylase following the procedure but never developed sepsis, their pain score was higher than the three not developing sepsis Figure 10.2.1. Unlike those developing sepsis their pain scores fell after 24 hours, and by 48 – 72 hours the blood amylase was back within the normal range. Like the sepsis patients the pain score pre-empted the blood and cytokine change, but with small numbers it is difficult to draw firm conclusions.

The patients were asked to rate their least and most expected and experienced pain, their response is shown in Figure 10.2.2. As with the biliary emergency patients the majority of patients under rated their expected most pain, compared to what they experienced. The patients in the significant pain experienced group expected to be in pain and unlike the other groups there prediction was not significantly different to their experience ($p = 0.910$ for the significant pain experienced patients in all arms, $p = 0.031$ for other ERCP, $p = 0.001$ for other laparoscopic and $p = 0.01$ for other open patients). As in the other arms they tended to score their least pain mid way along the VAS and their most at the right hand end for least and most pain respectfully. Of the six in the significant pain experienced group four had a successfully completed first ERCP, one underwent a second ERCP and developed sepsis, and the final one underwent a cholecystectomy after their first failed ERCP, and did not develop sepsis after cholecystectomy. Despite the diversity of outcomes in the group their expected and experienced pain score closely matched.

The least and most expected and recalled pain after ERCP

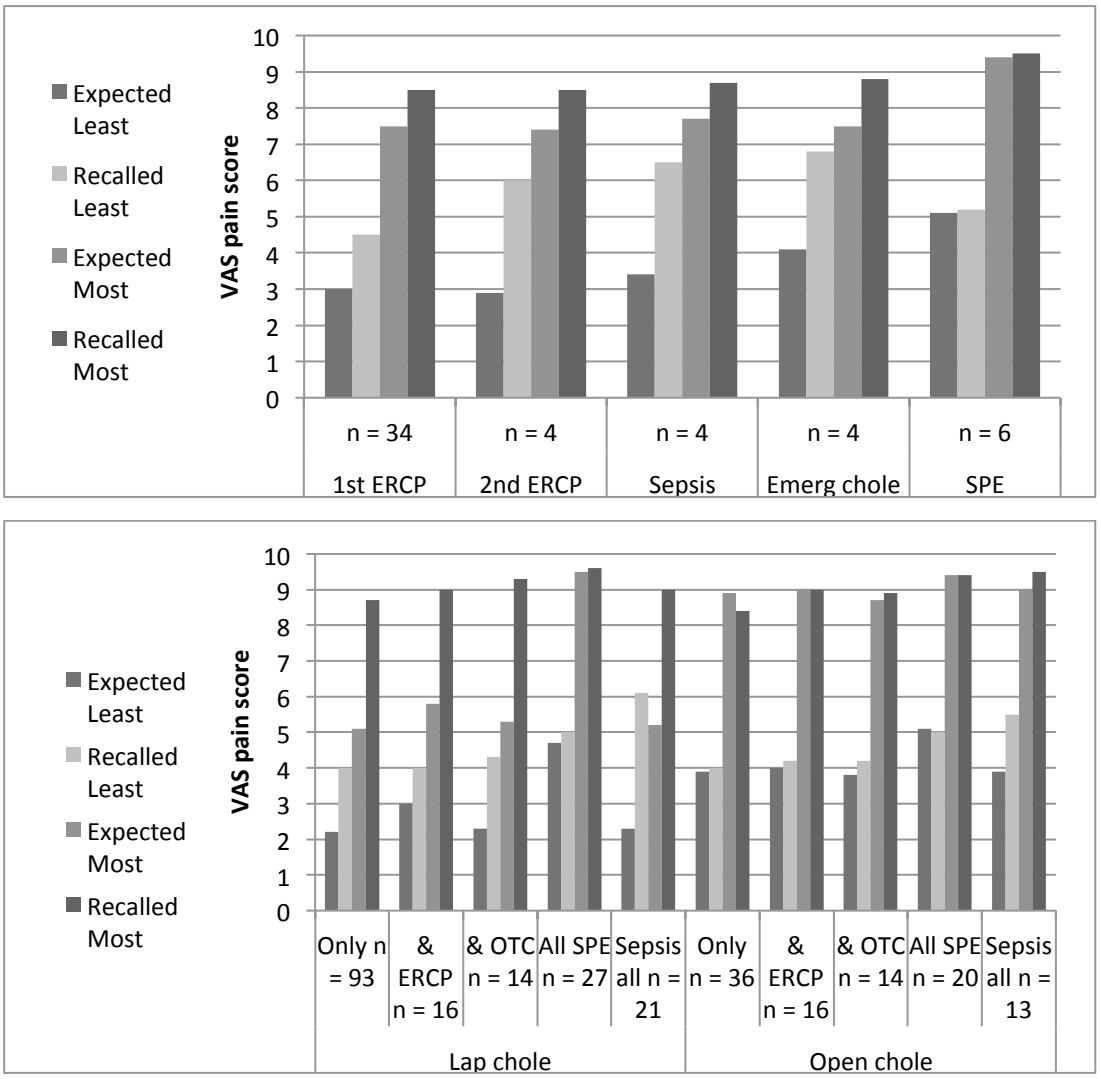


Figure 10.2.2: Demonstrates the patients expected and recalled pain after ERCP. One significant pain experienced (SPE) patient developed sepsis after a second ERCP, and one underwent a cholecystectomy. There pain scores are placed with the significant pain experienced group, because their expectation and recalled pain more closely matched this group, than the other sepsis and cholecystectomy patients. The Figure demonstrating the expected pain matching the recalled pain significantly more closely in the significant pain experienced group. This was also seen in the other arms of the study Figure 8.4.6. Chole – cholecystectomy.

10.3 Cholecystectomy patients – Pain

Ninety four (83%) of the laparoscopic cholecystectomy patients, who did not have ERCP or on table cholangiogram, were discharged on the day following surgery. The reason the other thirty one of this group was not discharged is demonstrated in Table 10.3.1. Eighty-one percent were not discharged due to pain. None of the patients developing sepsis were discharged at 24 hours, but had not been diagnosed with sepsis at this time point, but all were noted to be 'not quite right' (25%) or in pain (75%).

Two of the significant pain experienced group was discharged at 24 hours after cholecystectomy alone. Seventeen were not discharged due to pain in this group (77%), one developed sepsis. From Table 10.3.1 it can be seen that the significant pain experienced group can not be distinguished from the patients who went onto develop sepsis at the 24 hour time point. Only this group and the group developing sepsis remained as inpatients past 24 hours in the laparoscopic cholecystectomy only group. None of the laparoscopic approach patients who had had a recent ERCP or on table cholangiogram were discharged at 24 hours, principally due to pain.

Reason for patients not being discharged at 24 hours after laparoscopic cholecystectomy

Reason for not being discharged at 24 hours	Number not discharged (%)	Number in significant pain experienced group (%)	Notes
Sepsis group			
Laparoscopic cholecystectomy			
Pain	9 (75%)	1 (8%)	
Not being quite right	3 (25%)		2 bowel perforation, went to ITU, 1 respiratory infection went to HDU
ERCP laparoscopic cholecystectomy			
Pain	3 (75%)	-	
Not being quite right	1 (25%)		1 chest infection smoker
Laparoscopic cholecystectomy OTC			
Pain	4 (80%)	-	
Not being quite right	1 (20%)		1 Chest infection COPD went to HDU
No sepsis group			
Laparoscopic cholecystectomy			
Pain	16 (84%)	16 (72%)	
Not being quite right	3 (16%)	3 (14%)	
ERCP laparoscopic cholecystectomy			
Pain	16 (84%)	3 (100%)	
Not being quite right	3 (16%)	0 (0%)	
Laparoscopic cholecystectomy OTC			
Pain	15 (88%)	3 (100%)	
Not being quite right	2 (12%)	0 (0%)	

Table 10.3.1: The table demonstrates the reason for not being discharged at 24 hours after laparoscopic cholecystectomy. The open patients are excluded as it was not planned to discharge them at this time point. None of the ERCP and OTC patients were discharged at 24 hours principally due to pain. The OTC patients were not usually discharged at 24 hours.

In the laparoscopic patient group sepsis was diagnosed 25 to 60 hours following cholecystectomy. Open approach patients who developed sepsis were diagnosed 2 to 33 hours post operatively, seven being diagnosed prior to 24 hours, predominantly around 20 hours post-operatively and five diagnosed after 24 hours. This is significantly different to the laparoscopic group ($p = 0.002$). This fits with the findings of the systemic cytokine concentration and the SIRS not being significantly different until measured 48 hours after surgery in the laparoscopic surgical group developing sepsis, as demonstrated in Figure 9.3.2. Despite this none of the patients with sepsis were discharged and the principal reason for this being pain.

Figure 10.3.2 demonstrates the pain scores following surgery; the standard deviation is omitted for ease of interpretation. Figure 10.3.3 and 10.3.4 is the pain scores plotted by approach to cholecystectomy.

Pain scores for patients undergoing elective cholecystectomy

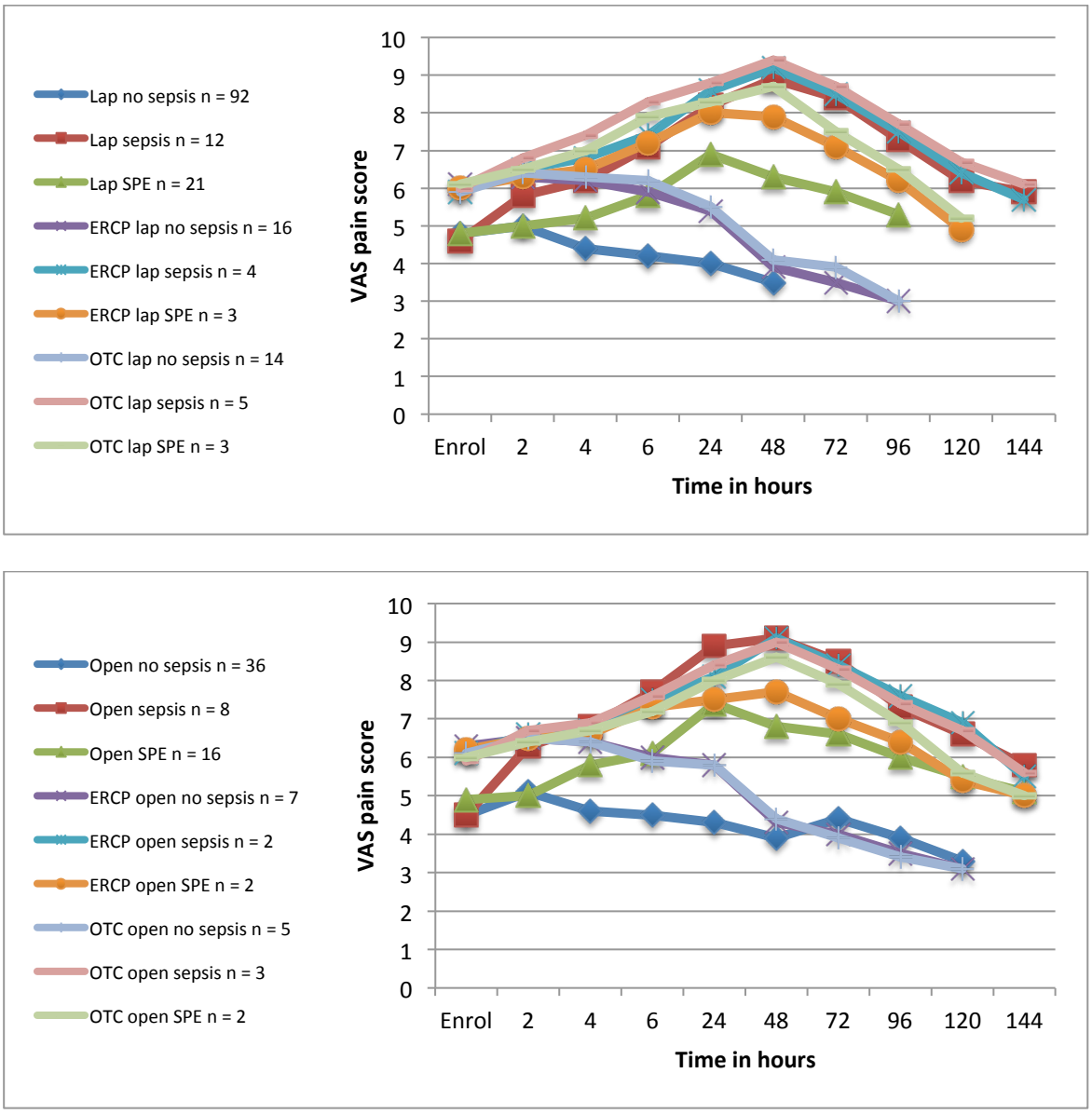


Figure 10.3.2: Demonstrates the pain score of the patients undergoing elective cholecystectomy, laparoscopic approach (above) and open (below). The standard deviation is omitted for ease of interpretation. Figures 10.3.3 and 10.3.4 demonstrate the pain scores with the standard deviation. SPE – significant pain experienced, Enrol - enrolment.

Pain scores in the elective laparoscopic cholecystectomy patients

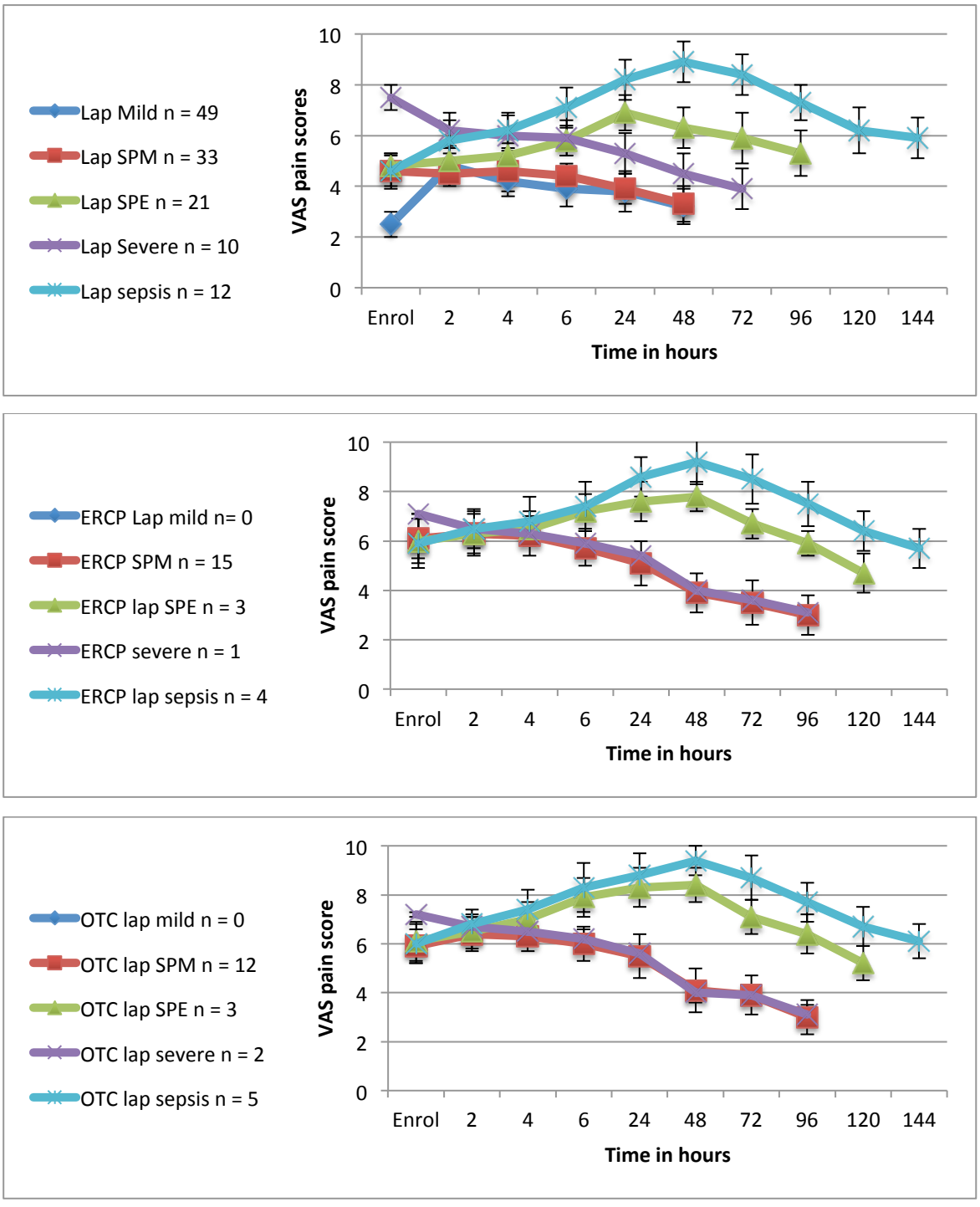


Figure 10.3.3: Demonstrates the pain score and the standard deviation for the laparoscopic approach patients undergoing, cholecystectomy alone (top), recent ERCP and cholecystectomy (middle), OTC and cholecystectomy (bottom). SPM – significant pain manageable, SPE – significant pain experienced, Enrol - enrolment.

Pain scores in the elective open cholecystectomy patients

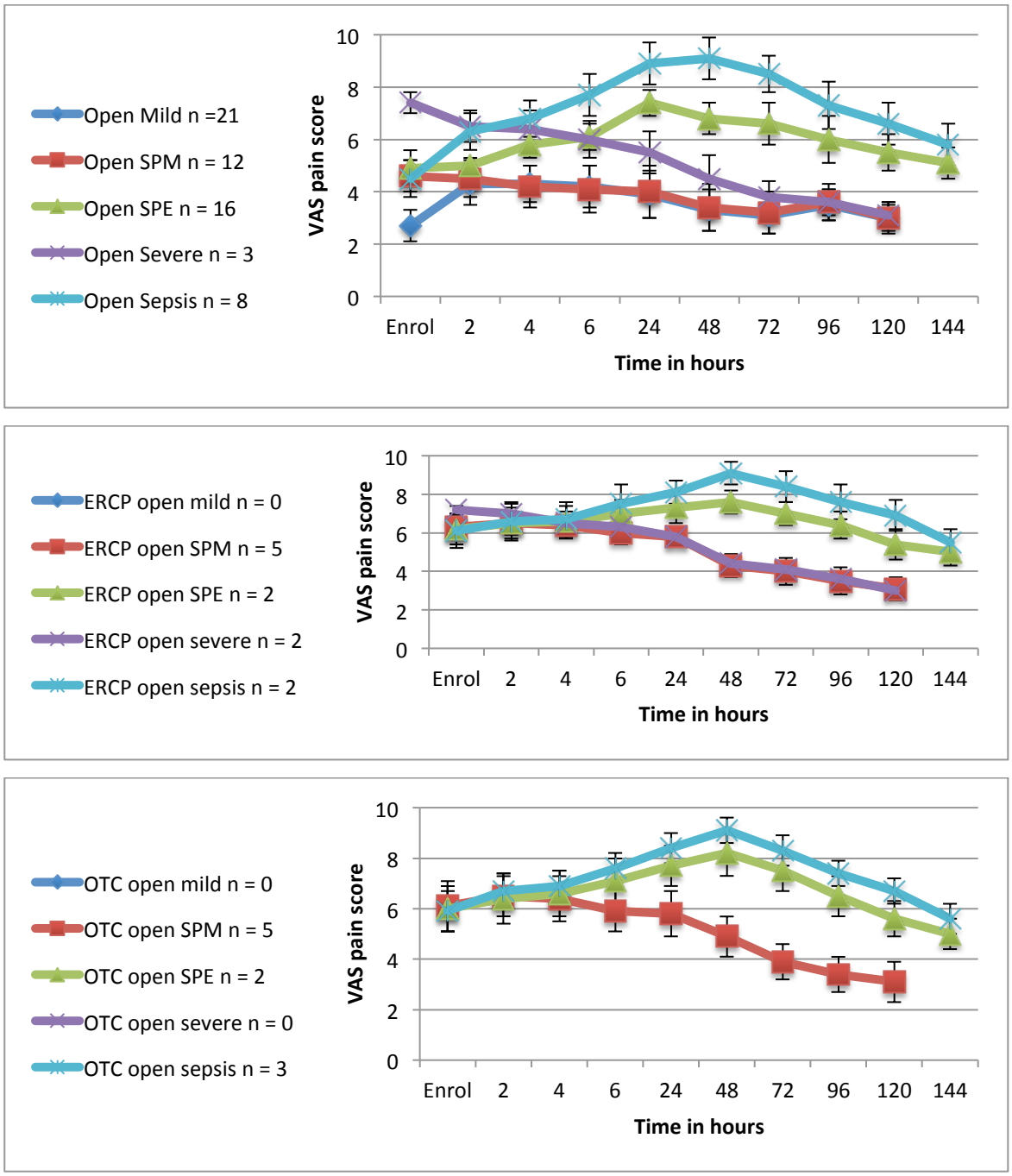


Figure 10.3.4: Demonstrates the pain score and the standard deviation for the open approach patients undergoing, cholecystectomy alone (top), recent ERCP and cholecystectomy (middle), OTC and cholecystectomy (bottom). SPM – significant pain manageable, SPE – significant pain experienced, Enrol - enrolment.

From Figures 10.3.2 – 4**,** it can be seen that the patients who have undergone a recent ERCP or had an OTC, at enrolment have significantly more pain than the majority of patients who just have a cholecystectomy alone. This is seen in both the laparoscopic and the open approach patients. The patients who are not in the significant pain experienced group for each approach, their pain score decreases post operatively. The severe pain group VAS scores falling the quickest after surgery. The mild pain groups pain score increases from enrollment to two hours after surgery, and then decreases, this probably is the response to surgical intervention.

Patients not diagnosed with sepsis - excluding significant pain experienced group

From early in the post operative period for both approaches the mild and the significant pain manageable patients pain scores are similar. The severe pain group patients pain scores drop to also be similar to the mild and significant pain manageable group at 48 hours. Although higher initially the patients who have an OTC or ERCP, their pain scores fall to be similar to the patients who have elective cholecystectomy alone, from 24 – 48 hours onwards. Whether this is when the post-operative oedema settles and the biliary drainage improves, is not known from this study.

Despite the perception of the difference in magnitude of the open and laparoscopic approach the pain scores are remarkably similar. This could be because we had difficulties getting patients to cough prior to measuring their pain score, to affect both visceral and parietal pain. Therefore with the laparoscopic approach we are measuring patients who are mobile and receiving as required analgesia, whereas the open approach patients they are frequently in bed and on PCA to 72 hours. This is a problem with the comparison of the pain scores throughout the study.

For the recent ERCP or OTC patients their pain score peak at twenty four to forty-eight hours after surgery, and then markedly drop. This drop in pain score is seen for all pain groups at 48 hours, regardless of surgical approach, in all the patients undergoing OTC or recent ERCP and who do not develop sepsis. These groups, not developing sepsis, have a higher peak in cytokine concentration than the cholecystectomy only patients regardless of surgical approach. The systemic cytokine concentration falls 48 hours onwards. The fall in pain score could be due to the systemic cytokine concentration falling, with the intraperitoneal concentration falling before the systemic level. This

would require a study measuring drain fluid levels. But it does support the pain scores responding to changes in cytokine concentration, and therefore the changes in pain scores being important in the group developing sepsis following surgery.

The problem with drawing conclusions is the small number of patients in the groups, particularly the significant pain experienced group, in the group who've had recent ERCP or have an OTC, as the study was not powered for analysing these subgroups.

Significant pain experienced group

Figures 10.3.3 and 4, demonstrate that the significant pain experienced pain group patients, increase their pain scores between four and six hours post operatively. By six hours their pain scores are diverging away from the other patients who do not develop sepsis. This is seen in both approaches and regardless as to whether the patients have had recent ERCP, on table cholangiogram or cholecystectomy alone. This corresponded to when the effects of surgery were decreasing, the patients were more alert, and was the time point when most visitors were present. Though patients were asked to complete the pain scores alone, visitors' presence may affect how the patients scored their pain. Up until six hours the sensory – discriminative dimension predominates, around this six-hour time point the affective – motivational and cognitive evaluative dimensions increase in importance.

In the open group with PCA to control of their pain, the pain scores in the significant pain experienced group are higher and similar to the laparoscopic approach patients. Unsuccessful attempts with the patient controlled analgesia, also increased from six hours onwards. The significant pain experienced group having significantly more unsuccessful attempts, in all three open groups.

The pain score for the significant pain experienced group just undergoing cholecystectomy peaks, for both approaches, at twenty-four hours and then gradually falls. The gradual fall mirroring the other pain groups not developing sepsis, undergoing cholecystectomy alone, but at a higher level. The cytokine concentration in this group also decreases from 24 hours onwards (Figure 9.3.2 and 3). The cytokine peak is not as marked as the recent ERCP and OTC groups' cytokine peak, and may explain the more gradual fall in pain score.

Patients developing sepsis

Excluding the significant pain experienced group, the laparoscopic approach patients developing sepsis pain score diverge significantly away from the group not developing sepsis between 6 and 24 hours after surgery. This pattern is seen for the recent ERCP, OTC and the cholecystectomy only patients. In contrast the laparoscopic cholecystectomy only patients who develop sepsis, their cytokines and the SIRS markers do not become significantly different until 24 – 48 hours after surgery, sepsis not being diagnosed till this time point. For the recent ERCP patients' the cytokines peak and the diagnosis of sepsis occurs at 24 to 48 hours, the OTC patients cytokines peak at 48 to 72 hours, sepsis being diagnosed closer to 48 hours in these groups. All the patients' pain scores continue to rise, peaking at 48 hours. This covers the period where the cytokine concentration is rising. The pain score decreased ahead of the reduction in the systemic cytokine concentration.

For the open approach patients, like the laparoscopic approach patients, the pain scores for the patients developing sepsis diverge away from the group not developing sepsis at 6 hours. Except for the significant pain experienced group. The cholecystectomy only patients' cytokine concentration peaks at 24 hours and their sepsis is diagnosed between 2 to 33 hours. The recent ERCP patients' cytokine concentration peaks between 24 to 48 hours, their sepsis being diagnosed 24 to 29 hours. The OTC cholecystectomy patients' cytokine concentration peaks at 48 hours, their sepsis being diagnosed 26 to 29 hours. As with the laparoscopic approach patients the pain score continues to rise to 48 hours, and then decreases as the cytokine concentration falls.

Given the variation in cytokine response between groups and between the two approaches to cholecystectomy the pain score response is remarkable similar, Figure 10.3.3 and 4. This could be that the pain response is generated by localised changes within the peritoneum, which are not detected by measuring systemic cytokines. The pilot study had three patients where drain fluid was measured and demonstrated the peritoneal cytokines rising ahead of the systemic cytokines, but this was not as early as the pain response. The factors that inhibit the cytokine response appear not to inhibit the pain response, and early on the pain response can differentiate between the group developing sepsis and the majority of patients not developing sepsis (significant pain experienced being the exception).

There was a trend towards the patients who required a higher level of care above the ward for their sepsis to have higher VAS form four hours after surgery, but this did not reach significance. This is potentially due to the small number of patients admitted to HDU and ITU, and the diverse causes of sepsis. We arbitrarily took the time from enrolment for the biliary emergency patients, and the start of ERCP or surgery for the other arms and measured the time to first mention of sepsis in the clinical notes. It is not possible in this study to have a definite time of the onset of sepsis, which may also affects the analysis of the VAS scores.

In conclusion the pain score increases as the cytokine concentration is significantly increasing, with the change in pain score appearing to precede the change in cytokine concentration in the majority of patients. The cytokine concentration falls with or 24 hours after the pain score. Figure 10.3.5 is a simplified model of the pain score response, the second Figure demonstrating the pain scores overlaying the cytokine concentration model from Figure 9.3.4.

Model of pain score change after cholecystectomy, lower figure demonstrates relationship to cytokine change

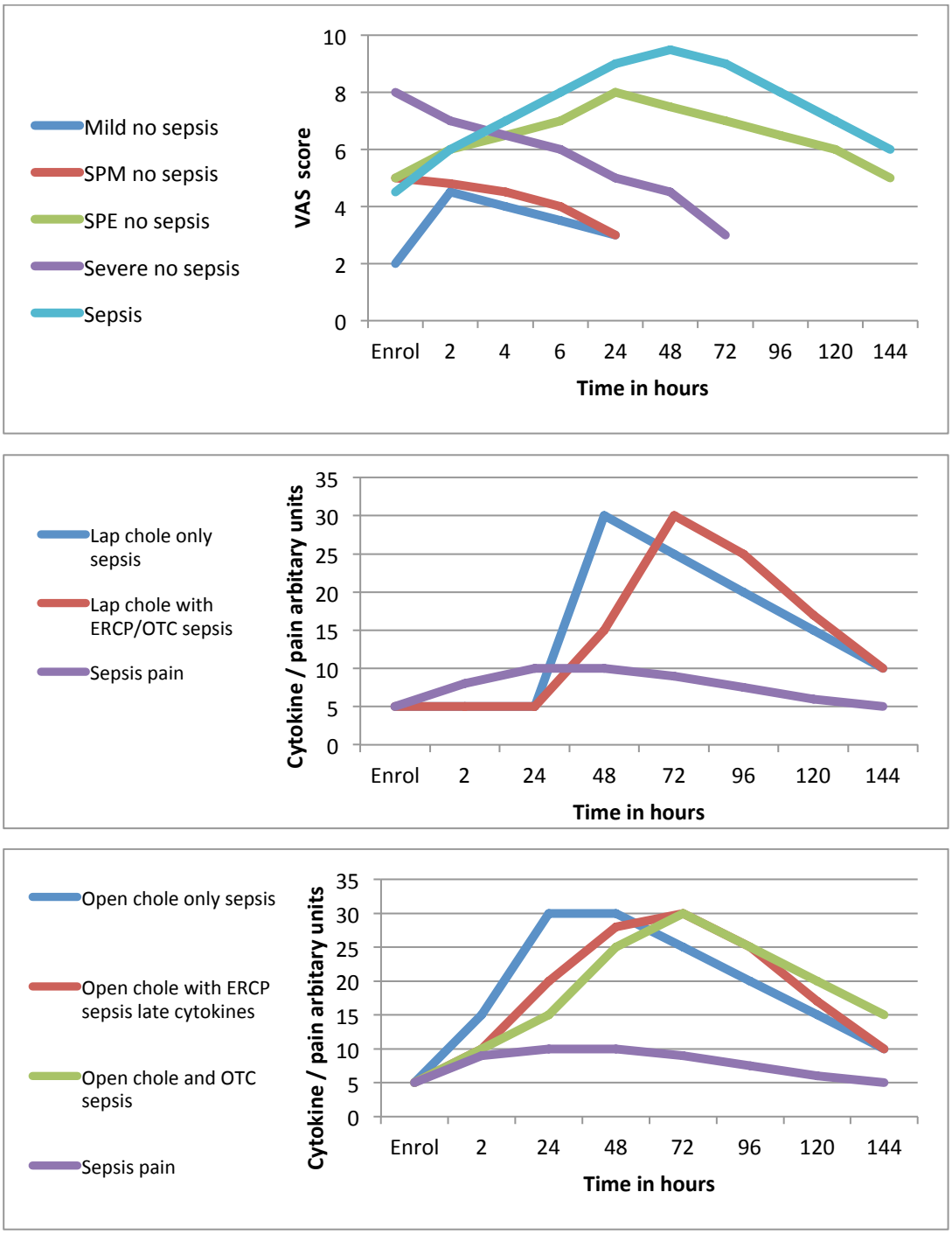


Figure 10.3.5 : Demonstrates the change in pain score following cholecystectomy (upper), for both approaches. The change in pain score for the group developing sepsis is shown with the model for change in cytokine concentration in those developing sepsis, laparoscopic approach (middle), open approach (lower). The units are different for pain and cytokine concentration but are over laid to demonstrate the relationship in timing. Due to difference in timing of recording pain score and cytokine concentration, 2 and 4 hours have been omitted.

Being able to distinguish the patients developing sepsis from the significant pain experienced group therefore becomes important to allow closer monitoring of the patients at risk of developing sepsis and initiate earlier treatment. Using the pain scores and quality of life questionnaires allowed us to distinguish the significant pain experienced group from the other patients prior to intervention, as will be discussed in Chapter **11**. This will help potentially identify patients developing sepsis earlier, though it should be remembered that some of the significant pain experienced patients developed post-operative sepsis. It will take a bigger study to look at a wider range of causes of sepsis and procedures to be able to confidently initiate treatment based on the patient's report of pain. From this study we are also unable to determine what is the source of the pain response, but we do demonstrate it is an indicator of potential post procedural problems.

It is difficult determining the sequencing of events, and the interaction between operative factors, biliary obstruction and stasis and analgesia, in a small study with diverse causes of sepsis. Many studies examining sepsis after laparoscopic surgery have focused on operative factors around the pneumoperitoneum. More recently studies, particularly after ERCP, it is becoming more apparent that pain is an early indicator of post-operative sepsis. In constructing future models of study we should try to examine multiple factors as part of the model.

Results

Chapter 11 – Quality of life score and Hospital Anxiety and Depression score.

11.1 Quality of Life and Hospital Anxiety and Depression Scale

From the pilot study it had been evident that there was a group of patients who scored their quality of life poorer than the main group of patients. This group of patients experienced a lot of pain post operatively. In Chapter 8.3 we demonstrated the significant pain experienced group had pain score indistinguishable from the group developing sepsis, but the significant pain experienced group did not develop post-procedural sepsis. I wanted to develop a method to be able to separate the two groups pre-operatively, to be able to recognise the patients who were at risk of sepsis and instigate early treatment to optimise the outcome of the septic event. Not overlooking the fact that the significant pain experienced group could develop postoperative sepsis (Table 8.3.5).

From the literature review Quintana *et al.,* (2003, 2005), using the SF-36, had identified a group of patients whose pain was possibly not related to gallstones and gained minimal benefit from cholecystectomy. Shi *et al.,* (2008, 2009) used the Gastro-intestinal Quality of Life Index (GIQLI) to identify patients in whom cholecystectomy did not achieve a 'Minimally clinically important difference – MICD' for each of the GIQLI domains. Ibrahim *et al.,* (2016) encouraging using the two questionnaires in combination to optimally assess quality of life, and advocating the use of the SF-36 and GIQLI.

The pilot highlighted a difference in quality of life in the significant pain group based on the VAS. Termed 'significant pain experienced' they scored their quality of life poorer than the other patients in the significant pain group, termed 'significant pain manageable'. We questioned the benefit significant pain experienced patients had from undergoing cholecystectomy.

For the first 100 patients recruited in the study I collected their data and followed them up observing their QoL data to their outcome. This allowed me to gain information about the mean scores in each domain for each of the pain group, and to determine the domains, which were the better indicators of their pain group. Table 11.1.1 demonstrates the distribution of pain scores for first 100 patients who I gathered quality of life and Hospital anxiety and depression data on.

The first 100 patients whose quality of life data was analysed to predict pain group pre-operatively

Enrolment arm and numbers in each group	Episodes of sepsis in first 100 pts analysed	Number in mild pain VAS <4	Number in significant pain manageable VAS ≥ 4 - <7	Number in significant pain experienced VAS ≥ 4 - <7	Number in severe pain VAS ≥ 7
Biliary emerg					
- BC (7)	0	1	1	5	0
- AC (9)	0	2	3	4	0
- Panc (5)	1	0	0	0	5
- OJ (4)	0	0	0	0	4
ERCP (13)	2	0	6	1	6
Lap. chole. (30)	2	13	9	5	3
Open chole. (15)	3	6	3	4	2
ERCP lap. (6)	1	0	4	0	2
ERCP open (3)	0	0	2	1	0
OTC lap. (5)	1	0	3	1	1
OTC open (3)	1	0	2	0	1

Table 11.1.1: Demonstrates the number of patients in each group in the analysis of the first one hundred patients quality of life data. The number of patients developing sepsis in the first 100 is also demonstrated. The biliary emergency group is split into the different types of biliary disease. This group was analysed as a separate group, to understand how the quality of life data allowed the pain groups to be distinguished as discussed in the text. Emerg. – emergency, BC – biliary colic, AC – Acute cholecystitis, Panc – pancreatitis, OJ – obstructive jaundice, Lap. Chole. – Laparoscopic cholecystectomy, OTC – on table cholangiogram.

In the pilot study the most useful distinguishing question, was on the SF-36, 'Compared to one year ago, how would you rate your general health now?', the significant pain experienced group consistently rating this poorer than the other groups, following surgery. At enrolment all groups rated this worse and it did not to help distinguish the significant pain experienced patients prospectively.

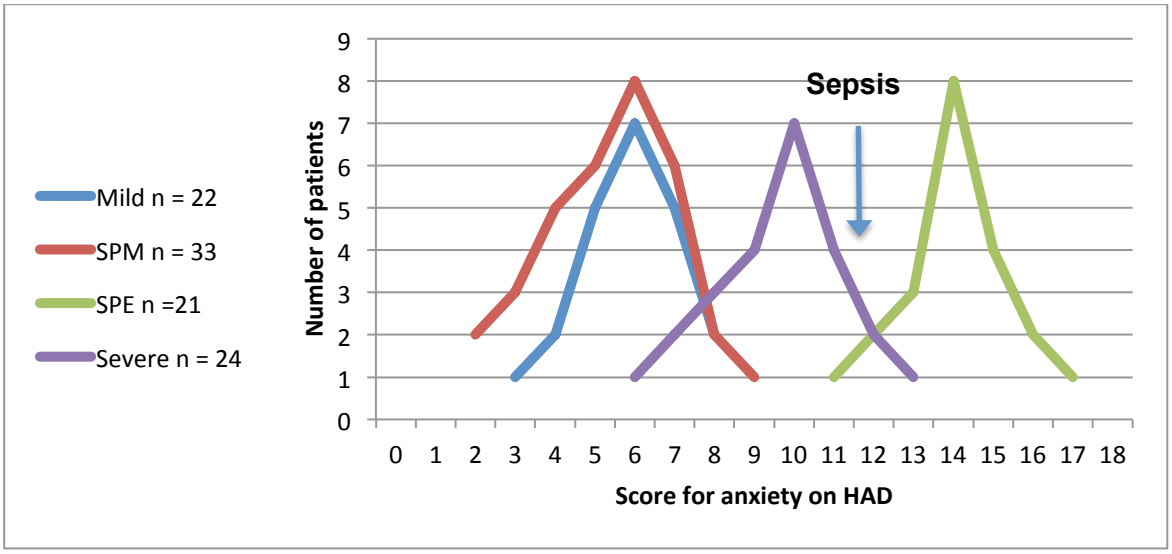
The mild and significant pain manageable group scored their quality of life in a similar way. The significant pain experienced group scored their quality of life poorer. It was harder to distinguish the severe pain group from the two significant pain groups, as the group straddled the significant pain groups as shown in Figure 11.1.2. The severe pain group patients developing sepsis tended to have greater overlap with the significant pain experienced group. In the early stages of data gathering I was concerned patients' scoring their quality of life like the significant pain experienced group, were also in the severe pain group at enrolment.

Analysing the first one hundred patients and trying to use the quality of life data to place the patients in pain groups

Figure 11.1.2 highlights the distribution of the HAD scores for the first 100 patients. Table 11.1.3 gives the problems which were encountered using the HAD questionnaire in these patients. Examining the SF-36 and GIQLI responses, I wondered whether the greater number of questions would provide greater clarity between the groups. Table 11.1.4 illustrates the problems encountered with the SF-36 and GIQLI being used to separate the pain groups pre-operatively. Figure 11.1.5 highlights the quality of life questionnaire domains that permitted the most discrimination between the groups after the first 100 patients.

The Hospital Anxiety and Depression Score

HAD classification	Anxiety score	Depression score
Normal	0 - 7	0 -7
Borderline	8 - 10	8 - 10
Abnormal	11 – 21	11 – 21



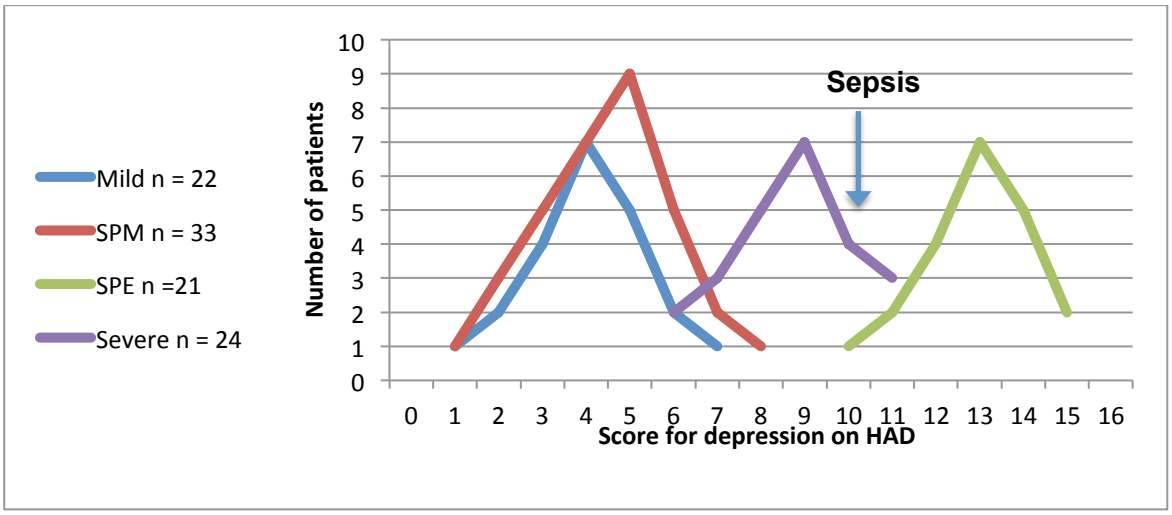


Figure 11.1.2: Demonstrates the classification of the groups the HAD score places the patients in. The middle figure is the scores of the first one hundred patients for anxiety, and the bottom figure is their score for depression. The arrow highlights the scores for the main group developing sepsis.

Problems encountered using the HAD with the first 100 patients recruited

Problems encountered using the HAD
The mild pain group and the significant pain manageable group overlapped their pain score – ***the two groups were indistinguishable***
The severe pain groups' scores lay between the significant pain manageable group, who scored lower on the HAD, and the significant pain experienced who scored higher on the anxiety and depression indices – ***Figure 11.1.2***
The group developing sepsis was predominantly within the severe pain group and scored the upper end of this groups domain – ***area of greatest overlap with the significant pain experienced group***
Ongoing observation demonstrated the HAD was more useful in distinguishing the groups when examining the change from pre to postoperatively, as the significant pain manageable and the severe pain groups both dropped their scores after surgery the greatest - ***this didn't give the ability to predict who was in the significant pain experienced group pre-intervention***
Fewer repeat HAD questionnaires at 12 weeks were collected for the biliary emergency and ERCP group as this was usually in the peri-operative period – ***this hampered the reflection on how the HAD score changed in the different groups***
The VAS for the biliary emergency and ERCP group also did not fall at 12 weeks, because of ongoing biliary problems – ***therefore their HAD and VAS scoring was seen to be like the significant pain experienced group leading to incorrect initial conclusions and premises for splitting the pain groups***
The mild, the significant pain manageable and the severe pain group scored highly pre-operatively on having worrying thoughts, being frightened and feeling panicky - ***post operatively these groups no longer scored highly on these questions and this helped begin to tease the groups apart***
The severe pain group scored poorly on feeling relaxed and finding enjoyment in life, reflecting more frequent episodes of right upper quadrant pain in this group pre-operatively - ***at 12 weeks, in patients who'd had surgery, the severe pain scored these questions like the mild and significant pain manageable group, helping distinguish groups***
The significant pain experienced group scored highly on feeling worried, being frightened and panicky pre and post operatively, as well as on other questions in the questionnaire. But there was less of a distinct pattern with the rest of the questionnaire – ***this pattern of answering was a start in distinguishing the groups***
The significant pain experienced group tended to score their feelings more extreme, for example the questions about feeling frightened they scored as 'quite often' or 'very often', whereas the other groups scored it as 'occasionally' or 'not at all' – ***pattern as above***
Patients admitted to ITU or HDU, their scores remained higher at 12 weeks, as it was a shorter time since their discharge, and they had had a prolonged recovery - ***this increased the complexity of the interpretation, because all the HAD scores dropped at 12 weeks, for all but the significant pain experienced group and the 2 patients requiring HDU / ITU were in the severe pain group at enrolment but behaving like the significant pain experienced patients, reinforcing the belief there was patients within the severe group like the significant pain experienced patients. This also widened the HAD scoring range for the significant pain experienced group***

Table 11.1.3: Demonstrates problems encountered using HAD to distinguish between the pain groups for first one hundred patients.

Problems encountered using the SF-36 and the GIQLI with the first 100 patients

Problems encountered with the SF-36 and GIQLI
Again in the quality of life questionnaires the significant pain manageable and mild pain groups were difficult to distinguish - ***taking the overall score did not allow me to reliably determine which group patients were in, and I realised it was important to look at individual domain scores in combination with the overall score. Even doing this the significant pain manageable and the mild pain group were difficult to distinguish, as their domain and overall scores overlapped significantly***
Severe pain group response – ***again the severe pain group's scores fell between and overlapped with the significant pain manageable group above and significant pain experienced group below***
The various domains on the quality of life questionnaires permitted more distinction between the groups, although the overall scores may not be different. The main domain that distinguished the mild and significant pain manageable from the severe pain group was the 'Bodily pain domain' of the SF-36, which contributes mainly to the 'Physical component score' (Table 4.8.3 page 121) - ***this tended to be scored significantly lower (p = 0.039) for the severe pain group, and reduced the physical component score***
The severe pain group score would improve the most from pre-operatively to 12 weeks after surgery - ***as with the HAD questionnaire patients who had prolonged admissions with sepsis their scores did not improve as greatly and this made it harder to distinguish the severe pain group from the significant pain experienced group on scores, and hence set boundaries on the scores between groups***
The significant pain experience group did tend to score lower on the physical domains contributing to the physical component score, but this was not significantly lower - ***there was a lot of overlap with the significant pain experienced and the severe pain group in particular, but also with the significant pain manageable and mild pain group***

Table 11.1.4: Demonstrates the problems encountered with SF-36 and GIQLI.

Problems encountered with the SF-36 and GIQLI (continued)
The principal physical domain which the significant pain experienced group scored poorly on was the 'Bodily pain domain' on the SF-36 – ***these scores overlapped significantly with the severe pain group, but helped separate the mild and significant pain manageable group***
The significant pain experienced group diverged from the other groups was in the domains, 'Role emotional functioning', 'Mental health' and 'Vitality'. What was surprising was the group scored better in the 'Social role functioning'. Retesting at 12 weeks the scores were similar, but the 'Social role functioning' had dropped significantly (p = 0.032) - ***discussion with the patient groups it became evident that when they had a lot of pain, or were being investigated or having surgery, family and friends gave support, which went again when they were 'recovered'. This happened in all the groups, but was particularly marked and important to the significant pain experienced group. This may explain the increased amount of contact with health services***
The biliary emergency and ERCP group who had not had surgery, did not see a significant improvement in score at 12 weeks – ***but tended to be in the peri-operative period leading to confusion as discussed above***
Patients with sepsis, particularly those admitted to ITU and HDU, saw the smallest improvement in score - **difficult to differentiate from the significant pain experienced group as described above**
GIQLI is a disease specific questionnaire and unsurprisingly there was a greater spread of scores in the 'gastrointestinal symptoms' domain, than there was on the SF-36 - ***the only domain that allowed a differentiation between the mild pain group and the significant pain manageable group. The mild pain group scoring this domain better than, Yu et al., (2018) study group, who were patients' undergoing cholecystectomy within 5 days of the onset of biliary symptoms. The significant pain manageable group scored their gastrointestinal symptoms at the same level as Yu's study group patients. Both groups demonstrating benefit from undergoing cholecystectomy***

Table 11.1.4 cont.: Demonstrates the problems encountered with SF-36 and GIQLI.

Problems encountered with the SF-36 and GIQLI (continued)
The severe pain group patients scored their gastrointestinal symptoms the lowest of any of the pain groups. The significant pain experienced patients scored this domain not significantly differently to the significant pain manageable group and some as highly as the mid pain group – ***this helped to distinguish the severe pain group from the other pain groups***
The 'emotional status' domain on the SF-36 permitted the greatest distinction between the significant pain experienced group and the other groups. The significant pain experienced group scoring the lowest here, and below **Yu's team (2018)** emergency cholecystectomy patients. As with the SF-36 the significant pain experienced group, scored their 'social function' domain higher than was expected, and as with the SF-36 this was the domain that changed the least or decreased at reassessment at 12 weeks, for the same reasons - ***this helped to distinguish the severe pain group from the other pain groups***
The GIQLI the gastrointestinal domain has 19 items and a score range of 0 -76, the physical 7 items 0 – 28, and the emotional and social functioning 5 items 0 – 20 each - ***this predominance of the gastrointestinal symptoms domains to the overall score meant using the overall score in isolation from the domains did not allow us to distinguish between the groups because of the weighting of this group***
Reassessing at 12 weeks allowed evaluation of the scores for each group. It was evident that the mild, significant pain manageable, and severe pain groups had achieved the increase in scores matching the 'minimum clinically important difference' (MCID) described by **Shi *et al.,* (2009)** - ***the significant pain experienced did not achieve the MCID in the emotional, and social function domains, and not consistently in the physical and gastrointestinal symptoms***

Table 11.1.4 cont.: Demonstrates the problems encountered with SF-36 and GIQLI.

Main conclusion encountered with the SF-36 and GIQLI with the first 100 patients (continued)
Due to the weighting of the questionnaire the above did not always give an overall score that was significantly higher at 12 weeks, particularly for the severe pain group and those who developed sepsis, or were in the peri-operative period at the second questionnaire for the biliary emergency and elective ERCP group – ***this made difficulties interpreting the differences between groups***
Analysis after the first one hundred patients I found the questionnaire overall scores with the domains were helping to distinguish the group. The significant pain manageable and mild pain groups together were the most easily distinguishable. But there was overlap with the severe patients, particularly the significant pain experienced group and upper end of the severe pain group, who tended to be the group who developed sepsis postoperatively

Table 11.1.4 cont.: Demonstrates the conclusions and problems encountered with using the SF-36 and GIQLI to distinguish between the pain groups at the analysis of the first one hundred patients.

Quality of life domains and their contribution towards distinguishing between the pain groups

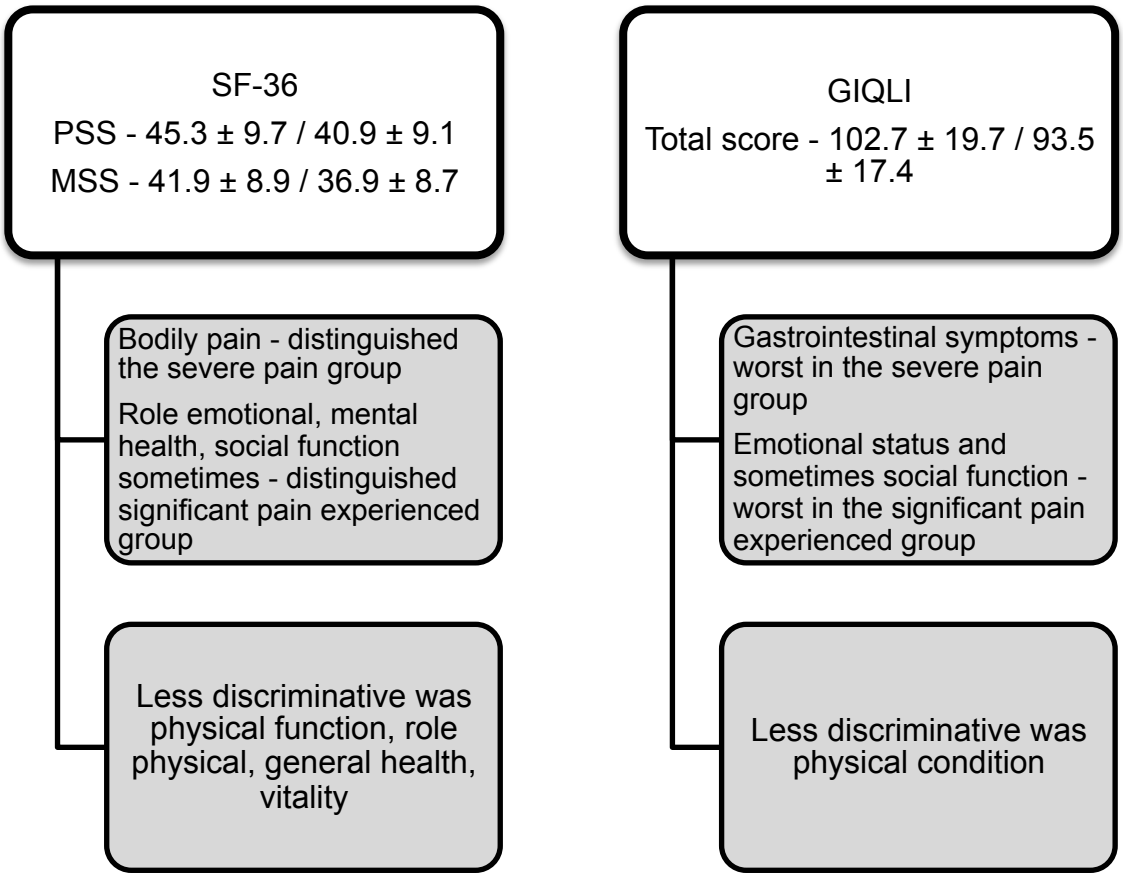


Figure 11.1.5: Analysis of the first one hundred patients' quality of life scores permitted learning about which were the important domains on the quality of life questionnaires, to distinguish the patients who would potentially have problems with pain post procedure or after their admission. PSS – is the physical summary score and MSS – is the mental summary score. The first figure for the PSS, MSS and Total score is for the mild and significant pain manageable group, the second is the significant pain experienced group. The severe pain group fell between these two groups.

Second hundred patients onwards

From the second hundred patients onwards I took the enrolment questionnaire and tried to use the scores across the three questionnaires to place them in pain group, without referencing the VAS. For this I used the domains in Figure 11.1.5. From early on it was evident that none of the questionnaires in isolation permitted identification with accuracy of the group, and by using all three questionnaires there was improved accuracy, but it was not totally accurate. Table 11.1.6 highlights the three groups of patients who were difficult to place when using the quality of life questionnaires. As Quintana *et al.,* (2003, 2005) and Shi *et al.,* (2008, 2009), the quality of life questionnaires could highlight the patients the patients who benefitted less from undergoing cholecystectomy. The group that they had identified was equivalent to the group we had termed significant pain experienced. Both the significant pain experienced and the group developing sepsis increased their pain scores post-operatively, separating the groups could not be based just on questionnaires, or the VAS, but on other peri-operative observations as well.

The three groups of patients who were difficult to place in groups based upon the quality of life questionnaires in the second hundred patients

Three groups of patients difficult to place based on quality of life questionnaires
Patients who developed post procedural sepsis but were not in the severe pain group, but the significant pain manageable group at enrolment – ***particularly biliary related complications because they scored their quality of life as the other significant pain manageable group patients, but their gastrointestinal symptoms were worse, but not significantly so, than the main group at enrolment***
Within this group were two patients developed unexpected sepsis, a bowel perforation patient and a clip becoming displaced off the cystic duct, these two patients were indistinguishable from the main group of significant pain manageable patients at enrolment – ***patients with intra-operative complications would always be difficult to distinguish without a high level of suspicion***
Difficult to distinguish, was two significant pain experienced patients. Their scores at enrolment were not significantly different to the rest of their group in any of the domains, and their pain scores were also not different. Both developed respiratory sepsis and were at all points difficult to distinguish from the other significant pain experienced group - ***except both had difficult procedures, one a laparoscopic cholecystectomy and the second an ERCP. This highlighted that it was imperative we did not dismiss the significant pain experience group's pain as not important, but investigate it completely***

Table 11.1.6: The table highlights the three groups of patients who were difficult to place in pain groups based upon their pre-operative quality of life questionnaires. This highlighted that distinguishing between the group developing sepsis and those who experienced significant pain but did not develop sepsis was based not just on questionnaires but on other peri-operative observations as well.

Towards the end of this group I was increasing in accuracy being able to distinguish the mild and significant pain manageable group out from the significant experienced group. The severe pain group remained the hardest to separate. It was also evident that the patients who required OTC or had had ERCP recently scored their pain as significant manageable, but had more bodily pain. This highlighted that for many patients it was the overall best fit across a range of domains, not matching the scores in all of the domains in the model in Figure 11.1.5. The more patients' scores I looked at the general health domain of the SF-36, and to a lesser extent the physical condition of the GIQLI increased in the discrimination between the significant pain experienced group and the other groups. Table 11.1.7 describes the main conclusions after analysing the second hundred patients.

Conclusions from the second hundred patients analysed

Conclusions from the second hundred patients analysed
There was not, within the severe pain group, a group of patients who rated their quality of life poorer than the average for the severe pain group, i.e. not an equivalent split as was seen in the significant pain group - ***the exact reason for this remained unclear, as the groups were not significantly different in size***
At the end of reviewing two hundred scores, I had improved in distinguishing all but the severe pain group. Up until then I had not used the patients enrolment VAS score - ***appreciating only the significant pain group, had two distinct groups I incorporated the VAS into the analysis, after making a preliminary decision on the quality of life data. This permitted separation of the pain groups more easily, and used the QoL and HAD to separate the significant pain group***
The SF-36 and GIQLI with their multiple domains to match was being shown to be more accurate, but no one questionnaire in isolation was completely accurate - ***using all the domains of all the questionnaires to give a best fit provided the greatest accuracy with the overall score. Particularly with the patients who had biliary obstruction or developed biliary related sepsis post operatively, as they scored their gastrointestinal symptoms worse than the patients who were not obstructed or didn't develop complications***

Table 11.1.7: Demonstrates the main conclusions from analysing the data at the end of the second hundred patients.

I drew up approximate scoring points for each domain to try and separate the mild and significant pain manageable groups from the significant pain experienced, shown in **Table 11.1.8 a – b**. The severe pain group straddled the other two groups. They were separable on their lower bodily pain and gastrointestinal symptoms, but scored their other domains similar to the mild and significant pain manageable group. Using this I scored each patient at enrolment and predicted their pain group, and reviewed the accuracy of scoring, for the final one hundred and eight one patient's.

Mean score in each domain in the SF-36 and the boundary set for each domain to separate the significant experienced group

	Mild and Significant pain manageable group mean score (± SD)	Boundary score	Significant pain experienced group mean score (± SD)
Physical function	76.8 ± 16.5	72.3	70.3 ± 18.9
Role physical	60.6 ± 18.1	56.2	52.7 ± 19.4
Bodily pain * (Severe pain group)	62.0 ± 17.4 (49.5 ± 18.7)	54.1	46.6 ± 20.1
General health*	62.3 ± 15.8	54.4	48.1 ± 17.9
Vitality	55.9 ± 20.1	51.1	47.1 ± 19.4
Social function*	78.5 ± 17.8	70.2	64.1 ± 22.1
Role emotional*	74.9 ± 19.1	67.9	60.3 ± 14.4
Mental health *	68.5 ± 16.5	58.9	50.4 ± 14.9
Physical summary score	45.7 ± 10.4	42.4	40. 2 ± 9.3
Mental summary score	43.7 ± 8.9	38.9	35.5 ± 8.7

Table 11.1.8 a: The mean (±SD) SF- 36 domain scores for the mild and the significant pain manageable group, and the severe pain score. After reviewing the quality of life scores for the first two hundred patients at enrolment, a boundary score was calculated above which the patients went into the mild and significant pain manageable group, below which they were placed in the significant pain experienced. The group placement was determined by the best fit of the number of domains scoring above and below. The * denotes the domains where the scores diverged the most. The severe pain group scored closest to the lower end of the significant pain manageable group except in the 'Bodily pain domain', there score is shown separately in the table.

Mean score in each domain in the GIQLI and HAD score and the boundary set for each domain to separate the significant experienced group

	Mild and Significant pain manageable group mean score (± SD)	Boundary score	Significant pain experienced group mean score (± SD)
GI symptoms (Severe pain group)	61.3 ± 8.5 (54.4 ± 6.1)	58.7	51.7 ± 7.9
Physical condition	19.9 ± 4.9	18.0	14.9 ± 5.1
Emotional status*	14.9 ± 3.3	12.1	9.7 ± 2.8
Social function*	15.4 ± 3.1	13.4	11.2 ± 4.1
Total score	108.9 ± 15.8	104.3	99.3 ± 12.2

	Mild and Significant pain manageable group mean score (± SD)	Boundary score	Significant pain experienced group mean score (± SD)
Anxiety	6 ± 4	10	14 ± 3
Depression	5 ± 4	9	13 ± 4

Table 11.1.8 b: The mean (±SD) GIQLI (upper) and HAD scores for the mild and the significant pain manageable group, and the severe pain score. After reviewing the quality of life scores for the first two hundred patients at enrolment, a boundary score was calculated above which the patients went into the mild and significant pain manageable group, below which they were placed in the significant pain experienced. The group placement was determined by the best fit of the number of domains scoring above and below. The * denotes the domains where the scores diverged the most. The severe pain group scored closest to the lower end of the significant pain manageable group except in the 'GI symptoms domain shown in the table.

Final group of patients

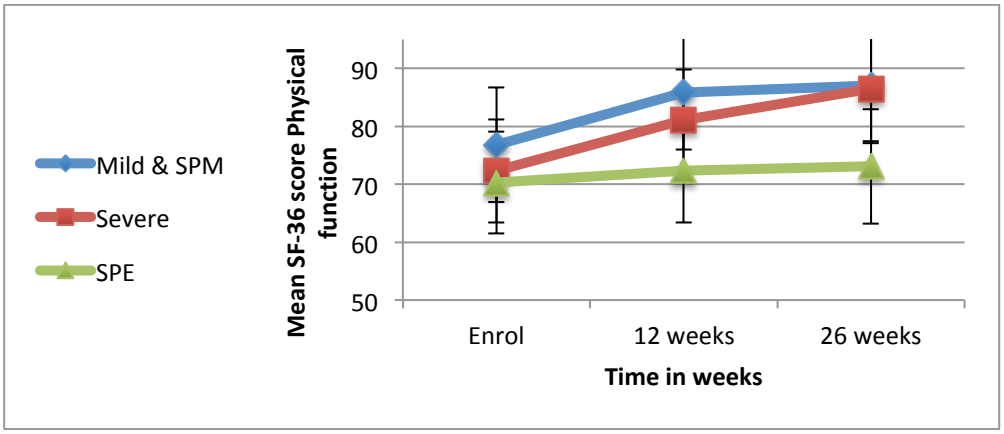
The accuracy of predicting the pain groups gradually increased and the boundary score between the significant pain group and the other pain groups gradually more refined, until I had a range of scores within which I expected each pain group to score their quality of life on the questionnaires. Not all of the patients fitted perfectly for each domain, but the number of domains matching increased in number as we improved in accuracy and built a score range for the severe pain group, between the other groups. At the end of the study the number placed in the correct pain group was 86%, the inaccuracy being in patients who were incorrectly not assigned to the severe pain group, prior to reviewing the VAS score.

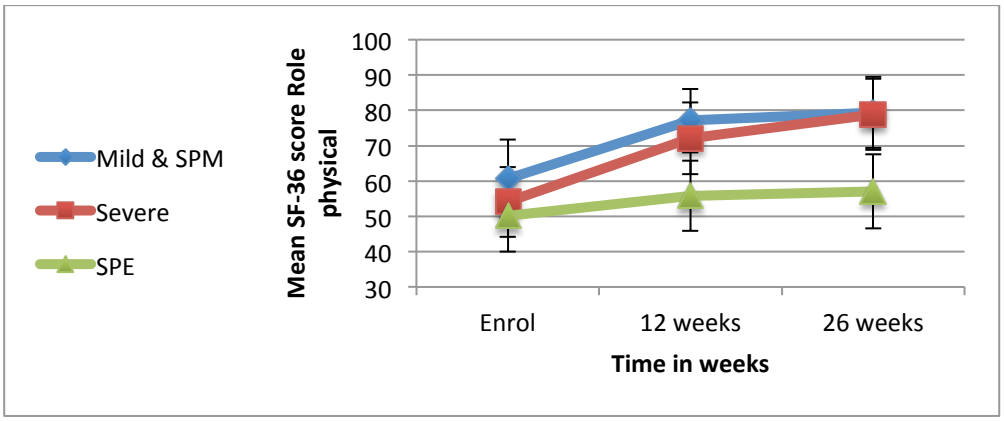
Mild and significant pain manageable group domain scores also increased in accuracy, with 89% of the patients correctly placed in the correct pain group, and 91% correctly excluded from the significant pain experienced group. The inaccuracy in the groups was particularly with the patients who were enroled prior to cholecystectomy and on table cholangiogram, as these patients had a low 'Bodily pain and GI symptom scores'. Their pain and their lower quality of life score increased the inaccuracy of their placement, and about appropriateness for surgery.

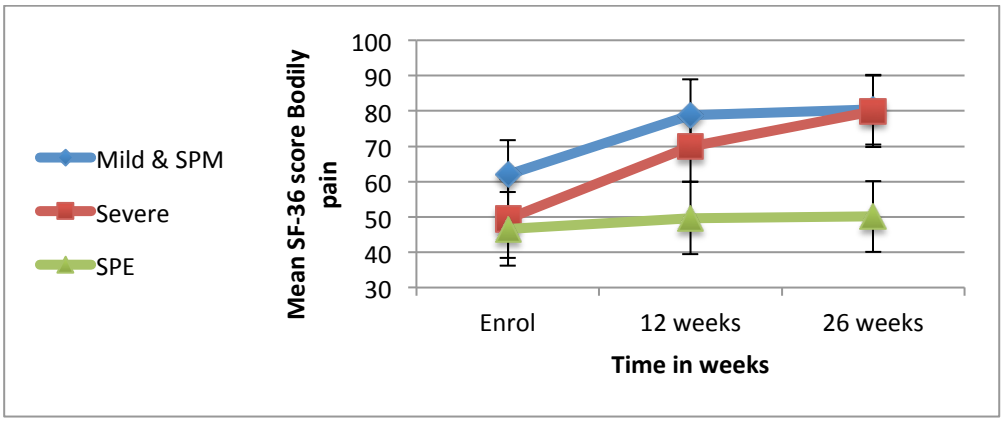
The severe pain group also became more predictable, although there remained overlap with the significant pain experienced group below and to a lesser the significant pain manageable and the mild pain group above. The accuracy for this group was 76%.

The enrolment, 12 and 26 week domain scores for each of the pain groups is shown in Figure 11.1.8 - 11.1.10. This demonstrates the change in pain scores that occurred with each pain group. The data at 52 weeks was similar to the 12 and 26 week scores. The main change at 26 weeks being in the scores for the 'General health', 'Bodily pain' and the 'GI symptoms' domain on the SF-36 and GIQLI respectively, in those admitted to ITU and HDU. Being lower at 12 weeks as they had more recently been discharged. There was no difference in quality of life score between the open and laparoscopic approach groups at 12 weeks or further out.

Change in SF-36 domain scores from Enrolment, 12 and 26 weeks







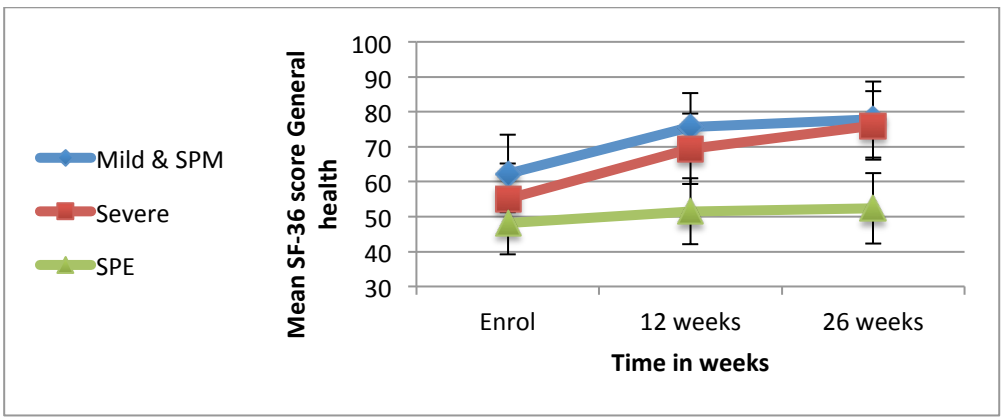


Figure 11.1.8: Demonstrates each of the domain scores (full description below).

Change in SF-36 domain scores from Enrolment, 12 and 26 weeks (cont.)

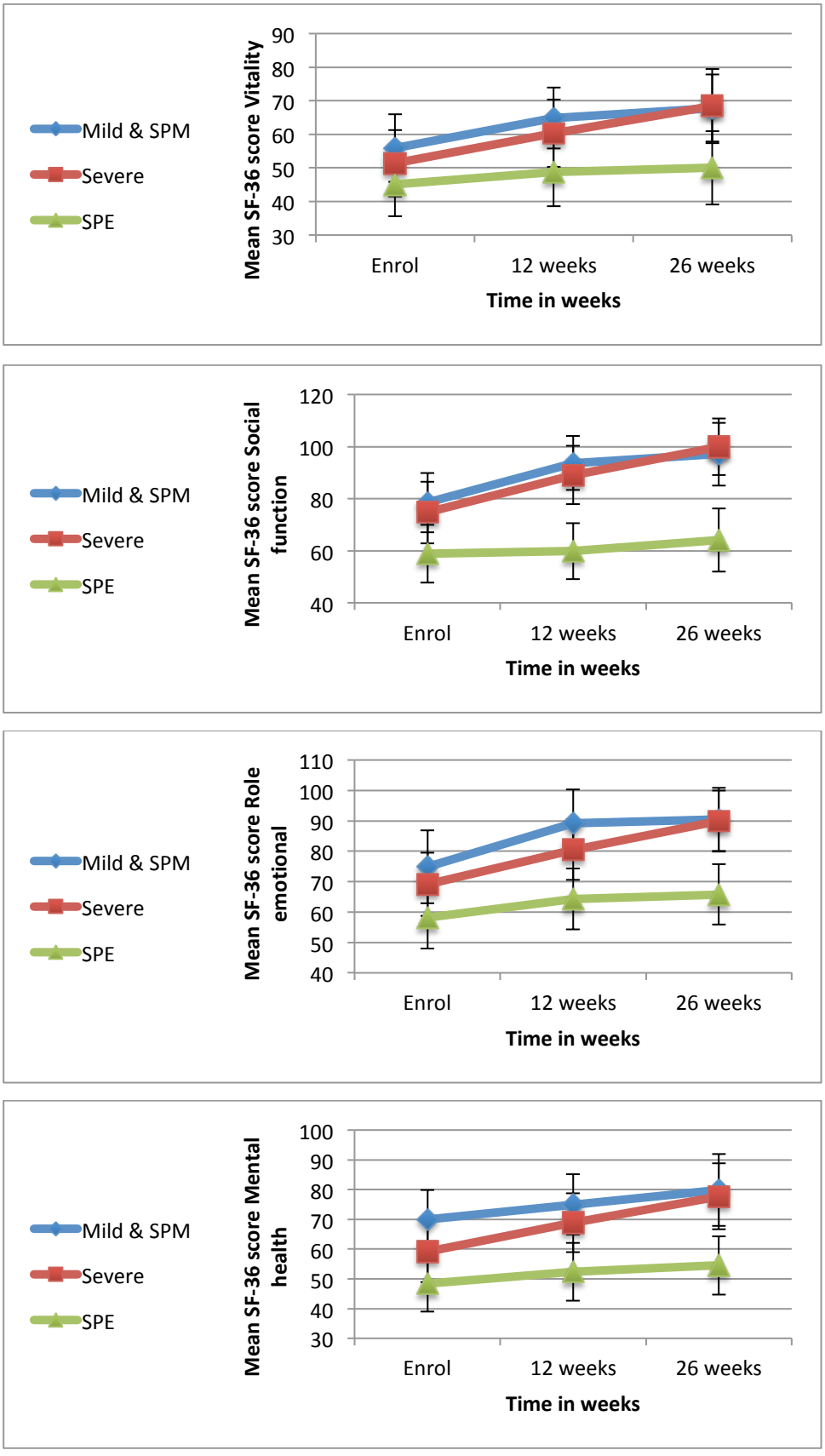


Figure 11.1.8 cont.: Demonstrates each of the domain scores (full description below).

Change in SF-36 domain scores from Enrolment, 12 and 26 weeks (cont.)

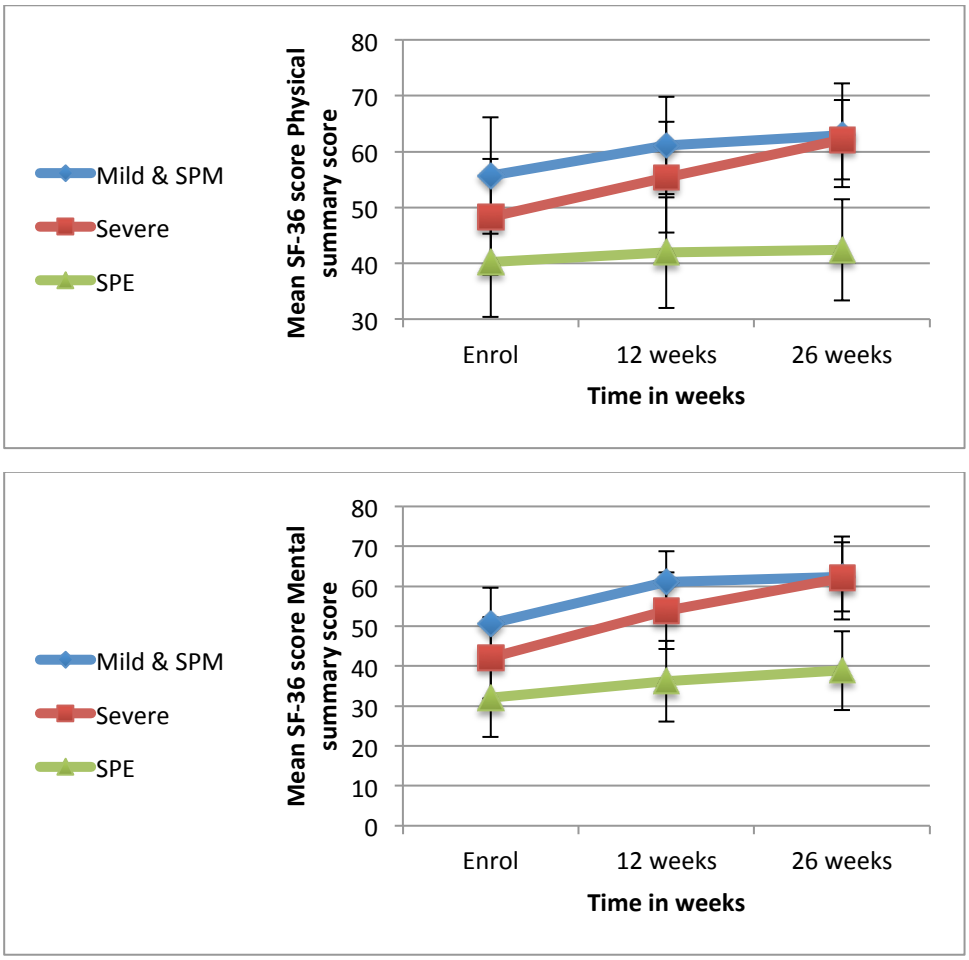


Figure 11.1.8 cont.: Demonstrates each of the domain scores and the mental and physical summary score. The scores are split into the mild with significant pain manageable (SPM), severe pain and the significant pain experienced (SPE) group. Each of the scores demonstrates the standard deviation for the score. The mild and SPM, have the highest score in each domain, and increases to 12 weeks, but does not increase significantly to 26 weeks. The severe group SF-36 score, at enrolment, falls between the mild group and the SPE. At 12 weeks their quality of life score has increased, but does not reach the same level as the mild pain group, as some of the patients had not long been discharged from hospital, following treatment of their sepsis. At 26 weeks, their quality of life scores increase to the level of the mild pain group. The SPE group's SF-36 score is the lowest at enrolment and does not increase at 12 or 26 week. The "Bodily pain' domain score for the severe pain group is particularly low (third Figure page 314).

Change in GIQLI domain scores from Enrolment, 12 and 26 weeks

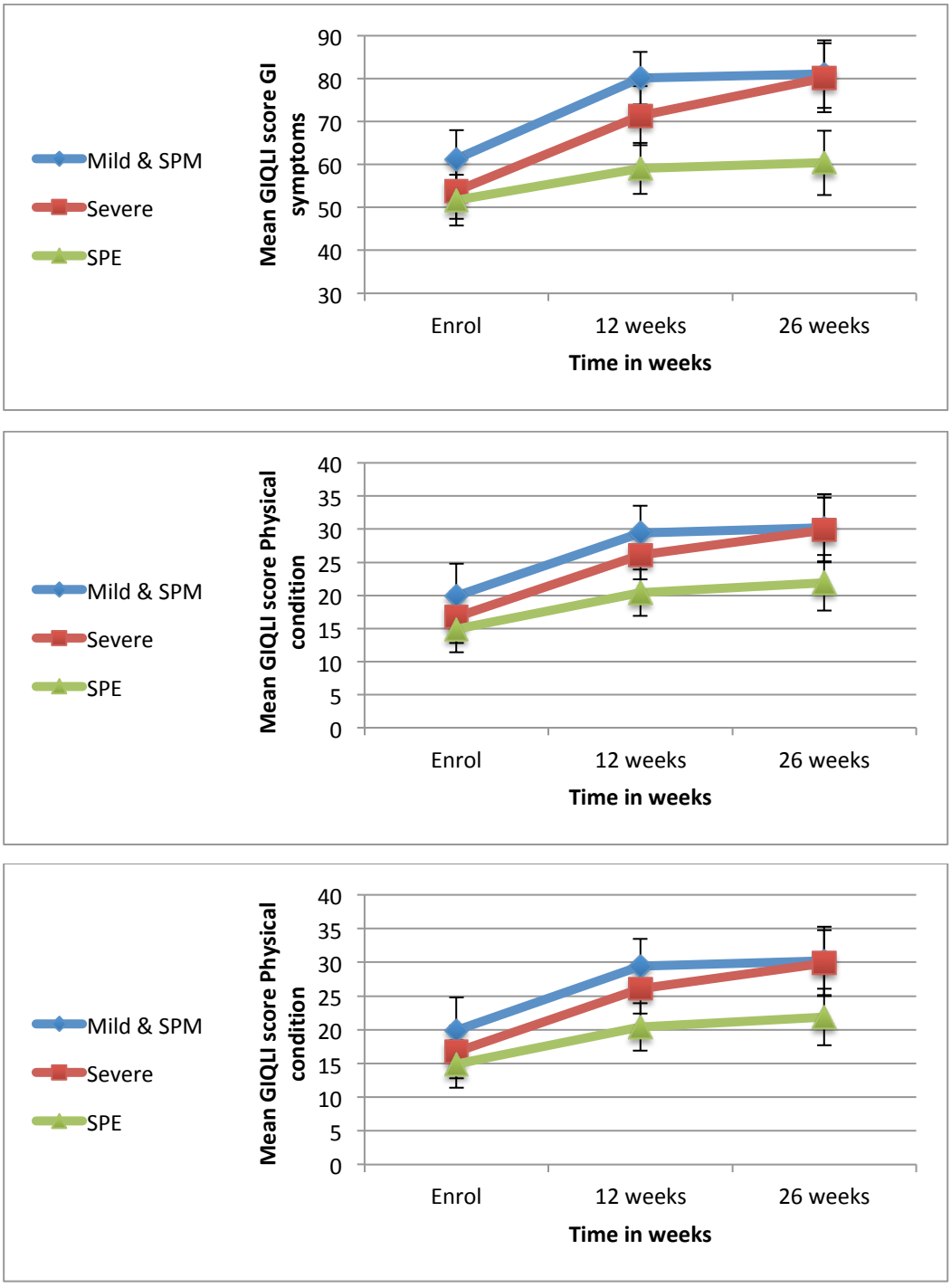


Figure 11.1.9: Demonstrates the GIQLI scores for the pain groups. Like the SF-36 scores, the mild and SPM group increase at 12 weeks, but not significantly further. The severe pain group's score increased at 12 weeks, but increase further at 26 weeks. The SPE don't increase significantly from the enrolment score. The severe pain group score particularly poorly in the GI symptoms domain (upper most Figure).

Change in GIQLI domain scores from Enrolment, 12 and 26 weeks continued

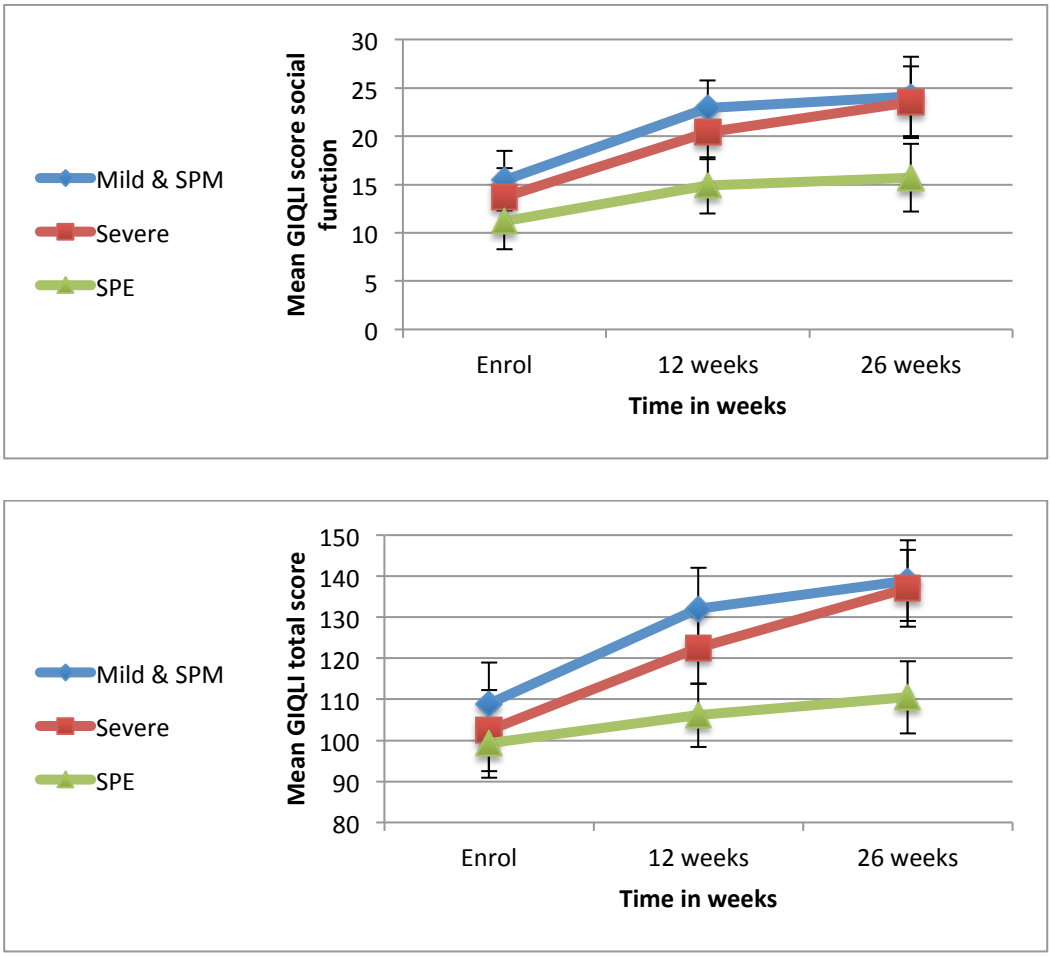


Figure 11.1.9 cont.: The GIQLI 'Social function' domain sore and the 'Total' score. The severe pain group GIQLI 'Total' score was low because of the weighting of the 'GI symptoms' score, compared to the other groups.

Change in HAD domain scores from Enrolment, 12 and 26 weeks

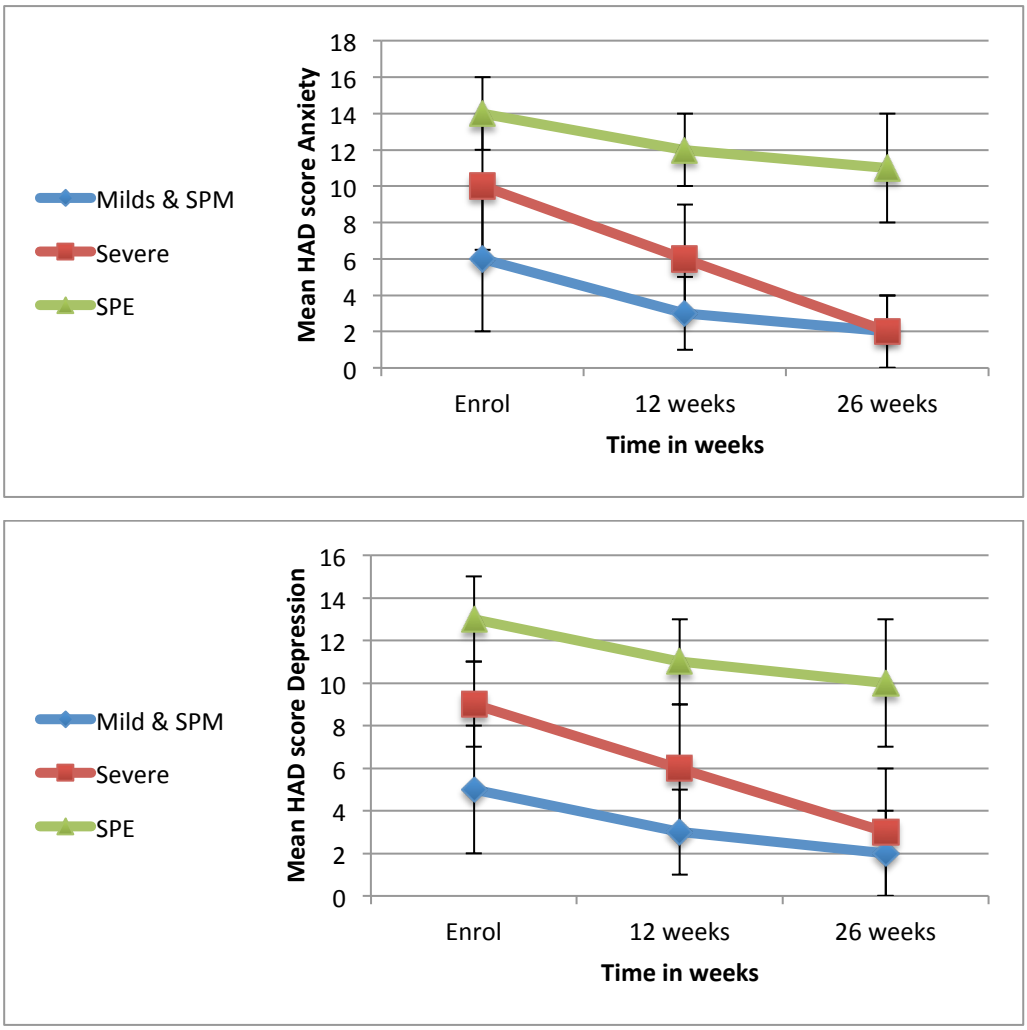


Figure 11.1.10: Demonstrates the HAD anxiety and depression score for the three pain groups. Unlike the quality of life scores, the HAD scores demonstrate less anxiety and depression with a lower score. As with the quality of life scores the severe pain group scored between the mild and SPM group and the SPE group. The severe pain group score decreases from enrolment to 12 weeks and then decreases further increases to 26 weeks. The SPE group does not significantly decrease following surgery.

Reviewing each of the pain groups demonstrated that the significant pain experienced group only achieved the Shi's teams (2008, 2009), minimum clinically important difference (MICD), in one, 'Physical function' of the GIQLI domains. Scoring their change in quality of life at a level which Quintana's team (2003, 2005) termed 'Inappropriate or of uncertain indication for cholecystectomy'. The other pain group's patients satisfied the MICD score and the Quintana's level for appropriateness for cholecystectomy. This level not being reached until 26 weeks in those admitted to ITU or HDU with sepsis. This was particularly apparent in the physical domains of the questionnaires.

For the final group of 181 patients, I attempted to place the patients in the pain groups initially with two of the quality of life questionnaires, then added in the VAS, and then the final questionnaire. This demonstrated that with two questionnaires I could maintain the accuracy identifying the mild and significant pain manageable. Those who were scoring at the lower end of the QoL range, I accurately placed in the significant pain experienced group. Where I was inaccurate was with the severe group patients in the central overlap area. Particularly for the severe pain group there was compensation occurring between domains, for example they were scoring poorly in the 'Bodily pain' domain, but people were coming to visit and they were compensating this poor score with a higher 'Social function' score.

Table 11.1.11 demonstrates the accuracy of scoring with only 2 quality of life questionnaires, compared to three and the VAS. The table demonstrates the most accurate combination of two questionnaires was the SF-36 and GIQLI, due to the increased number of domains to allow fitting to the range of scores for each of the pain groups.

The accuracy of using two versus three quality of life scores to predict the group the patients were in at enrolment

	n	Accuracy on using 2 quality of life questionnaires	Accuracy on adding the VAS and the third quality of life questionnaire
SF-36 and HAD	60	43 (72%)	52 (87%)
GIQLI and HAD	60	41 (68%)	51 (85%)
SF-36 and GIQLI	61	46 (75%)	53 (87%)

Table 11.1.11: Demonstrates the accuracy of placement of the patients into pain groups using two and three quality of life questionnaires.

Compared to one year ago, how would you rate your health in general now?

The SF-36 includes the above question. As mentioned previously this was a good discriminative question. The results for 12, 26 and 52 weeks after surgery; are shown in Table 11.1.12. By fifty-two weeks all the patients who were enroled in the study had undergone cholecystectomy. Except for the one patient who had had been readmitted elsewhere, following successful ERCP, with gall stone pancreatitis and died from this, they had been deemed to frail for surgery at the first admission. It can be seen from this table a third of the significant pain experienced group believe that they have not benefited from surgery. Three significant pain experienced patients developed sepsis and it is these patients who rate their health the worst at 12 months. The other patient is the person who sustained a bile duct injury.

The response to the question 'Compared to one year ago, how would you rate you general health now' at 12 and 52 weeks, based on enrolment pain group

	VAS pain score				
	Mild pain	**SPM**	**SPE**	**Severe pain**	**Sepsis**
Much better (%)					
12 weeks	37.4%	38.2%	10.5%	54.3%	14.8%
52 weeks	44.9%	43.1%	12.9%	56.9%	41.1%
Somewhat better (%)					
12 weeks	34.7%	38.2%	15.8%	28.6%	33.3%
52 weeks	43%	47.8%	14.2%	34.8%	37.5%
About the same (%)					
12 weeks	22.5%	20.6%	57.9%	14.3%	25%
52 weeks	12.1%	9.1%	41.8%	8.3%	19.8%
Somewhat worse (%)					
12 weeks	4.1%	2.9%	10.5%	0%	8.3%
52 weeks	0%	0%	19.1%	0%	1.2%
Much worse (%)					
12 weeks	0%	0%	5.3%	0%	16.7%
52 weeks	0%	0%	12%	0%	0.4%

Figure 11.1.12: The patient's response to the supplementary question on the SF-36 about rating their health to one year ago.

11.2 Patients recruited to multiple parts of the study

Patients were recruited to multiple parts of the study, their data was kept separate, only pairing it up after the data had been analysed. This permitted a check on accuracy and examined the question of whether patients moved between pain groups. For the biliary emergency group significantly more went on to have cholecystectomy within the six-month study period, in the significant pain experienced group ($p = 0.049$). There was also significantly more contact between the primary care physician and the consultant for this group (81% versus 12% $p = 0.003$), and they were more likely to have opted for surgery with the consultant with the shortest waiting list, which was usually the open approach consultant.

Analysis of the assignment to groups demonstrated a 70% agreement in assigning them to pain groups. Table 11.2.1 demonstrated the patients who went onto have cholecystectomy in the study period. The group with the greatest inaccuracy being the patients initially placed in the severe pain group. Admitted with obstructive jaundice or pancreatitis, or with obstruction for elective ERCP this was treated and they were readmitted in a lower pain group. Or they had had biliary colic or acute cholecystitis and when admitted for cholecystectomy required an OTC for biliary obstruction.

Pain group patients were independently placed in when they participated in a second part of the study

	Patients going on to have cholecystectomy	Independently placed in the same pain group
	Biliary emergency admissions n = 78	
Mild n = 10	4 (30%)	4 (100%)
Significant pain manageable n =13	5 (39%)	4 (80%)
Significant pain experienced n = 27	20 (74%)	20 (100%)
Severe pain n = 28	8 (28%)	2 (25%)
	Elective ERCP n = 52	
Significant pain manageable n = 20	8 (40%)	7 (88%)
Significant pain experienced n = 6	5 (83%)	5 (100%)
Severe pain n = 26	14 (54%)	3 (21%)

Table 11.2.1: Demonstrates the number of the biliary emergency and ERCP patients, who went onto have a cholecystectomy during the study period. All consented to taking part again in the study. The significant pain experienced patient in the ERCP group had an emergency cholecystectomy performed.

We have demonstrated that the quality of life questionnaires can distinguish the pain groups and in particular the significant pain experienced group. This allows patients potentially developing sepsis to be recognised earlier. Secondly it allows the surgical team to discuss the benefits of surgery and set realistic expectations about benefit and pain management.

Chapter 12 – Discussion

12.1 Cytokine response to biliary intervention

Concepcion – Martin *et al.,* (2016), found pain was the only indicator of post ERCP sepsis and pancreatitis in the first 24 hours. In this study we have demonstrated the cytokine concentration increase to be most impaired after laparoscopic surgery. Analysis of these results indicates an interaction of factors, rather than a single factor inhibiting the increase in cytokine concentration. To our knowledge this is the first study that has examined the interaction of a number of factors, proposing they interact together to delay cytokine response. Previous studies have used animal or *in-vitro* models, and have mainly examined bowel surgery. This study examined the effect of the disease and treatment Figure 12.1.1 highlights factors considered potentially to be involved.

Proposal for factors, which could interact, to affect the timing of the cytokine response to sepsis

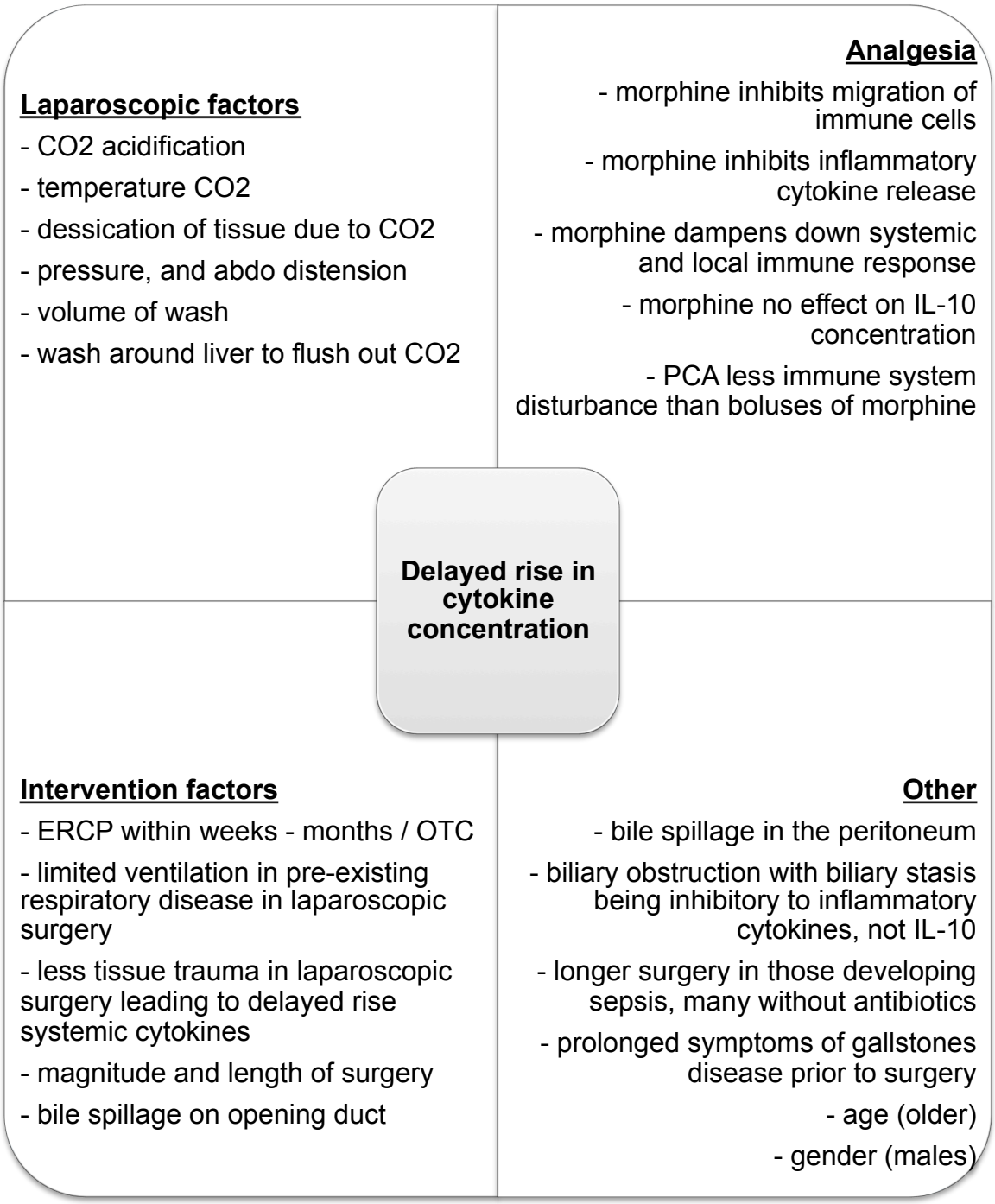


Figure 12.1.1: Proposed factors, from this study and the work of others (from the introduction), which potentially delay the rise in inflammatory cytokine concentration after surgery. The impact of each factor is unknown, and if they do contribute how they interact together. Each factor is discussed in the discussion. CO2 – carbon dioxide, PCA – patient controlled analgesia, OTC – on table cholangiogram.

Factors around laparoscopic surgery

Matsumoto *et al.,* (2001), demonstrated the laparoscopic approach to surgery attenuated the rise in the cytokine concentration in the early post-operative period. Demonstrated in laparoscopic colorectal and splenectomy surgery (Sammour *et al.,* 2010, Kvanstom *et al.,* 2013 and Wu *et al.,* 2012). This is the first time the attenuation of cytokine concentration has been demonstrated after laparoscopic cholecystectomy.

Factors implicated in contributing to the delayed cytokine increase include carbon dioxide, inhibiting the cytokine production and decreasing the cellular and humoral response (Yahara *et al.,* 2002, Watson *et al.,* 1995, and West *et al.,* 1997). The pneumoperitoneum dropping the core temperature, causing desiccation and acidification and the length of time the pressure is over 12 mmHg are all implicated (Hanly *et al.,* 2007 b). This is mediated locally by the cells within the peritoneum and systemically. These factors were implicated in the study in the group developing sepsis after surgery and in the severe pain group. Longer procedures, difficult procedures, volume of wash and in the open group longer incisions, were also important (Figure 8.4.1 a, b and c). We demonstrated patients weight and BMI was also important in predicting sepsis after ERCP and cholecystectomy by either approach (Figure 8.4.2).

Obviously a number of these factors interplay together, but this fits with individual factors identified by others. Two factors implicated, which we did not measure, is the decompression of the pneumoperitoneum at the end of the procedure. And the volume of wash used to suction the gas from around the liver at the end of the procedure. This would be useful to measure in a further study.

Peritoneal acidification correlated with the fall in the serum and peritoneal inflammatory cytokines (Hanly *et al.,* 2007) Peritoneal acidification stimulates an increase in IL-10, even in the absence of lipo-polysaccharide on the bacterial coat. We demonstrated IL-10 concentration in the laparoscopic group rising before the inflammatory cytokines, even in those not developing sepsis. IL-10 concentration peaking concurrently with the inflammatory cytokines peaking (Figure 9.3.2 and 9.3.3). The IL-10 concentration, in this study, was not significantly greater in the recent ERCP / OTC patients, despite their longer pneumoperitoneums. Neither was there a delay in the rise in IL-10 concentration, despite the delay in the inflammatory cytokines

increasing after surgery by either approach. There was no detectable difference in concentration between the two approaches. Whether this reflects IL-10 not being inhibited by the pneumoperitoneum, as opposed to not being stimulated by the acidification of the pneumoperitoneum is unclear Hanly *et al.,* (2007). Possibly the early IL-10 concentration increase is stimulated by the pneumoperitoneum, later on the increase in concentration could be maintained by the increase in inflammatory cytokine concentration, as the effect of the pneumoperitoneum decreases. TNF-α and IL-1 being the standard regulators of IL-10 concentration (Oberholzer *et al.,* 2002a). Certainly the decline in IL-10 concentration mirrors these cytokines returning to baseline concentration. This potential interplay is difficult to interpret without measuring peritoneal cytokines.

The peak cytokine concentration for the group not developing sepsis after cholecystectomy, was similar for both approaches. Given the difference in the size of the wounds it could be expected the open approach would have a greater cytokine rise. Lin *et al.,* (2000) and Sammour *et al.,* (2010), in a meta-analysis of cytokines concentration after colorectal surgery, found the inflammatory cytokine concentration reflected the magnitude of the surgery, out weighing the approach to surgery. Hernia repair, and gynaecological surgery, are shorter procedures and do not demonstrate an increase in cytokines in the laparoscopic approach, due to the magnitude of the operation is less (Sammour *et al.,* 2010). No studies have compared approach to cholecystectomy, to report a difference in cytokine concentration, we have demonstrated the magnitude of surgery being sufficient to evoke a cytokine response

Factors around analgesia

Opiates, particularly strong opiates, are known to inhibit the migration of inflammatory mediators and immune cells and the release of inflammatory cytokines (Laing *et al.,* 2016). Principally acting to inhibit B and T cell and monocyte function, including migration, differentiation and mediator release and dampening down local and systemic inflammatory response (Sacerdote and Panerai, 2012, Schafer and Zollner 2013). Morphine is thought to act by binding to the opioid receptor and inhibiting the response at the level of transcription, in myeloid and lymphoid cells (Roeckel, 2016). There are very few studies examining the interaction of analgesia and the type of surgery.

Patients with a recent ERCP or undergoing an OTC with their cholecystectomy received a higher dose of morphine compared to those who underwent cholecystectomy alone (Table 8.4.3 and 8.4.4). The former patients displaying a delayed increase in cytokine concentration compared to the cholecystectomy alone patients (Figure 9.3.2). Overall these patients cytokine concentration was greater, even for the patients not developing sepsis, than the patients just underwent cholecystectomy alone. These patients also had longer operations, and hence longer pneumoperitoneum, greater volume of wash and more difficult procedures. They did demonstrate a delayed rise in inflammatory cytokine concentration.

The open patients all had morphine patient controlled analgesia (PCA) and received on average more morphine than the equivalent laparoscopic approach patient. The open patients did not demonstrate as delayed cytokine response as the laparoscopic approach patients undergoing equivalent surgery (Figure 9.3.4). Comparisons of bolus morphine versus PCA, has demonstrated that PCA is less disruptive to the immune system (Sacerdote and Panerai, 2012, Schafer and Zollner 2013). This may also partially explain the difference between the open and laparoscopic approach patients seen in this study.

For the surgical and ERCP patients the morphine equivalent dose received by the patients developing sepsis was greater, regardless of the approach to surgery (Table 8.4.4). Despite this these patient's cytokine concentration increased and peaked at the same time as patients undergoing surgery by the same intervention but not developing sepsis (Figure 9.3.2 and 9.3.3). This response could be being driven by the septic insult.

Laing (2016), demonstrated less immune modulation with paracetamol and NSAID's, and decreased septic events, Amodeo *et al.,* (2018), demonstrated tramadol inhibited the immune system than morphine and codeine. Poor compliance to the analgesia protocol and the unavailability of intravenous paracetamol, and tramadol in this study, do not allow us to comment on this.

Sepsis was seen more frequently in the group who scored their pain as severe at enrolment, regardless of approach to surgery ($p = 0.001$). But sepsis was not seen in all patients in the severe pain group, despite this group receiving more analgesia (Table

8.4.4). The analgesia received by the significant pain experienced group was equivalent and often greater than the group developing sepsis but their rate of sepsis was less ($p = 0.0009$). This probably is the lack of chronicity of biliary disease in the significant pain experienced group, but it does illustrate the likely interaction of factors affecting the cytokine response.

Intervention factors - Elective surgery compared to ERCP and emergency cholecystectomy

In the main study the patient's cytokine concentration is greater than the healthy controls cytokine concentration at enrolment. In the biliary emergency arm the cytokine concentration reflected the severity of the biliary disease (Figure 9.1.1). Those developing sepsis after elective ERCP had a greater cytokine concentration at enrolment but unlike the biliary emergency patients the difference was not significant except for IL-6 (Figure 9.2.1).

The elective ERCP patients going on to have an emergency cholecystectomy do not demonstrate a slower rise in their cytokine concentration compared to the rise after open cholecystectomy alone (Figure 9.2.1 and Figure 9.3.3). Despite receiving morphine boluses, before and after ERCP and then a morphine PCA after emergency surgery, their cytokine response is not inhibited even in the laparoscopic approach patients (Figure 9.2.1). With only four patients in this group it is difficult to draw conclusions. One possibility is the systemic cytokine concentration is already increased at enrolment, the systemic concentration was not significantly greater (Figure 9.3.2 and 9.3.3), but there could be localised inflammation within the peritoneum and the immune cells already recruited are less inhibited by morphine, particularly if it principally inhibits immune cell recruitment. Similar to the elective cholecystectomy patients the peak cytokine concentration occurred at 24 hours in the open approach and 48 hours in the laparoscopic emergency cholecystectomy group.

Intervention factors - ERCP or OTC and surgery

Concepción-Martin *et al.,* (2016), reported a general non-specific rise in cytokine concentration four hours after elective ERCP. The group developing complications only being reliable differentiated from the other patients from 8 - 12 hours onwards for IL-6 and 24 hours after for TNF-α. We also demonstrated a non-specific increase at 2 hours,

with the group developing sepsis or emergency cholecystectomy not having a significantly higher TNF-α and IL-6 concentration until 24 hours after ERCP.

Concepción-Martin *et al.,* (2016), proposed an early inhibition of the cytokine response secondary to the instrumentation of the biliary tree. Also described by Chen *et al*., (2003), in their model of post ERCP pancreatitis. Pro-inflammatory cytokines not rising until 8 hours after pancreatic injury, and not being detected systemically until 24 to 48 hours after the procedure. This would fit the delay demonstrated for both approaches in the rise in cytokine concentration for patients who had recent ERCP or OTC and surgery. Potentially factors around laparoscopic surgery delaying the cytokine increase further. This delayed cytokine response after recent ERCP or OTC has not previously been reported. The exact reason is unclear it is hypothesised the obstruction of the system and the biliary stasis could inhibit cytokine response. Recent ERCP causing oedema of the tract sub-clinically affecting drainage. Bile is known to inhibitory to the increase in concentration of the inflammatory cytokines, but not inhibitory to cytokines such as IL-10 (Nesseler *et al.,* 2012). Table 8.2.3 demonstrates a greater rate of sepsis in those who had recent ERCP or OTC with cholecystectomy than those who underwent cholecystectomy alone, regardless of the approach to surgery. Alternatively a 'septic nidus' with biliary stasis could proliferate without, or eliciting a minimal, inflammatory cytokine response. Only following surgery is the systemic cytokine concentration increased. It is not supported by the immediate rise in cytokine concentration seen in the biliary emergency patients undergoing emergency ERCP and the elective ERCP patients developing sepsis demonstrating an increase in inflammatory cytokine concentration at two hours following ERCP.

Other factors - Type of sepsis and co-morbidities

Laparoscopic approach patients developed significantly more respiratory sepsis following surgery (Table 8.2.7 and 8.4.1), except the laparoscopic cholecystectomy with OTC group. This could be related to the pneumoperitoneum. The OTC patients developed biliary and haematological sepsis possibly because the bile stasis, spillage and instrumentation being more important than the length of the pneumoperitoneum. Potentially they may have pre-procedural biliary sepsis, as they had positive bile cultures. As we did not measure it directly, it is not possible to say whether the bile spillage was sufficient to cause T-cell anergy and decreased proliferation and cytokine

release, seen with peritonitis with macrophages and T-cells (Green and Beere, 2000, Heffernan *et al.,* 2013).

The open approach patients had significantly more positive bile and blood cultures (p = 0.036) (Table 8.2.7). Possibly the open cholecystectomy procedure releases bacteria and localised inflammatory cytokines into the systemic circulation, earlier due to the increased trauma of gaining access. Whereas the generation of the pneumoperitoneum inhibits normal ventilation of the lungs, increasing the risk of chest infections; particularly in those with pre-existing respiratory disease. Post-operative pain limits normal ventilation, predisposing to respiratory complications, this would be expected in the open approach patients, these patients received PCA, and did not have significantly higher pain scores.

Patients for both approaches developing respiratory sepsis tend to require a higher level of care, and tend to be diagnosed later than those with positive blood cultures (Table 8.2.5). Suggesting the sepsis developed post-operatively, supported by the lack of recording of clinical concern on the anaesthetic chart, about current respiratory problems, whereas pre-existing respiratory disease was recorded. Both the respiratory sepsis and the positive bile / blood culture patients scored their pain score in the severe pain group, from enrolment onwards. Therefore the enrolment pain score could be indicating a potential problem or likely hood of longer surgery. Therefore making a case for consideration of prophylactic antibiotics.

We would expect the patients with positive bile cultures to demonstrate an earlier cytokine increase, but there was a uniform delay in the laparoscopic approach patients, and no delay with respiratory sepsis in the open patients (Table 8.2.7). Surgical factors appearing to be more important.

Antibiotic prophylaxis does not appear to be required in the majority of laparoscopic approach patients, who do not develop sepsis after surgery. But in patients with co-existing co-morbidities, particularly respiratory problems, diabetes and smokers this does become important (Table 8.2.6). These patients have significantly more sepsis (p = 0.005). The patients who developed sepsis were also significantly older and more males

developed sepsis in each group in the study (p = 0.004). But overall the men also received more morphine.

Kishimoto study (2009), demonstrated that cytokine concentration decreases with age, and is lower in all males compared to premenopausal females. We did not see a lower cytokine level in the males, but the male patients and the older patients had a higher rate of sepsis, and required a higher level of care to treat their sepsis (Table 8.2.5). But this could also be secondary to the cause of sepsis and a bigger study is required to examine this further. Sacrerdote and Franchi, (2012), found the immunocompromise caused by opiates was more of a significant problem in older patients. Amodeo *et al.,* (2018), demonstrated older patients taking longer to recover from the post-operative immunosuppression, particularly when morphine is used for postoperative analgesia. We had too few episodes of secondary sepsis to be able to comment on this, but we did have more sepsis in the older patient group (Figure 8.2.4).

Potentially age is a confounder because the group developing sepsis were older, and the males were older. Another potential confounder is common bile duct stones are commoner in older patients, which increases the likely hood of recent ERCP or on table cholangiogram, but our patients mean age was not significantly greater in these groups (Table 8.2.3).

Hypothetical model

Previous research, has focused upon individual factors, or factors only around the generation of the pneumoperitoneum, or analgesia. Single factors alone fail to explain all the variation in finding found in this study and by others. We attempted to describe a model which described the interplay of factors Figure 12.1.2 - 3. The current model still cannot entirely explain the full range of clinical responses seen in this observational study.

This being a single centre observational study with the small numbers and multiple causes of sepsis, it is difficult to draw firm conclusions, this requires a multi-centre approach to have sufficient power to draw firm conclusions. Further clarity would be gained by measuring peritoneal as well as systemic cytokine concentration, but placing a drain is not a routine clinical procedure and patients declined this in the pilot study.

Therefore alternative approaches are required for example measuring cytokine concentration in the bile aspirated for culture, or measuring the cytokine concentration in the bile of all gall bladders removed at cholecystectomy.

Hypothesis for the interaction of factors influencing the cytokine response in patients undergoing ERCP alone (Part a)

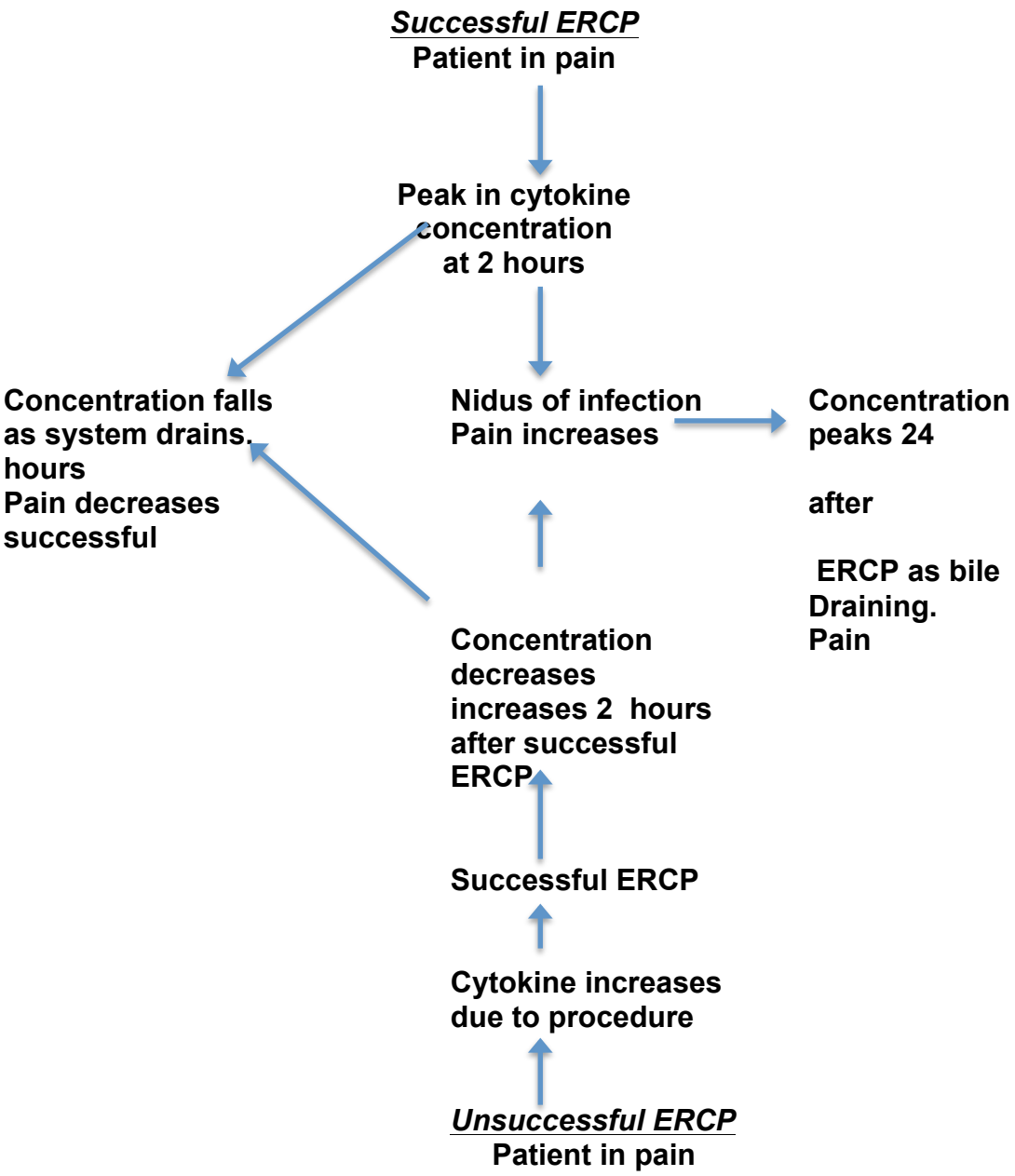


Figure 12.1.2 a: Hypothesis for cytokine and pain response demonstrated in the study. This Figure should be read in conjunction with Figure 12.1.2 b on the following page. Unsuccess – unsuccessful, pt – patient, lap. chole. – laparoscopic cholecystectomy, Op. – operative.

Hypothesis for the interaction of factors influencing the cytokine response in patients undergoing ERCP (Part b)

ERCP patients going on to have emergency cholecystectomy

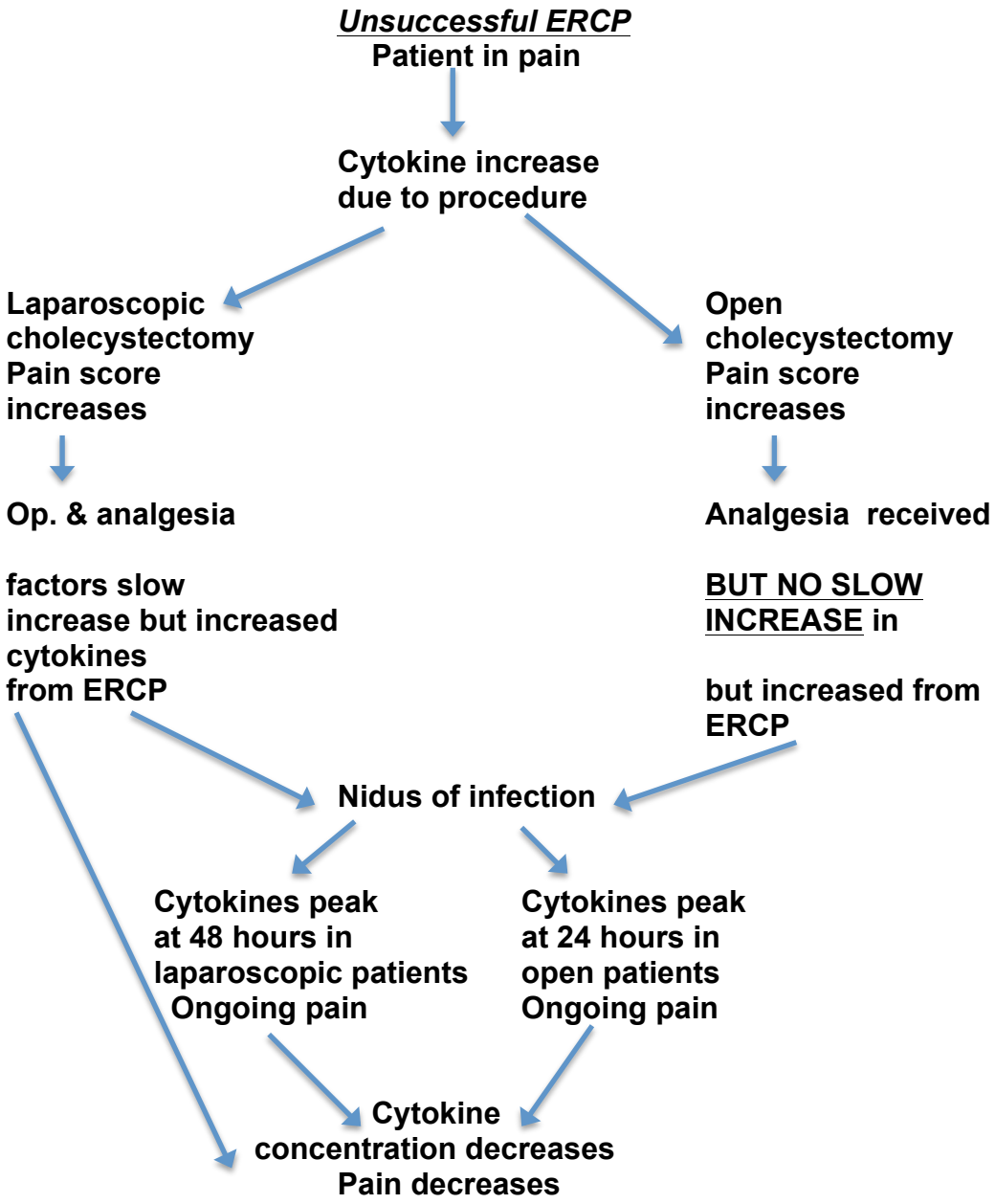


Figure 12.1.2 b: Hypothesis for cytokine and pain response demonstrated, underlined in capitals is the unexplained factor, where analgesia received increases due to the failed ERCP, but this didn't appear to delay the rise in the cytokine concentration. This should be read in conjunction with Figure 14.1.2 a on the previous page. Op. – operative.

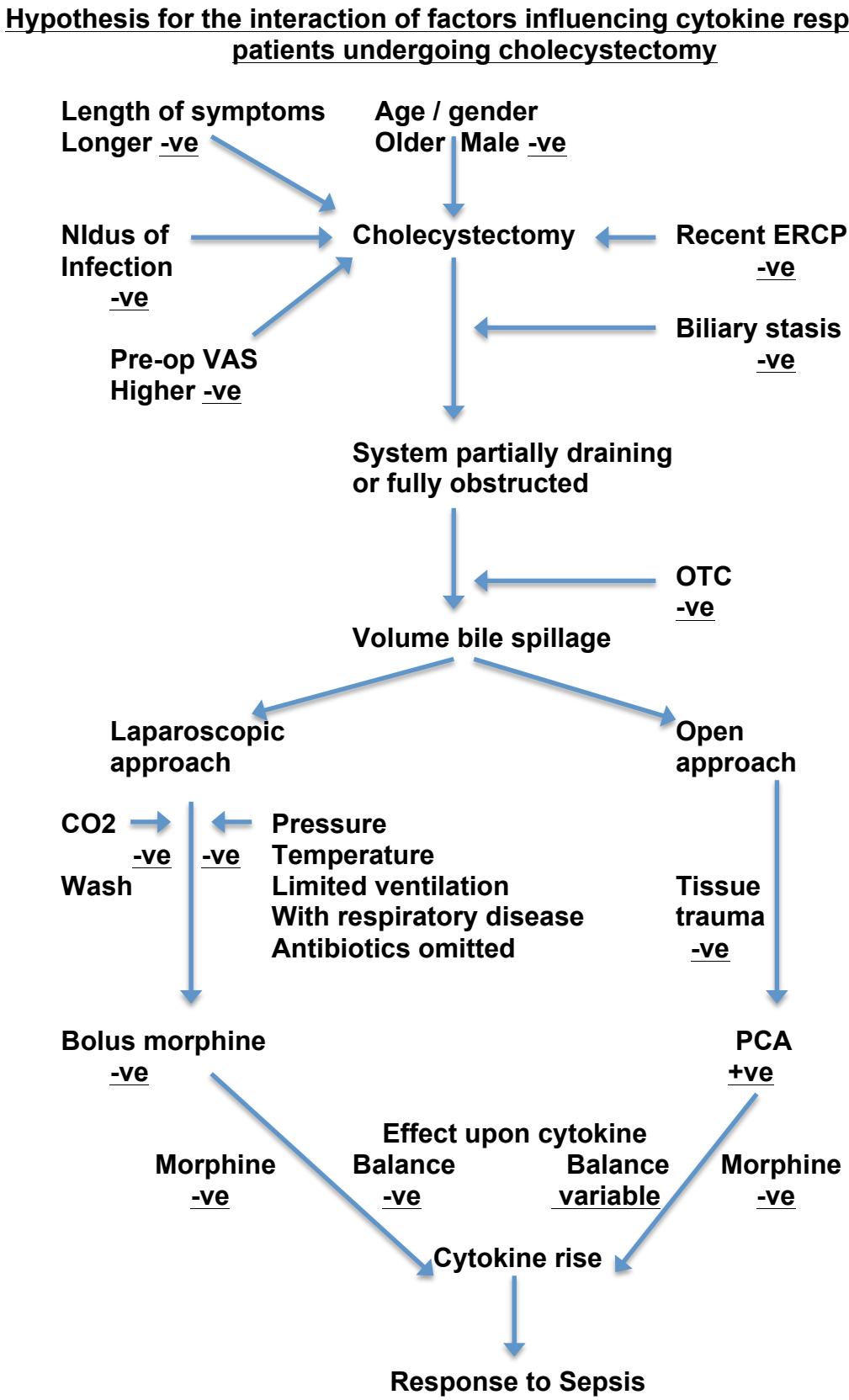


Figure 12.1.3: Hypothesis of factors interplaying to alter the timing of the cytokine response after cholecystectomy. Balance is the contribution of negative (-ve) and positive (+ve) factors illustrated above, delaying rise in the laparoscopic approach.

12.2 Pain as an early indicator of post procedural sepsis

It has been well demonstrated that the early recognition and treatment of sepsis improves the overall outcome of sepsis. The aim of this study was to find a way to identify sepsis earlier in patients undergoing laparoscopic cholecystectomy. From the pilot study we were aware the cytokine response was not reliable and the pain scores gave an earlier indication of post procedural problems.

The majority of work examining reported pain as a marker of post procedural sepsis, has been following ERCP or elective colorectal surgery. Cote *et al.,* (2015) and Concepción - Martin *et al.,* (2016), recognised pain following ERCP as a good indicator of potential post procedural problems and used it as an indicator for admission. No studies had examined cholecystectomy, or a surgical model where pain was part of the disease course. I was also interested to establish if it were a marker of problems after ERCP, would it be a marker at other points in the gall bladder disease treatment.

Correlating patients pain score, with their cytokine concentration and their outcome of surgery, revealed that as early as four to six hours after cholecystectomy or ERCP those developing sepsis reported significantly more pain. Examining their cytokine concentration at this time point revealed no significant difference in cytokine concentration to patients not developing sepsis in the same arm (Figure 9.3.2 and 3 and 10.3.3 and 10.3.4 for the cholecystectomy patients and Figure 9.2.1 and 10.1.2 for the ERCP patients). In the cytokine discussion we have highlighted the cytokine concentration increase was affected by multiple different factors. In contrast the timing of the pain score becoming significantly greater for the group developing sepsis was remarkably constant. This was seen irrespective of approach to surgery, regardless of recent ERCP or OTC, and in the elective ERCP patients. The timing of this increase in pain score being similar to those demonstrated by Cote *et al.,* (2015) and Concepción - Martin *et al.,* (2016).

<u>Pain and the immune system</u>

Watkins and Maier (2005), reported that postoperative pain contributes to immunosuppression, with decreased cell mediated immunity and a reduction in the non-specific immune response. This could be interpreted as explaining the results found in the laparoscopic approach patients, but we see similar reported levels of pain in the

open patients without the delayed rise in cytokine concentration. This supports the hypothesis of other factors, discussed above, having a greater role in delaying the immune response, and post procedural pain being an indicator and not a cause of sepsis.

Nicholson and Hall (2011), discussed the pivotal role that IL-1 has in producing the mediators of the pain response, demonstrating blockade of IL-1 production and it's receptor, improving post-operative pain. Ren and Dubner (2010) reporting IL-1β acting peripherally on the primary afferent neurons to synthesis and release substance P. This study did demonstrate an increase of IL-1 in the open approach and the ERCP patients, at 2 hours, which potentially could contribute to the pain response between 4 and 6 hours. The intra-peritoneal IL-1 levels being reduced by factors around the pneumoperitoneum in the laparoscopic approach patients may explain the reduced post-operative pain after laparoscopic surgery. Systemically we could not demonstrate a significant rise in IL-1 concentration in the laparoscopic approach patients until 48 hours, well after the pain score became significantly different at 4 – 6 hours.

Hsing and Wang (2015), report the recovery in the intra-peritoneal IL-1 concentration at four to six hours following laparoscopic surgery, with this corresponding to an increase in post-operative pain after laparoscopic surgery, and a more significant increase in those with post-operative complications. This would correspond with the timing of the increase in the pain scores in the laparoscopic approach patients, but the same timing was seen in the open approach patients. It has alternatively been proposed that the pain response is secondary to localised increases in inflammatory cytokines within the central nervous system. Watkins and Maier (2005), established that the peripheral cytokines had a minor effect upon the central nervous system, compared with the cytokines produced within the central nervous system.

Factors discussed in Section 12.1, limit the local cytokine concentration accumulation. In those developing sepsis a certain concentration level of cytokine could invoke a pain response within the peritoneum. Further time being required for the intra-peritoneal concentration to increase and reach the level to spill over into the systemic circulation. Matsuzaki and colleagues (2014), reported that carbon dioxide used to generate the pneumoperitoneum suppressed the inflammatory and metabolic response of peritoneal neutrophils and macrophages until 4 ½ hours after surgery. This has been proposed to

correspond with the increase in pain score in those developing sepsis after laparoscopic surgery. This theory should mean we should be able to recognise sepsis earlier in the open compared to the laparoscopic approach patients but this was not the case.

Concepción – Martin *et al.,* (2016), note an increase in pain score at six hours in those developing post ERCP complications, but no detectable change in systemic cytokine concentration until 12 to 24 hours after ERCP. They report the suppression of the cytokine response after ERCP means that it is not until four to six hours have elapsed for the local concentration to be sufficient to stimulate local nerve fibres within the peritoneum. The increase in intra-peritoneal cytokine concentration not being sufficient to be detected systemically until 12 to 24 hours after intervention. This limits the earlier recognition of sepsis but fits the findings found in this study.

Watkins and Maier, (2005) found localised inflammatory response enhance the sensory nerve terminals expression of opioid receptors, in those developing sepsis after surgery. Suggesting the increased analgesia requirement in the patients developing sepsis occurs as a result of the sepsis, rather than pre-disposing the patient to developing sepsis. Wordliczek *et al.,* (2000) demonstrated local nerve damage following elective surgery increased immune cells within the dorsal root ganglia, which in turn causes a measurable pain response. In this study the patients developing sepsis had longer more difficult procedures, requiring increased dissection (Table 8.4.1).

Wordliczek *et al.,* (2000), demonstrated inhibiting systemic cytokine synthesis prior to laparoscopic cholecystectomy, particularly IL-1, there was decreased requirement for opiates in the post operative period. This implicates the increasing cytokine concentration in those developing sepsis being responsible for the pain, rather than post-operative other factors such as post-operative analgesia causing the sepsis. Mayes and colleagues (1993) described an exaggerated pain response when cytokines flooded over onto healthy neurons, or when they were exposed to bile, or bowel contents, bacteria, fungi or viruses.

Cook *et al.,* (2018), has highlighted the role of other inflammatory cytokines such as TNF-α and IL-6. But this is in inflammatory conditions, such as arthritis, atherosclerosis and Alzheimer's, in which the cytokines are acting directly to increasing responsiveness

to stimulation. The role of cytokines in surgery and sepsis being less clear, and potentially being an interaction between multiple factors. This would fit with the variation in the timing of the cytokine response that we observed, and the constancy of the pain response.

The study did demonstrate that pain is a marker of post-operative sepsis, and although the clinical team did not objectively score the patients pain they did not discharge patients with sepsis, principally because of 'not being quite right or having a lot of pain' (Table 10.3.1). The timing of the diagnosis of each type was consistently later in patients who had undergone laparoscopic surgery compared to those who had open surgery, or ERCP (Table 8.2.5). The patients who required the higher level of care for each approach had their sepsis diagnosed later than those cared for on the ward. As an observational study it is important to highlight that the exact time point for the onset of sepsis was not possible to verify. Particularly as the causes of sepsis were diverse, and we were using systemic cytokine concentration as a proxy for peritoneal cytokine concentration.

Measuring patient's pain would not only permit earlier treatment or closer observation, with potential improvement in outcome, for those with potential septic complications. It would also allow the discharge of those at low risk of complications. Why the patients developing sepsis score their pain higher in the early post-operative period is difficult to fully determine from this observational study. Figure 12.2.1 is the hypothesis we developed to try and explain the results we observed, It is based around the Figures we developed for the changes in cytokine concentration dependent upon the approach to surgery (Figure 12.1.2 a and b and 12.1.3).

Hypothesis for the findings of the pain score increasing prior to the cytokine concentration in those developing sepsis

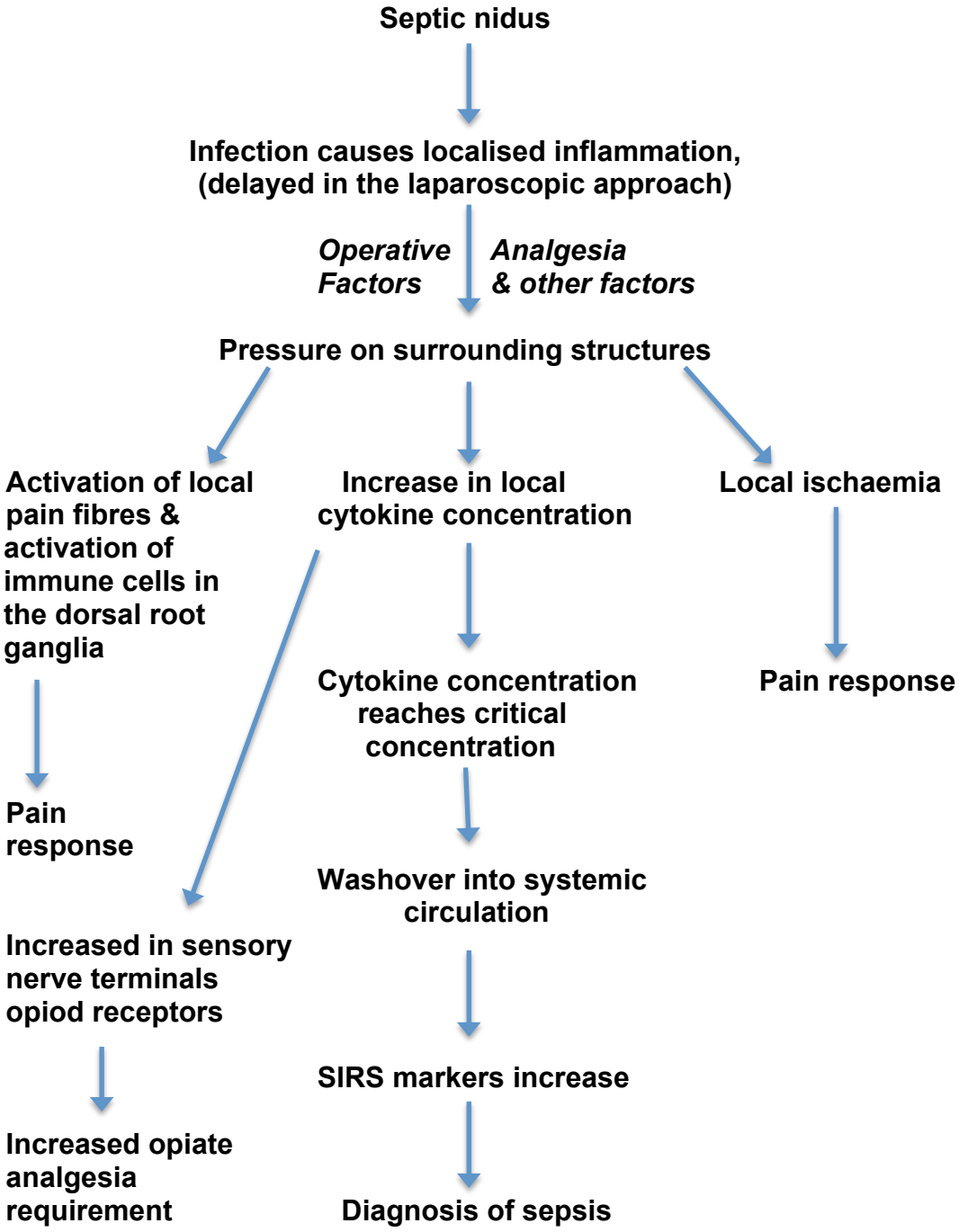


Figure 12.2.1: Demonstrates a potential hypothsis to explain the findings of this study. The factors in italics are those discussed in Figure 12.1.1 and act as limiters on the increase in the local cytokine concentration. This is the step which determines when the diagnosis of sepsis is made. As this was an obsevational study it was not possible to test the hypothesis.

Analgesia

Excluding the significant pain experienced group, men rate their pain higher following cholecystectomy or ERCP. Male participants had a higher rate of sepsis, but this experience of increased pain is seen regardless of developing sepsis. The men received more morphine during their admission, irrespective of the arm they were in. Lloyd *et al.,* (2008), demonstrated a higher concentration of μ – opioid receptors in the male mid – brain, correlating with them experiencing greater benefit from the morphine they received. Matching female and male patients they found the female patients scored their pain as high but they benefited less from the morphine administered. Whether the greater use of morphine in the male patients is related to the increased number of cases of sepsis, or the morphine requirements increased due to sepsis, cannot be definitively determined from this study.

The open approach patients all had PCA, whereas the laparoscopic approach patients and the patients in the other arms had analgesia available on request. We asked patients to measure their pain after coughing, important maneuvers to decrease sepsis (Lynch *et al.,* 1997), an action that assesses parietal and visceral pain (Joris *et al.,* 1995). Patients were not very compliant with this. Patients with PCA were less mobile, and this increases the complexity of making comparisons between the groups. Particularly at 24 hours after ERCP or surgery, the mild and significant pain manageable patients not on PCA were mobilising and preparing for discharge, whereas the PCA patients were not.

12.3 Significant pain experienced group and the VAS

Within the patients not developing sepsis, in each arm of the study, were a group of patients who experienced more pain than the other patients admitted for the same reason. This group of patients termed 'Significant pain experienced group'.

Pain is obviously subjective and the significant pain experienced patients developed sepsis significantly less frequently ($p = 0.008$); but expressed similar levels of pain on their VAS scores. The group received equivalent amounts of analgesia to the group developing sepsis. Their reduced rate of sepsis, was due to their shorter history of symptoms and their younger age. The procedures were rated as less complex (Figure 8.4.1), with reduced likely hood of localised inflammation.

Based on the VAS alone it was not possible to distinguish the significant pain experienced group from those developing post-procedural sepsis. The QoL and HAD questionnaires in combination with the VAS permitted us to identify the significant pain experienced patients pre-operatively (Table 11.1 11). This will permit more targeting of treatment to those who are likely to be developing sepsis and those with significant issues with pain management. The non-significant pain experienced patients with lower pain scores after intervention it permits us to consider planning for day surgical procedures, and early discharge if pain is controlled.

Gebhart (2000), stated 'pain is real for the person experiencing it and should not be dismissed but thoroughly investigated.' The significant pain experienced patients' rated their pain higher in the peri-operative period, expected and recalled being in more pain. Up until fours after surgery the significant pain experienced group VAS scores were equivalent to the other pain groups (Figure 10.3.2 – 10.3.4). After this their analgesia requirements, and pain scores increased as the importance of operative factors decreased. This corresponded to when the patients became more alert, and the relevance of psychological factors increased, as did the presence of visitors. This was also the time point the VAS scores in those developing sepsis diverged significantly from the other patients not developing sepsis.

The significant pain experienced group believed their pain was unrecognised (Table 8.4.7), despite receiving significantly more opiate analgesia (Table 8.4.3 and 8.4.4). It

would appear from their VAS scores the benefit they receive from morphine is less, and those on PCA had more unsuccessful attempts recorded. This may be secondary to the increased analgesia they have received prior to admission (Table 8.4.3), with studies highlighting pre-operative analgesia causing hyperalgesia, making it harder to attain adequate post-operative pain relief (Carroll *et al.,* 2004). Their pain should not be dismissed as just their experience of pain, but it was less likely to be due to sepsis.

The expectation was for a group experiencing more pain but not developing sepsis to be found in the severe pain group as well. Careful analysis failed to demonstrate this, and poor rating of QoL was only found in the group within the significant pain group. Discussion with significant pain experienced patients highlighted the chronicity of their pain, with patients potentially becoming habituated to it, and therefore rating their pain score at enrolment as significant but not severe.

The significant pain experienced group was over represented in the biliary colic group. This is a subjective diagnosis, with the majority who rated their pain as mild and significant pain manageable at enrolment being discharged prior to 24 hours and therefore excluded from the study. The group were also over represented in was the open cholecystectomy group. This was unexpected, as the significant pain experienced patients were younger, and younger patients tended to move from open to laparoscopic surgery (Table 8.2.3). Discussion around their decision-making about surgery demonstrated that they were anxious about swapping consultant, in case they had to wait longer and the open surgeon had a shorter waiting list, and they wanted to undergo surgery to reduce their pain. Six patients swapped to the laparoscopic approach, two mild and four significant pain manageable group. Their data was excluded from the analysis in case their preference of approach biased their response. Separate analysis demonstrated they were mainly self employed and wanted to return to work earlier.

It was postulated that the higher dose of analgesia required delayed the diagnosis of sepsis. Principally the additional morphine in the group developing sepsis slowed the rise in cytokine concentration and the development of abnormal SIRS markers. There were no increased cases of sepsis in the significant pain experienced, despite more frequently receiving analgesia prior to admission (Table 8.4.3 and 8.4.4). They also did

not have delayed presentation of sepsis compared to the other groups receiving less analgesia.

The study highlights the divergence between the health professionals' assessment of pain and the patient's experience of pain, and the value of assessment of the effectiveness of analgesia intervention in patients. The value of education about pain management at pre-assessment, during admission and at discharge cannot be underestimated in improving all patients' experiences. Clinic letters indicated there was a paucity of discussion around alternative diagnosis for patients right upper quadrant pain, and alternatives to surgery for its management. Particularly in the significant pain experienced group we have demonstrated a third rated their health as worse, and continued to experience significantly pain up to a year after surgery (Table 8.4.9 and 11.1.12). This raises the question of the benefit they received from cholecystectomy.

12.4 Using quality of life measures distinguishing groups of patients

Somasekar *et al.,* (2002), demonstrated biliary disease negatively impacts upon patients QoL and HAD scores. Cholecystectomy improves QoL scores on patient rated scores Someasekar *et al.,* (2002) and Yu *et al.,* (2018), and in this study. Within the group undergoing cholecystectomy we, and others, have found a group of patients who do not benefit from surgery (Quintana *et al.,* 2003, 2005). Quintana *et al.,* (2005) study included the SF-36 question 'Compared to one year ago, how would you rate your health in general now?' This was a useful discriminator question for the appropriateness for surgery, but was only beneficial following surgery.

The pilot study demonstrated a group of patients who scored their quality of life poorer, and experienced more pain post-operatively. The study aimed to distinguish this group pre-operatively, allowing us to recognise those developing sepsis that scored their pain in a similar pattern, from this group who did not develop sepsis. This would allow us to commence treatment for sepsis earlier for those who required it. Secondary it would permit discussion around alternatives to surgery, the benefits of surgery and the optimisation of pain management.

Shi *et al.,* (2009) and Quintana *et al.,* (2005 and 2008), have both used QoL measures to identify those who benefitted and did not benefit from cholecystectomy. Shi *et al.,* (2009), calculated the 'Minimal clinically important difference' (MICD) for improvement in QoL, permitting evaluation of whether cholecystectomy had been beneficial to patients. Both have attempted to identify patients pre-operatively where cholecystectomy may not be beneficial.

In this study the mild and significant pain manageable group, QoL score was equivalent to Shi *et al.,* (2009), group 'much better' after cholecystectomy, and Quintanna *et al.,* (2005 and 2008) group of 'appropriate surgical candidates'. The severe pain group scored similar to Shi *et al.,* (2009), 'somewhat better' group and Quintanna *et al.,* (2005 and 2008) group of 'uncertain indication or benefit' group at three months. At six months scoring as 'much better' or Quintanna *et al.,* group of 'appropriate for cholecystectomy'. In this study the severe pain group had more patients developing sepsis than the other groups, and at 3 months those requiring ITU and HDU had not long been discharged.

The significant pain experienced group's quality of life scores did not change significantly pre to post operatively up to twelve months after surgery. Scoring their QoL at the level of Shi *et al.,* (2009) and Quintanna *et al.* (2005 and 2008), groups described as 'inappropriate' for surgery.

Patients identified as appropriate candidates for cholecystectomy improved maximally in body pain, symptom score, vitality and social function. The patients described as 'inappropriate for cholecystectomy' scored minimal improvements in their body pain and psychological domains scores. The severe pain group score was lower in the 'Bodily pain' and the 'Symptom score' domains at 3 months, these domains taking longer to improve. The mental health domains were not uniformly lower at enrolment in the severe pain group, possibly because being unwell garnered more social contact, and had improved at 3 months. This was a distinguisher from the significant pain experienced patients who scored poorer in all domains at enrolment and at 3 months.

The study demonstrated the QoL scores were comparatively reproducible with patients participating in multiple arms of the study being independently reassigned to the same group by their QoL scores with an accuracy of over 70%. The severe pain group was less accurately reassigned to the same group, as the cause of their pain had been treated and they had a lower pain score on readmission. This improved pain was reflected in the QoL scores, and is not seen as a failure of the questionnaires.

Predicting pre-operatively pain groups

The aim was to develop the findings of Shi *et al.,* (2009) and Quintanna *et al.* (2005 and 2008), and not only discriminate the patients who would not benefit from cholecystectomy prior to surgery; but identify those with postoperative pain which was potentially an indicator of a septic event, from those who required more support with pain management. This we were able to do with questionnaires with good accuracy by the end of the study (Table 11.1.11).

We tried to refine the number of questionnaires required to discriminate the patient's benefit from cholecystectomy, finding the pre-operative VAS permitted identification of the mild and severe pain groups. This left a smaller group, the significant pain group, where the QoL questionnaires were required to sub-divide the group into the significant

pain manageable and experienced group. Removing one questionnaire, particularly the SF-36 and GIQLI, reduced the accuracy. The multiple domain scores in the two questionnaires permitted greater accuracy, by allowing an overall best fit of domain scores (Table 11.1.11). This was important because there was compensation between domains. For example the 'Social function' domain in the severe pain group compensated poor scores in 'Bodily pain', as people visited them due to being off sick. This discriminated them from the significant pain experienced group who scored poorly in both domains. The HAD score, was the quickest to complete, but the reduced number of domains reduced its discrimination power between overlapping groups.

We have also demonstrated the work of Quintana *et al.,* (2005, 2008) and Shi *et al.,* (2009), with cholecystectomy patients, is also valid for patients undergoing OTC or have had a recent ERCP and are undergoing cholecystectomy, and with elective ERCP patients.

12.5 Conclusions

The study has the limitations of being an observational study, with a small number of patients, and hence small number of cases of sepsis, and an imbalance in genders in some groups. The causes of sepsis were diverse, from being related to biliary sepsis, procedural related, or related to co-morbidities, and the level of care required was not standard.

From the pilot to the main study compromises were made to increase the acceptability of the study to patients. This included measuring systemic and not peritoneal cytokines, and reducing and adjusting the timing of measuring the cytokine concentration. This caused a loss of clarity in the timing of the cytokines rising and peaking, and prevented a comparison of the response between the peritoneal cytokine and the systemic cytokines.

We found the significant pain experienced patients experience significant amounts of long-term pain, leading to the question of allocation of resources and the pre-operative counseling patients prior to surgery. Poor adherence to analgesia protocols required adjustment in the results analysis. This lead to the initiation of regular VAS assessment of pain and response to analgesia, and staff and patient education around analgesia, and counseling about pain becoming an integral part of the pre-assessment and discharge pro formas. The QoL results has lead to a more robust assessment of patients pain prior to them being listed for cholecystectomy.

Particularly at 24 hours, there was significant variation between patients mobilising and preparing for discharge and those on PCA. Repeating this study I would tighten the assessment of pain, to enable a more robust comparison between groups. The results analysis would have benefited from the use of a statistics package to highlight areas where the results indicated a significant difference between groups. The study highlighted the difference between the significant pain experienced patients and the other groups. Comparison of this group to others requires caution because of these patients' different psychology and expectations of surgery. The VAS in combination with QoL questionnaires permits identification of this group from the other groups.

Patients developing sepsis after laparoscopic surgery demonstrated a delay in their cytokine response, with interventions such as OTC or having had a recent ERCP also delaying the cytokine response. Notwithstanding the variation in cytokine response, those developing sepsis score their pain significantly higher from six hours onwards.

Previous studies have examined single factors around laparoscopic surgery, or analgesia. This study has examined multiple factors and developed an evidenced based model of how these multiple factors potentially interact to delay the rise in the cytokine concentration following intervention. A higher rate of sepsis has been demonstrated in for example male and older patients, and raises the question of other potential factors interplaying to determine the cytokine response. This includes genetic variation to factors including BMI, co-morbidities and the microbial moiety encountered.

The study permits us to clinically identify those who benefit less from surgery, and in whom alternative diagnosis should be considered and alternative approaches to managing their pain discussed. The quality of life data allows us to distinguish these patients from those developing postoperative complications such as sepsis. This is important with limited health care resources, and central in the appropriate use of antibiotics and early recognition of sepsis.

Appendix

Appendix 1 – Quality of life questionnaires

36-Item Short Form Survey Instrument (SF-36)

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- 1 - Excellent
- 2 - Very good
- 3 - Good
- 4 - Fair
- 5 - Poor

2. **Compared to one year ago**, how would you rate your health in general **now**?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day.
Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3

	Yes, limited a lot	Yes, limited a little	No, not limited at all
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	1	2	3
5. Lifting or carrying groceries	1	2	3
6. Climbing **several** flights of stairs	1	2	3
7. Climbing **one** flight of stairs	1	2	3
8. Bending, kneeling, or stooping	1	2	3
9. Walking **more than a mile**	1	2	3
10. Walking **several blocks**	1	2	3
11. Walking **one block**	1	2	3
12. Bathing or dressing yourself	1	2	3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

13. Cut down the **amount of time** you spent on work or other activities	1	2
14. **Accomplished less** than you would like	1	2

15. Were limited in the **kind** of work or other activities ○ ○

16. Had **difficulty** performing the work or other activities (for example, it took extra effort) ○ ○ 1 2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
. Cut down the **amount of time** you spent on work or other activities	1	2
. **Accomplished less** than you would like	1	2
. Didn't do work or other activities as **carefully** as usual	1	2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

○ 1 - Not at all

○ 2 - Slightly

○ 3 - Moderately

○ 4 - Quite a bit

○ 5 – Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

○ 1 - None

○ 2 - Very mild

○ 3 - Mild

○ 4 - Moderate

○ 5 - Severe

○ 6 - Very severe

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

○ 1 - Not at all

○ 2 - A little bit

○ 3 - Moderately

○ 4 - Quite a bit

○ 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
24. Have you been a very nervous person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
25. Have you felt so down in the dumps that nothing could cheer you up?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
26. Have you felt calm and peaceful?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- 1 - All of the time
- 2 - Most of the time
- 3 - Some of the time
- 4 - A little of the time
- 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5

GIQLI Survey Questionnaire

The Gastrointestinal Quality of Life Index (GIQLI) Please circle one choice for each question.

1. How often during the past 2 weeks have you had pain in the abdomen?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

2. How often during the past 2 weeks have you had a feeling of fullness in the upper abdomen?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

3. How often during the past 2 weeks have you had bloating (sensation of too much gas in the abdomen)?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

4. How often during the past 2 weeks have you been troubled by excessive passage of gas through the anus?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

5. How often during the past 2 weeks have you been troubled by strong burping o r belching?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

6. How often during the past 2 weeks have you been troubled by gurgling noises from the abdomen?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

7 How often during the past 2 weeks have you been troubled by frequent bowel movements?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

8. How often during the past 2 weeks have you found eating to be a pleasure?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

9. Because of your illness, to what extent have you restricted the kinds of food you eat?

1. Very much
2. Much
3. Somewhat
4. A little
5. Not at all

10. During the past 2 weeks, how well have you been able to cope with everyday stresses?

1. Extremely poorly
2. Poorly
3. Moderately
4. Well
5. Extremely well

11. How often during the past 2 weeks have you been sad about being ill?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

12. How often during the past 2 weeks have you been nervous or anxious about your illness?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

13. How often during the past 2 weeks have you been happy with life in general?

1. Never
2. A little of the time
3. Some of the time
4. Most of the time
5. All of the time

14. How often during the past 2 weeks have you been frustrated about your illness?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

15. How often during the past 2 weeks have you been tired or fatigued?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

16. How often during the past 2 weeks have you felt unwell?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

17. Over the past week, have you woken up in the night?

1. Every night
2. 5-6 nights
3. 3-4 nights
4. 1-2 nights
5. Never

18. Since becoming ill, have you been troubled by changes in your appearance?

1. A great deal
2. A moderate amount
3. Somewhat
4. A little bit
5. Not at all

19. Because of your illness, how much physical strength have you lost?

1. A great deal
2. A moderate amount
3. Somewhat
4. A little bit
5. Not at all

20. Because of your illness, to what extent have you lost your endurance?

1. A great deal
2. A moderate amount
3. Somewhat
4. A little bit
5. Not at all

21. Because of your illness, to what extent do you feel unfit?

1. Extremely unfit
2. Moderately unfit
3. Somewhat unfit
4. A little unfit
5. Fit

22. During the past 2 weeks, how often have you been able to complete your normal daily activities (school, work, household)?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

23. During the past 2 weeks, how often have you been able to take part in your usual patterns of leisure or recreational activities?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

24. During the past 2 weeks, how much have you been troubled by the medical treatment of your illness?

1. Very much
2. Much
3. Somewhat
4. A little
5. Not at all

25. To what extent have your personal relations with people close to you (family or friends) worsened because of your illness?

1. Very much
2. Much
3. Somewhat
4. A little
5. Not at all

26. To what extent has your sexual life been impaired (harmed) because of your illness?

1. Very much
2. Much
3. Somewhat
4. A little
5. Not at all

27. How often during the past 2 week, have you been troubled by fluid or food coming up into your mouth (regurgitation)?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

28. How often during the past 2 weeks have you felt uncomfortable because of your slow speed of eating?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

29. How often during the past 2 weeks have you had trouble swallowing your food?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

30. How often during the past 2 weeks have you been troubled by urgent bowel movements?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

31. How often during the past 2 weeks have you been troubled by diarrhea?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

32. How often during the past 2 weeks have you been troubled by constipation?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

33. How often during the past 2 weeks have you been troubled by nausea?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

34. How often during the past 2 weeks have you been troubled by blood in the stool?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

35. How often during the past 2 weeks have you been troubled by heartburn?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

36. How often during the past 2 weeks have you been troubled by uncontrolled stools?

1. All of the time
2. Most of the time
3. Some of the time
4. A little of the time
5. Never

Hospital Anxiety and Depression Scale (HADS)

Instructions: Read each item and circle the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate than a long thought out response.

I feel tense or 'wound up':	
Most of the time	3
A lot of the time	2
Time to time, occasionally	1
Not at all	0

I feel as if I am slowed down:	
Nearly all of the time	3
Very often	2
Sometimes	1
Not at all	0

I still enjoy the things I used to enjoy:	
Definitely as much	0
Not quite so much	1
Only a little	2
Not at all	3

I get a sort of frightened feeling like 'butterflies in the stomach':	
Not at all	0
Occasionally	1
Quite often	2
Very often	3

I get a sort of frightened feeling like something awful is about to happen:	
Very definitely and quite badly	3
Yes, but not too badly	2
A little, but it doesn't worry me	1
Not at all	0

I have lost interest in my appearance:	
Definitely	3
I don't take as much care as I should	2
I may not take quite as much care	1
I take just as much care as ever	0

PTO	

I can laugh and see the funny side of things:	
As much as I always could	0
Not quite so much now	1
Definitely not so much now	2
Not at all	3

I feel restless as if I have to be on the move:	
Very much indeed	3
Quite a lot	2
Not very much	1
Not at all	0

Worrying thoughts go through my mind:	
A great deal of the time	3
A lot of the time	2
From time to time but not too often	1
Only occasionally	0

I look forward with enjoyment to things:	
A much as I ever did	0
Rather less than I used to	1
Definitely less than I used to	3
Hardly at all	2

I feel cheerful:	
Not at all	3
Not often	2
Sometimes	1
Most of the time	0

I get sudden feelings of panic:	
Very often indeed	3
Quite often	2
Not very often	1
Not at all	0

I can sit at ease and feel relaxed:	
Definitely	0
Usually	1
Not often	2
Not at all	3

I can enjoy a good book or radio or TV programme:	
Often	0
Sometimes	1
Not often	2
Very seldom	3

Appendix

Appendix 2 – Conclusions from the pilot study group meeting

Patients' concerns from the review study group meeting

Point	Decision
Patients' believed their reported pain was not addressed	Discussion around how to be more clear in the study recruitment that the study team was separate from the clinical team and did not relay information between them Patients had felt let down by this ***Decision made about written and repeated verbal information about the study group being separate at each clinical encounter***
Too frequent blood tests	Aware we had had problems recruiting because of this and patients believed they were a significant block to participation ***Decision made to perform blood test at enrolment, one following the procedure and then every 24 hours. I was concerned we'd miss peaks in cytokine concentration but wanted a representative group to participate***
Discussion with patients about analgesia	Patients highlighted minimal discussion with them about analgesia, even at discharge and frequent delays in medication ***Decision made to educate staff with posters and presentations by myself and the pharmacist at ward, departmental meetings and staff induction. Information about the study included***

Appendix Table 2.1.1: Detailing the patients' principal concerns and the conclusions, which were reached, and adjustments made to the study protocol. The decision is in italics.

Procedural concerns from the review study group meeting

Point	Decision
Drain fluid cytokine concentration rose ahead of the systemic cytokine concentration	Discussion around drain insertion and reassurance that it would be placed in a port site in the laparoscopic approach patients Clinicians felt it inappropriate to place drains, the ERCP and biliary emergency patients would only have systemic cytokines and drains would only be placed in high risk patients therefore was the comparison valid ***Decision made to measure systemic cytokines only***
VAS or VRS	The VRS scores had demonstrated digit preference, particularly for whole or ½ integers. The group experiencing a lot of pain had scored their least and worst pain 5 or 10. The relationship was good between VAS and VRS but VAS gave a greater scatter of results ***Decision made to use the VAS patient preference to use the simpler VAS in part b of Figure 4.7.2 page 117 as the others confusing in the early period after anaesthesia. To also use this for least and worst pain***
Timing and administration of the VAS	The majority of the 11 and 17 hours VAS administered by the nurses were incomplete or completed at 24 hours. Patients were happy to measure the VAS more frequently than blood tests, but staff and patients did not want their, or other patients, rest disturbed. Not all patients were mobile and patients had been poorly compliant with the request to cough immediately before completing the questionnaire. We also discussed about the questionnaire and analgesia ***Decision made to complete additional VAS at 4 and 6 hours, to encourage the patients to be compliant with coughing. To score the VAS twice to measure reproducibility***
Analgesia protocol	Poor adherence to the pain protocol, in particularly the administration of NSAID's, but also paracetamol. Study was conducted prior to i.v. paracetamol being on the hospital formulary. Discussed about the other routes that were available and the other forms of analgesia, other than strong opiates ***Decision made to educate staff with posters and presentations by myself and the pharmacist at ward, departmental meetings and staff induction. Information about the study included***
Trainees performing the procedures	Trainees performed ERCP and cholecystectomy and were mainly first year trainees ***Decision made to perform the study in the second six months of the higher trainees attachment to the firm, which would also coincide with the junior doctors second six months of foundation year and therefore people should be more experienced and proficient***
Standardise the local anaesthesia approach and anaesthesia protocol	No decision could be reached ***Decision made to differ and to look if one route of local analgesia was optimal***
Lack of space and time on the morning of surgery	Everyone was aware there was a lack of space and time for everyone to see the patients and consent them for the theatre and the study and for them to complete the questionnaires. Offered to complete the QoL and consent in advance, but still concern over this ***Decision made to complete consent, VAS, bloods and QoL at pre-assessment, but ERCP patients could be seen on the day of the procedure due to the list time***
Taking cytokine concentration at the time of diagnosis of sepsis	This had failed as the lead investigator had not been called, no feasible solution to this ***Decision made to record from the notes the time of diagnosis and SIRS and continue cytokine concentration measurements at the set times in the study***

Appendix Table 2.1.2: Details procedural concerns and decisions of protocol changes.

Experimental concerns from the review study group meeting

Point	Decision
Financial concerns	Reducing the number of blood tests and not sampling drain fluid would reduce the number of ELISA kits required ***Decision made to not sample drain fluid and reduce the number of kits***
Variation between ELISA plates	Concerns about differences between plates and whether the differences were due to changes in cytokine concentration or differences between plates ***Decision made to run the control patients samples on multiple plates to act as internal controls but this would require more blood to be taken from the controls***
Taking one control sample and number of controls, gender and ethnic variation	There was interest whether there was diurnal variation in cytokine concentration ***Decision made to enrol 15 controls and commence one control in the study every hour from 8am to 10pm. We enroled 5 men and included 5 non Caucasian controls to look for variation between groups. There was no diurnal variation, found therefore subsequent samples could have been standardised to the times of the main theatre list. But it allowed diurnal variation to be excluded from differences in the biliary emergency group***.

Appendix Table 2.1.3: Detailing the concerns and the conclusions around the experimental work, and adjustments made to the study protocol. The decision is in italics.

Appendix

Appendix 3 - Patient Information Forms

Patient information forms for

Patient information sheet for those admitted with biliary emergencies

Introduction

You have been asked to take part in the study because the doctors looking after you think that gallstones are the cause of the pain you have in your abdomen (tummy).

The study is asking people who have gallstones causing problems to take part to see how the body responds to the gallstones. We are looking at markers of infection and seeing what happens to them when you have pain or problems from your gallstones. We are also looking at your level of pain to see if it can show us earlier when you have gallstone problems.

What will happen if I take part?

The research doctor will explain the study to you and then ask you to read this leaflet. What will happen to you is shown in a plan of the study on page 3 of this leaflet. If you decide you want to take part, you will be asked to sign a form (consent form) to say you agree to take part.

The infection markers are found in your blood so we will be asking to take some blood samples as in the diagram. At each time about 5 – 10mls of blood (one - two teaspoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. Blood tests will be at the start, two and 24 hours. We will try and combine the blood tests with other blood tests you have to investigate the gallstones so you don't need to have too many blood tests. If you are worried about the blood tests you can just complete the pain scores and questionnaire, please tell the researcher.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. If you are still in hospital the following day we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours being scored. Pain will be scored at the start, 2, 4, 6 and 24 hours after starting the study.

We also want to know how the pain affects your normal daily life and so we will also be asking you to fill in a questionnaire about this at the start. We are interested to see how this changes so will ask your permission to post or complete by telephone these questionnaires at 3, 6 and 12 months after being in hospital.

We also ask your permission to look at your hospital notes and occasionally contact your GP (family doctor) to see how many times you

have needed to see a doctor for about your gallstones and your general health.

What will happen to my information and my blood?

When you sign the form to take part your information will be given a code to identify it. All your information will be marked identified by this code, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part it will not change how the doctors look after you care for you. Just tell the research doctor you don't want to take part; you don't have to give a reason.

What happens if I change my mind?

If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered out of the trial. Alternatively you can allow us to use the information we already have, but not gather any more information. This will not affect your care. We will destroy all your blood samples in storage.

Do I need to know anything else?

If you have certain tests or have an operation to have your gallbladder taken out (a cholecystectomy), we will ask your permission again to collect information about these tests or surgery. You do not have to take part in the other parts of the research.

The research doctor is a different doctor to the doctors looking after you for your gallstones. The information the research doctor has about your pain will not be available to the doctors looking after you. They will not assess you for pain, or give you pain killers, the research doctor will ask you to tell the team looking after you so they can assess you. You will need to explain to the doctors looking after you about your pain. The reason for this is we are trying to make your as normal as possible.

With your permission the research doctor will let your GP (family doctor) know you are taking part in the research in case we need to ask them information from your GP notes. This information will be about how long you have had problems with your gallstones, what problems and treatment you have had. Please tell us if you don't want us to contact your family doctor.

The Study Plan

Discuss with the reserach doctor the trial, and read this information leaflet. If you want to take part you will be asked to sign the consent form to agree to take part in the study.

Research doctor will give you a form with a line on it and ask you to mark on the line the amount of pain you have at that time. They will leave you alone to do this.

The research doctor will then ask your permission to take the blood sample.

The research doctor will then give you another pain score sheet and ask you to complete it as before.

The research doctor will also leave you the forms on how the gallstones affect your daily life (quality of life). Don't think too long about the answer, there is no right or wrong answer we are interested in your experience.

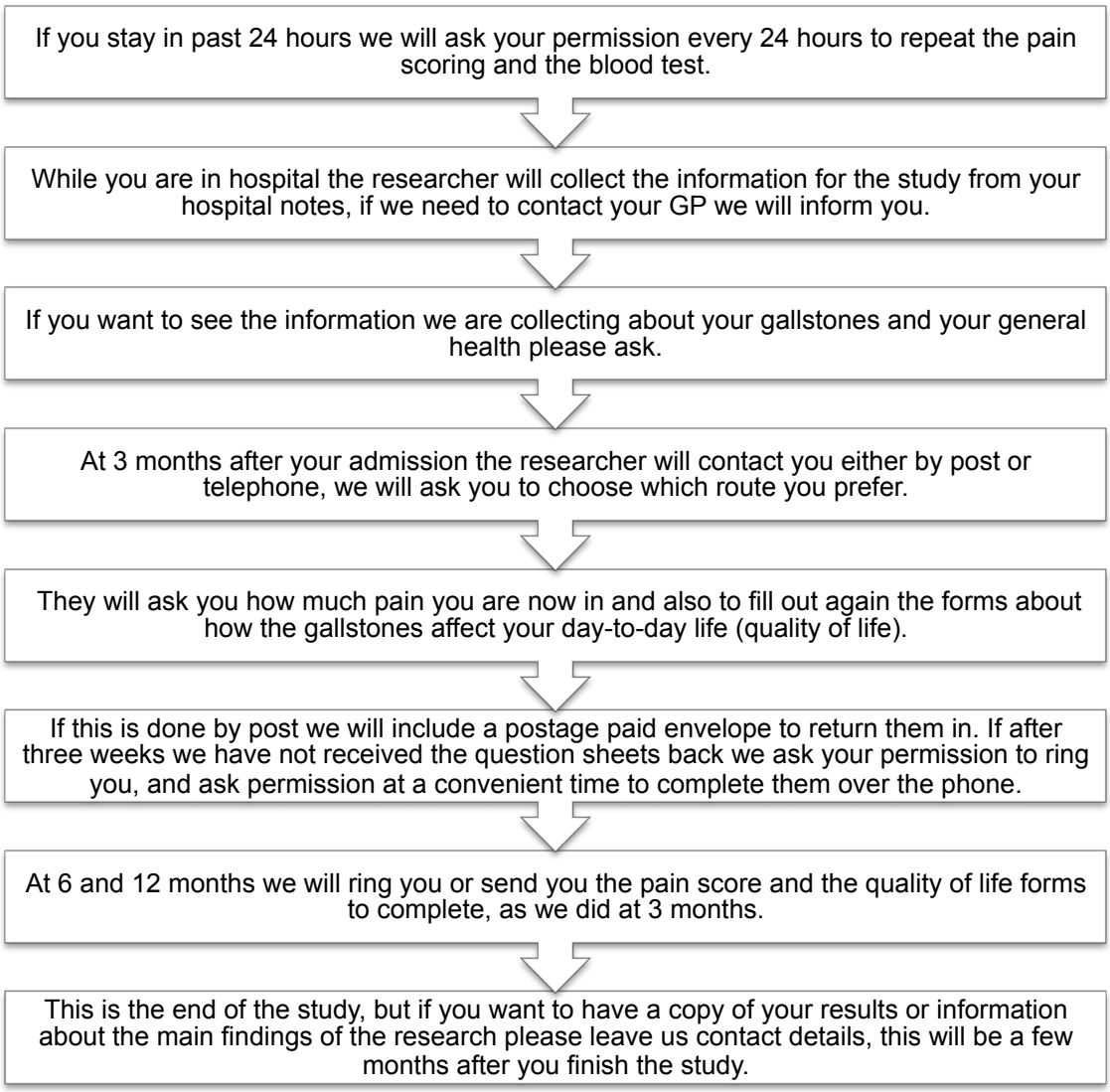
At 2 hours after joining the study the researcher will come back and repeat the pain score and take your blood test, and repeat the pain scoring again.

At 4 and 6 hours the researcher will ask you to fill out how much pain you have at that moment. They will ask you to repeat it 15 minutes later. There will be no blood test at 4 and 6 hours.

At 24 hours the researcher will come back and ask you to mark again how much pain you have. They will also ask the most and the least pain you have had over the last 24 hours, this is marked on a separate score chart.

Blood samples will be taken with your permission. After the blood test the researcher will ask you again how much pain you have at the moment, and least and most pain you have had over the last 24 hours.

The researcher will also collect the forms about the impact of pain on your life (the quality of life forms), if you have not already returned them.



How do I contact the research team?

Please keep this information sheet as it tells you about the study. If there are questions, change in details or you wish to withdraw then please contact as below and ask for Doctor Rachel Soulsby.

Telephone number **0115 969 1169** (this maybe an answer phone, please leave your contact details and a preferred time to return the call).

Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Patient information sheet for those attending for planned ERCP

Introduction

You have been asked to take part in the study because the doctors looking after you think that gallstones are the cause of the pain you have in your abdomen (tummy), and possibly the problem with your blood test that is causing your skin to be a yellowish colour (jaundice).

The study is asking people who have gallstones causing problems to take part to see how the body responds to the gallstones. We are looking at markers of infection and seeing how they change when you have pain, problems from your gallstones or investigations for your gallstones. We are also looking at your level of pain to see if it can show us earlier when you have gallstone problems or any problems after tests for your gallstones.

What will happen if I take part?

The research doctor will explain the study to you and then ask you to read this leaflet. What will happen to you is shown in a diagram on page 3 of this leaflet. If you decide you want to take part, you will be asked to sign a form (consent form) to say you agree to take part.

The infection markers are found in your blood so we will be asking to take some blood samples as in the diagram. At each time about 5 – 10mls of blood (one - two teaspoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. Blood tests will be at the start, two and 24 hours after the ERCP test. We will always try and combine the blood tests with blood tests required for the ERCP test so you don't need to have too many blood tests. If you are worried about having blood tests you can just complete the pain scores and questionnaire, please tell the research doctor.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. If you are still in hospital the following day we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours being scored. Pain will be scored at the start, 2, 4, 6 and 24 hours after the ERCP test.

We also want to know how the pain affects your normal daily life and so we will also be asking you to fill in a questionnaire about this. We are interested to see how this changes so will ask your permission to post or complete by telephone these questionnaires in 3, 6 and 12 months after your ERCP test.

What will happen to my information and my blood?

When you sign the form to take part your information will be given a code to identify it. All your information will be marked identified by this code, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part it will not change how the doctors look after you care for you. Just tell the research doctor you don't want to take part; you don't have to give a reason.

What happens if I change my mind?

If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered out of the trial. Alternatively you can allow us to use the information we already have, but not gather any more information. This will not affect your care. We will destroy all your blood samples in storage.

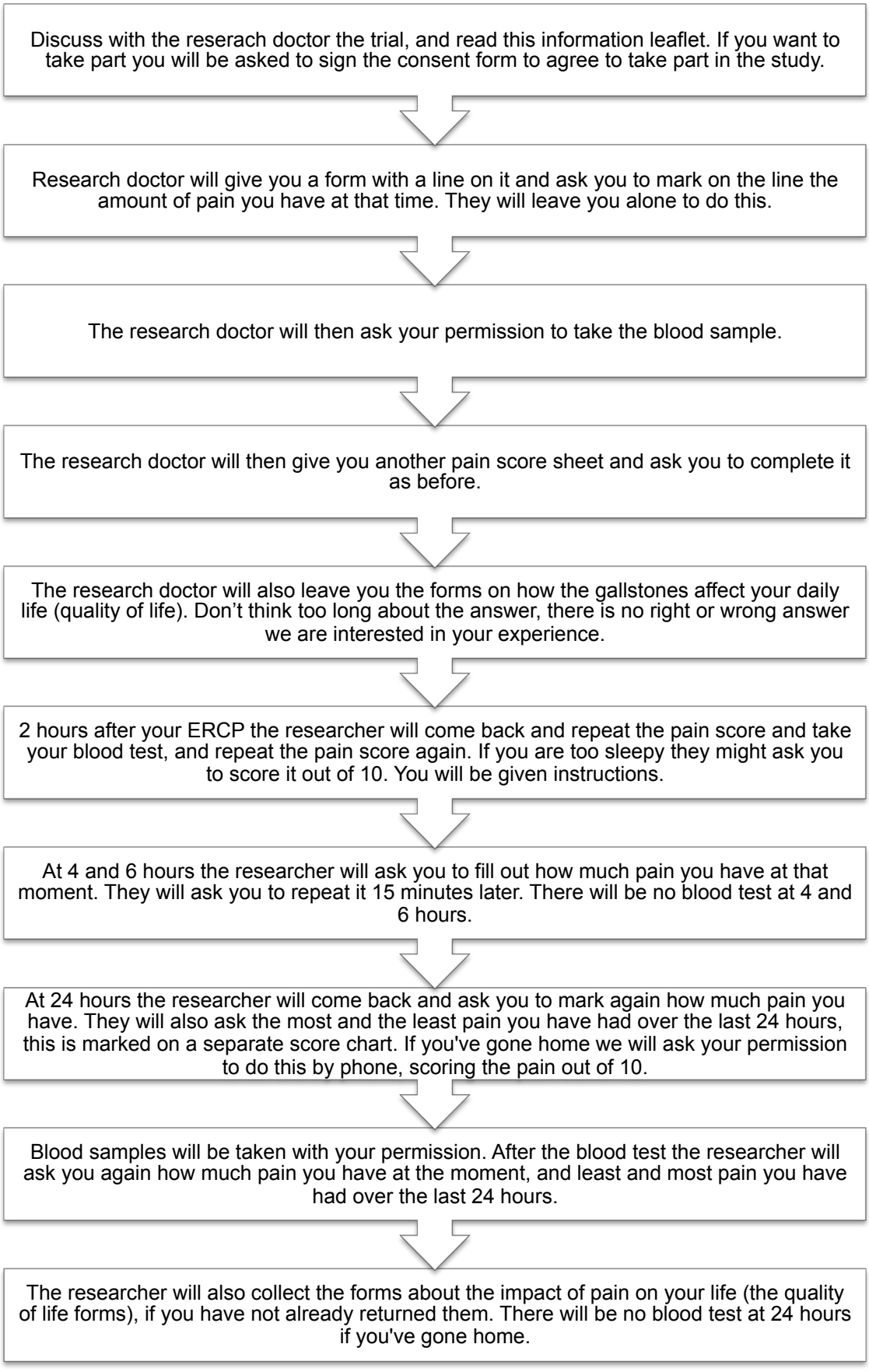
Do I need to know anything else?

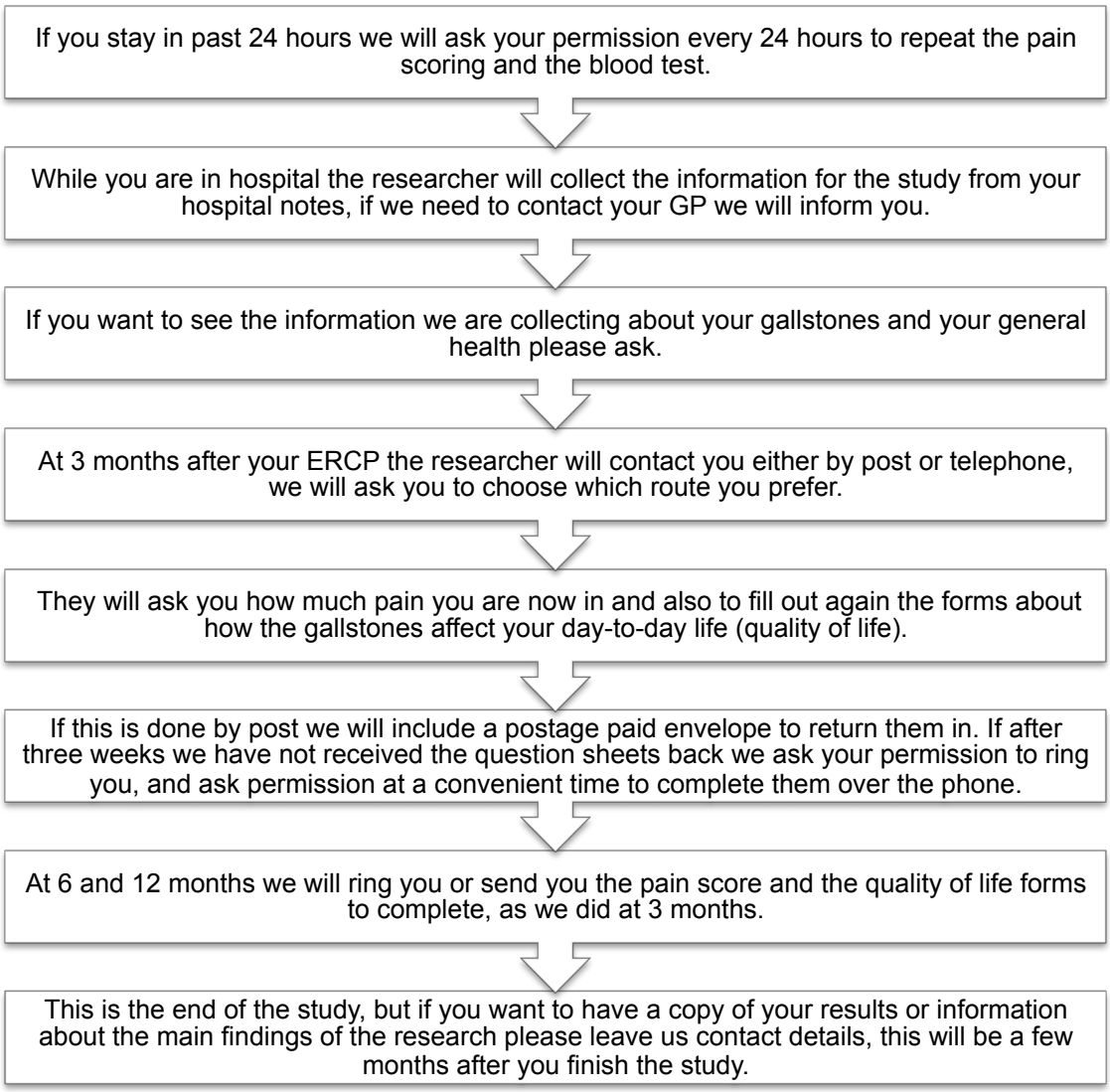
If you are admitted to hospital with problems from your gallstones or have an operation to have your gallbladder taken out (a cholecystectomy), we will ask your permission again to collect information about these tests or surgery. You do not have to take part in the other parts of the research.

The research doctor is a different doctor to the doctors looking after you for your gallstones. The information the research doctor has about your pain will not be available to the doctors looking after you. They will not assess you for pain, or give you pain killers, the research doctor will ask you to tell the team looking after you so they can assess you. You will need to explain to the doctors looking after you about your pain. The reason for this is we are trying to make your as normal as possible.

With your permission the research doctor will let your GP (family doctor) know you are taking part in the research in case we need to ask them information from your GP notes. This information will be about how long you have had problems with your gallstones, what problems and treatment you have had. Please tell us if you don't want us to contact your family doctor.

The Study Plan





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Telephone number **0115 969 1169** (this maybe an answer phone, please leave your contact details and a preferred time to return the call).

Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Patient information sheet for those admitted for elective cholecystectomy (surgery to remove the gallbladder)

Introduction

You have been asked to take part in the study because the doctors looking after you are planning an operation to remove your gallbladder. This is because they think the gallstones are the cause of the pain you have in your abdomen (tummy).

The study is asking people who have gallstones causing problems to take part to see how the body responds to the gallstones. We are looking at markers of infection and seeing what happens to them when you have pain or problems from your gallstones, or surgery for your gallstones. We are also looking at your level of pain to see if it can show us earlier when you have gallstone problems, or any problems after surgery for your gallstones.

What will happen if I take part?

The research doctor will explain the study to you and then ask you to read this leaflet. What will happen to you is shown in a diagram on page 3 of this leaflet. If you decide you want to take part, you will be asked to sign a form (consent form) to say you agree to take part.

The infection markers are found in your blood so we will be asking to take some blood samples as in the diagram. At each time about 5 – 10mls of blood (one - two teaspoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. Blood tests will be at the start, two, and 24 hours after the surgery. We will always try and combine the blood tests with blood tests required for the surgery (cholecystectomy) so you don't need to have too many blood tests. If you are worried about having blood tests you can just do the pain score and the questionnaires, please tell the research doctor.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. If you are still in hospital the following day we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours being scored. Pain will be scored at the start, 2, 4, 6 and 24 hours after the surgery (cholecystectomy).

We also want to know how the pain affects your normal daily life and so we will also be asking you to fill in a questionnaire about this. We are interested to see how this changes so will ask your permission to post or complete by telephone these questionnaires in 3, 6 and 12 months after being in hospital.

We also ask your permission to look at your hospital notes and occasionally contact your GP (family doctor) to see how many times you have needed to see a doctor about your gallstones and your general health.

What will happen to my information and my blood?

When you sign the form to take part your information will be given a code to identify it. All your information will be marked identified by this code, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part it will not change how the doctors look after you care for you. Just tell the research doctor you don't want to take part; you don't have to give a reason.

What happens if I change my mind?

If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered out of the trial. Alternatively you can allow us to use the information we already have, but not gather any more information. This will not affect your care. We will destroy all your blood samples in storage.

Do I need to know anything else?

If you have certain tests following surgery for gallstones, we will ask your permission again to collect information about these tests or surgery. You do not have to take part in the other parts of the research.

The research doctor is a different doctor to the doctors looking after you for your gallstones. The information the research doctor has about your pain will not be available to the doctors looking after you. The research doctor also will not assess you for pain, or give you pain killers, the research doctor will ask you to tell the team looking after you so they can assess you. You will need to explain to the doctors looking after you about your pain. The reason for this is we are trying to make your as normal as possible.

With your permission the research doctor will let your GP (family doctor) know you are taking part in the research in case we need to ask them information from your GP notes. This information will be about how long you have had problems with your gallstones, what problems and treatment you have had. Please tell us if you don't want us to contact your family doctor.

The Study Plan

Discuss with the reserach doctor the trial, and read this information leaflet. If you want to take part you will be asked to sign the consent form to agree to take part in the study.

Research doctor will give you a form with a line on it and ask you to mark on the line the amount of pain you have at that time. They will leave you alone to do this.

The research doctor will then ask your permission to take the blood sample.

The research doctor will then give you another pain score sheet and ask you to complete it as before.

The research doctor will also leave you the forms on how the gallstones affect your daily life (quality of life). Don't think too long about the answer, there is no right or wrong answer we are interested in your experience.

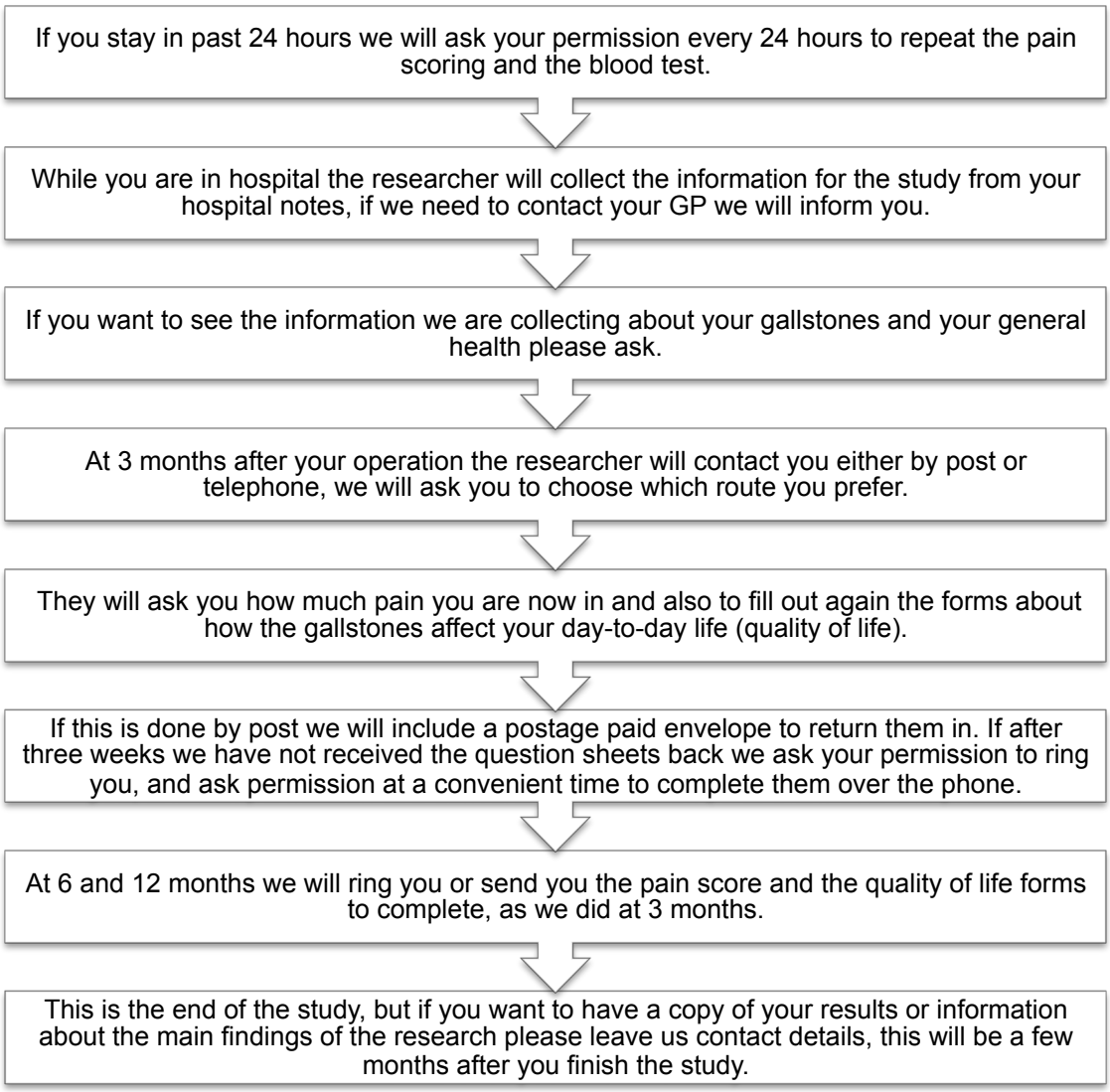
2 hours after your operation the researcher will come back and repeat the pain score and take your blood test, and repeat the pain score again. If you are too sleepy they might ask you to score it out of 10. You will be given instructions.

At 4 and 6 hours the researcher will ask you to fill out how much pain you have at that moment. They will ask you to repeat it 15 minutes later. There will be no blood test at 4 and 6 hours.

At 24 hours the researcher will come back and ask you to mark again how much pain you have. They will also ask the most and the least pain you have had over the last 24 hours, this is marked on a separate score chart. If you've gone home we will ask your permission to do this by phone, scoring the pain out of 10.

Blood samples will be taken with your permission. After the blood test the researcher will ask you again how much pain you have at the moment, and least and most pain you have had over the last 24 hours.

The researcher will also collect the forms about the impact of pain on your life (the quality of life forms), if you have not already returned them. There will be no blood test at 24 hours if you've gone home.



How do I contact the research team?

Please keep this information sheet as it tells you about the study. If there are questions, change in details or you wish to withdraw then please contact as below and ask for Doctor Rachel Soulsby.

Telephone number **0115 969 1169** (this maybe an answer phone, please leave your contact details and a preferred time to return the call).

Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Patient information sheet for admissions for elective cholecystectomy (gallbladder removal surgery) whom have had previous ERCP

Introduction

You have been asked to take part in the study because the doctors looking after you are planning an operation to remove your gallbladder. They think that gallstones are the cause of the pain you have in your abdomen (tummy).

The study is asking people who have gallstones causing problems to take part to see how the body responds to the gallstones. We are looking at markers of infection and seeing what happens to them when you have pain or problems from your gallstones, or surgery for your gallstones. We are also looking at your level of pain to see if it can show us earlier when you have gallstone problems, or any problems after surgery for your gallstones. We are interested to see if your previous ERCP (telescope test to investigate your stones) affects how your body responds to surgery, either with blood markers or pain.

What will happen if I take part?

The research doctor will explain the study to you and then ask you to read this leaflet. What will happen to you is shown in a diagram on page 3 of this leaflet. If you decide you want to take part, you will be asked to sign a form (consent form) to say you agree to take part.

The infection markers are found in your blood so we will be asking to take some blood samples as in the diagram. At each time about 5 – 10mls of blood (one - two teaspoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. We will try and combine blood tests with those required for surgery (cholecystectomy) so you don't need to have too many blood tests. If you are worried about having blood tests you can just do the pain score and the questionnaires, please tell the research doctor.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. If you are still in hospital the following day we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours being scored. Pain will be scored at the start, 2, 4, 6, and 24 hours after the surgery (cholecystectomy).

We also want to know how the pain affects your normal daily life and so we will also be asking you to fill in a questionnaire about this. We are interested to see how this changes so will ask permission to post or complete by telephone these questionnaires in 3, 6 and 12 months after surgery. We ask

permission to look at your hospital notes and occasionally contact your GP (family doctor) to see how the gallstones affect you and about your general health. This includes information about your ERCP.

What will happen to my information and my blood?

When you sign the form to take part your information will be given a code to identify it. All your information will be marked identified by this code, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part it will not change how the doctors look after you care for you. Just tell the research doctor you don't want to take part; you don't have to give a reason.

What happens if I change my mind?

If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered. Alternatively you can allow us to use the information we already have, but not gather any further information. This will not affect your care. We will destroy all your blood samples in storage.

Do I need to know anything else?

If you have certain tests following surgery for gallstones, we will ask your permission again to collect information about these tests or surgery. You do not have to take part in the other parts of the research.

The research doctor is a different doctor to the doctors looking after you for your gallstones. The information the research doctor has about your pain will not be available to the doctors looking after you. The research doctor also will not assess you for pain, or give you pain killers, the research doctor will ask you to tell the team looking after you so they can assess you. You will need to explain to the doctors looking after you about your pain. The reason for this is we are trying to make your as normal as possible.

With your permission the research doctor will let your GP (family doctor) know you are taking part in the research in case we need to ask them information from your GP notes. This information will be about how long you have had problems with your gallstones, what problems and treatment you have had. Please tell us if you don't want us to contact your family doctor.

The Study Plan

Discuss with the reserach doctor the trial, and read this information leaflet. If you want to take part you will be asked to sign the consent form to agree to take part in the study.

Research doctor will give you a form with a line on it and ask you to mark on the line the amount of pain you have at that time. They will leave you alone to do this.

The research doctor will then ask your permission to take the blood sample.

The research doctor will then give you another pain score sheet and ask you to complete it as before.

The research doctor will also leave you the forms on how the gallstones affect your daily life (quality of life). Don't think too long about the answer, there is no right or wrong answer we are interested in your experience.

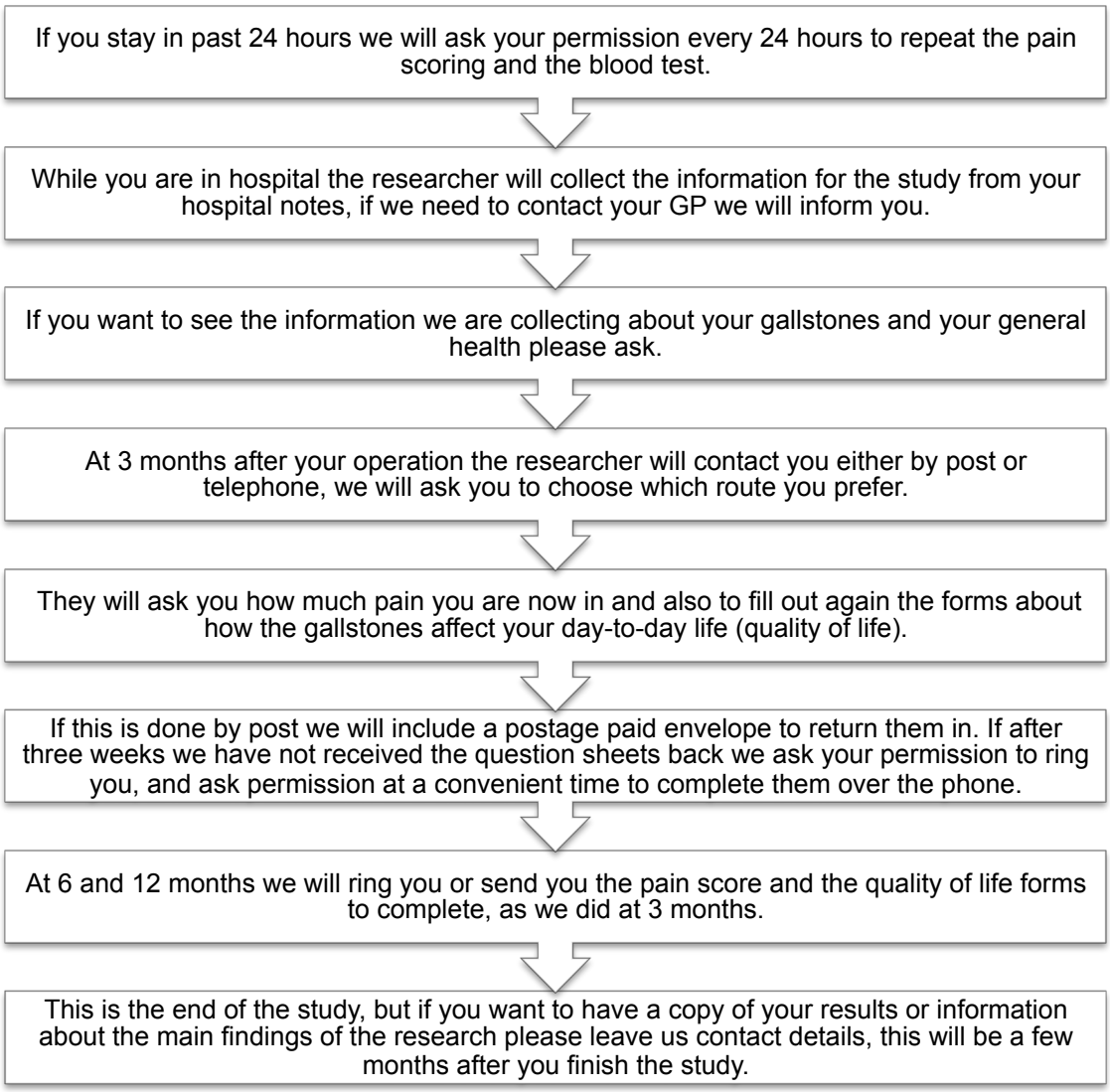
2 hours after your operation the researcher will come back and repeat the pain score and take your blood test, and repeat the pain score again. If you are too sleepy they might ask you to score it out of 10. You will be given instructions.

At 4 and 6 hours the researcher will ask you to fill out how much pain you have at that moment. They will ask you to repeat it 15 minutes later. There will be no blood test at 4 and 6 hours.

At 24 hours the researcher will come back and ask you to mark again how much pain you have. They will also ask the most and the least pain you have had over the last 24 hours, this is marked on a separate score chart. If you've gone home we will ask your permission to do this by phone, scoring the pain out of 10.

Blood samples will be taken with your permission. After the blood test the researcher will ask you again how much pain you have at the moment, and least and most pain you have had over the last 24 hours.

The researcher will also collect the forms about the impact of pain on your life (the quality of life forms), if you have not already returned them. There will be no blood test at 24 hours if you've gone home.



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Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Patient information sheet for admissions for elective cholecystectomy (gallbladder removal surgery) with on table cholangiogram (OTC) (investigation of the bile duct)

Introduction

You have been asked to take part in the study because the doctors looking after you are planning an operation to remove your gall bladder at the same time they will check no stones are blocking the pathway from the gallbladder to the bowl. They think that gallstones are the cause of the pain you have in your abdomen (tummy). Possibly a stone in the pathway to the bowel has caused your liver blood tests to be altered.

The study is asking people who have gallstones causing problems to take part to see how the body responds to the gallstones. We are looking at markers of infection and seeing what happens to them when you have pain or problems from your gallstones, or surgery for your gall stones. We are looking at your level of pain to see if it can show us earlier when you have gallstone problems, or any problems with surgery for your gallstones. We are interested to see if the bile duct exploration affects how your body responds to surgery, either with blood markers or pain.

What will happen if I take part?

The research doctor will explain the study to you and then ask you to read this leaflet. What will happen to you is shown in a diagram on page 3 of this leaflet. If you decide you want to take part, you will be asked to sign a form (consent form) to say you agree to take part.

The infection markers are found in your blood so we will be asking to take some blood samples as in the diagram. At each time about 5 – 10mls of blood (one to two teaspoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. We will try and combine blood tests with those required for surgery (cholecystectomy so you don't need to have too many blood tests. If you are worried about having blood tests you can just complete the pain scores and questionnaire, please tell the research doctor.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. If you are still in hospital the following day we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours. Pain will be scored at the start, 2, 4, 6, and 24 hours after the surgery (cholecystectomy).

We also want to know how the pain affects your normal daily life and so we will also be asking you to fill in a questionnaire about this. We are interested

to see how this changes so will ask your permission to post or complete by telephone these questionnaires in 3, 6 and 12 months after being in hospital. We ask your permission to look at your hospital notes and occasionally contact your GP (family doctor) to see how gallstones affect you and about your general health.

What will happen to my information and my blood?

When you sign the form to take part your information will be given a code to identify it. All your information will be marked identified by this code, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part it will not change how the doctors look after you care for you. Just tell the research doctor you don't want to take part; you don't have to give a reason.

What happens if I change my mind?

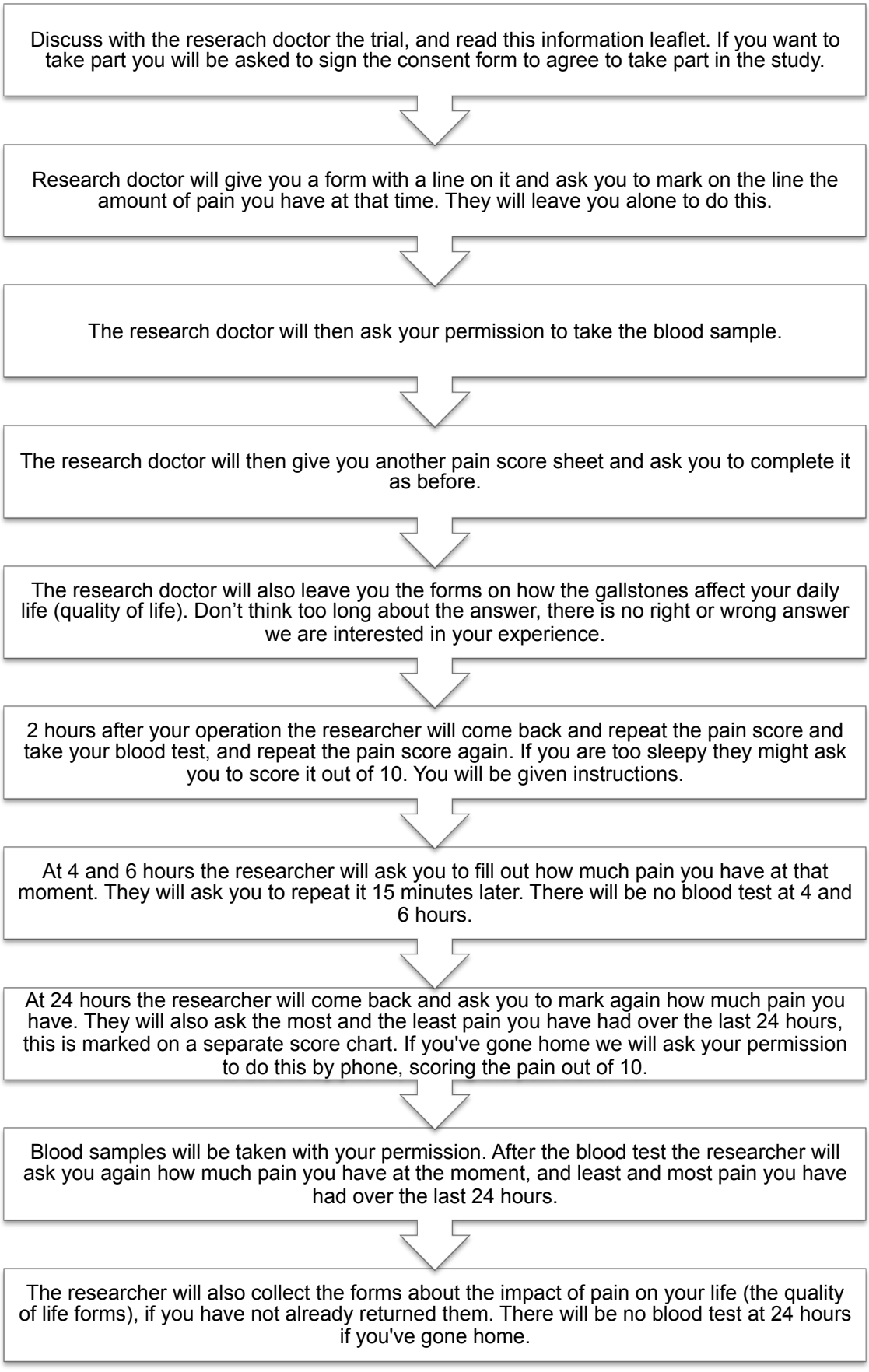
If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered out of the trial. Alternatively you can allow us to use the information we already have, but not gather any more information. This will not affect your care. We will destroy all your blood stored samples.

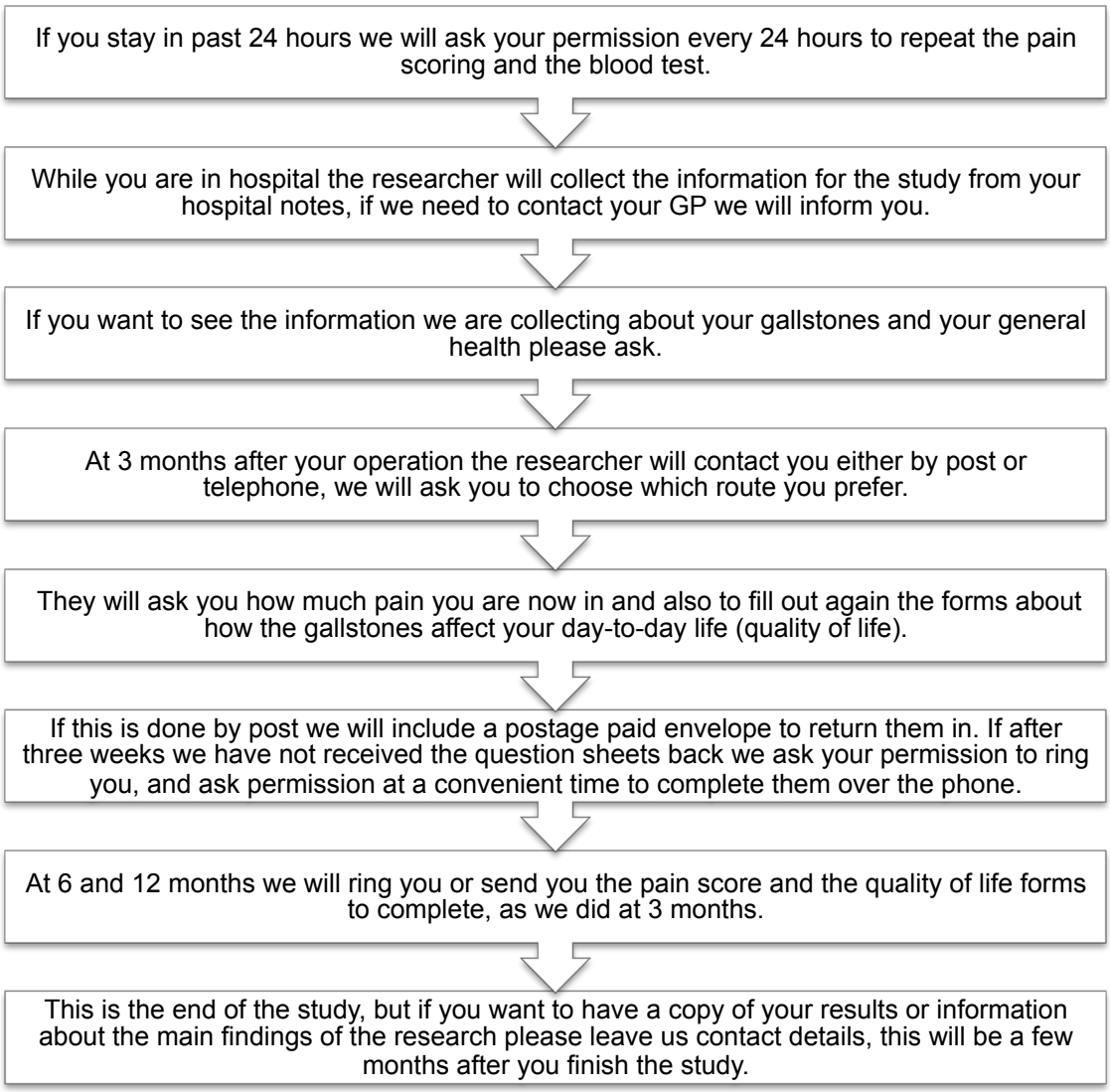
Do I need to know anything else?

The research doctor is a different doctor to the doctors looking after you for your gallstones. The information the research doctor has about your pain will not be available to the doctors looking after you. The research doctor also will not assess you for pain, or give you pain killers, the research doctor will ask you to tell the team looking after you so they can assess you. You will need to explain to the doctors looking after you about your pain. The reason for this is we are trying to make your as normal as possible.

With your permission the research doctor will let your GP (family doctor) know you are taking part in case we need to ask them information from your GP notes. This information will be about how long you have had problems with your gallstones, what problems and treatment you have had. Please tell us if you don't want us to contact your family doctor.

The Study Plan





How do I contact the research team?

Please keep this information sheet as it tells you about the study. If there are questions, change in details or you wish to withdraw then please contact as below and ask for Doctor Rachel Soulsby.

Telephone number **0115 969 1169** (this maybe an answer phone, please leave your contact details and a preferred time to return the call).

Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Patients information sheet for those having urgent ERCP / urgent cholecystectomy / post cholecystectomy surgery

Introduction

You kindly took part in the research study looking at how the body responds to gallstones disease. Particular seeing the markers of infection change with gallstones, and examining if pain is a marker of problems, particularly infections in patients with gallstones.

The team looking after you are wishing to investigate your gallstone problems further with a special telescope test / with surgery / with another operation to see if they can confirm what the problem is and treat the problem. The research study team are asking if we can continue following you with blood tests and pain scoring to see how these change with the next investigations and treatment.

What will happen if I take part?

Like last time the research doctor will explain the study to you and then ask you to read this information leaflet with the study plan on page 3. If you decide to take part you will be asked to sign another form (consent form) to say you agree to continuing in the trial now there has been a change in what is happening to you.

The research doctor will ask your permission to take a blood sample when you sign the form. This will be repeated two hours after the telescope test / surgery, and 24 hours afterwards. If you stay in over 24 hours we would like permission to take a blood test every 24 hours until you are discharged or up to one week. If you are still in at one week we will with your permission take the blood test every 48 hours.

At each time about 5 – 10mls of blood (one - two teaspoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. Blood tests will be at the start, two and 24 hours. We will try and combine the blood tests with other blood tests you have to investigate the gallstones so you don't need to have too many blood tests. If you are worried about the blood tests you can just complete the pain scores and questionnaire, please tell the researcher.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. If you are still in hospital the following day we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours being scored. Pain will be scored at the when you sign this form, 2, 4, 6 and 24 hours after the telescope test / surgery.

As before, we ask your permission to collect information from your notes about your telescope test / your surgery.

What will happen to my information and my blood?

When you signed the first consent form to take part you were given a unique identification code. We will continue to use this code to keep your information under, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part it will not change how the doctors look after you care for you. Just tell the research doctor you don't want to take part; you don't have to give a reason. We will ask if you want us to destroy all data and samples you have already given, or whether we can use these but not collect any further data on you.

What happens if I change my mind?

If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered out of the trial. Alternatively you can allow us to use the information we already have, but not gather any more information. This will not affect your care. We will destroy all your blood samples in storage.

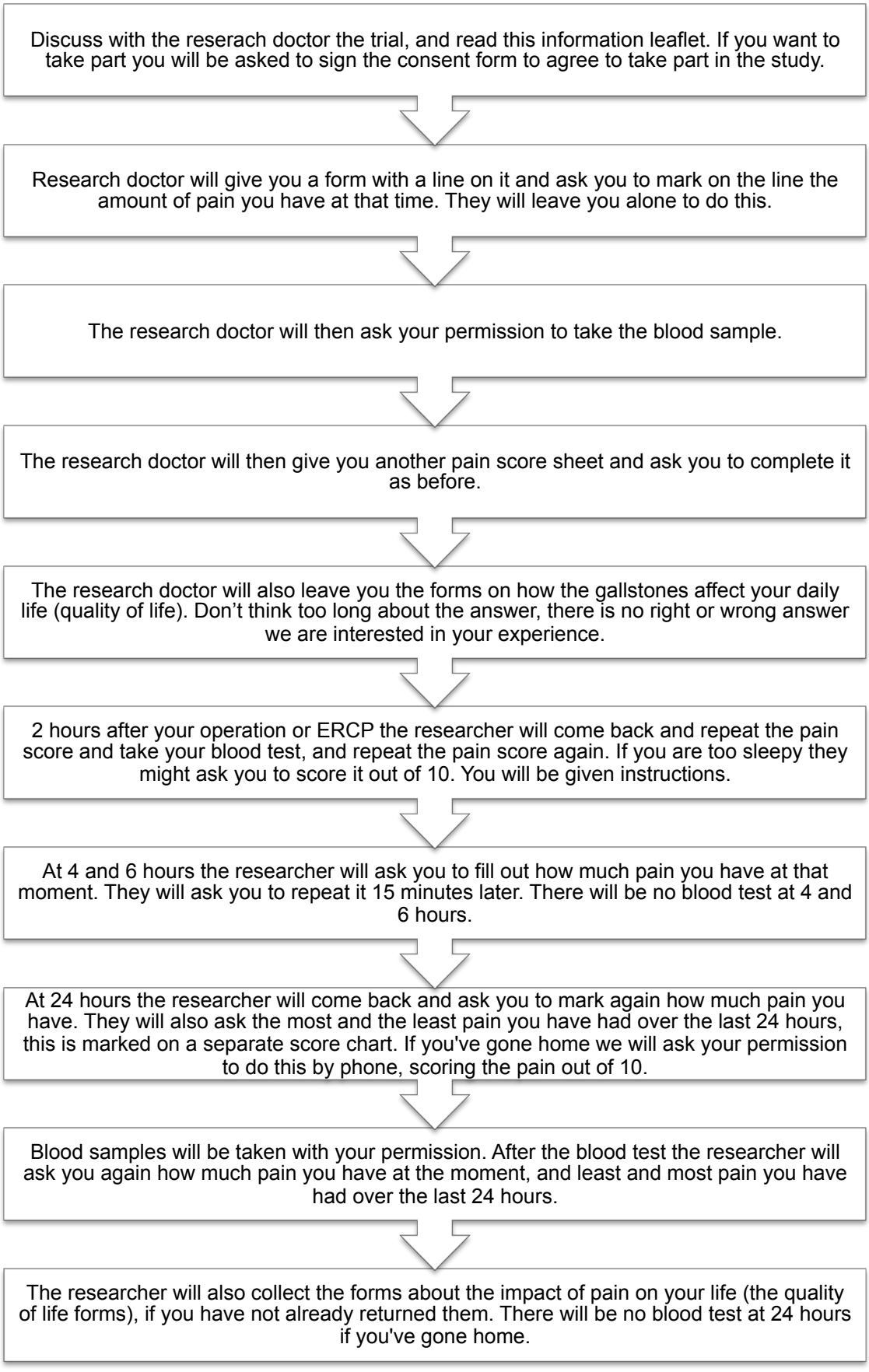
Do I need to know anything else?

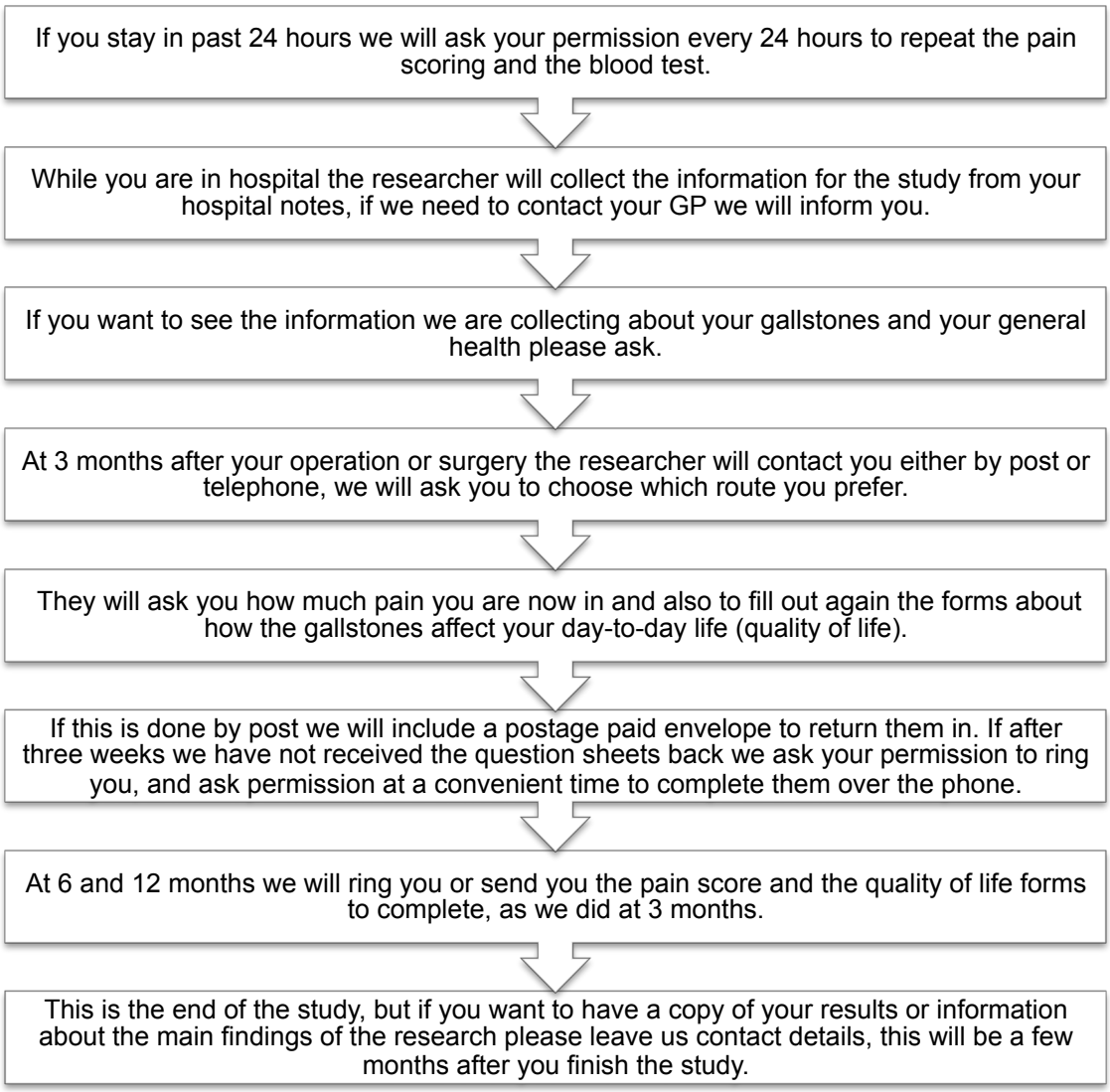
We are grateful that you have taken part in the research trial, you don't have to take part in this next part, but we appreciate you considering doing so. We may ask your permission to take part again if they go on to do surgery / further surgery.

The research doctor is a different doctor to the doctors looking after you for your gallstones. The information the research doctor has about your pain will not be available to the doctors looking after you. They will not assess you for pain, or give you pain killers, the research doctor will ask you to tell the team looking after you so they can assess you. You will need to explain to the doctors looking after you about your pain. The reason for this is we are trying to make your as normal as possible.

We ask your permission for the research doctor to contact your GP (family doctor) for information on support and problems you had after discharge from hospital. Please tell us if you don't want us to contact your family doctor.

The Study Plan





How do I contact the research team?

Please keep this information sheet as it tells you about the study. If there are questions, change in details or you wish to withdraw then please contact as below and ask for Doctor Rachel Soulsby.

Telephone number **0115 969 1169** (this maybe an answer phone, please leave your contact details and a preferred time to return the call).

Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Healthy controls information sheet

Introduction

We are carrying out a study looking at what happens to the markers of inflammation when people have gallstones. We are also looking at what happens when they have certain tests to investigate the gallstones or when they have surgery for the gallstones. We also want to know how much pain they have, and does the pain change with having tests, over time or if there are problems.

We can see how a person results change, and we can compare them to other people with similar problems or having similar tests or surgery. But we would also like to compare them to people who do not have gallstones, who are fit and well and do not have investigations or surgery and this is why you are being asked to take part.

What if I do have gallstones or other medical problem?

You can just say no to taking part, you don't have to give a reason. The researcher is a doctor and if you are happy to share your medical problem you can ask the research doctor and she will keep anything you tell her confidential. A lot of people who are well do have gallstones and if they are not causing you a problem you can take part, we will just note it down.

What will happen if I take part?

The research doctor will explain the study to you and then ask you to read this leaflet. What will happen to you is shown in a diagram on page 3 of this leaflet. If you decide you want to take part, you will be asked to sign a form (consent form) to say you agree to take part.

The infection markers are found in your blood so we will be asking to take some blood samples as in the diagram. At each time about 50mls of blood (just under three tablespoons) is taken. It maybe a little uncomfortable having the blood test taken and you can sometimes get a bruise afterwards. If you are worried about having blood tests you can just do the pain score and the questionnaires, please tell the research doctor. We will also perform the blood tests the patients have at enrollment, two and twenty-four hours, this includes full blood count, urea and electrolytes, liver function tests and C-reactive protein.

At the same time as having the blood test the research doctor will ask you to put a mark on a line to show how much pain you have at that moment. This will be repeated after the blood test, in case being worried about your blood test changes how much pain you have. At 24 hours we will ask you to tell us the most and the least pain you had experienced over the 24 hours. The researcher will tell you if it is the pain now or over the 24 hours being scored. Pain will be scored at the start, 2, 4, 6, and 24 hours after you sign to take part in the study.

We also want to know how pain affects normal daily life of the people with gallstones. We would like to compare it to people without gallstones, and so we will also be asking you to fill in a questionnaire about this. We are interested to see how this changes so will ask permission to post or complete by telephone these questionnaires in 3, 6 and 12 months after surgery.

We ask permission to look at your hospital notes and occasionally contact your GP (family doctor) to look at your medical problems. If you do not wish to take part in this part of the study you can opt out of this part and we won't collect this information.

What will happen to my information and my blood?

When you sign the form to take part your information will be given a code to identify it. All your information will be marked identified by this code, and not your name to make it anonymous (no one can identify it as you). Information will be kept on secure computers and will just be kept under the code number. One list of names with code numbers will be kept securely on a separate computer, this will allow us to contact you to send the questionnaires out.

What happens if I don't take part?

If you decide you do not want to take part just tell the research doctor you don't want to take part; you don't have to give a reason.

What happens if I change my mind?

If you take part, but then decide you no longer want to carry on in the trial, just contact the research doctor and tell us. Again you don't have to give a reason for no longer taking part. We will ask you if you want us to remove all your information already entered. Alternatively you can allow us to use the information we already have, but not gather any further information. This will not affect your care. We will destroy all your blood samples in storage.

Do I need to know anything else?

We are grateful for yours and the patient's feedback about how you find the trial and any information to improve it is gratefully received.

If you are diagnosed with gallstones within three months of taking part, and are happy to inform the researcher, please let me know, contact details are on page 4.The researcher is a doctor, but she is not able to give you advice about medical problems, and she will ask you to see your GP (family doctor) to discuss medical problems if necessary. This is because it is your GP (family doctor) who will be providing ongoing care. If one of your blood tests is abnormal the researcher will give you your results in a letter it is your choice to go to your GP (family doctor).

The Study Plan

Discuss with the reserach doctor the trial, and read this information leaflet. If you want to take part you will be asked to sign the consent form to agree to take part in the study.

Research doctor will give you a form with a line on it and ask you to mark on the line the amount of pain you have at that time. They will leave you alone to do this.

The research doctor will then ask your permission to take the blood sample.

The research doctor will then give you another pain score sheet and ask you to complete it as before.

The research doctor will also leave you the forms on how the gallstones affect your daily life (quality of life). Don't think too long about the answer, there is no right or wrong answer we are interested in your experience.

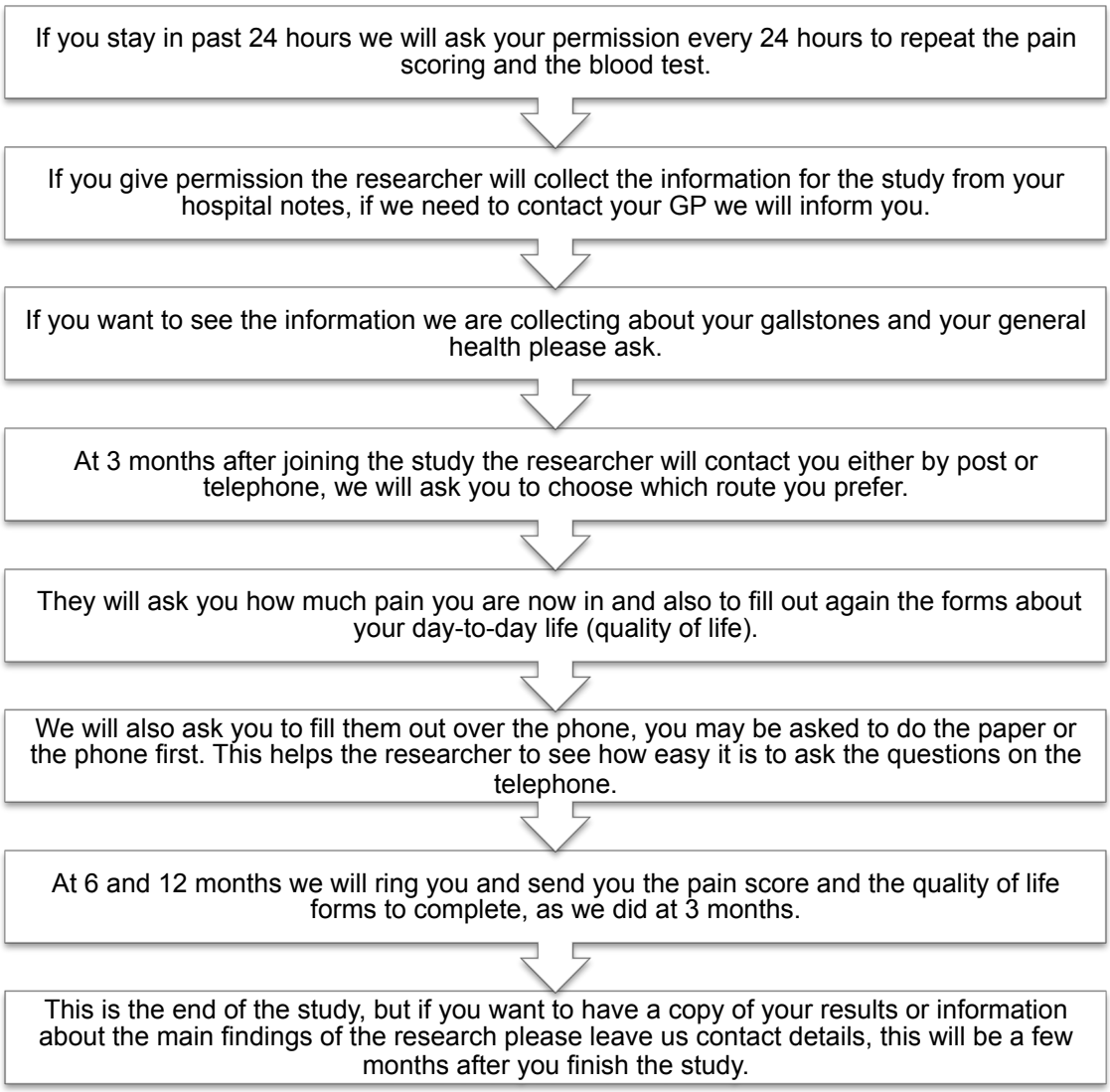
After 2 hours the researcher will come back and repeat the pain score and take your blood test, and repeat the pain score again.

At 4 and 6 hours the researcher will ask you to fill out how much pain you have at that moment. They will ask you to repeat it 15 minutes later. There will be no blood test at 4 and 6 hours.

At 24 hours the researcher will come back and ask you to mark again how much pain you have. They will also ask the most and the least pain you have had over the last 24 hours, this is marked on a separate score chart.

Blood samples will be taken with your permission. After the blood test the researcher will ask you again how much pain you have at the moment, and least and most pain you have had over the last 24 hours.

The researcher will also collect the forms about the impact of pain on your life (the quality of life forms), if you have not already returned them.



How do I contact the research team?

Please keep this information sheet as it tells you about the study. If there are questions, change in details or you wish to withdraw then please contact as below and ask for Doctor Rachel Soulsby.

Telephone number **0115 969 1169** (this maybe an answer phone, please leave your contact details and a preferred time to return the call).

Or email nuhgallstonestudy@nuh.nhs.uk

Or write
Nottingham City Hospital, Hucknall Road, Nottingham, Nottinghamshire NG5 1PB

Thank you for considering taking part the researcher will come back and ask if you have any questions and to sign the consent form.

Appendix

Appendix 4 - Consent Forms

Patient consent forms for

Consent form for those admitted with biliary emergencies

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to start the trial sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Consent form for those attending for planned ERCP

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to start the trial sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Consent form for those attending for elective cholecystectomy

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to start the trial sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Consent form for those attending for elective cholecystectomy who have had previous ERCP

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to start the trial sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team including my previous ERCP _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Consent form for those attending for elective cholecystectomy with on table cholangiogram

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to start the trial sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Consent form for those having urgent ERCP / urgent cholecystectomy / post cholecystectomy surgery

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to stay in the trial for this new investigation sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Consent form for the healthy controls

We ask you to initial every statement you agree with. If you do not wish to take part in one part of the trial **do not** initial that statement. If happy to start the trial sign and date the bottom of the form with the research doctor. Please feel free to withdraw consent at any point if you change your mind.

1) I have read and understand the information leaflet _____
2) I have been able to ask the questions I wish to _____
3) I am happy to have the blood tests taken as in the research information sheet

_____

4) I am happy to score my pain _____
5) I am happy to complete the quality of life questionnaires about how gallstones affects my day to day life _____
6) I understand there are forms to fill out at 3, 6 and 12 months, I am happy to complete these _____
7) I wish them to be posted / to complete by telephone (delete as appropriate).
8) My contact details are:-
9) If I am discharged before 24 hours I am happy to be rung at home for my pain score _____
10) My preferred number is:- as above OR
11) My preferred contact time is _____
12) I give permission for information from my hospital notes to be recorded by the research team _____
13) I give my permission for the research team to contact my GP (family doctor) for information described in the information leaflet _____
14) I wish to see the information collected about me _____
15) I would like to see a summary about the research findings _____

I have completed all the points for the part of the trial I wish to take part in

Name _____ Signature _____ Date ____/___/___

Researcher I have explained the research, given the information sheet, answered the questions asked, and given the contact details card.

Name _____ Signature _____ Date ____/___/___

Appendix

Appendix 5 – Pro forma For Data Collection

Proforma for data collection for data from patients in the following groups

Data for the group who had had an ERCP previously, for those who underwent on table cholangiogram, or who had an urgent ERCP were recorded upon the ERCP form. Those who had urgent cholecystectomy or post cholecystectomy surgery was recorded on the cholecystectomy form. The data for the healthy controls was recorded upon the biliary admission patients form. Separate forms were not designed to try and aim for standardisation of data collected.

Biliary admission patients

Patient unique Identifier_____

Time – Enrollment / 2 hours / 24 hours / Other

Date - ____/_____/____

Demographics

Patient identifier - _____

Age at first enrollment - _____ years Sex – M / F

Biliary emergency patients Date: / / .

Diagnosis

- Biliary colic
- Acute Cholecystitis
- Obstructive jaundice
- Pancreatitis

Length of symptoms prior to admission hours

Length of time from admission to enrollment

Previous episodes of right upper quadrant pain

Number of AE attendance with RUQ pain

Number of prior admissions with RUQ pain

	Biliary colic	**Cholecystitis**	**Obstructive jaunduce**	**Pancreatitis**
Date				
Date				

Previous ERCP

Highest level of care during admission Ward / HDU / ITU

US results

	This admission	Previously - Date
Stones present		
• **Single**		
• **Multiple**		
• **Sludge**		
GB wall thickness		
Bile duct dimensions		
Stones in biliary tree		
Grade of sonographer		

CT results

	This admission - Date	Previously - Date
Evidence pancreatitis		
Pancreatic necrosis		
Pseudocyst		
Other pathology		

ERCP performed Y / N Time from enrolment

Findings

Cholecystectomy performed Y / N Time from enrolment

Findings

PMH –

Patient weight kgs Patient Height cms

BMI

Respiratory disease Y / N Type:
Cardiac disease or hypertension Y / N Type:

Diabetes Type I / II Diet / Tablet / Insulin

Thyroid disease Hypo / Hyper

IBS / Non specific pain / Sphincter of Oddi dysfunction

Previous surgery

- Non abdominal
- Abdominal (mark approach, reason and all which apply)
    - Laparoscopic elective
    - Laparoscopic emergency
    - Open elective
    - Open emergency
    - Appendicectomy
    - Gynaecological surgery
    - Adhesions
    - Bowel
    - Other

More than five emergency admissions in the last five years

Reflux medication

Self medication / Ranitidine prescribed / Proton pump inhibitor prescribed

Anxiety / Depression

SSRI prescribed Y / N

Pre-existing pain problem Y / N

Long term analgesia Y / N

- Medication?
- Medication
- Immunosuppression
- Steroids
- Recent blood transfusion

Smoker Y / N / Quit in the last 6 months

Alcohol

Allergies

Social

Full time / Part time / No paid employment / Caring for family member

Able bodied partner or parent or child >14 years Y / N

Non-planned contact with health care professional

Additional analgesia prescribed Y / N

Mean return to employment / usual activities days

Analgesia –

	Prior to admission	Admission to enrollment
Paracetamol		
Ibuprofen		
Diclofenac		
Codeine		
Tramadol		
Morphine		
Other		

	2 hours	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Paracetamol					
Ibuprofen					
Diclofenac					
Codeine					
Tramadol					
Morphine					
Other					

Use second grid if in > 96 hours

Time from enrolment	
Paracetamol	
Ibuprofen	
Diclofenac	
Codeine	
Tramadol	
Morphine	
Other	

Antibiotics –

Type - Date commenced / /

Length of course - days

Microbiology results

Observations –

	Prior to admission	**Admission to enrolment**	**2 hours**	**24 hours**
Pulse				
BP				
Temperature				
Respiratory rate				
WBC				

	48 hours	**72 hours**	**96 hours**
Pulse			
BP			
Temperature			
Respiratory rate			
WBC			

Bloods –

	At enrolment	2 hours if not previously available	24 hours	48 hours	72 hours	96 hours
Hb						
Haematocrit						
WBC						
- neutrophils						
Plt						
Coag if available						
Na						
K						
Ur						
Cr						
eGFR						
Bilirubin						
ALP						
AST						
GGT						
Albumin						
CRP						
Glucose						
LDH						
Ca						
Base deficit						
Partial pressure O2						
Fluid sequestration						

Ranson's criteria for pancreatitis patients -

Cytokine results –

	Enrolment	2 hours	24 hours
TNF-α			
IL-1			
IL-6			
IL-10			

	48 hours	72 hours	96 hours
TNF-α			
IL-1			
IL-6			
IL-10			

Pain scores

	Enrolment Pre / Post analgesia	2 hours	4 hours	6 hours	24 hours
VAS					

	48 hours	72 hours	96 hours
VAS			

	Expected VAS pain score	Actual VAS pain score	Time of this
Least pain			
Most pain			

	VAS score	HAD score	SF-36 score	GIQLI score
Pre-operative				
12 weeks				
26 weeks				
52 weeks				

Compared to 3 months ago question

Pre-operative	
12 weeks	
26 weeks	
52 weeks	

Semi-structured pain questions

Pain questions

- Experienced problems with pain? Y / N
- Has pain been discussed with you? Y / N
- Was analgesia discussed with you? Y / N
    - Including declining prescribed medication Y / N
- Was the information?
    - Helpful Y / N
    - Sufficient Y / N
    - Understandable Y / N

If you experienced pain

- Was your level of pain assessed? Y / N
- Did you receive analgesia within 15 minutes of requesting it? Y / N
- If you were not prescribed analgesia was your pain assessed? Y / N
- Was alternative methods of managing pain discussed with you? Y / N

At 12 weeks

- How long did you take analgesia for after discharge? days
- Did you see a doctor after discharge? Y / N
    - For infection
    - For problems with pain
    - For other pain pronlems
    - My GP invited me for review
- Post-operative patient - Would you consider having laparoscopic / open (as appropriate) surgery again?Y / N

ERCP patients

Patient unique Identifier_____

Time – Enrollment / 2 hours / 24 hours / Other

Date - ____/_____/____

For those biliary emergencies going on to have ERCP this section was completed, enrolment being taken as time of ERCP and confirmation the patient wished to continue in study. If did not want bloods and pain scoring completed permission to record information was going to be sought but no patient opted to not have bloods and pain score.

Demographics

Patient identifier - _____

Age at first enrollment - _____ years Sex – M / F

Participated in emergency section - Y / N

Date of ERCP

Reason for ERCP

Diagnosis

- Obstructive Jaundice
- Pancreatitis
- Bile duct dilatation on MRCP

Previous MRCP Y / N

Position of stone

Past biliary history

	Biliary colic	Cholecystitis	Obstructive jaunduce	Pancreatitis
Date				
Date				

Length of symptoms prior to admission days

Length of time from admission to enrollment

Previous episodes of right upper quadrant pain

Number of AE attendance with RUQ pain

Number of prior admissions with RUQ pain

US results

	This admission	Previously - Date
Stones present		
GB wall thickness		
Bile duct dimensions		
Stones in biliary tree		
Grade of sonographer		

CT results

	This admission - Date	**Previously - Date**
Evidence pancreatitis		
Pancreatic necrosis		
Pseudocyst		
Other pathology		

PMH –

Patient weight kgs Patient Height cms BMI

Respiratory disease Y / N Type:

Cardiac disease or hypertension Y / N Type:

Diabetes Type I / II Diet / Tablet / Insulin

Thyroid disease Hypo / Hyper

IBS / Non specific pain / Sphincter of Oddi dysfunction

Previous surgery

- Non abdominal
- Abdominal (mark approach, reason and all which apply)
  - Laparoscopic elective
  - Laparoscopic emergency
  - Open elective
  - Open emergency
  - Appendicectomy
  - Gynaecological surgery
  - Adhesions
  - Bowel
  - Other

More than five emergency admissions in the last five years

Reflux medication

Self medication / Ranitidine prescribed / Proton pump inhibitor prescribed

Anxiety / Depression

SSRI prescribed Y / N

Pre-existing pain problem Y / N

Long term analgesia Y / N

- Medication?

Smoker Y / N / Quit in the last 6 months

Alcohol

Allergies

Social

Full time / Part time / No paid employment / Caring for family member

Able bodied partner or parent or child >14 years Y / N

Non-planned contact with health care professional

Additional analgesia prescribed Y / N

Mean return to employment / usual activities days

ERCP

Antibiotic cover Y / N

- Type
- Length of course

Sedation Y / N

- Type
- Amount
- Antispasmodic
- Anti-emetic

Length of procedure mins

Grade of endoscopist

ERCP successfully completed Y / N

Ease of procedure

- Easy / Medium / Difficult but completed Abandoned

If not why not?

- Failure to tolerate procedure
- Failure to canulate sphincter
- Failure to negotiate stricture
- Pain
- Allergic reaction
- Other

Sphincterotomy

- Diverticulum Y / N
- Oedema Y / N
- Sphincter canulation
  - Easy / Medium / Difficult but completed Abandoned
- Sphincterotomy performed Y / N
- Stone retrieved Y / N
- Stent insertion Y / N / Abandoned

- Stricture present Y / N
- Stricture dilated Y / N
- Biopsy taken Y / N
- Biopsy result

Post ERCP complication

- Pain
- Bleeding
- Pancreatitis
- Sepsis

Repeat ERCP for failed procedure

- Time after abandoned procedure
- Length of procedure
- Grade of endoscopist
- Successfully completed

Time from ERCP to cholecystectomy being performed

Laparoscopic or open cholecystectomy

Discharge on day of procedure Y / N

Returned at 24 hours for review Y / N

Scoring by phone Y / N

Time from ERCP to discharge

Analgesia –

	Prior to admission	**Admission to procedure**
Paracetamol		
Ibuprofen		
Diclofenac		
Codeine		
Tramadol		
Morphine		
Other		

	2 hours	24 hours	48 hours	72 hours	96 hours
Paracetamol					
Ibuprofen					
Diclofenac					
Codeine					
Tramadol					
Morphine					
Other					

Antibiotics –

Type - Date commenced / /

Length of course - days

Microbiology results

Observations –

	Prior to admission	Admission to enrolment	2 hours	24 hours
Pulse				
BP				
Temperature				
Respiratory rate				
WBC				

	48 hours	72 hours	96 hours
Pulse			
BP			
Temperature			
Respiratory rate			
WBC			

Bloods –

	At enrolment	2 hours if not previously available	24 hours	48 hours	72 hours	96 hours
Hb						
Haematocrit						
WBC						
- neutrophils						
Plt						
Coag if available						
Na						
K						
Ur						
Cr						
eGFR						
Bilirubin						
ALP						
AST						
GGT						
Albumin						
CRP						
Glucose						
LDH						
Ca						
Base deficit						
Partial pressure O2						
Fluid sequestration						

Ranson's criteria for pancreatitis patients -

Cytokine results –

	Enrolment	2 hours	24 hours
TNF-α			
IL-1			
IL-6			
IL-10			

	48 hours	72 hours	96 hours
TNF-α			
IL-1			
IL-6			
IL-10			

Pain scores

	Enrolment	2 hours	4 hours	6 hours	24 hours
VAS					

	48 hours	72 hours	96 hours
VAS			

	Expected VAS pain score	Actual VAS pain score	Time of this
Least pain			
Most pain			

	VAS score	HAD score	SF-36 score	GIQLI score
Pre-operative				
12 weeks				
26 weeks				
52 weeks				

	Compared to 3 months ago question
Pre-operative	
12 weeks	
26 weeks	
52 weeks	

Semi-structured pain questions

Pain questions

- Experienced problems with pain? Y / N
- Has pain been discussed with you? Y / N
- Was analgesia discussed with you? Y / N
    - Including declining prescribed medication Y / N
- Was the information?
    - Helpful Y / N
    - Sufficient Y / N
    - Understandable Y / N

If you experienced pain

- Was your level of pain assessed? Y / N
- Did you receive analgesia within 15 minutes of requesting it? Y / N
- If you were not prescribed analgesia was your pain assessed? Y / N
- Was alternative methods of managing pain discussed with you? Y / N

At 12 weeks

- How long did you take analgesia for after discharge?
  days
- Did you see a doctor after discharge? Y / N
    - For infection
    - For problems with pain
    - For other pain pronlems
    - My GP invited me for review
- Post-operative patient - Would you consider having laparoscopic / open (as appropriate) surgery again? Y / N

Cholecystectomy patients

Patient unique Identifier_____

Time – Enrollment / 2 hours / 24 hours / Other

Date - ____/_____/____

For those biliary emergencies and ERCP patients going on to have cholecystectomy this section was completed, enrolment being taken as time of cholecystectomy and confirmation the patient wished to continue in study. If did not want bloods and pain scoring completed permission to record information was going to be sought but no patient opted to not have bloods and pain score.

Demographics

Patient identifier - _____

Age at first enrollment - _____ years Sex – M / F

Participated in emergency section - Y / N

Participated in the ERCP section – Y / N

Date of surgery / /

Past biliary history

	Biliary colic	Cholecystitis	Obstructive jaunduce	Pancreatitis
Date				
Date				

Length of symptoms prior to admission days

Length of time from admission to enrollment

Previous episodes of right upper quadrant pain

AE attendance with RUQ pain

Number of prior admissions with RUQ pain

Previous ERCP Y / N

- Date of procedure / /

US results

	This admission	Previously - Date
Stones present		
GB wall thickness		
Bile duct dimensions		
Stones in biliary tree		
Grade of sonographer		

CT results

	This admission - Date	Previously - Date
Evidence pancreatitis		
Pancreatic necrosis		
Pseudocyst		
Other pathology		

PMH –

Patient weight kgs Patient Height cms

BMI

Respiratory disease Y / N Type:

Cardiac disease or hypertension Y / N Type:

Diabetes Type I / II Diet / Tablet / Insulin

Thyroid disease Hypo / Hyper

IBS / Non specific pain / Sphincter of Oddi dysfunction

Previous surgery

- Non abdominal
- Abdominal
- Laparoscopic elective
- Laparoscopic emergency
- Open elective
- Open emergency
- Appendicectomy
- Gynaecological surgery
- Adhesions
- Bowel
- Other

More than five emergency admissions in the last five years

Reflux medication
Self medication / Ranitidine prescribed / Proton pump inhibitor prescribed

SSRI prescribed Y / N

Pre-existing pain problem Y / N

Long term analgesia Y / N

- Medication?

Smoker Y / N / Quit in the last 6 months

Alcohol

Allergies

Social

Full time / Part time / No paid employment / Caring for family member

Able bodied partner or parent or child >14 years Y / N

Non-planned contact with health care professional

Additional analgesia prescribed Y / N

Mean return to employment / usual activities days

Cholecystectomy

ASA grade I / II / III / IV

Pre-op

Referred to consultant who performed procedure Y / N

Reason to swap consultant

- Wanted laparoscopic surgery
- Wanted open surgery
- Length of waiting time
- Reason not stated
- Performed as emergency

ERCP previously Y / N Date / /

- Successfully completed Y / N / Post procedural complications
- Stent / Sphincterotomy
- LFT's returned to normal Y / N

OTC performed Y / N

- Successfully completed Y / N
- T – tube placed Y / N

Surgical access

Type of surgery Laparoscopic / Open / Converted

Previous abdominal surgery Y / N

Length of surgery mins

Length of pneumoperitoneum mins

Length of incision mm

Laparoscopic incision

- 2 x 10 mm and 2 x 5 mm
- 1 x 10 mm 2 x 5 mm
- 2 x 10mm 1 x 5 mm

Incision lengthened for gall bladder removal mm

Which port removed from

- Umbilical
- Upper central

Open port insertion / Verres needle

Volume of gas used mls

CO2 temperature Warm / Room temperature / Cold

CO2 pressure

- Maximal mmHg
- Lowest mmHg
- Length of time at each pressure
  - All at maximal / Majority at maximal / Half and half / Mainly at an intermediate pressure / Majority at low / All at low
- Anaesthetist asked for pressure to be reduced

Operative findings

Adhesions

- None / Few filmy / Many but not requiring division / Many requiring division / Dense

Calot's triangle

- Easily identified
- Moderately difficult to find
- Required a lot of dissection to identify

Gall bladder (mark all which apply)

- Distended with mucus
- Distended with pus
- Aspirated
- Thick walled
- Necrotic
- No stones
- One large stone
- One stone and few small stones
- Multiple small stone

Stones (mark all which apply)

- Stones in duct retrieved and cholangiogram performed
- Stones in duct not retrieved cholangiogram performed
- Stones in duct retrieved no cholangiogram performed
- Stones in duct not retrieved no cholangiogram performed
- Ducts not checked for stones
- Stones spilt when duct divided
- Stones spilt in gall bladder dissection
- Stones spilt in gall bladder extraction
- Stones all retrieved / Stones mostly retrieved / Some stones retrieved / Stones not retrieved

Bile

- No bile contamination
- Bile aspirated from gall bladder
- Bile spilt at division of duct
- Bile spilt dissecting gall bladder
- Bile spilt removing from abdominal cavity

- Bile well washed out / Bile partially washed out / Bile not washed out
- Drain placed

Dissection gall bladder

- Easy dissection
- Moderate dissection
- Difficult dissection
- Significant bleeding
- Gall bladder wall left on liver bed

Wash (mark all which apply)

- Volume used mls
- Wash used for dissection
- Good wash at end / Some wash / No wash
- Patients position on table altered to wash out Y / N

Abdomen decompressed at the end of the procedure Y / N

Local anaesthetic

- Type used including percentage
- Diluted Y / N Volume used to dilute mls
- Volume of local anaesthetic used
- When used
  - All at start / Mainly at start / Half and half / Mostly at end / All at end
- Site of infiltration (tick all which apply)
  - Skin only
  - Skin mainly
  - Small amount skin
  - Gall bladder bed
  - Around peritoneum
  - Right hemidiaphragm
  - Sprayed into peritoneum

Surgical histology

Report

Post-operative

Highest level of post operative care

- ITU Length of stay N/A
- HDU Length of stay N/A
- Ward Length of stay N/A
- Day surgery Length of stay N/A

Time from cholecystectomy to discharge hours

- Reason for not being discharged at 24 hours
- Pain
- Infection
- Not being well enough for discharge
- Social reasons
- Not stated
- Not applicable as discharged

Developed post operative complication Y / N

- Positive blood culture
- Positive bile cultures
- Chest infection
- Bile leak
- Bile duct injury
- Trocar injury
- Other

Secondary infection Y / N

- Time from cholecystectomy to diagnosis of sepsis
  hours
- What
- Other infections

Second surgery

Second surgery performed Y / N

Time from cholecystectomy hours or days

Open / Laparoscopic

Findings

Analgesia –

	Prior to admission	**Admission to enrollment**
Paracetamol		
Ibuprofen		
Diclofenac		
Codeine		
Tramadol		
Morphine		
Other		

	Received in theatre	**Received in recovery**	**Time to first dose when back on ward**
Paracetamol			
Ibuprofen			
Diclofenac			
Codeine			
Tramadol			
Morphine			
Other			

	2 hours	**24 hours**	**48 hours**	**72 hours**	**96 hours**
Paracetamol					
Ibuprofen					
Diclofenac					
Codeine					
Tramadol					
Morphine					
Other					

Time from enrolment
Paracetamol
Ibuprofen
Diclofenac
Codeine
Tramadol
Morphine
Other

Antibiotics –

Type - Date commenced / / Induction

Length of course - days

Microbiology results

Developed secondary infections Y / N

Observations –

	Prior to admission	Admission to enrolment	2 hours	24 hours
Pulse				
BP				
Temperature				
Respiratory rate				
WBC				

	48 hours	72 hours	96 hours
Pulse			
BP			
Temperature			
Respiratory rate			
WBC			

Bloods –

	At enrolment	2 hrs if not previously available	24 hrs	48 hrs	72 hrs	96 hrs
Hb						
Haematocrit						
WBC						
- neutrophils						
Plt						
Coag if available						
Na						
K						
Ur						
Cr						
eGFR						
Bilirubin						
ALP						
AST						
GGT						
Albumin						
CRP						
Glucose						
LDH						
Ca						
Base deficit						
Partial pressure O2						
Fluid sequestration						

Cytokine results –

	Enrolment	2 hours	24 hours
TNF-α			
IL-1			
IL-6			
IL-10			

	48 hours	72 hours	96 hours
TNF-α			
IL-1			
IL-6			
IL-10			

Pain scores

	Enrolment Pre / Post analgesia	2 hours	4 hours	6 hours	24 hours
VAS score					

	48 hours	72 hours	96 hours
VAS score			

	Expected VAS pain score	Actual VAS pain score	Time of this
Least pain			
Most pain			

	VAS score	HAD score	SF-36 score	GIQLI score
Pre-operative				
12 weeks				
26 weeks				
52 weeks				

	Compared to 3 months ago question
Pre-operative	
12 weeks	
26 weeks	
52 weeks	

Semi-structured pain questions

Pain questions

- Experienced problems with pain? Y / N
- Has pain been discussed with you? Y / N
- Was analgesia discussed with you? Y / N
  - Including declining prescribed medication Y / N
- Was the information?
  - Helpful Y / N
  - Sufficient Y / N
  - Understandable Y / N

If you experienced pain

- Was your level of pain assessed? Y / N
- Did you receive analgesia within 15 minutes of requesting it? Y / N
- If you were not prescribed analgesia was your pain assessed? Y / N
- Was alternative methods of managing pain discussed with you? Y / N

At 12 weeks

- How long did you take analgesia for after discharge?
  days
- Did you see a doctor after discharge? Y / N
  - For infection
  - For problems with pain
  - For other pain pronlems
  - My GP invited me for review
- Post-operative patient - Would you consider having laparoscopic / open (as appropriate) surgery again? Y / N

Apppendix

Appendix 6 – VAS Scoring Sheet

VAS Scoring Sheet

Patient unique Identifier_____
Part of study – Biliary emergency / ERCP / Cholecystectomy / Further intervention
Time – Enrollment / 2 hours / 24 hours / Other
Date - ____/_____/____
Form – A / B / C

{above completed by research doctor, A was before the bloods, B afterwards C if third one if patient had recently had analgesia}

Please record what your pain is now by making a cross on the line below and sign underneath the line.

No pain at all Worst possible pain

I_____I

VAS scoring sheet for LEAST and WORST pain

Patient unique Identifier_____

Part of study – Biliary emergency / ERCP / Cholecystectomy / Further intervention

Time – 24 hours / Other

Date - ____/_____/____

Form – A / B / C

Expected / Experienced

{above completed by research doctor, A was before the bloods, B afterwards C if third one if patient had recently had analgesia}

Please record what your pain is now by making a cross on the line below and sign underneath the line.

What is the **LEAST** (little / lowest / most comfortable) amount of pain

No pain at all Worst possible pain

I_____I

What is the **MOST** (worst / largest / most uncomfortable) amount of pain

No pain at all Worst possible pain

I_____I

Appendix

Appendix 7 - Detailed instructions for cytokine ELISA

ELISA instructions

For TNF-α

- Standards 0-5 were supplied as lypophilized samples which required reconstituting with high quality distilled water. Dilution carried out only when the standards had reached room temperature.
- Standards concentration was 0, 14.9, 43, 130, 428 and 1385pg/ml respectfully.
- Each standard vial was diluted by the volume recommended on each vial, with Gilson micropipettes (Biosphere filter tips, Starstedt, Biosphere. For each dilution a clean disposable plastic tip was used. Once diluted mixing was carried out by gentle agitation or swirling, vortexing was not used as it risked denaturing the protein.
- Controls 1 and 2 were reconstituted with 2mls of distilled water.
- Standards and controls were stable once diluted for a maximum of 4days at 2-8°C, or frozen a maximum of twice, to -20°C (stable for a maximum of 2 months) or -70°C (stable until expiration date).
- Wash solution was stored in a clean plastic container. 2mls of wash solution concentrate was added to 400mls of distilled water for the wash solution. Distilled water being measured in a volumetric flask. Prepared wash solution was stable until expiry date, but to avoid contamination we prepared fresh solution each time we performed an assay.
- Incubation buffer with preservatives, anti-TNF-α-HRP conjugate (in buffer) with preservatives, conjugate buffer with preservatives, concentrated chromogen (tetramethylbenzidine (TMB) in DMF), substrate buffer (H_2O_2 in acetate/citrate buffer) and stop solution (H_2SO_4 1.8N) came ready to use.
- The concentrated chromogen should be kept out of direct sunlight.
- Horizontal micro titre plate shaker capable of 700rpm±100rpm (Titertek Flow Laboratory) was turned on prior to preparing the plates to allow it to achieve optimum function.
- Ensure prior to using reagents, standards, controls or samples they are thoroughly mixed.
- Into all wells pipette 50µl of incubation buffer.
- Into the appropriate wells pipette 200µl of standard control or sample.
- Pipetting should take no longer than 30minutes to avoid drift and ensure accuracy in the results.
- The plate was covered with a Press apply Adhesive sealing film for Microwell plates, (Anachem, Bedfordshire, UK) plate cover, and incubate for 2hours at room temperature on a horizontal plate shaker at 700±100rpm. Ensure the plate is secured in place.
- Towards the end of the incubation dilute the concentrated conjugate as shown in the table of dilution shown below. The dilated anti TNF-α-HRP

conjugate should be made up in a clean test tube (Scientific Laboratory Supplies, Nottingham, UK).

Number of wells	Concentrated conjugate	Conjugate buffer	Working volume
8	50µl	500µl	550µl
16	100µl	1000µl	1100µl
24	150µl	1500µl	1650µl
32	200µl	2000µl	2200µl
48	300µl	3000µl	3300µl
96	600µl	6000µl	6600µl

- At the end of incubation aspirate the liquid from each well, and dry the plate.
- The plate should then be washed three times as follows: -
    - Pipette 400µl of wash solution into each well (fills the well completely).
    - Aspirate the fluid from each well.
    - Dry the plate.
- Into each well pipette 100µl of standard 0.
- Then into each pipette 50µl of appropriately diluted anti-TNF-α conjugate. Again pipetting should take place over a maximum of 30minutes.
- Cover the plate with a fresh plate cover and incubate for 2hours on a horizontal shaker set at 700±100rpm.
- After 2hours aspirate all the liquid from each well, and dry the plate.
- Wash the plate three times following the three steps given above.
- For pipetting the chromogenic solution and stop solution avoid using pipettes with metal parts.
- Pipette 200µl of chromogenic solution into 1 vial of substrate buffer. This must be used within 15minutes of preparation. If a blue colour develops within a few minutes of preparation prior to use, then the chromogenic solution is unstable and should be discarded.
- Pipette 200µl of the freshly prepared chromogenic solution into each well.
- Cover the plate, and incubate for 30minutes at 700±100rpm on the horizontal plate shaker. During this time ensure the plate is out of direct sunlight to ensure the chromogen is not affected and the accuracy of the results.
- Pipette 50µl of stop solution into each well.
- Read each plate at 450nm and 490nm as described below, within 15minutes of applying the stop.
- Where samples generated values higher than the highest standard the original serum sample was dilated with standard 0, and the dilated sample reanalysed on a subsequent plate.

For IL-1β

- The six standards (0-5) once at room temperature should each be diluted with 2mls of high quality distilled water, measured in a Gilson micropipette. The vial should be gently agitate or swirled to ensure all the lyophilised sample is dissolved. Vortexing should be avoided because of the risk of denaturing the proteins.
- The concentrations of the standards was 0, 33, 100, 335, 670, 1400pg/ml respectfully.
- Controls 1 and 2 were also reconstituted with 2mls of distilled water, once they had reached room temperature.
- Both the standards and controls once reconstituted could be stored at 2-8°C for a maximum of 4days, or at -20°C for 2months (with two cycles of defrosting and re-freezing, although we never refroze samples), or at -70°C until the expiry date.
- Volumetric flask was used to measure 400mls of distilled water, to this 2mls of wash solution was added. This was stored in a clean plastic container, and made up on the day of the assay.
- All the following were made up ready to use anti-IL-1β-HRP conjugate (in buffer) with preservatives, substrate buffer (H_2O_2 in acetate/citrate buffer), concentrated chromogen tetramethylbenzidine (TMB) in DMF and stop solution (H_2SO_4 1.8N).
- To achieve optimal speed the Horizontal micro titre plate shaker (Titertek Flow Laboratory) was turned on prior to preparing the plates to allow it to achieve speed of 700rpm±100rpm.
- Concentrated chromogen should be protected from direct sunlight.
- Ensure all reagents, standards, controls and samples are thoroughly mixed prior to use.
- Aim to completely pipette all standards, controls, samples and anti-IL-1β within 30minutes to avoid drift and ensure accuracy.
- Pipette 200µl of standard, control or sample into the pre-designated wells.
- To each well pipette 50µl of anti-IL-1β conjugate.
- Cover the plate with a Press apply Adhesive sealing film for Microwell plates, (Anachem, Bedfordshire, UK) plate cover, and incubate for 2hours at room temperature on a horizontal plate shaker at 700±100rpm.Ensure the plate is secured in place.
- At the end of 2hours remove the plate from the shaker and aspirate the liquid from each well, dry the plate.
- Into each well pipette 400µl of wash (completely fills the well).
- Aspirate the wash from each well.
- Dry the plate.
- Repeat the last 3 steps to wash the plate 3 times.
- Within 15minutes of its use prepare the chromogen solution. This is done by pipetting 200µl of chromogen into 1 vial of substrate buffer. The appearance of a blue colour before the chromogen is added to the plate indicates the chromogenic solution is unstable and should be discarded.
- To each well add 200µl of the freshly prepared chromogenic solution.
- Cover the plate again with a fresh plate cover, and incubate for 15minutes on the horizontal shaker at 700±100rpm, at room temperature. To avoid the chromogenic solution degrading the plate should be kept out of direct sunlight during this incubation step.

- At the end of this incubation period add 50µl of stop solution to each well.
- The plate was read as described below at 450 and 490nm.
- Samples with absorbance higher than the highest standard were treated as follows; -
    - Human serum diluent vials were warmed from 2-8°C to room temperature (18-25°C), and then reconstituted with 6mls of distilled water to each vial.
    - Dilute the original serum sample to a ratio of 1:4 with diluent.
    - Repeat the analysis of the sample on a subsequent plate.

For IL-6

- Allow the standards (0-5) and two controls to reach room temperature. Then reconstitute with 1ml of high quality distilled water, and gentle agitation or swirling to ensure the entire lyophilised sample is dissolved. Avoid vortexing the sample, as there is a risk of denaturing the proteins.
- The standards concentrations are 0, 16, 45, 147, 462, 1690pg/ml.
- Standards and controls can be stored for a maximum of 4days at 2-8°C once diluted. Freezing to –20°C allow the sample to be kept for 2months, and to -70°C for preservation to the expiry date. Once frozen the sample can be defrosted a maximum of twice.
- Wash solution is prepared in a clean plastic container, by adding 2mls of wash solution concentrate to 400mls of distilled water. Although wash solution was stable until the expiry date, for this research fresh wash solution was prepared for each assay.
- Solution's A and B, anti-IL-6-RP conjugate (in a buffer), chromogen (tetramethylbenzidine (TMB) in DNF) and stop solution (H_2SO_4 1.8N) came ready to use.
- The chromogen should be kept out of direct sunlight.
- The Horizontal micro titre plate shaker (Titertek Flow Laboratory) was turned on prior to preparing the plates to allow it to achieve speed of 700rpm±100rpm.
- Ensure that all samples and reagents are at room temperature and mixed well before starting to pipette onto the plate. Pipetting should take no longer than 30minutes to avoid drift and accuracy of the results
- Into each well pipette 50µl of solution B.
- Into the first six wells in columns' A and B pipette 100µl of the standards in ascending order of concentration. Into the final two wells pipette 100µl of controls 1 and 2 respectfully. Into the remaining wells pipette 100µl of sample in duplicate.
- When pipetting is complete cover the plate with a Press apply Adhesive sealing film for Micro well plates, (Anachem, Bedfordshire, UK) plate cover.
- Incubate for 1hour at room temperature on a horizontal plate shaker (Titertek Flow Laboratory) at 700±100rpm. Ensure the plate is secured in place.
- From each well aspirate the fluid and dry the plate.
- To each well add 400µl of wash solution, filling the well.
- Aspirate each well and dry the plate.
- Repeat the last two steps twice more.
- To each well add 100µl of anti IL-6 conjugate.
- Then add 50µl of solution A to each well in turn.
- The pipetting steps should take no longer than 30 minutes, to avoid drift.
- Cover the plate with a new Press apply Adhesive sealing film for Micro well plates (Anachem, Bedfordshire, UK).
- Secure in place on the horizontal plate shaker (Titertek Flow Laboratory), and incubate for 1hour at 700±100 rpm.
- Aspirate each well and dry the plate.
- Add 400µl of wash to each well, then aspirate each well and dry the plate.
- Wash and dry the plate two further times.

- Over a maximum of 15minutes pipette 200µl of the chromogen into each well using a non-metallic pipette.
- Again cover the plate with a new sealing film (Press apply Adhesive sealing film for Microwell plates Anachem, Bedfordshire, UK).
- Once the plate is secured on the horizontal plate shaker (Titertek Flow Laboratory), incubate for 15minutes at 700±100rpm. During this incubation stage the plate should be kept out of direct sunlight to avoid degradation of the chromogen.
- At the end of incubation add 100µl of stop solution to each well.
- The plate was read at 450 and 490nm as described below.
- Where the absorbances were off the scale then the original sample was diluted in a ratio of 1:2 with solution A. The diluted sample was then analysed on a subsequent plate.

For IL-10

- All the vials in the kit were allowed to warm to room temperature.
- When the standards (0-5) and controls (1-2) reached room temperature they were reconstituted by adding 1ml of distilled water to each vial. Then gently agitated by swirling to ensure all the lyophilised sample is dissolved. Avoid vortexing the sample, as there is a risk of denaturing the proteins.
- Solution A was reconstituted with distilled water the volume dependent upon the amount indicated on the vial.
- A volumetric flask should be used to measure 400mls of distilled water, to this should be added 2ml concentrate washing solution. The prepared wash solution should be kept in a clean plastic container. Despite being stable until the kits expiration date, in this work fresh wash solution was prepared on the day of analysis to avoid contamination.
- Solution B, Anti-IL-10-HRP conjugate (in a buffer), and stop solution (H_2SO_4) do not require dilution.
- The chromogen requires dilution as the point indicated below and should be kept out of direct sunlight to avoid degradation.
- To achieve optimal function the Horizontal micro titre plate shaker (Titertek Flow Laboratory) was turned on prior to preparing the plates.
- Into each well pipette 100µl of solution B.
- Into appropriate wells pipette 100µl of standard in ascending order of concentration. Into the remaining wells pipette 100µl of either the two controls or the samples. All standards, controls and samples should be plated in duplicate.
- The above two steps should take no longer than thirty minutes to avoid sample drift.
- Cover the plate with a Press apply Adhesive sealing film for Microwell plates, (Anachem, Bedfordshire, UK) plate cover, and secure the plate on the shaker.
- The horizontal plate shaker (Titertek Flow Laboratory) should be at 700±100rpm, and the plate left to incubate for two hours at room temperature on the shaker.
- At the end of the incubation period aspirate the fluid from each well and dry the plate.
- Into each well pipette 400µl of wash solution, which should fill the well to the brim.
- After filling each well aspirate the wash from each and dry the plate.
- These two steps should be repeated a further two times.
- To each well add 100µl of solution A.
- Following this add 50µl anti-IL-10 to each well. These two steps should be completed within thirty minutes.
- Cover the plate with the plate cover (Press apply Adhesive sealing film for Microwell plates, Anachem, Bedfordshire, UK).
- Secure in position on the horizontal plate shaker (Titertek Flow Laboratory) and incubate at 700±100rpm, for two hours at room temperature.
- At the end of the incubation period aspirate the fluid from each well and dry the plate.

- To each well in turn add 400µl of wash solution, then aspirate each well and dry the plate.
- Repeat this step twice more.
- Into one of the vials of substrate buffer add 200µl of concentrated chromogen (TMB in DMF). Mix by gentle agitation.
- If the chromogenic solution develops a blue colour, it should be discarded. The chromogenic solution should be used within 15minutes of preparation.
- To each well add 200µl of chromogenic solution.
- Cover the plate with a further adhesive cover (Press apply Adhesive sealing film for Microwell plates, Anachem, Bedfordshire, UK).
- Out of direct sunlight and at room temperature incubate the plate on the horizontal shaker at 700±100rpm for 30minutes.
- After 30minutes add 50µl of stop solution to each well.
- Read the plate at 450 and 490nm, as soon as possible after adding the stop solution.
- If a sample generates a value higher than the highest standard the original sample should be diluted with solution A in a ratio of 1:2.

Reading the plate.

- Plates should be read immediately after stop solution to avoid degradation of the chromogen. If this is not feasible then the plate should be kept out of the light and read within a maximum of 3hours.
- A standard curve is constructed at 450nm, for samples or controls with absorbances above the standard read at 450nm, a second reading at 490nm. This allows a high sensitivity of assay at the 450nm wavelength and an extended standard range using the absorbencies at 490nm.
- Readings at 490nm do not replace the 450nm readings, and should only be used where the values are off the scale at 450nm.
- For each control and sample the average value of absorbance of the two wells is used to measure concentration.
- Various software programmes exist for reading the plates, for this research the Stingray programme (Stingray Pharmaceuticals) was used with a Rosys anthos 2001 mass spectrometer attached.
- At the first screen choose 'Create a new profile'.
- Mark out the plate template with the position of standards, controls, samples, blanks and unused wells.
- Under Stingray assay master, name the assay, and the assay type, highlighting curve fitting, GLP.
- Under Transformation manager, highlight Calculate concentration.
- The following screen is Define transformation highlighting type curve fit, and output name concentration.
- Under Input matrix highlight 'Raw 1 Anthos AN2001 450, 490 pre concentration.
- On the curve fit method screen choose 'Polynomial order 2'.
- The following screen allows the standard graph to be titled and axis labelled, in this research we used Cytokine concentration in pg/ml linear scale for the x-axis, and OD at 450nm linear scale for the y-axis.
- The summary screen should display 'Curve fit uses x-values defined in standard set, y-values on matrix Raw 1.
- The values for the standards screen depend on the cytokine measured and are shown in the table below.

Standard	TNF-α	IL-1β	IL-6	IL-10
1	0	0	0	0
2	14.9	33	19	11
3	43	100	55	40
4	130	335	194	120
5	428	670	607	420
6	1385	1400	2350	1335

- When the plate was ready to be read press the 'read to run' button.

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