UNIVERSITY OF HULL

Influence of gut function, on SIRS and clinical outcomes in surgical patients.

being a Thesis submitted for the Degree of Doctor of Medicine (MD)

in the University of Hull

by

Ramana Reddy Kallam

MBBS, MRCS (Glasgow)

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DECLARATION:

I confirm that this thesis contains original research, composed entirely by Ramana R Kallam.

The work contained in this thesis has not been presented for any other degree.

All the study design, data collection, laboratory work and subsequent analysis have been performed by Ramana R Kallam. Any contribution from other individuals has been acknowledged clearly in relevant sections.

All sources of information have been specifically acknowledged and referenced.

Ramana R Kallam.

THESIS ABSTRACT

Background: The GI tract is a highly complex organ system with multitude of functions. Gastrointestinal dysfunction remains an unrecognised clinical entity in day to day clinical practice hence it is possible many patients are inadequately managed resulting in poorer clinical outcomes. Recognising gut failure and early initiation of gut directed therapies may influence clinical outcomes.

Aims: This thesis aims to review available literature for importance of gut function and its influence on SIRS and clinical outcomes and investigate the state of gut function and its influence on SIRS and clinical outcomes in surgical patients, further develop a method to optimise gut function and test the influence of this optimisation package on clinical outcomes in elective surgical patients.

Methods: A series of clinical studies in elective surgical patients to investigate the influence of gut function on clinical outcomes.

Results: Inadequate gut function was common in patients with pancreatitis and the persistent gut failure was associated with SIRS, MODS and poorer clinical outcomes. Critically ill patients with gut failure had increased prevalence of SIRS however this has not resulted in increased mortality or poorer clinical outcome. Elective GI surgical patients developed gut dysfunction in the post operative period more commonly than patients who underwent breast surgery and this was associated with increased prevalence of SIRS and septic morbidity. Optimisation of gut function was associated with early return of gut function and improved clinical outcomes in elective surgical patients.

Conclusion: Recognition of gut failure is important in day to day clinical practice and gut failure is associated with poorer outcomes in surgical patients. Gut directed therapy to optimise gut function is associated with improved clinical outcomes.

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TABLE OF CONTENTS:

ABBREVIATIONS:

Several abbreviations have been used throughout this thesis. These are summarised here in alphabetical order. The first time an abbreviation was used, it is preceded by the words for which it stands.

APACHE	Acute physiological and chronic health evaluation (score)
ASA	American society of anaesthesiologists (grading)
BCFA	Branched Chain Fatty Acids
BMI	Body mass index
BT	Bacterial translocation
CRP	C-reactive protein
EN	Enteral nutrition
ERAS	Enhanced recovery after surgery
GALT	Gut-associated lymphoid tissue
GIT	Gastrointestinal Tract
GP	General Practitioner
GSN	Gut-specific nutrient
ICU	Intensive care unit
IF	Intestinal failure
IGF	Inadequate gut function
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-1β	Interleukin-1β
IQR	Interquartile range
LREC	Locally organized research ethics committee
M: F	Male to Female ratio
MLN	Mesenteric lymph node
MODS	Multiple organ dysfunction syndrome
MOF	Multiorgan failure
NG	Nasogastric
NGT	Nasogastric tube
NJ	Nasojejunal
PEG	Percutaneous endoscopic gastrostomy

PN	Parenteral nutrition
POSSUM	Physiological and operative severity score for the enumeration of mortality and morbidity.
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SGD	Selective gut decontamination
SOFA	Sequential organ failure assessment (score)
TNF-α	Tumour necrosis factor-α
TPN	Total parenteral nutrition

CHAPTER ONE

INTRODUCTION, REVIEW OF LITERATURE AND THESIS RATIONALE

PART 1: THE GASTROINTESTINAL TRACT

1.1 .1 FUNCTIONS OF GASTROINTESTINAL TRACT:

The gastro intestinal tract is a highly complex organ system which has many functions. Even though the primary function of gut is nutrition, gut is the largest immunological organ in human body, periodically mounting measured immunological response to the antigenic stimulus. Gut also acts as a barrier against living organisms and other antigens within the lumen. In addition gut is central to a host of other vital homeostatic mechanisms in the human body.

In view of its many functions, in health it is essential to have a normally functioning gastrointestinal tract. In other words inadequate gut function is deleterious to individual's outcome in illness. However historically little attention has been given to the state of gastrointestinal function in illness. Traditional teachings have promoted the dogma that the gut is dormant, metabolically inactive, and of little physiologic and pathologic significance in illness. Recent literature evidence has refuted these long standing beliefs.

Gastrointestinal dysfunction remains an unrecognised clinical entity in day to day clinical practice; hence it is possible many patients are inadequately managed, resulting in poor clinical outcomes.

In recent years, interest in role of gut function is increasing and clinicians are paying due attention to the functional state of the gut. Current circumstantial evidence suggests that the inadequate gut function or gut failure may be associated with onset of systemic inflammatory response syndrome, sepsis, multi organ failure and even death amongst critically ill and surgical patients. Common mechanisms by which gut dysfunction has been implicated in poor clinical outcomes in disease are related to gut barrier disruption leading to

immunological response setting up a vicious cycle of SIRS, Sepsis MOF and death. However, due to the complexity of gut function and limited availability of methods to accurately diagnose gut failure, therapies directed specifically at preventing gut failure and treating recognised gut failure are yet to be commonly available.

This thesis aims to review the available literature and attempt to relate gut function and failure to clinical outcomes in day to day clinical practice and develop methods to optimise gut function and test the effects of this optimised gut function in clinical setting in subsequent chapters.

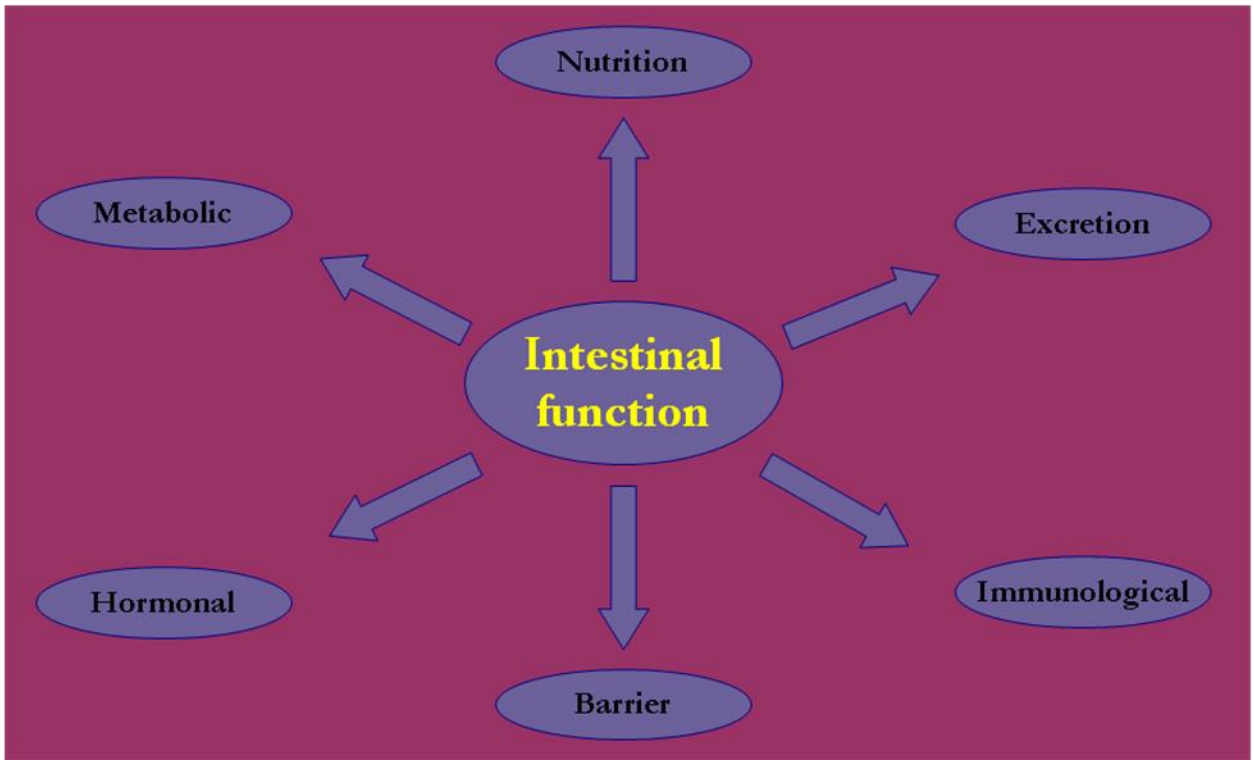


Figure: 1.1: Various functions of gut: Primary function being nutrition.

Very little attention has been given in the literature to the overall functional state of the gastrointestinal tract. This is exemplified by the fact that, unlike other single organ failures which refer to inadequate function of that organ system as a whole, the term 'gut failure' is commonly used in the literature to refer to patients with short gut syndrome or those requiring home parenteral nutrition due to lack of gut mass. In this series of studies, the terms 'gut failure', 'inadequate gut function' and 'gut dysfunction' are used interchangeably to refer to any insufficiency of the gastrointestinal tract mediated by attenuation of its various functions. In this sense, patients with short gut syndrome or those requiring home parenteral feeding are only a small minority. The term is more commonly applicable to the transient attenuation of gut function that is commonly seen in day to day clinical practice, for example, the immediate postoperative period, the setting of ileus, or that of critical illness, as is applied to other organ systems. There is circumstantial evidence which implicates gut failure with disease, and in particular, with the onset and propagation of delayed sepsis, MOF, and death. This support for the importance of gut function to patient outcome can be drawn from different areas of the medical literature including that pertaining to human nutrition, the gut origin of sepsis hypothesis and enhanced recovery after surgery (ERAS). The common mechanisms by which gastrointestinal dysfunction cause disease are thought to relate, at least in part, to a disruption of gut barrier, immunological and cytokine producing functions. This in turn instigates ingress of microorganisms and other antigens into the internal milieu described as bacterial translocation, in the setting of an otherwise immunocompromised host, resulting in overwhelming SIRS, sepsis, MOF, and even death (Marshall et al., 1993; Cohen *et al.*, 2004; MacFie *et al.*, 2006). Even though there is circumstantial evidence supporting the significance of gut failure in disease, definitive proof for the prognostic importance of gastrointestinal dysfunction remains elusive in day to day clinical practice.

1.1.2 HISTORICAL PERSPECTIVE:

"The large intestine with its teeming myriads of bacteria is a source of chronic poisoning, the removal of which would indefinitely prolong life"

Nobel laureate Ely Metchnikoff, 1097

For many centuries, philosophers and scientists have been fascinated by the role of the gastrointestinal tract in health and disease. Historically, Physicians such as Arbuthnot Lane, subjected many patients to colonic lavage or colectomy in the belief that the colon was the cause of multiple diseases as diverse as diabetes and pes planus (Chapman 2001). The idea that peritonitis could result from the passage of bacteria from adjacent organs to the peritoneal cavity evolved in the late 19th century. In Germany this was referred to as "*durchwanderungs-peritonitis*", literally meaning "*wandering through peritonitis*". Fraenkel (1891) for the first time hypothesised that viable bacterium could pass through the intact gut wall *in vivo*. These findings were confirmed after the Second World War when Schweinburg *et al*. (1953) were able to show viable bacteria in the peritoneal cavity of dogs suffering from haemorrhagic shock. Schatten *et al*. (1954) demonstrated that bacteria could migrate from the gut into the portal circulation in the absence of an infective process in humans. However, this notion of gut origin of sepsis fell out of favour until the late 1970's when the concept of bacterial translocation was proposed as a potential mechanism to explain systemic infection in patients with organ failure. Berg and Garlington (1979) defined this phenomenon of passage of live bacteria from adjacent organs to peritoneal cavity as "*Bacterial Translocation*". It was later realised that, in addition to bacteria, endotoxins and fungi can also cross the intact mucosal barrier in humans.

1.1.3 GUT BARRIER AND GUT ORIGIN OF SEPSIS:

Humans have evolved a complex gut-mucosal barrier to enable the digestion and absorption of nutrients and water, whilst excluding vast numbers of bacteria from the systemic circulation. The gut-mucosal barrier consists of immunological and non-immunological components, the interactions of which are essential to normal barrier function. Endogenous bacteria contribute to barrier function through a variety of mechanisms including "colonisation resistance" and altering mucus production.

Perhaps the most cited component of the gut-mucosal barrier is the epithelial layer. The intestine is covered by a single layer of columnar epithelial cells, arranged into villi and crypts. The cells are bound together by tight junctions, to prevent the movement of luminal contents through the paracellular channels to the sub-epithelial lamina propria. (Dayal 1989) Until recently, the epithelium has been viewed as an inert surface onto which bacteria can adhere. Studies have shown, however, that intestinal epithelial cells can express glycoconjugates onto their cell surface, in response to specific indigenous bacteria. (Lu 2001) This facilitates the adherence of the non-pathogenic bacteria to the cell surface. This bacterial-epithelial "crosstalk" is generally beneficial to the host as it prevents the adherence of pathogenic organisms (Walker 2000). However, enteropathogenic *Escherichia coli* can exploit this situation, as it inserts its own receptor into the host cell membrane to facilitate adhesion (DeVinney 1999).

The mucus layer forms another integral structural component of the gut-mucosal barrier. It has a number of important functions, acting as a medium for protection, lubrication and transport between the luminal contents and the intestinal lining. (Fostener 1995) The mucus layer is also important in preventing the adherence of bacteria to the epithelium. (Matsuo 1997) It is suggested that acidic mucins predominate in the colon and may prevent bacterial

translocation. (Roberton 1997) The composition and quality of mucin released by goblet cells can be influenced by the composition of the faecal flora.

The Gut Associated Lymphoid Tissue (GALT) forms the immunological component of gut barrier and is the largest immunological organ in the body. It is comprised of intraepithelial lymphocytes, lamina propria lymphocytes, Payer's patches, specialised M cells and the mesenteric lymph nodes. The function of GALT is not only to prevent systemic infection from luminally derived microorganisms, but also to sample luminal antigens and induce immunotolerance (Erickson 2000). Payer's patches (PP) are lymphoid follicles found in the mucosal and sub-mucosal layers within the small bowel and are the site of B cell differentiation and IgA production (Langkamp-Henken 1992). Payer's patches carry specialised epithelial cells on their surface, called M (microfold) cells. M cell sample luminal antigens and transport them across the epithelium. Once the antigen is presented, B cells produce IgA and enter the systemic circulation. The specific immunity, which is generated locally, is disseminated throughout the GALT by stimulated B and T cells, which move between PP by means of adhesion molecules on the lymphocytes and the endothelium. Majority of secretory IgA in humans is derived at the mucosal level and not from the circulation. The sensitised B cells then migrate to the lamina propria and mature into plasma cells. Secretory IgA is produced in a polymetric form and binds to the secretory component expressed on the basolateral membrane of the mucosal epithelium. The secretory component is important as it prevents proteolysis by bacteria. In this way, bacteria are prevented from adhering to the mucosa.

Intraepithelial lymphocytes and lamina propria lymphocytes are predominately T cells. (Johnson 1999) When presented with a specific antigen, the intraepithelial lymphocytes, which express CD8 activity, are stimulated and produce a variety of cytokines to promote IgA production. They also have a direct cytolytic activity against pathogens. Lamina propria

lymphocytes have a predominately CD4 action and produce a cytokine response in response to antigens. If bacteria or endotoxin manage to penetrate the GALT, circulating systemic lymphocyes and portal Kupffer cells act as a second line of defence.

The fact that luminal contents in the caecum have a bacterial concentration of the order of 10^{12} organisms per ml of faeces (Simon and Gorbach, 1986) whilst portal blood, mesenteric lymph nodes (MLN) and indeed tissues one cell deep to the intact intestinal mucosa are usually sterile, illustrates the efficacy of this gut barrier (Baumgart and Dignass, 2002).

The gut's barrier function serves to manage luminal antigens, allows for the safe ingestion of foodstuffs, and encourages the symbiotic relationship between human host and enteric bacteria, while constantly ensuring that the internal milieu remains sterile. Breakdown of this barrier may result in the ingress of viable bacteria and their antigens with the development of sepsis, initiation of a cytokine-mediated systemic inflammatory response syndrome (SIRS), multi-organ failure (MOF), and even death. It was Berg and Garlington in1979 who first defined the phenomenon of bacterial translocation (BT) as the passage of viable resident bacteria from the gastrointestinal tract, across the intact mucosa, to normally sterile tissues such as the MLN and other internal organs. The term also applies to the passage of inert particles and other antigenic macromolecules, such as lipopolysaccharide endotoxins and peptidoglicans, across the intestinal mucosal barrier. This role of the gut as 'the motor of multiple organ failure' (Carrico *et al*., 1986) may help to explain the absence of a discreet focus of infection in most patients with delayed SIRS and MOF. The process of gut barrier failure and associated BT describes the gut origin of sepsis hypothesis (Deitch and Goris, 1996).

It is possible to think that any bacteria or endotoxin passing through the intestinal barrier might cause septic complications in the host, there is growing evidence to suggest that translocation may in fact be a normal phenomenon. It is possible that translocation occurs to allow the alimentary tract to be exposed to and sample antigens within the lumen such that the gut can mount a controlled local immune response helping to keep these antigens away from the internal milieu. This process is known as ‘oral tolerance’ (Song and Whitacre, 2001). It is only when the ingress of micro-organisms and other antigens is above a certain threshold, and the host’s immune defences are compromised that septic complications arise.

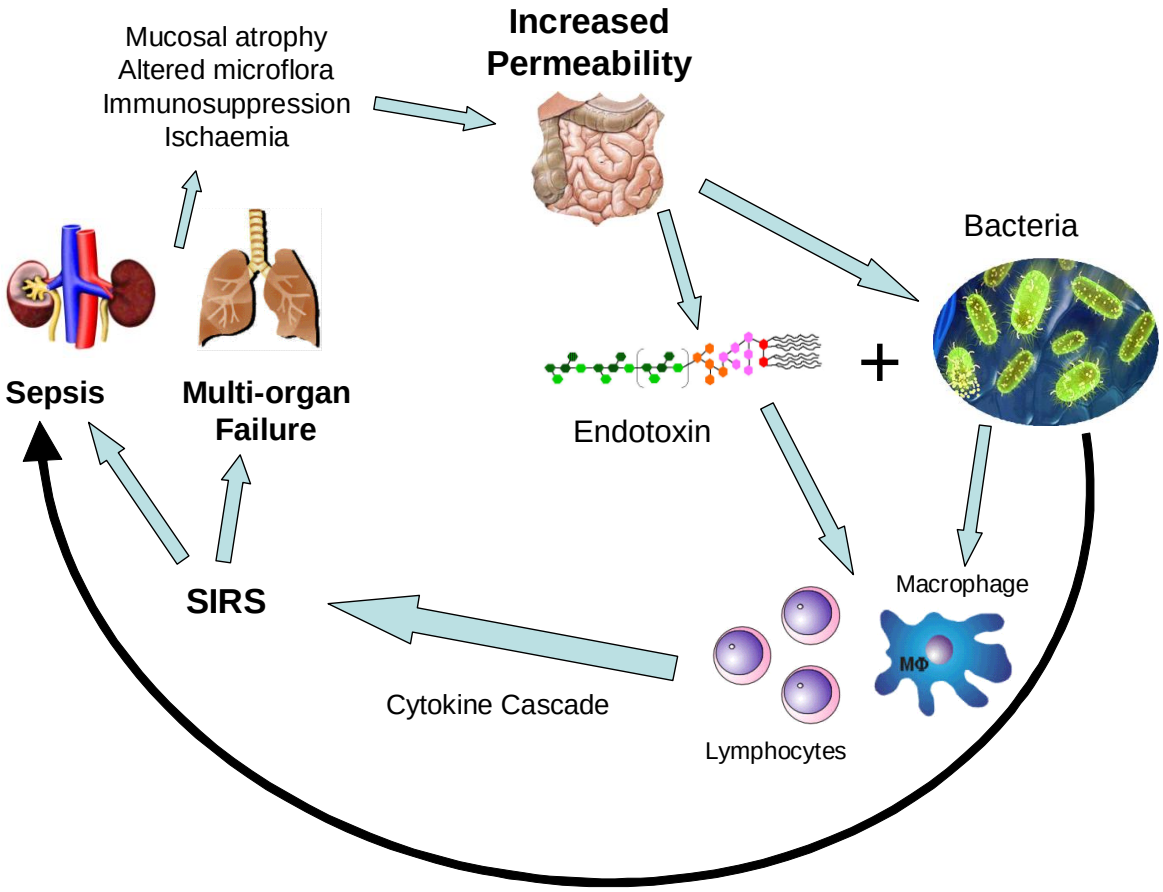


Figure: 1.2 Schematic representation of the gut origin of sepsis hypothesis.

Figure modified and adapted from *Soderholm JD, Perdue MH*. Stress and the gastrointestinal tract: Stress and intestinal barrier function. *Am J Physiol* 2001; **280**: G7-G13.

Several modifications to "the gut origin of sepsis" hypotheses have been put forward to define this process of gut-derived sepsis. Deitch proposed the 'three-hit hypothesis' (Deitch, 2002). In this model, an initial insult results in splanchnic hypoperfusion (first hit) with the gut becoming a major site of proinflammatory factor production. Resuscitation results in reperfusion which leads to an ischaemia-reperfusion injury to the intestine (second hit) with a resultant loss of gut barrier function and an ensuing enhanced gut inflammatory response, without the need for translocation of microbes as far as the MLN or beyond. Once bacteria or endotoxin cross the mucosal barrier, they can trigger an augmented immune response such that the gut becomes a proinflammatory organ, releasing chemokines, cytokines and other pro-inflammatory intermediates which affect both the local as well as the systemic immune systems (third hit), finally resulting in SIRS and MOF.

Another modification of "the gut origin of sepsis" hypothesis is known as the "gut-lymph Theory" (Deitch;2001) which proposes that macrophages and other immune cells in the submucosal lymphatics of the gut wall or the MLN trap the majority of translocating bacteria. However, those that survive, or the cell wall and protein components of the dead bacteria (including lipopolysaccharides and peptidoglycans) along with the cytokines and chemokines generated in the gut, travel via the mesenteric lymphatics to the cysterna chilli, and via the thoracic duct empty into the left subclavian vein to reach the right side of the heart. These inflammatory products then enter the pulmonary circulation and activate the alveolar macrophages. In so doing, they contribute to acute lung injury and the progression to adult respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS). This theory corroborates earlier work published by Moore and co-workers who failed to demonstrate bacteria or endotoxins in portal venous blood of polytrauma patients (Moore *et al*., 1991). However, the mechanisms by which translocating bacteria, their antigenic

components or cytokines generated in the gut set about causing SIRS, sepsis and MODS remains unclear.

Luminal bacteria that manage to breach intestinal barrier defences can cross the mucosal epithelium by taking either the transcellular or the paracellular route, or a combination of the two (Wells and Erlandsen, 1996). On entering the lamina propria, most bacteria are destroyed by macrophages; those that are not, enter the portal venous system and associated solid organs, pass to the MLN, or transgress the peritoneal cavity directly Confirmation of BT therefore necessitates the identification of bacteria in one or more of these sites, making assessments of BT difficult in humans as it necessitates invasive tissue sampling. The occurrence of BT has been identified in several animal studies. The majority of these studies have involved the culture of MLN to demonstrate BT (Barber *et al.,* 1991; Deitch *et al.,* 1995). Using a similar technique, studies in humans have demonstrated BT often associated this with sepsis (Ambrose *et al.,* 1984; Sagar *et al.,* 1995; MacFie *et al.,* 2006). Major limitation of this technique will be that BT can only be assessed in surgical patients who are undergoing laparotomy. However, recent advances in molecular microbiological techniques have opened new opportunities to assess BT by non invasive methods like PCR and DNA finger printing. These newer techniques are still in research stage and not yet established.
Available literature evidence for BT supports the gut origin of sepsis hypothesis but proof is still elusive.

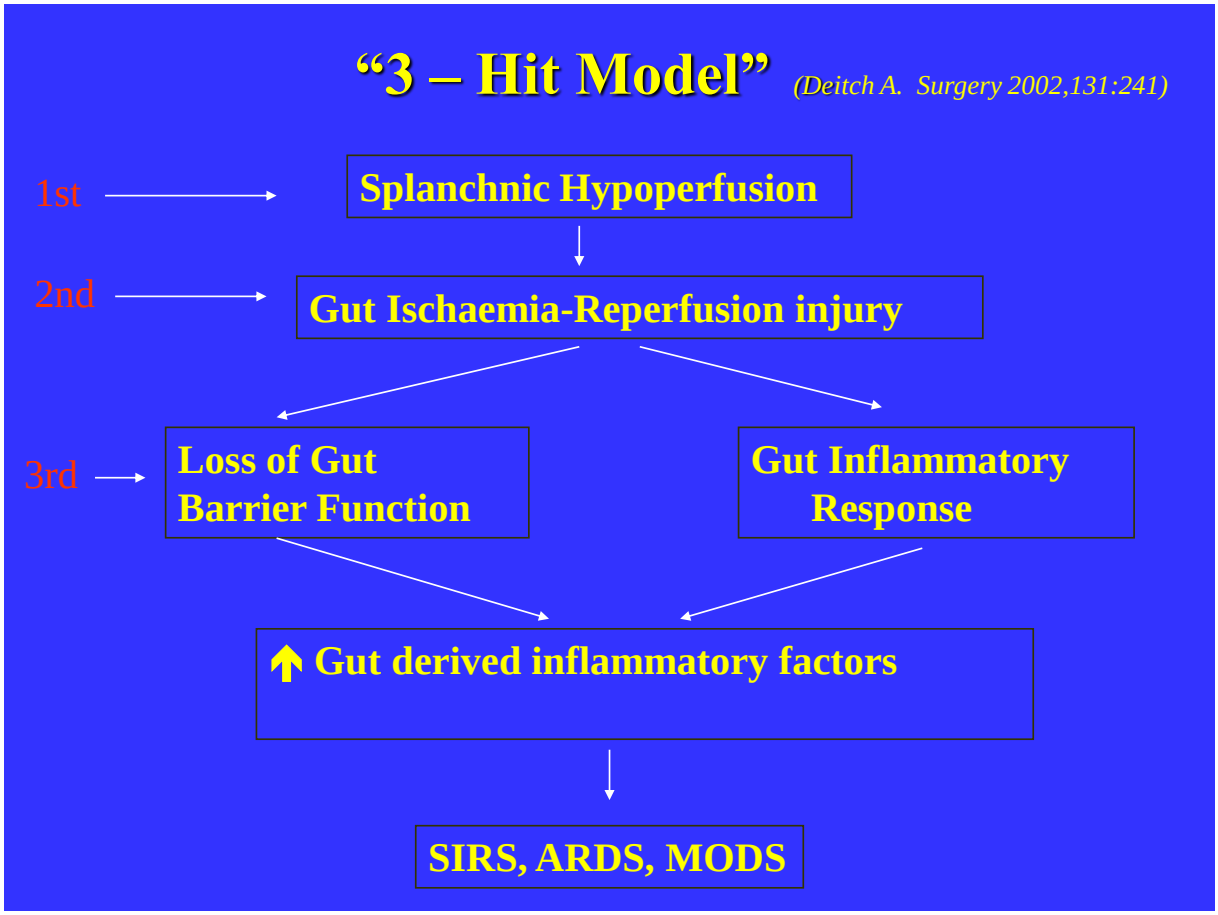


Figure 1.3: Schematic diagram representing Deitch's three hit hypothesis for 'gut origin of sepsis' hypothesis.

Adapted and redrawn from surgery 2002.

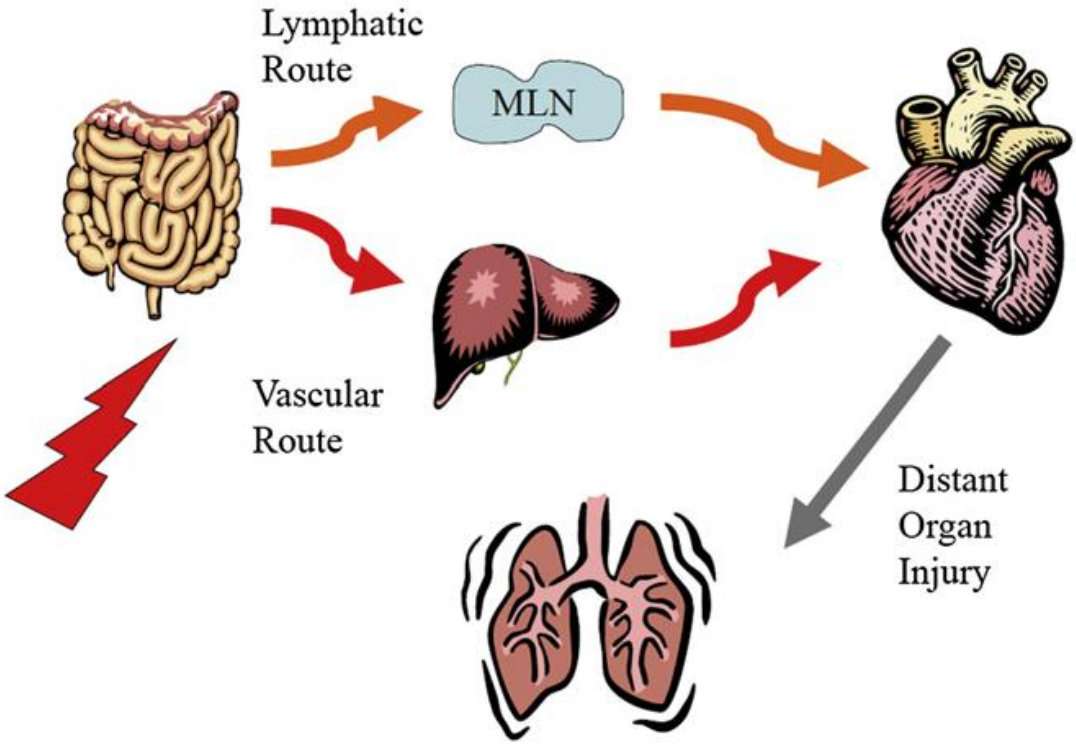


FIGURE: 1.4: Schematic illustration of lymphatic and portal pathways by which gut-derived factors and/or bacteria can reach the systemic circulation.

Adapted from Edwin A Deitch; Gut origin of sepsis evolution of concepts. The surgeon doi:10.1016/j.surge.2012.03.003

1.1.4: DEFINING GUT FUNCTION AND GUT FAILURE:

Clinicians conventionally resort to the use of traditional surrogate markers of gut function such as the auscultation of bowel sounds, the passage of flatus and faeces or the tolerance of an unquantified enteral challenge (Seidner and Ramasamy, 2005). These observations are all subjective and have long been known to be of limited clinical value in assessing underlying gut function (Harper and Catchpole, 1963). One of the difficulties in establishing a definition for gut function or failure is that, unlike other single organs (e.g. the heart, lungs and kidneys), the gut is functionally much more complex, being involved in a multitude of metabolic processes. Numerous tests have been described to assess isolated aspects of gastrointestinal function including investigations which assess intestinal anatomy and length, intestinal motility, gut absorption and permeability, nutritional status, gastrointestinal hormone production, as well as barrier and immunological function. Despite a multitude of metabolic functions, the primary role of the gut remains that of nutrition. In common with other organ systems, its state of function or failure should therefore, theoretically at least, be defined in terms of its primary role. There have been numerous attempts at characterising the state of gut function but none have been easily applicable clinically or widely accepted. Following examples are a few of many defining gut failure available in medical literature:

"...a reduction in functioning gut mass below the minimal amount necessary for adequate digestion and absorption of nutrients."

Fleming and Remington, 1981

"Intestinal failure occurs when there is reduced intestinal absorption so that macronutrients and/or water and electrolyte supplements are needed to maintain health and/or growth. Undernutrition and/or dehydration result if no treatment is given or if compensatory mechanisms do not occur."

Nightingale, 2001

A pragmatic approach towards establishing a clinically applicable description of adequate or inadequate gut function would be to formulate a definition based on its primary function that is nutrition in terms of tolerance to daily requirements, or multiples thereof, as this would facilitate any necessary calculations and facilitate bedside application.

Recently Gatt *et al*., from Scarborough has defined and validated gut function based on its primary function, nutrition and enteral tolerance as

"Oral/enteral tolerance of ≥80% of calculated nutritional requirements for a continuous period of ≥48 hours"

As this definition is more practical, measurable and reproducible, this definition has been adapted in all the studies this thesis describes.

PART 2

1.2.1 EVIDENCE FOR IMPORTANCE OF GUT FUNCTION:

Indirect or circumstantial evidence can be drawn from medical literature suggesting gut function is important and functioning gut is associated with improved clinical outcomes based on nutritional studies, studies related to enhanced recovery after surgery and so on. However a direct link of gut failure and poorer clinical outcomes is lacking. This section will briefly review the circumstantial evidence.

1.2.2 EVIDENCE FROM NUTRITIONAL STUDIES:

Current thinking about nutritional support favours the use of enteral nutrition over parenteral feeding and the instigation of early enteral nutrition over delayed initiation (Kreymann *et al*., 2006). It is a commonly held belief that feeding into the gut is beneficial to patients, and is associated with improved outcomes, particularly when instituted early, as it is more physiological than TPN. The idea that well nourished patients do better than their malnourished counterparts is well established.

Moore *et al*. in1992 investigated 230 patients receiving adjuvant feeding by the enteral or parenteral route, reported that those receiving TPN were more than twice as likely to develop septic morbidity when compared to those patients receiving EN.

Lewis *et al*. in 2001 published the merits of early enteral feeding *versus* 'nil-by-mouth' in patients after gastrointestinal surgery. In this study, authors found that early enteral feeding was associated with a decrease in overall sepsis despite an increase in the risk of vomiting. There was also a trend towards decreased mortality with early enteral feeding, but, this did not achieve statistical significance.

In a systemic review by Heyland *et al*. published in 2003 which compared early *versus* delayed nutrient intake and their relationship to outcome, the authors reported that early EN was associated with a trend towards decreased mortality and decreased sepsis, even though neither reached statistical significance.

Results from these and several other similar studies even though level of evidence is grade B or below have been incorporated in to several established organisations like ASPEN feeding guidelines.

These guidelines promote the use of enteral feeding in preference to parenteral administration, and the use of early enteral feeding over delayed commencement of nutrition.

It may be that it is actually underlying gut function which is important, as it is this that then conditions the timing and method of patient feeding? Is it conceivable that if a patient's gut does not work adequately (such that the patient necessitates parenteral nutrition), then any predisposition to septic complications arises by virtue of the fact that the patient has impaired gut immunological function and not as a direct result of the intravenous administration of nutrients?

In a landmark pragmatic study, Woodcock et al in 2001 including over 550 patients reported that no differences between patients fed enterally and those fed parenterally with respect to septic morbidity in contradistinction to the majority of observational studies in the literature. Patients receiving EN in this study had a higher absolute mortality than patients assigned to TPN feeding. Many have interpreted this study as definitive proof that TPN is, contrary to popular belief, better in many ways than EN.

However, there is more important interpretation of these results that the authors allude to in their conclusion.

"If adequate volumes (*of feed*) are tolerated then TPN is clearly not required and the absence of intestinal failure is probably a favourable prognostic indicator."

Following this landmark study few other studies have drawn similar conclusions. These results suggest that the state of underlying gut function, and the ensuing tolerance or intolerance of feed administered to the gut, has a prognostic significance.

1.2.3: ENHANCED RECOVERY AFTER SURGERY PROTOCOLS AND GUT FUNCTION:

Enhanced recover after surgery (ERAS) programmes or multimodal optimization (MMO) packages are now standard perioperative care all over the world primarily in elective surgical setting. These packages include number of interventions aimed at optimizing perioperative care. Studies of this nature have repeatedly been shown to result in improved post-operative outcomes in patients undergoing elective surgery (particularly colorectal surgery) by reducing morbidity, expediting recovery and reducing hospital stays in the optimized groups (Anderson *et al*., 2003; Kehlet and Wilmore, 2005; Fearon *et al.,* 2005;), When compared to conventional perioperative care.

Benefits of ERAS programmes have been attributed to a reduction in the stress response to surgery. Mechanisms involved in improved stress response are believed to be mediated via neuroendocrine mechanisms leading to alterations in protein homeostasis altered carbohydrate metabolism and increased lipolysis (Weissman, 1990; Desborough, 2000; KehletH, 2002). In the short term, the stress response can be advantageous, but over a longer period it can lead to organ dysfunction, loss of lean body mass, reduced muscle power, and fatigue (Henriksen, 2000). Optimization packages are thought to work by preserving postoperative organ function, including the attenuation of transient postoperative cardiac, respiratory and renal failure (Kehlet and Wilmore, 2005).

It has also been suggested that the earlier return of normal gut function, noticed by some investigators, is pivotal in bringing about the benefits of these packages (Gatt *et al*., 2005, Wind *et al*., 2006). This understanding is based on several observations. Firstly, optimization is associated with an earlier tolerance of food (Anderson *et al*., 2003, Gatt *et al*., 2005), curtailed postoperative ileus, and a lower prevalence of postoperative nausea and vomiting (Kehlet and Dahl, 2003). Secondly, many of the treatment strategies included in optimization packages, such as early mobilisation, synbiotics, opiate avoidance, use of epidurals, high inspired oxygen concentrations, and early enteral challenge, primarily affect the gut. Thirdly, the gastrointestinal system with its GALT constitutes more than 50 percent of the body's immunological cell mass and plays a central role in orchestrating the stress response to surgery.

In summary exact mechanisms responsible for the improvements in outcome noted with ERAS packages remain unclear. Enhanced recover could, in part, be due to improvements in early return of gut function, but this cannot be ascertained from the published literature because of the difficulties when assessing gut function and lack of standardisation (Elia, Stroud and Itobi, 2006).

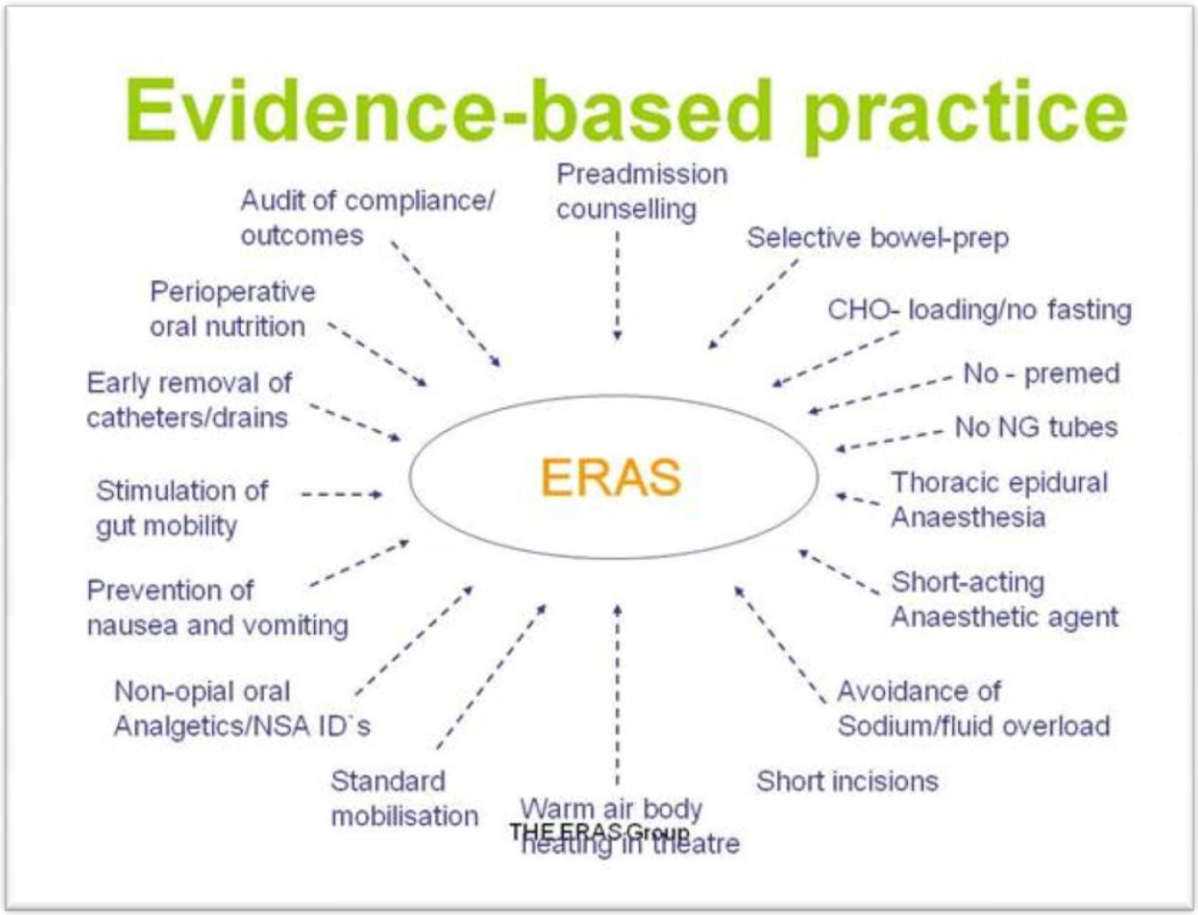


Figure: 1.5 Key components of ERAS package.

Figure adapted from ESPEN congress 2010 highlight topics.

1.2.4: ELECTIVE SURGERY AND GUT FUNCTION:

Tolerance of an enteral diet is one of the fundamental components of postoperative wellness, along with the ability to mobilize freely without supplemental oxygen and a readiness to be discharged home as soon as possible. Accordingly, postoperative gastrointestinal (GI) tract dysfunction is best defined as intolerance of an enteral diet after having been tolerant of one preoperatively.

Post operative gut dysfunction is common and is most common cause for delayed discharge from hospital following surgery and is implicated in excessive costs.

In a large prospective cohort study conducted at Duke University Medical Centre USA by Bennett-Guerrero et al in 1999 Postoperative Morbidity was surveyed to document complications following major non-cardiac surgery (i.e. anticipated duration > 2 hours and anticipated blood loss > 500 ml). Hospital discharge was delayed in 27% of the study's 438 patients as a result of a postoperative complication, and GI dysfunction was the most common complication overall. Patient population included in this study was mainly common teaching hospital elective surgical patients undergoing Gastro intestinal surgery, revision hip and knee surgery, spinal surgery, Gynaecological cancer debulking and uro-oncological procedures. Most of the post operative morbidity in this study was not directly related to the type or site of surgery. Hence the authors have has concluded that the post operative morbidity including GI dysfunction is likely to be due to systemic inflammatory response.

Mythen et al in 2005 classified post operative GI dysfunction with respect to Onset, Duration and severity. This classification was adapted by various authors subsequently.

Previous study was validated in UK setting by Grocott et al., in 2006 including 450 patients and similar patient population. This study has reported GI dysfunction as common post

operative morbidity unrelated to the type or site of surgery. In this cohort Post operative GI dysfunction was recorded in 47% of patients at various time points before their discharge.

From these studies it is reasonable to argue that GI dysfunction in post operative period is common irrespective of type or site of surgery and this may be related to systemic inflammatory response or other unidentified mechanism.

PART 3: GUT, SIRS/MODS AND CLINICAL OUTCOMES.

With emerging animal and human study literature, it is increasingly being recognised in clinical practice that gut function like other organ function is important and influence clinical outcomes. However mechanisms through which gut function influences clinical outcome is poorly understood and warrants further research. Gut origin of sepsis hypothesis postulates gut derived cytokines and pro-inflammatory mediators in response to injury/insult driving the motor of SIRS/MODS and sepsis resulting in poorer clinical outcomes.

1.3.1 PATHO PHYSIOLOGY OF SIRS, SEPSIS, MODS AND SEPSIS SYNDROME:

The initial response to any injury or infective stimulus in humans is the production of a local inflammatory response. Cytokines, which are small proteins produced de-novo in response to an infective focus, mediate the inflammatory response. Polymorpho-nucleocytes (PMNs), monocytes, macrophages and endothelial cells are the effector cells of the inflammatory response. Leukocyte activation and adhesion to endothelial cells stimulate further cytokine release and the secretion of secondary inflammatory mediators, such as prostaglandins, leukotrienes, platelet activating factor, oxygen free radicals and nitric oxide. This inflammatory process is beneficial to the host in that it localises and eliminates the infective focus. If this inflammatory response is sustained, however, the overspill of cytokines into the

systemic circulation can result in widespread tissue injury and organ dysfunction. (Blackwell 1996)

The main pro-inflammatory cytokines indicated in the development of SIRS are TNFα, IL-1β, IL-6 and IL-8. TNFα is produced by many cells of the reticuloendothelial system and is important for neutrophil migration and macrophage activation. It induces IL-1β expression. The administration of TNFα and IL-1β to animals will reproduce the pathophysiological events seen in patients with sepsis. IL-6 is a 21kDa glykoprotein with a wide range of biological functions, including activation of B and T lymphocytes and the induction of acute phase protein production. Levels of plasma IL-6 are an indicator of cytokine cascade activation in sepsis and are predictive of subsequent MOF and death. The primary function of IL-8 is to activate and chemo-attract neutrophils to sites of inflammation. Neutrophil activation by IL-8 in the lung is also thought to be important in the development of ARDS. (Miller 1992)

Given the profound biological effects of these cytokines, it is of no surprise that their production is tightly regulated at the level of gene transcription and the expression of cytokine mRNA. Specific transcription regulating proteins, called transcription factors, bind to receptor sites on the cytokine genes. In particular, the transcription factor nuclear factor κB (NF κB) is thought to have a central role in regulating the cytokine cascade. This is activated in many cell types in response to endotoxin and other stimuli. In addition, inflammatory stimuli also stimulate the production of anti-inflammatory cytokines and cytokine neutralising molecules, such as IL-10 and IL-1 receptor antagonist. It is thought, therefore, that following an inflammatory stimulus, the body induces a pro-inflammatory response (SIRS), a compensatory anti-inflammatory response (CARS) and an intermediate mixed antagonist response syndrome (MARS). (Davies 1997) The aim of these responses are to re-

establish Homeostasis, but other outcomes are possible including Cardiovascular shock, Apoptosis, multiple Organ dysfunction, Suppression of the immune system and sepsis syndrome.

1.3.2: DEFINING SIRS/MODS/SEPSIS:

In order to facilitate research into the pathogenesis of sepsis, the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee proposed a set of definitions for sepsis and the clinical syndromes associated with it. (American College 1992) Sepsis was defined as a Systemic Inflammatory Response Syndrome (SIRS) which results from infection, which can be bacterial, paracytic, protozoan or viral. The diagnosis of SIRS is made by two or more of the following:

1) Temperature of > 38°C (100.4°F) or < 36°C (96.8°F).

2) Heart rate >90 beats/min.

3) Respiratory rate > 20 breaths/min or PaCO2 <4.3 kPa (32 mm Hg),

4) White blood count >12,000 cells/mm^3, < 4,000 cells/mm^3 or 10% immature band forms.

The clinical outcome of sepsis syndrome appears to be related to the intensity of the host response. Nearly all patients with sepsis develop failure of one organ system, the most common being respiratory failure (ARDS), acute renal failure and disseminated intra-vascular coagulation (DIC). Approximately 30% will develop multiple organ failure (MOF), which can be defined as the failure to maintain physiological homeostasis without intervention.

The management of sepsis and its systemic sequelae is a challenge to physicians worldwide. The incidence of sepsis syndrome has been estimated at 500,000 cases per year in United

States alone, with an overall mortality rate of around 40%. The majority of cases of sepsis are secondary to Gram Negative bacteria, such as *Escherichia Coli* and *Klebsiella* species, with gram positive organisms accounting for an increasing proportion. At present, the mainstay of treatment of septic shock is antibiotics and supportive measures, but there has been little improvement in the outcome of this syndrome in the last 50 years.

PART 4:

1.4.1: GUT DIRECTED THERAPIES AND OPTIMISATION OF GUT FUNCTION:

The possibility that gut dysfunction or failure drives disease processes suggests that gut directed therapies to attenuate gut failure or preserve gut function may improve patient outcomes in day to day clinical practice.

There are a number of methods by which at least in theory gut function might be modulated favourably these include:

- Physical methods such as early post operative mobilisation
- Drugs such as Prokinetics and avoidance of opiates
- Immuno nutrients such as glutamine, arginine, fish oils, antioxidants and trace elements
- Modulation of GI microflora by using synbiotics and selective gut decontamination.
- Combination approaches.

There are many other individual and combined approaches that may modulate or optimise gut function.

1.4.2: Gut directed therapy immune nutrition Glutamine:

It is generally accepted that a significant proportion of hospitalised patients are malnourished and that malnutrition impairs immune function (McWhirter 1994). The capacity for nutrients to modulate the actions of the immune system and to affect clinical outcomes has thus become an important issue in clinical practice. Glutamine was extensively investigated in various clinical conditions in the recent past to understand its influence on various outcomes.

Glutamine is the most abundant non-essential amino acid in plasma (Darmaun 1986). Glutamine can be reclassified as a conditionally essential amino acid because of the body's inability to synthesize glutamine in sufficient quantities under certain circumstances such as major surgery, shock, traumatic injury and severe sepsis (Smith R J, 1990). Although glutamine constitutes >50% of the unbound amino acid pool in human skeletal muscle, rapid reduction in blood and tissue glutamine have been noted following catabolic events such as major surgery (Vinnars, 1975), trauma (Askanazi, 1978) and sepsis (Roth, 1985). Glutamine exhibits extremely rapid cellular turnover rates and is a source of oxidative energy. It also serves as a metabolic precursor in the biosynthesis of nucleotides, glucose, amino sugar, as well as in glutathione homeostasis and protein synthesis. It is a key link between carbon metabolism of carbohydrates and proteins in mammalian cells playing an important role in the growth of fibroblasts, lymphocytes and enterocytes (Cao, 1998: Neu, 1996). Glutamine synthesis occurs in skeletal muscle and liver when the plasma level is insufficient to satisfy the body's requirement. Decreased glutamine availability for macrophages and lymphocytes correlated with low plasma glutamine levels (Windmuller, 1981). Glutamine acts as the preferred respiratory fuel for lymphocytes, hepatocytes and intestinal mucosal cells and is metabolized in the gut to citrulline, ammonium and other amino acids (Boelens, 2001). Reduced arginine levels observed after trauma, can be restored to physiological levels using

glutamine supplementation whereas, the physiological levels of glutamine are only partly restored (Houidjk, 1994). Glutamate is a component of the antioxidant glutathione. Glutamine and cysteine are direct precursors in the production of glutathione. Animal studies have shown that glutathione depleted rats benefited from glutamine supplements (Hong, 1992).

Glutamine function	Mechanism
Function in metabolism	Nitrogen shuttle: urea and ammonia clearance. • Takes up excess ammonia and forms urea. • Nitrogen from ammonia is transferred to alanine via transamination with glutamate formed by reductive amination of alpha-ketoglutarate. Direct source of cell energy
Anabolism: anticatabolism	Decreases protein breakdown Rate-limiting factor or muscle growth Stimulates release of human growth hormone
Effect on wound healing	Direct fuel for fibroblast and macrophages Indirectly by preserving lean body mass
Preserves gut integrity	Primary fuel for gut enterocytes via glutamate via glutathione antioxidant action.
Immune function	Improves neutrophil bacterial killing and is a lymphocyte fuel
Antioxidant	Substrate for the key cellular and plasma glutathione via glutamate.

Table:1.1 Key functions of glutamine.

Adapted from Robert H. Demling, M.D., Patrick Seigne, M.D., World J. Surg. 24, 673-680, 2000.

1.4.2.2: Glutamine and the Immune System:

A recent *in vitro* study by Furukawa, Saito et al., (2000) evaluating the effect of glutamine supplements on phagocytosis and reactive oxygen intermediates (ROI) production by neutrophils and monocytes in postoperative patients concluded that glutamine enhances both phagocytosis and ROI production by neutrophils. They observed a dose dependent response most effective at concentrations >2000 μM. This is in keeping with the findings of Wilmore, et al., (1998) who concluded that immune function will be jeopardized at plasma glutamine levels <400 μM in post-op period amongst surgical patients (Wilmore; 1998).

1.4.2.3: Clinical Evidence for the use of Glutamine

There have been several studies showing the beneficial effect of glutamine on complications and clinical outcome. Many of such studies were done on trauma, burns, abdominal surgery and critically ill septic patients. Demling et al., 1998, demonstrated that glutamine therapy reduced hospital stay and improved patient outcome In a randomized trial of sixty patients grouped to either a standard enteral feeding or one containing 14.2 g glutamine/l, within 48 hr of admission, observed a significant reduction in the infection rate in the supplement group - 17% vs. 45% ($p<0.02$). A randomised prospective study using glutamine dipeptide as total parenteral nutrition concluded that the supplemented group had shorter hospital stay, improved immune status after abdominal surgery (Morlion; 1998). In another double blind randomised controlled trial in 168 patients' glutamine therapy was significantly associated with a reduction in hospital stay among surgical patients (Powell-Tuck; 1999).

1.4.2.4: Glutamine Administration in the Elective Surgery Patients:

A mild catabolic response has been well documented in patients following elective surgery. By standardizing the operation and anesthesia provided and by excluding patients with associated disease (such as diabetes mellitus or those requiring steroids), the effects of

nutritional manipulations such as glutamine administration on postoperative catabolic response have been investigated. No outcome studies are available in which to evaluate the effect of postoperative enteral nutrition with and without glutamine. However, Dechelotte et al. (1998) studied protein turnover in 16 patients receiving tube feeding after esophagectomy with enteral glutamine supplementation. The endogenous de novo synthesis of glutamine was reduced by 32% ($p < 0.03$) and phenylalanine oxidation was reduced by 26% ($p > 0.05$). Protein turnover was similar in both groups. Aosasa et al. (1999) gave oral glutamine to patients receiving preoperative parenteral nutrition and compared their findings with nonsupplemented individuals. After surgery, they harvested blood mononuclear cells and stimulated production of tumor necrosis factor and interleukin-10. In patients receiving standard parenteral nutrition, there was an increase in cytokine production; this was greatly attenuated in the glutamine group, supporting the concept that glutamine may modulate the proinflammatory cytokine response. Fish et al. (1997) compared the effects glutamine-enriched parenteral nutrition with enteral feedings of similar composition administered to patients after gastric or pancreatic resections. Nitrogen balance and plasma protein concentrations were comparable between the two groups. Plasma concentrations of glutamine did not differ significantly between the two groups although these data tended to suggest that glutamine levels, which fell postoperatively, recovered more slowly in the enterally fed group. It should be noted, however, that nutrient delivery was increased only gradually to 100% of recommendations in the postoperative period in both groups. This gradual increase in the intravenous solution is inconsistent with clinical practice, and administering the usual quantity of total parenteral nutrition on the first postoperative day may have resulted in higher glutamine concentrations by day 5 post-surgery when measurements were made.

1.4.2.5: Glutamine Administration in Critically ill:

Glutamine is a major fuel and nucleotide substrate in both enterocytes and the gut associated lymphoid tissue (Ziegler; 2000). During critical illness the gut mucosal cells, deprived of glutamine, cease to perform their barrier function and allow entry of luminal toxins and bacteria directly into the portal bloodstream (Ziegler; 2000). Conventional total parenteral nutrition (TPN), which is glutamine-free, can actually worsen mucosal permeability. The body then breaks down muscle protein to release glutamine and aminoacids for the gut and immune system

Either enteral or parenteral provision of glutamine can maintain the intracellular or extracellular glutamine pool (Stehle; 1989). Parenteral glutamine supplemented nutrition promotes growth and nitrogen retention and there is less muscle loss during stress (Stehle; 1989). Glutamine lessens pancreatic atrophy and hepatic steatosis associated with TPN or elemental enteral diets (Karner; 1989).

In the critically ill patient on the intensive-care unit, parenteral nutrition is used only when enteral feeding route is unsuccessful or impractical. Conventional enteral feeds provide some glutamine (protein-based enteral products 6–8 g/day, and peptide-based products 1–5 g/day), but this is insufficient for the critically ill patient and some researchers argued for supplements of 10–20 g/day (Kuhn; 1996). 50–80% of free glutamine is absorbed by the gut during routine enteral feeding and plasma glutamine can be seen to rise with supplementation. Supplemented enteral feeds can reverse the changes in intestinal permeability associated with parenteral feeds, possibly by yielding a high glutamine concentration (>2.5 mmol/L) in the gut lumen(Beier-Holgersen; 1996, Houdijk;1998). Some researchers find that addition of glutamine to enteral nutrition formulas in critically ill patients reduces rates of pneumonia, sepsis and bacteraemia, with shortened hospital stays

and lower hospital costs(Houdijk; 1998), but others report no benefit (Hall; 2003). In a systematic review of work on enteral glutamine supplementation in critically ill patients, Novak *et al.. 2003,* have concluded there was no reduction in mortality, complications or hospital stay.

Significant improvement in net nitrogen balance in the glutamine supplemented TPN subgroup was associated with maintenance of the intracellular glutamine pool In critically ill surgical patients (Wischmeyer; 2001), TPN supplemented with Glutamine improved nitrogen economy, had an immunostimulatory effect on lymphocytes, maintained plasma glutamine concentration and shortened hospital stay (Stehle; 1989, Pastores; 1994). Since then, glutamine-enriched TPN has been found beneficial in several other patients with critical illness (Lacey; 1996). Glutamine supplementation can help to maintain the structure and function of the small bowel in patients undergoing bone marrow transplantation(Ziegler; 1992). After bone marrow transplantation the treatment has been associated with increased percentages of lymphocytes and improved markers of T-cell function together with a degree of hepatic protection, possibly due to its effects on tissue glutathione concentrations (Ziegler; 1992); moreover, glutamine-supplemented patients showed advantages in psychosocial wellbeing, whether through some direct action in the brain or through its effects on protein status. Intravenous glutamine supplementation in severely burned patients reduced Gram-negative bacteraemia, with a trend towards lower mortality (Griffiths;1997). In a meta-analysis, Novak *et al*. 2002, combined two large studies of critically ill patients who had gastrointestinal failure and received parenteral nutrition with glutamine supplementation. Their conclusion was that, after six days of TPN, glutamine-supplemented patients had a moderately (but significantly) lower mortality rate than the non-supplemented. They also concluded that a glutamine dose higher than 0.20 g/kg per day has a greater effect than a lower dose (Novak; 2002).

1.4.2.6: Glutamine Summary:

Two decades ago, Ziegler *et al.* indicated the potential benefits of glutamine supplementation, still many of their predictions are in research state with no clear cut use in day to day clinical practice. In enteral feeds, glutamine supplementation has not yielded clear benefit. One reason may be the difficulty of administering high doses, especially during the early course of the illness. With parenteral glutamine, the patients most likely to benefit are the critically ill and those at high risk of gut dysfunction. In such patients, a dose of 15–25 g per day may be sufficient. The availability of stable preparations of glutamine dipeptide opens the way to better management of the subgroups of patients most at risk of glutamine depletion in day to day clinical practice.

1.4.3: ARGININE:

Arginine is a nonessential amino acid in the normal physiological state that becomes conditionally essential during periods of hypermetabolic stress. Current literature is conflicting on arginine use in the clinical setting, with some proposing it as a panacea, whereas others report it as poison. The use of immune-modulating nutrients and formulas has gained popularity in recent years all over the world in many clinical conditions, where by patients are managed by specialist nutrition teams. Several immunemodulating formulas are currently commercially available for clinical use. Some combination of the nutrients arginine, n-3 fatty acids, glutamine, antioxidants, and nucleic acids are those most commonly found in these formulas. Arginine, one of the key components of these formulas, has gained specific attention.

1.4.3.1:Arginine metabolism:

Arginine synthesis and catabolism in specific body tissues is conditioned by the presence of arginosuccinase and arginase respectively, but only periportal hepatocytes and, to some extent, certain brain areas possess all the enzymes required for arginine recycling and urea

synthesis. Gut acts as a user of arginine because it possesses arginase (isoenzyme II) and ornithine carbamoyltransferase. Enterocytes also express ornithine decarboxylase and an NADPH2 dependent arginine deiminase which respectively lead to local production of aliphatic polyamines and nitric oxide.

L-Arginine is available to the host from endogenous synthesis (via citrulline conversion in kidney), endogenous protein breakdown, and dietary protein sources (diet only contributing; 20–25% of total arginine supply). Arginine is a prominent intermediate in polyamine synthesis (helps in cell growth and proliferation) and proline synthesis (helps in wound healing and collagen synthesis) and is the only biosynthetic substrate for nitric oxide (NO) production.

NO is a potent intracellular signalling molecule that influences virtually every mammalian cell type. Arginine also serves as a potent modulator of immune function via its effects on lymphocyte proliferation and differentiation (Morris *et al.*; 2004) as well as its benefits in improved bactericidal action via the arginine NO pathway (Marin *et al.*; 2006).

In critical illness the de novo synthesis and dietary intake of arginine is markedly reduced. In spite of reduced supply, the cellular demand for arginine is increased. This increased demand in trauma, surgery, sepsis, and critical illness is driven mainly by the upregulation of arginase yielding urea and ornithine, and inducible NOS (iNOS) yielding NO and citrulline (Luiking *et al.;* 2004). This up regulation of arginase has been the focus of attention in the recent literature, for its importance in potentially reducing NO levels, presumably by reducing the available arginine (Morris *et al.;* 2004). Elevated levels of arginase, as reported in acute trauma and surgery, resulted in inhibition of NO synthesis and alterations of gene expression. The increased arginase levels within the activated macrophage results in limiting T-cell

proliferation.These changes in arginase activity resulted in impaired immune function at multiple levels of the immune response (Bansal *et al.*; 2003).

1.4.3.2: Arginine clinical evidence:

Both animal and human data are available to support the argument that arginine is potentially beneficial in some models, whereas others have argued that arginine is toxic and could potentially have an adverse influence on clinical outcomes (Luiking *et al.;* 2004, Marik *et al.;* 2006).

Animal models of supplemental arginine in sepsis have yielded a variety of results. Reviewing the rodent, rabbit, guinea pig, dog, sheep, and pig data, the results are very mixed, again depending on the model of infection or sepsis, dose, method of delivery, and species. The results are an almost equal mix of benefit, no change, or adverse effect (Minhao Zhou *et al.;* 2007). Using a canine model of *Escherichia coli* sepsis, intravenous L-arginine was studied as monotherapy by Kalin *et al* in 2006. This study showed that, the arginine supplementation resulted in higher mortality. The main problem with extrapolation of this study results to a human model is that the this study used supraphysiologic doses of i.v arginine at 10–20 times greater than the highest levels given in any of the human studies. Arginine delivered via the GI tract undergoes metabolism to the order of 40% before ever reaching the portal circulation (Marik *et al.;* 2006). The liver further metabolizes the arginine delivered to it from the portal circulation. Hence these results cannot be extrapolated to human models.

Arginine i. v infusion in septic humans has been recently reported by Luiking *et al*., in 2006. This prospective randomized double-blind placebo control study evaluated 18 severely septic patients. This study showed no adverse effects on overall clinical outcome of these severely septic and critically ill patients. Galban et al., in 2000 reported that addition of an immune-

modulating formula that contained arginine was beneficial in improving outcome. This benefit was observed in only the sub-group with a low APACHE score of <15.

The arginine controversy revolves around interpretation of very few well known studies. Galban *et al.*, showed benefit in the moderately septic patient using a high arginine-containing formula (supplemental arginine 12.5 g/L). Dent *et al.*, reported a placebo-controlled study showing a higher mortality in the experimental group that received a low arginine formula (supplemental arginine 5.5 g/L). The other study that reported adverse effects of supplemental arginine was Bertolini *et al.* This group compared a low arginine-containing enteral formula to total parenteral nutrition. The study was discontinued prior to completion and was poorly stratified, with most patients having pneumonia on entry into the study. Heyland *et al.*, in 2001, concluded in a meta-analysis, that arginine was potentially toxic in the medical ICU population. This conclusion was based mainly on a quality score rate given to each study in the meta-analysis.

1.4.3.3: Dosing of arginine:

The optimal dose of supplemental arginine is yet to be determined. It is well recognised that arginine plasma levels rapidly decline in critical illness, trauma, and sepsis. This decrease in plasma levels is thought to result from decreased intake, increased tissue uptake, and increased metabolism. Arginine transport in catabolic states is accelerated in several tissue beds including liver, intestine, and endothelium. It can be extrapolated from available studies that the 15–30 g of enteral supplemental arginine is safe and meets requirement.

Cytotoxic effects of NO and arginine	Protective effects of NO and arginine
• Metabolic pathway inactivation • Damage to cell structure • Lipid peroxidation • Nitration of tyrosine • Oxidation of sulfhydryl groups • DNA mutations • DNA strand breaks • Activates poly(ADP-ribose) polymerases • Alterations in gene expression • Possible detrimental hypotension in sepsis	• Hepatic damage following septic insult • GI injury and splanchnic permeability • Pulmonary HTN • Lung neutrophil infiltration • Myocardial ischemia • Secondary sinus infections • Inhibits apoptosis • Antiinflammatory mediator • Downregulator of intercellular adhesion molecule • Decreases neutrophil adhesion • Prevents endothelial damage • Platelet aggregation • Leukocyte adherence • Free radical scavenger • Enhances anastomotic healing • iNOS inhibitor in sepsis is harmful

TABLE:1.2: Cytotoxic and protective effects of arginine and NO

Table: Adapted from Minhao Zhou and Robert G. Martindale the journal of nutrition: 2007: (S): 1687-1692.

1.4.3.4: ARGININE SUMMARY:

With this available evidence, arginine controversy will continue until more robust studies have consistently confirmed either benefit or detriment of arginine supplementation. Before any firm conclusion or consensus is made on arginine use in the day to day clinical setting, one must first define the patient's injury or illness; establish the level of nutrients to be delivered and state of gut. The numerous potential beneficial effects of arginine include: stimulation of immune function via its influence on lymphocyte, macrophage, and dendritic cells, improved wound healing, increased net nitrogen balance, increased blood flow to key vascular beds, and decreased clinical infections. The potential that arginine may have detrimental effects in patients at the doses delivered in clinical conditions must also be considered, although this appears to be supported more by theoretical concepts rather than clinical data.

1.4.4:Fish oils:

Since 1970s, much epidemiologic evidence has demonstrated that diets high in ω-3 fatty acids decrease the incidence of cardiovascular disease and improve the outcome of hypertension and coronary diseases. The ω -3 fatty acid supplements also seems to have beneficial effects on certain kinds of auto immune diseases like arthritis, lupus, and thromboembolism. Consequently, the diet fat spectrum alteration is becoming one of the principal prevention strategies for these disorders. Since the 1990s, the therapeutic role of ω -3 fatty acids has been recognized in clinical nutrition. A few enteral nutrition products that contain fish oil have been used in surgical patients with clinical conditions like malignancy and severe sepsis. In the late 1990s, commercial fish oil emulsion became available in clinical settings for parenteral use. Several experimental and clinical studies have demonstrated the effects of fish oil emulsion in inflammation regulation, membrane lipid metabolism regulation, and organ function preservation.

Han *et al.*, in 2012 reported the effect of ω-3 fatty acids on immune and inflammatory modulation in surgical intensive care patients. In this RCT they have reported reduced serum inflammatory cytokine levels, reduced post operative liver dysfunction and reduced infective complications following major surgery associated with intravenous ω-3 fatty acid supplemented TPN.

Bin Liang et al., in 2008 reported the effects of ω-3 fatty acids supplemented TPN in a RCT including forty two patients undergoing elective colo-rectal surgery for cancer. This trial has concluded Postoperative supplementation of TPN with ω -3 fatty acids have a favourable effect on the outcomes in colorectal cancer patients undergoing radical resection by lowering the magnitude of inflammatory responses and modulating the immune response.

Recently, several small studies have shown nutritional supplementation with fish oil is associated with improved clinical outcomes in chronic hyper-inflammatory diseases such as Crohn's disease (Yao et al., 2005) rheumatoid arthritis (Berbert et al., 2005), cancer cachexia

(Larsson et al., 2004), and as an adjunct therapeutic measure for trauma, and sepsis (Mayer et al., 2006: Berger et al., 2007). Although several studies have demonstrated the beneficial effects of omega-3 fatty acid supplementation on patient outcome or immune competence, randomized controlled clinical trials focusing on the use of parenteral fish oil in critically ill and surgical patients are very few in number.

1.4.5: OXIDATIVE STRESS, ANTI OXIDANTS:

Oxidative stress is increasingly being recognized as central to the underlying pathophysiology of various illnesses, especially organ injury and the development of organ failure in critically ill. Reactive oxygen species (ROS) and reactive nitrogen-oxygen species (RNOS) are capable of causing cellular dysfunction and tissue destruction by several well described pathways. In critical illness ROS can be produced from mitochondrial dysfunction, seen during ischemia reperfusion injury as is classically observed in septic shock.

In humans there is a complex endogenous defence system designed to protect tissues from ROS/RNOS induced cell injury. Special antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase (including their cofactors selenium, zinc, manganese, and iron), and vitamins (i.e., vitamins E, C, and b-carotene) form a network of functionally overlapping defense mechanisms. The circulating antioxidant levels decrease rapidly after insult, trauma, or surgery and remain below normal levels for several days or even weeks (Metnitz et al., 1999). Reduced endogenous stores of antioxidants are associated with an increase in free radical generation, an augmentation of the systemic inflammatory response, subsequent cell injury, increased morbidity, and even higher mortality (Goode 1995, Metnitz 1999).

The association between increased oxidative stress and poor outcomes in the critically ill is well documented (Neve, 2002, Paterson, 2003). Current literature is still not clear whether antioxidant supplementation is beneficial, and the trials until now have not enabled definitive conclusions as they generally included small patient populations. None of the reported trials using antioxidant as intervention reported deleterious effects of their administration. One can argue that the antioxidant administration is safe in clinical setting. The administration of antioxidant therapy in many reported studies was generally initiated upon admission to the ICU, suggesting that timing of the intervention is important. Given the heterogeneous nature of the populations included in trials showing clinical benefit like trauma, surgical, burns, pancreatitis, SIRS, head injury, one can argue that the antioxidant therapy is effective in wide

ranging clinical conditions. Daren et al., in 2005 have summarised the clinical benefit of antioxidant supplementation in their systematic review and concluded that the antioxidant supplements are safe and associated with reduced morbidity and mortality in critically ill patients. However this needs to be established in larger clinical trials to better understand benefits and the mechanisms involved.

1.4.6: PREBIOTICS, PROBIOTICS AND SYNBIOTICS:

There is strong symbiotic relationship between the GI microflora and the human host. Luminal nutrients, which serve as an energy source for the GI microflora, are further metabolised to compounds required to sustain intestinal homeostasis. The colonic microflora is also a fundamental component of the gut-mucosal barrier. This barrier separates the luminal contents from the sterile extra-intestinal tissues.

The GI microflora has been implicated in the pathogenesis of variety of clinical conditions, ranging from infective diarrhoea, atopic eczema, pneumonia and sepsis.

The GI microflora in humans can be modulated by ingesting large number of live bacteria called probiotics or taking prebiotic substances which stimulate endogenous bacterial populations. When pre and pro biotics are used in combination it is described as synbioti (Collins 1999).

There is considerable evidence from animal studies to suggest that synbiotics can modulate the mucosal and systemic immune system and this modulation is beneficial to the host.

Synbiotics have been experimented in clinical practice to modulate GI microflora for host health benefit. Several human studies in surgical patients, patients with Crohn's disease, patients with irritable bowel syndrome and critically ill patients have reported equivocal results. There are sound theoretical reasons to support the use of synbiotics to modulate GI microflora for health benefit.

1.4.7: SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT (SDD):

Selective decontamination of the digestive tract (SDD) has been advocated as a method of reducing nosocomial infections in people at high risk for opportunistic infections, particularly for surgical patients in the intensive care unit. SDD involves the application of topical, non-absorbable antibiotics to the gastrointestinal tract, often including a course of intravenous antibiotics to control early systemic infections. The goal is to eradicate yeast and Gram-negative aerobic bacteria without harming natural anaerobic flora.

In several human trials various combinations of antibiotics have been used and reviewing those combinations and controversies surrounding this issue is beyond the scope of this thesis work. There is reasonable evidence from several reviews to suggest SDD is associated with reduced infective complications, and improved clinical outcomes in terms of morbidity and mortality amongst surgical and critically ill patients.

1.5: THESIS HYPOTHESIS:

After reviewing the literature there is ample evidence to suggest that the state of gut function conditions the integrity of the intestinal barrier, influences BT and affects the development of SIRS, sepsis, MOF and even death. Even though inadequate gut function or gut failure has been implicated in poor clinical outcomes, definitive evidence is elusive.

The central aim of this thesis is that the gut failure is associated with SIRS, MODS and poorer clinical outcomes of patients. As a result, gut directed therapies that optimise gut function by attenuating the gut failure could possibly improve patient outcomes. This thesis will investigate the poorly recognised phenomenon of gut dysfunction and its effects on SIRS/MODS, sepsis and patient outcomes.

CHAPTER TWO

AIMS AND METHODS

2.1 Aims of this thesis:

The gut is not just an organ of digestion. It is the largest immunological organ in the body, has an essential antigen recognition role, maintains a stable ecoflora which is essential to our health and well being and maintains the critically important intestinal barrier which prevents escape from the lumen of the gut of potentially pathogenic enteric bacteria.

Although it is now widely accepted, that the gut plays a major role in the pathogenesis of SIRS, MODS and sepsis. It is possible that unrecognised gut failure or inadequate gut function may have adverse consequences for patient prognosis in day to day clinical practice. Clinical interventions to monitor and treat this organ system remain very limited.

After reviewing the literature in Chapter one, the main aims of this thesis will be:

1. To investigate gut function and its influence on SIRS, MODS, sepsis and clinical outcomes in patients admitted with acute pancreatitis (chapter 3).

2. To assess the surgical stress, state of gut function and clinical outcomes in patients undergoing non gastrointestinal surgery (chapter 4).

3. To investigate gut function and its influence on clinical outcomes amongst critically ill intensive care patients receiving adjuvant nutritional support (chapter 5).

4. To propose a gut directed therapy with a view to optimising gut function and to investigate the influence of this intervention on systemic inflammatory response and clinical outcomes amongst patients undergoing elective colo-rectal surgery for cancer (chapter 6).

2.2 General Introduction:

This chapter details various studies and technical aspects of laboratory-based assays performed as part of this thesis. Justification for the methods used, including an assessment of their limitations, will be discussed individually in relevant chapters of this thesis.

2.3 Series of Clinical studies:

All the studies described in this thesis were performed at Combined Gastroenterology Research unit, Scarborough general Hospital. All the work towards this including writing up of research protocols, submission of these for research and ethical approvals, Recruitment of patients in to these trials, appropriate sample collection and laboratory analysis was performed by the author. Some of the laboratory work was performed at Department of Biological Sciences, The University of Hull. Due recognition to the work and contribution of others has been given where appropriate.

2.3.1 Patients with acute pancreatitis:

In this prospective observational study, thirty patients admitted to Scarborough general hospital with acute pancreatitis were investigated for state of their gut function and its influence on SIRS, MODS, Sepsis and clinical outcomes. This study is described in detail in chapter 3.

2.3.2 Critically ill patients receiving adjuvant nutritional support:

This was a prospective observational study including forty critically ill patients admitted to intensive care unit at Scarborough general hospital. Twenty of these patients had inadequate gut function hence were receiving total parenteral nutrition and the remaining twenty patients were assessed to have adequate gut function and were on enteral nutritional support. These

patients were studied to understand the relationship between the gut function and its influence on clinical outcomes with a view to design a gut directed therapy and test the therapy in a clinical setting. This study has been described in detail in chapter 4.

2.3.3 Patients undergoing elective surgery:

In this prospective observational study, twenty patients undergoing elective breast surgery for breast carcinoma at Scarborough general hospital were investigated to assess surgical stress response, state of their gut function and their influence on clinical outcomes. These patients were compared with twenty elective colo-rectal surgical patients undergoing elective right hemi colectomy for cancer. This ground work was essential to understand relationship between the surgical stress response and clinical outcomes in relation to the gut function. This study is described in detail in chapter 5.

2.3.4 Patients undergoing elective colo-rectal surgery for cancer:

This study was a prospective randomised controlled clinical trial including one hundred patients with colonic carcinoma undergoing elective open colorectal surgery. Patients were randomised to two groups, control and intervention group. Control group received standard perioperative care and intervention group received the proposed intervention (gut directed therapy) over and above the standard perioperative care received by the control group. These groups were compared and contrasted with respect to various clinical and laboratory outcomes to assess whether the proposed gut directed therapy (optimised gut function) provides any clinical benefit. This study has been described in detail in chapter 6.

2.4: Measured clinical outcomes:

Numerous clinical outcomes were prospectively recorded in the series of clinical trials presented in this thesis. This section describes the clinical outcomes measured in more than one trial and further details where relevant were described in relevant chapters.

2.4.1: Systemic inflammatory response syndrome:

Systemic inflammatory response syndrome was defined as the presence of two or more of the following criteria.

Temperature	>38^{o} C or <36^{o}C.
Heart rate	>90 beats/min.
Respiratory rate	>20 or $PaCO_2$ <32 mm Hg.
WBC count	>12K or <4K or >10% immature neutrophils

Table 2.1: SIRS criteria.

2.4.2: Septic morbidity:

Septic morbidity was defined as the presence of pathogens in the body tissues that are normally sterile, confirmed by result of culture and supported by clinical, radiological or haematological evidence.

Wound Infection: clinical evidence of inflammation e.g. erythema, tenderness and discharge from the wound.

Chest Infection: Isolation of pathogens in purulent sputum with or without evidence of Pneumonia on Chest X-ray.

Intra-abdominal abscess**:** Clinical or radiological evidence of intra abdominal collection.

Urinary Tract Infection**:** Isolation of bacteria in urine samples upon culture with Concentration $\geq 10^4$/ml.

These isolates will be identified by standard laboratory techniques, as part of the standard patient care.

2.4.3: Length of hospital stay:

Hospital stay was recorded in days prospectively. Any readmission to hospital within thirty days of discharge was also recorded and this data was analysed appropriately in relevant chapters and presented as part of final analysis.

2.4.4: State of gut function:

Extensive research work was carried out in the recent past in our unit to define the state of gut function by *Gatt, et al.*. Gut function was defined based on the primary function of gut that is nutrition. Normal gut function was defined as the oral/enteral tolerance of ≥80% of calculated nutritional requirements for a continuous period of ≥48 hours. Anything less than this was defined as attenuated or inadequate gut function. This definition was adopted in all the clinical trials and the state of gut function was studied in relation to clinical outcomes. Data was analysed in appropriate chapters and presented as final results.

2.4.5: Mortality as clinical outcome:

Any death during hospital stay or within thirty days of discharge was prospectively recorded and analysed as data point in appropriate studies.

2.5: Measured laboratory outcomes:

As part of series of clinical trials described in this thesis numerous laboratory tests were conducted both at Scarborough hospital and also at the University of Hull. Those tests were used in more than one trial are described in this section. Further details are described in relevant chapters.

2.5.1: Assessment of Gut Barrier Function:

The human gut-mucosal barrier separates the sterile extra-intestinal tissues from the vast bacterial load in the gastrointestinal tract, whilst enabling the digestion and absorption of nutrients and water. Given the complex physiochemical composition of the gut-barrier, no one investigative technique can claim to assess gut barrier function completely. Commonly quoted markers of gut barrier function include intestinal permeability, bacterial translocation and gastric colonisation.

Confirmation of bacterial translocation by microbiologic identification of organisms in normally sterile, extra-intestinal tissues remains the ‘gold standard’ for assessing the integrity of the gut barrier. The technique of mesenteric lymph node sampling as a measure of bacterial translocation has been shown to be technically feasible and reproducible in both animal and human research. (Ambrose 1984, Deitch 1990, Sedman 1994) Lymph node harvest may, however, under estimate the true prevalence of bacterial translocation in humans. In this study mesenteric lymph node was harvested after complete mobilisation of

right or left colon as per standard surgical technique before ligation of vascular pedicle from ileocolic (for right colectomy) or inferior mesenteric (for left sided colonic resections) chain using a sterile surgical scalpel. Bacterial translocation can also be detected by sampling portal venous blood or thoracic duct lymph. Although this is commonly performed in animal studies, the morbidity associated with these techniques in elective surgical patients made their use unethical (Moore 1991, Duce 1996). Bacterial translocation is thought to occur by transcellular or paracellular route via the enterocyte tight junctions (Berg 1999, Botterill2000). One other method of assessing bacterial translocation is by culturing serosal biopsies. One limitation of serosal biopsies is that transmural inflammatory conditions of the bowel may allow bacteria to enter serosa resulting in falsely higher levels of bacterial translocation (Ambrose 1984). In this series, bacterial translocation was assessed by mesenteric lymph node sampling.

2.5.1.1 Bacterial translocation:

Once mesenteric lymph node samples were collected, they were transported to the laboratory immediately in a sterile universal containing normal saline. Mesenteric lymph node samples were homogenised with 0.5 to 1ml of peptone water in a stomacher (Seward Medical, London, UK). Direct culture for bacteria from the homogenate was carried out on Columbia blood agar and cysteine lactose electrolyte deficient (CLED) media (Oxoid, Basingstoke, UK) for aerobic culture and incubated in air with 5% carbon dioxide. Wilkins-Chalgren blood agar with neomycin and Columbia blood agar were used for anaerobic culture in Don Whitley modular atmosphere controlled system (MACS) cabinet in an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. Aerobic cultures were performed at 37°C for 2 days and anaerobic cultures were performed at 37°C for 5 days. Isolates grown on culture plates were identified by characteristic colonial and microscopic appearances (*Stokes* and *Ridgeway*, 1980 and *Barrow* and *Feltham*, 1993) and by using the API 20E bacterial

identification strips (Bio Merieux, France). The API-20E test strip has 20 separate test compartments on the strip. Bacterial suspension was used to rehydrate each well on the strip. Some of the wells have colour changes due to pH, others products were identified with reagents provided by the manufacturer. A profile number was determined from the sequence of positive and negative test results. A codebook showing correlation between numbers and bacterial species was used to identify bacteria. Fungi were identified by the Auxacolor colorimetric identification system (AUX) (Sanofi Diagnostics Pasture, France). The AUX micro well plate was inoculated according to the manufacturer's instructions by using 2 drops of the test strain suspended in the medium supplied with the kit and incubated at 30°C. Color changes were noted, and a code was developed, this was compared to the database provided by the manufacturer in order to identify the fungus.

2.5.1.2: Gastric colonisation:

Mucosal surface area of the human gastrointestinal tract is about 200-300 m^2 and is colonised by about 400-500 different species of organisms. Savage (1986) classified gastrointestinal microflora into indigenous flora and transient flora. Indigenous microorganisms colonise particular habitats i.e., physical spaces in the gastro intestinal tract, where transient organisms cannot reside, except under abnormal conditions. Most pathogens are transient to the human body; but small number of pathogens can be indigenous to the ecosystem and live in harmony with the host, till the eco system is disturbed. The prevalence of bacteria in different parts of the gastro intestinal tract appears to depend on several factors, such as pH, peristalsis, redox potential, bacterial adhesion, bacterial co-operation (synergism), bacterial antagonism (colonisation resistance), mucin secretion and specific nutrient availability (*Hao & Lee*, 2004). Due to low pH and swift peristalsis in the stomach, bacterial concentrations are very low and consist of mainly acid tolerant lactobacilli and streptococci. The presence of *Enterobacteriaceae*, multiple organisms or Fungi in the proximal gastro intestinal tract is

unusual and may represent a disturbance in the normal ecosystem of the gastro intestinal micro flora. Gastric colonisation was included in this study as an indicator of gut-barrier function.

All patients were fasted for a minimum of six hours for solids and two hours for clear fluds prior to insertion of nasogastric tube. A nasogastric tube was inserted and aspirates were taken immediately after induction of general anaesthesia and before commencement of surgery. The first 5 ml of aspirate was discarded, and a further 10 ml was then aspirated using a sterile syringe and transported to the laboratory in a sealed sterile container. These samples were submitted for microbiological culture and pH analysis. This technique has been shown to yield reliable and reproducible results, compared to those obtained by direct sampling of gastric contents at laparotomy (Gorbach 1967). The nasogastric tube was removed at the end of the surgical procedure.

Initially pH of the sample was determined using a hand held pH meter (pH Boy K5071, Camlab, Cambridge, UK). Following this the aspirate was cultured. Aerobic cultures were inoculated onto Columbia blood agar and CLED medium and incubated in air plus 5 per cent carbon dioxide at 37°C for 48 hours, whilst anaerobic cultures were inoculated onto Columbia blood agar and Wilkins-Chalgren blood agar with neomycin and incubated at 37°C for 72 hours. A Sabouraud dextrose media was also utilised to ascertain the presence of any yeasts or fungi in the sample. All isolates were stored in a bead recovery system at -72°C. The isolated organisms were each identified using standard techniques as before.

Both mesenteric lymph node and nasogastric samples were analysed by Mr. P Sudworth, chief medical microbiology technician at Scarborough Hospital.

2.5.2 Systemic inflammatory response:

To quantify the systemic inflammatory response, serum levels of C-reactive protein (CRP), Albumin, Interleukin 6 (IL-6), Interleukin 10 (IL-10), Tumor necrosis factor α (TNF-α) were measured.

2.5.2.1 Serum C-reactive protein:

C-reactive protein (CRP) is a member of the pentraxin family of proteins. In response to tissue damage or infection, increased levels of acute phase proteins are produced by the liver and can be measured in the peripheral blood. CRP, fibrinogen and complement proteins increase, whilst plasma albumin concentrations decrease. The CRP protein combines with bacterial polysaccharides or phospholipids released from the damaged tissue to become an activator of complement pathway. Serum CRP levels increase rapidly in response to acute inflammation and is useful as a marker of infection (Mayne 1994).

For purposes of this thesis, appropriate blood samples were collected at predetermined intervals in various studies. All the samples were analysed for serum CRP levels by the staff at Department of Biochemistry, Scarborough Hospital, by using turbidometric method (Synchron CX Systems, Beckman Coulter Inc., Fullerton, CA, USA). The process of CRP level estimation was fully automated and involved mixing the serum with a reagent containing specific anti-CRP antibodies, forming insoluble antigen-antibody complexes. This caused a change to the optical density of the mixture. This could be detected by a change in the absorbance measured at a wavelength of 340 nm. This change was proportional to the CRP concentration in the specimen. The intra-assay coefficient of variance (CV) for this test was 4.7 per cent, while the inter-assay CV was 6.9 per cent.

2.5.2.2 Serum albumin:

Human albumin is the most abundant protein in blood plasma. It is produced in the liver. Albumin constitutes about half of the blood serum protein. It is soluble and monomeric. Albumin transports hormones, fatty acids, and other compounds, buffers pH, and maintains osmotic pressure, among other functions. Albumin is synthesized in the liver as pre-proalbumin, which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin. Albumin represents one of the 'negative' acute phase proteins, with a reduction in serum levels occurring as a consequence of the acute phase response. Serum levels were considered to be within the reference range between the concentrations of 38 and 47 g/L. For purposes of this thesis, blood samples were collected appropriately at pre determined intervals and details are discussed in appropriate sections. Serum albumin assays were performed by the laboratory staff in the Biochemistry Department at Scarborough Hospital using routine auto-analysis (Cobas Integra 800, Roche Diagnostics Ltd, Lewes, UK). As part of this automated process, the serum sample was mixed with the anionic dye bromcresol green at a pH of 4.3. This binds to albumin to form a blue/green coloured complex imparting a similar colour to the reagent mixture which can be assayed using light absorbance methods. The absorbance of the mixture was measured at a wavelength of 629 nm, with the intensity of the blue/green colour being directly proportional to the serum albumin concentration, which was calculated automatically based on a non-linear calibration curve. The intra-assay CV was 1.2 per cent, while the inter-assay CV was 1.5 per cent.

2.5.2.3: Serum Interleukin 6 (IL-6) ELISA:

Interleukin-6 is a multi-functional cytokine that regulates immune responses, acute phase reactions and haematopoiesis and may play a central role in host defence mechanisms. The gene for human IL-6 has been localized to chromosome 7p21. IL-6 is usually not produced constitutively by normal cells, but its expression is readily induced by a variety of cytokines, lipopolysaccharide or viral infections. IL-6 is a pleiotropic cytokine produced by a variety of cells. It acts on a wide range of tissues, exerting growth-induction, growth-inhibition, and differentiation, depending on the nature of the target cells. IL-6 is involved in induction of B-cell differentiation, induction of acute phase proteins in liver cells, induction of IL-2 and IL-2 receptor expression, proliferation and differentiation of T cells, enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells and induction of maturation of megakaryocytes as a thrombopoietic factor. Levels of IL-6 have previously been shown to be an indicator of cytokine cascade activation in sepsis and are a predictor of subsequent multi organ failure and death (Blackwell 1996). IL-6 is involved in the induction of acute phase proteins and induction of fever. Elevated serum levels of IL-6 are also found in patients with severe burns, in serum and plasma as a marker for predicting postoperative complications.

Principles of ELISA for IL-6:

Serum levels of IL-6 were determined using a quantitative enzyme-linked immunosorbent assay technique (Bender MedSystems GmbH). The principle of this assay is as follows. A monoclonal anti-human antibody specific for IL-6 has been adsorbed onto the micro wells of test kit plate. Standards and samples are pipetted into the wells and any human IL-6 present in the test sample is bound by the immobilised antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is

added to the wells and colour develops in proportion to the amount of IL-6 bound in the first step. The colour reaction is terminated by acid (stop solution) and the intensity of colour is measured using a micro plate reader at a wavelength of 450nm.

Serum collection

10mls of venous blood was obtained from patients and decanted into a plain tube. Samples were sent to the laboratory at Scarborough Hospital within one hour of venesection. Serum was obtained by centrifuging the sample at 1000 rpm for 10 minutes. The serum was alliquotted into four epindorph vials and stored at -80°C. This method of storage was used to avoid loss of bioactive human IL-6 from the samples. These samples were batched in groups of forty. All patient details were removed from these vials and coded with unique identification number. Each set of forty samples were transported on dry ice to the University of Hull Laboratory for ELISA. Samples were defrosted for two hours before use.

Reagent preparation

Wash Buffer: 50mls of wash buffer concentrate was diluted in 950mls of distilled water to produce 1000mls of wash buffer.

Substrate solution: Colour reagents A and B were mixed together in equal volumes 15 minutes prior to use, and wrapped in foil to protect from light activation.

Assay Buffer: 5ml of provided assay buffer concentrate was mixed with 95ml of distilled water to make 100ml of assay buffer.

Streptavidine-HRP: 0.12ml of streptavidine-HRP concentrate was mixed with 11.88ml of Assay buffer 30 minutes prior to use.

IL-6 standard: The IL-6 standard was reconstituted with 5ml of distilled water to produce a stock solution of 200pg/ml. Serial dilutions of 100, 50, 25, 12.5, 6.25 and 3.12pg/ml were made using assay buffer. Distilled water served as zero standard.

Assay procedure

100µl of Assay buffer was added to each well. A further 50µl of standard or sample were added in duplicate. 50µl of biotin-conjugate was added to all the wells and incubated at room temperature for two hours. The microplate was then washed six times with wash buffer using a micro plate auto washer. Excess fluid was removed by blotting the plate on dry paper towel. 100µl of streptavidin-HRP was added to each well. The microplate was again incubated at room temperature for one hour on a microplate shaker set at 100rpm. A further six washes with buffer solution were performed before the addition of 100µl of amplification solution I. The plate was protected from light for fifteen minutes and incubated on microplate shaker set at 100rpm. Plate is washed further six cycles and dried. 100µl Amplification solution II was added to all the wells and incubated on microplate shaker at room temperature for thirty minutes. Further six cycles of plate wash was carried out on auto plate washer. 100µl of TMB solution was added to all the wells and incubated for ten minutes avoiding exposure to direct light. Once highest standard has developed dark blue colour 100µl of stop solution was added to each well. The optical density of each well was determined using an automated plate reader set at a wavelength of 450nm as primary wavelength. A standard curve was obtained and the concentration of IL-6 (pg/ml) was calculated for each sample. Samples with values out-with the standard curve were pooled and further diluted and repeated the process.

Assay Precision

The minimum detectable dose of IL-6 is 0.7pg/ml. Intra and inter- assay precision has been estimated to be between 1.6 - 8%. The average recovery of IL-6 from serum samples ranged from 72% to 95% with an overall mean recovery of 85%. There is no significant cross reactivity with factors related to IL-6 or any other cytokines.

B	B	1	1	9	9	17	17	25	25	33	33
S1	S1	2	2	10	10	18	18	26	26	34	34
S2	S2	3	3	11	11	19	19	27	27	35	35
S3	S3	4	4	12	12	20	20	28	28	36	36
S4	S4	5	5	13	13	21	21	29	29	37	37
S5	S5	6	6	14	14	22	22	30	30	38	38
S6	S6	7	7	15	15	23	23	31	31	39	39
S7	S7	8	8	16	16	24	24	32	32	40	40

Figure2.1: Standard IL-6 ELISA microplate lay out.

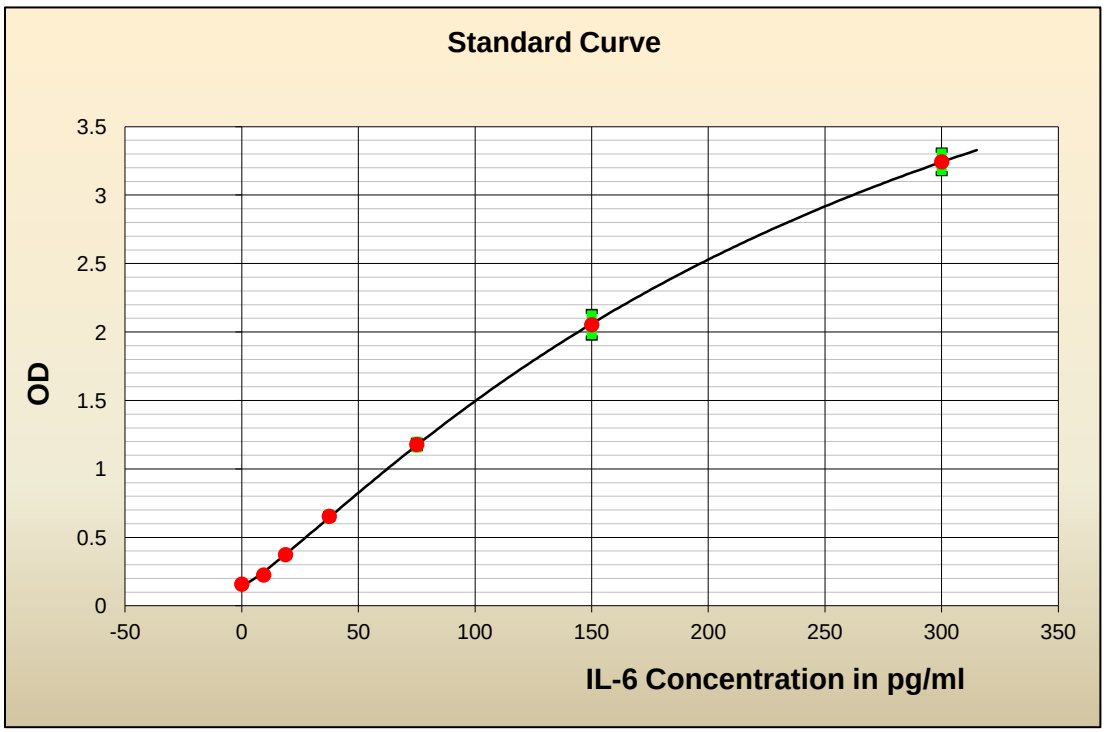
S1-S7: Standards.

B: Blank.

1-40 Test samples 1-40.

B	B	0.211	0.197	0.272	0.226	0.818	0.408	0.667	0.667	1.441	1.443
3.304	3.183	0.19	0.182	0.284	0.28	3.304	3.303	1.243	1.243	1.446	1.442
2.122	1.985	0.667	0.665	0.238	0.241	0.978	0.979	1.565	1.834	1.211	1.199
1.2	1.157	0.28	0.298	0.178	0.199	0.152	0.152	0.377	0.376	3.201	3.2
0.668	0.64	0.601	0.486	0.381	0.421	0.649	0.668	3.04	3.01	0.233	0.242
0.377	0.371	0.225	0.226	0.13	0.181	0.162	0.199	0.226	0.226	0.668	0.668
0.226	0.225	1.137	1.243	0.204	0.204	1.137	1.138	0.171	0.294	2.122	2.122
0.162	0.155	0.805	0.579	0.345	0.239	1.19	1.249	1.985	1.985	1.243	1.243

Figure 2.2: Optical density readings at 450nm for IL-6



X-axis: Concentration of IL-6 in pg/ml. Y-axis: Absorption at 450nm(optical density)

Figure 2.3: Standard curve for IL-6

B	B	6.421	5.261	11.183	7.629	49.497	21.034	39.061	39.061	93.776	96.347
S1	S1	4.665	3.969	12.084	11.785	310.72	309.77	80.314	80.314	101.33	95.753
S2	S2	39.061	39.037	8.576	8.81	59.882	60.893	105.716	128.834	79.317	76.371
S3	S3	11.785	13.123	3.614	5.429	1.089	1.089	18.84	17.34	297.34	293.71
S4	S4	34.444	26.488	19.124	21.949	37.13	39.021	231.71	226.87	8.183	8.888
S5	S5	7.55	7.629	3.88	3.881	2.121	5.429	7.629	7.629	39.071	39.071
S6	S6	72.388	80.314	5.845	5.845	72.388	74.441	2.977	12.827	156.016	156.016
S7	S7	48.587	32.925	16.55	8.654	76.327	80.768	142.736	142.736	80.314	80.314

Figure: 2.4: Calculated IL-6 Values from the samples 1-40

B. Blank

S1 to S7 Standards

1-40 test Samples calculated Il-6 values in duplicate. (Mean of the two values represents concentration of IL-6 in pg/ml)

2.5.2.4 Serum IL-10 ELISA:

Interleukin-10 is a pleiotropic cytokine playing an important role as a regulator of lymphoid and myeloid cell function. Due to the ability of IL-10 to block cytokine synthesis and several accessory cell functions of macrophages this IL-10 is a potent suppressor of the effector functions of macrophages, T-cells and NK cells. In addition, IL-10 participates in regulating proliferation and differentiation of B-cells, and mast cells. The primary structure of human IL-10 has been determined by cloning primary structure, IL-10 is a member of the four-helix bundle family of cytokines.

The human IL-10 exhibits strong DNA and amino acid sequence homology to the murine IL-10. The immunosuppressive properties of IL-10 suggest a possible clinical use of IL-10 in suppressing rejections of grafts after organ transplantations. IL-10 can furthermore exert strong anti-inflammatory activities.

IL-10 inhibits the production of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) from macrophages and monocytes.IL-10 also has been reported to be produced during sepsis and to possibly control the systemic responses in infection and inflammation. Inflammatory cytokines IL-6, IL-8, and granulocyte colony-stimulating factor (GCSF) are up-regulated during and after major abdominal surgery. However, it remains unclear whether abdominal surgery induces the production of antiinflammatory cytokines such as IL-10. We speculated that, during and after major abdominal surgery, the plasma levels of the antiinflammatory cytokines, as well as the inflammatory cytokines, would also increase. Therefore, we measured the serum IL-10 levels in this thesis work.

Serum collection

10mls of venous blood was obtained from patients and decanted into a plain tube. Samples were sent to the laboratory at Scarborough Hospital within one hour of venesection. Serum was obtained by centrifuging the sample at 1000 rpm for 10 minutes. The serum was

alliquotted into four epindorph vials and stored at -80°C. This method of storage was used to avoid loss of bioactive human IL-10 from the samples. These samples were batched in groups of forty. All patient details were removed from these vials and coded with unique identification number. Each set of forty samples were transported on dry ice to the University of Hull Laboratory for ELISA. Samples were defrosted for two hours before use.

Reagent preparation

Wash Buffer: 50mls of wash buffer concentrate was diluted in 950mls of distilled water to produce 1000mls of wash buffer.

Assay Buffer: 5ml of provided assay buffer concentrate was mixed with 95ml of distilled water to make 100ml of assay buffer.

Biotin-conjugate: 0.06ml of concentrated biotin conjugate solution was mixed with 5.94ml of assay buffer to make up 6ml of 1:100 dilute biotin conjugate.

Streptavidin-HRP: 0.04ml of streptavidin-HRP concentrate was mixed with 11.96ml of Assay buffer 30 minutes prior to use.

IL-10 standard: The IL-10 standard was reconstituted with 5ml of distilled water to produce a stock solution of 50pg/ml. Serial dilutions of 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39pg/ml were made using assay buffer. Distilled water served as zero.

Assay procedure

100µl of Assay buffer was added to each well. A further 50µl of standard or sample were added in duplicate. 50µl of biotin-conjugate was added to all the wells and incubated at room temperature for two hours. The microplate was then washed six times with wash buffer using a micro plate auto washer. Excess fluid was removed by blotting the plate on dry paper towel.

100µl of streptavidin-HRP was added to each well. The microplate was again incubated at room temperature for one hour on a microplate shaker set at 100rpm. A further six washes with buffer solution were performed before the addition of 100µl of amplification solution I. The plate was protected from light for fifteen minutes and incubated on microplate shaker set at 100rpm. Plate is washed further six cycles and dried. 100µl Amplification solution II was added to all the wells and incubated on microplate shaker at room temperature for thirty minutes. Further six cycles of plate wash was carried out on auto plate washer. 100µl of TMB solution was added to all the wells and incubated for ten minutes avoiding exposure to direct light. Once highest standard has developed dark blue colour 100µl of stop solution was added to each well. The optical density of each well was determined using an automated plate reader set at a wavelength of 450nm as primary wavelength and 620nm as reference wavelength. A standard curve was obtained and the concentration of IL-10 (pg/ml) was calculated for each sample. Samples with values out-with the standard curve was pooled and further diluted and repeated the process.

Assay Precision

The minimum detectable dose of IL-10 is 0.39pg/ml. Intra and inter- assay precision has been estimated to be between 2 and 7.5%. The average recovery of IL-10 from serum samples ranged from 78% to 111% with an overall mean recovery of 94%. There is no significant cross reactivity with factors related to IL-10 or any other cytokines.

B	B	1	1	9	9	17	17	25	25	33	33
S1	S1	2	2	10	10	18	18	26	26	34	34
S2	S2	3	3	11	11	19	19	27	27	35	35
S3	S3	4	4	12	12	20	20	28	28	36	36
S4	S4	5	5	13	13	21	21	29	29	37	37
S5	S5	6	6	14	14	22	22	30	30	38	38
S6	S6	7	7	15	15	23	23	31	31	39	39
S7	S7	8	8	16	16	24	24	32	32	40	40

Figure: 2.5: Standard IL-10 micro plate lay out

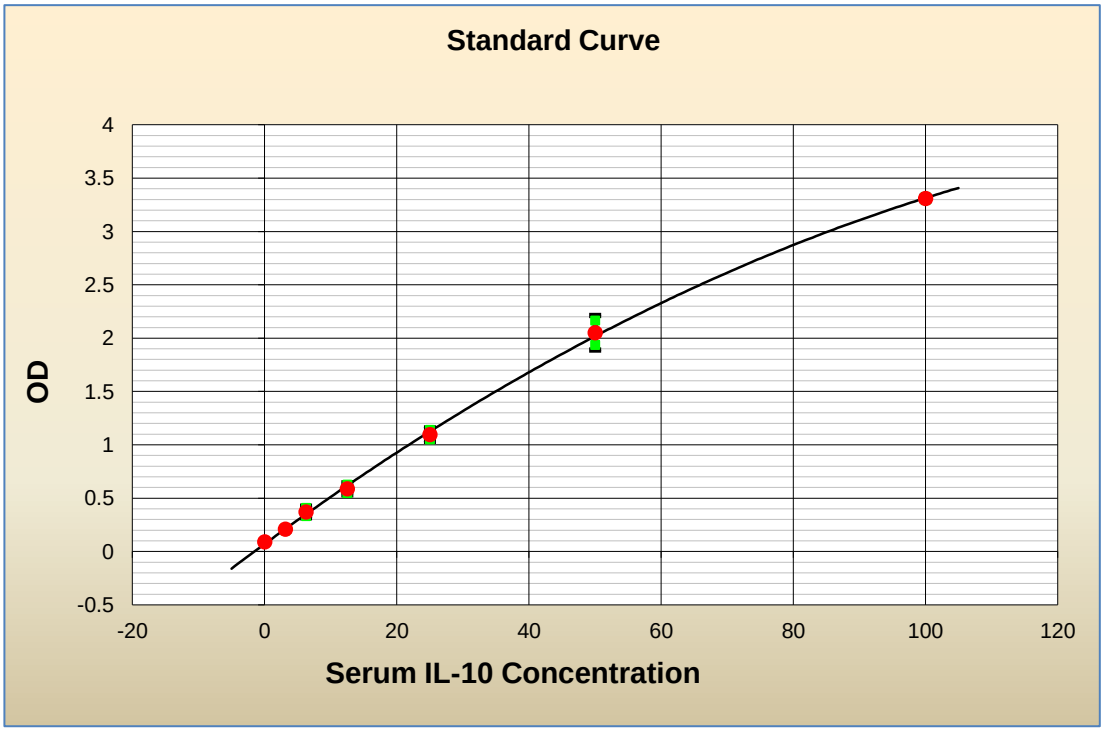
B. Blank

S1 to S7 Standards

1-40 test Samples in duplicate

B	B	0.143	0.148	0.099	0.094	0.075	0.075	0.096	0.09	0.15	0.158
3.323	3.293	0.172	0.167	0.09	0.089	0.122	0.115	0.324	0.296	0.091	0.099
1.935	2.164	0.259	0.258	0.382	0.339	0.174	0.17	0.345	0.276	0.189	0.223
1.05	1.139	0.267	0.303	0.215	0.217	0.2	0.18	0.153	0.143	0.162	0.187
0.547	0.626	0.108	0.101	0.096	0.1	0.102	0.107	0.752	0.852	0.098	0.117
0.331	0.408	0.225	0.215	0.121	0.129	0.174	0.167	0.702	0.848	0.251	0.275
0.197	0.219	0.351	0.365	0.276	0.346	0.345	0.297	1.088	1.037	0.464	0.51
0.081	0.097	0.428	0.539	0.256	0.269	0.301	0.254	0.971	1.049	0.372	0.384

Figure: 2.6: Optical density readings at 450nm wavelength primary (IL-10).



X-axis: Concentration of IL-10 in pg/ml. Y-axis: Absorption at 450 nm(optical density)

Figure 2.7 Standard curve for Il-10

B	B	3.208	3.43	1.258	1.037	0.2	0.2	1.126	0.861	3.519	3.875
S1	S1	4.499	4.276	0.861	0.817	2.276	1.966	11.345	10.073	0.905	1.258
S2	S2	8.401	8.356	13.994	12.028	4.588	4.409	12.302	9.168	5.258	6.781
S3	S3	8.761	10.391	6.422	6.511	5.75	4.856	3.652	3.208	4.053	5.168
S4	S4	1.656	1.347	1.126	1.303	1.391	1.612	31.414	36.288	1.214	2.054
S5	S5	6.87	6.422	2.232	2.586	4.588	4.276	29.004	36.092	8.04	9.123
S6	S6	12.576	13.215	9.168	12.347	12.302	10.119	48.104	45.511	17.776	19.916
S7	S7	16.111	21.273	8.265	8.852	10.3	8.175	42.189	46.119	13.536	14.086

Figure: 2.8: Calculated IL-10 Values in pg/ml.

B. Blank

S1 to S7 Standards

1-40 test Samples calculated Il-10 values in duplicate. (Mean of the two values represents concentration of IL-10 in pg/ml)

2.5.2.5 **Serum TNF-α ELISA:**

Tumor Necrosis Factor (TNF-α), also known as cachectin, is a polypeptide cytokine produced by monocytes and macrophages. It functions as a multipotential modulator of immune response and further acts as a potent pyrogen. TNF-α circulates throughout the body responding to stimuli (infectious agents or tissue injury), activating neutrophils, altering the properties of vascular endothelial cells, regulating metabolic activities of other tissues, as well as exhibiting tumoricidal activity by inducing localized blood clotting. TNF-α also inhibits lipoprotein lipase activity resulting in cachexia. TNF-α production is mediated by the action of lymphokines and endotoxins on the macrophage. Tumor necrosis factor-α can be produced ectopically in the setting of malignancy and parallels parathyroid hormone in causing secondary hypercalcemia.

Serum collection

10mls of venous blood was obtained from patients and decanted into a plain tube. Samples were sent to the laboratory at Scarborough Hospital within one hour of venesection. Serum was obtained by centrifuging the sample at 1000 rpm for 10 minutes. The serum was alliquotted into four epindorph vials and stored at -80°C. This method of storage was used to avoid loss of bioactive human TNF-α from the samples. These samples were batched in groups of forty. All patient details were removed from these vials and coded with unique identification number. Each set of forty samples were transported on dry ice to the University of Hull Laboratory for ELISA. Samples were defrosted for two hours before use.

Reagent preparation

Wash Buffer: 50mls of wash buffer concentrate was diluted in 950mls of distilled water to produce 1000mls of wash buffer.

Assay Buffer: 5ml of provided assay buffer concentrate was mixed with 95ml of distilled water to make 100ml of assay buffer.

Biotin-conjugate: 0.06ml of concentrated biotin conjugate solution was mixed with 5.94ml of assay buffer to make up 6ml of 1:100 dilute biotin conjugate.

Streptavidin-HRP: 0.03ml of streptavidin-HRP concentrate was mixed with 11.97ml of Assay buffer 30 minutes prior to use, making total volume of 12ml.

TNF-α standard: The TNF-α standard was reconstituted with 5ml of distilled water to produce a stock solution of 40pg/ml. Serial dilutions of 20, 10, 5, 2.5, 1.25, 0.63, and 0.31pg/ml were made using assay buffer. Distilled water served as zero.

Assay procedure

100µl of Assay buffer was added to each well. A further 50µl of standard or sample were added in duplicate. 50µl of biotin-conjugate was added to all the wells and incubated at room temperature for two hours. The microplate was then washed six times with wash buffer using a micro plate auto washer. Excess fluid was removed by blotting the plate on dry paper towel. 100µl of streptavidin-HRP was added to each well. The microplate was again incubated at room temperature for one hour on a microplate shaker set at 100rpm. A further six washes with buffer solution were performed before the addition of 100µl of amplification solution I. The plate was protected from light for fifteen minutes and incubated on microplate shaker set at 100rpm. Plate is washed further six cycles and dried. 100µl Amplification solution II was added to all the wells and incubated on microplate shaker at room temperature for thirty minutes. Further six cycles of plate wash was carried out on auto plate washer. 100µl of TMB solution was added to all the wells and incubated for ten minutes avoiding exposure to direct light. Once highest standard has developed dark blue colour 100µl of stop solution was added

to each well. The optical density of each well was determined using an automated plate reader set at a wavelength of 450nm as primary wavelength and 620nm as reference wavelength. A standard curve was obtained and the concentration of TNF-α (pg/ml) was calculated for each sample. Samples with values out-with the standard curve was pooled and further diluted and repeated the process.

Assay Precision

The minimum detectable dose of TNF-α is 0.13pg/ml. Intra and inter- assay precision has been estimated to be between 2 and 7.5%. The average recovery of TNF-α from serum samples ranged from 81% to 101% with an overall mean recovery of 91%. There is no significant cross reactivity with factors related to TNF-α or any other cytokines.

B	**B**	**1**	**1**	**9**	**9**	**17**	**17**	**25**	**25**	**33**	**33**
S1	**S1**	**2**	**2**	**10**	**10**	**18**	**18**	**26**	**26**	**34**	**34**
S2	**S2**	**3**	**3**	**11**	**11**	**19**	**19**	**27**	**27**	**35**	**35**
S3	**S3**	**4**	**4**	**12**	**12**	**20**	**20**	**28**	**28**	**36**	**36**
S4	**S4**	**5**	**5**	**13**	**13**	**21**	**21**	**29**	**29**	**37**	**37**
S5	**S5**	**6**	**6**	**14**	**14**	**22**	**22**	**30**	**30**	**38**	**38**
S6	**S6**	**7**	**7**	**15**	**15**	**23**	**23**	**31**	**31**	**39**	**39**
S7	**S7**	**8**	**8**	**16**	**16**	**24**	**24**	**32**	**32**	**40**	**40**

Figure: 2.9: TNF-α ELISA microplate lay-out

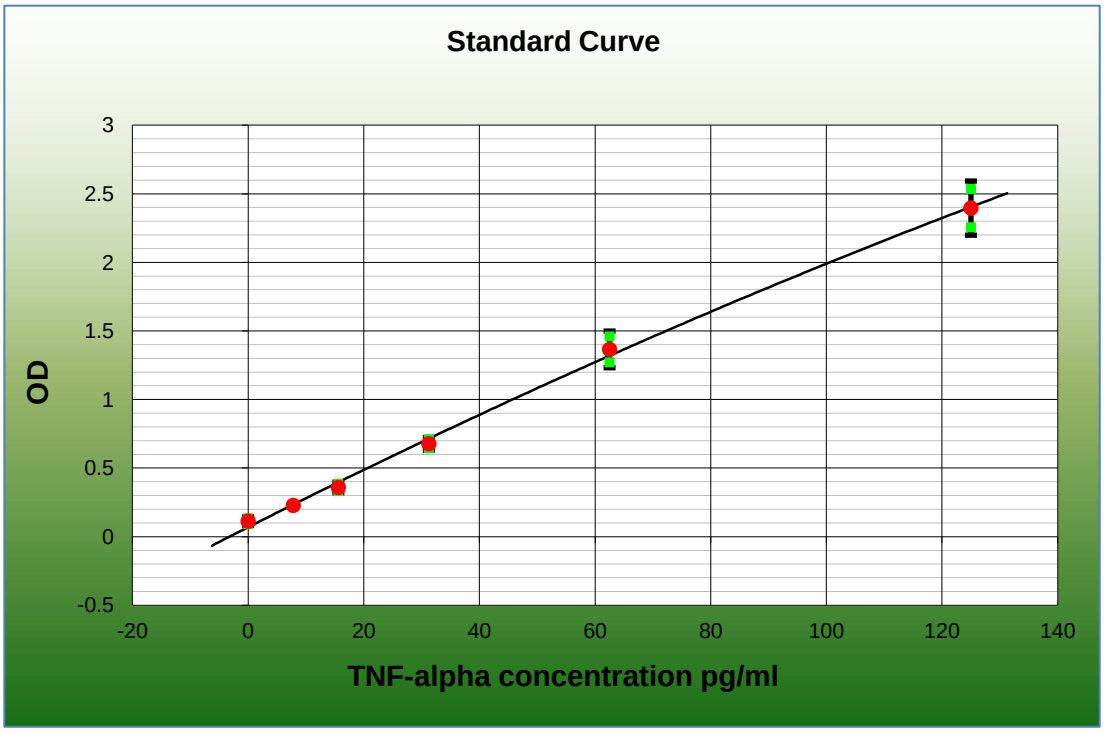
B. Blank

S1 – S7 Standards

1-40 represents test samples in duplicate.

B	B	0.164	0.164	0.077	0.077	0.08	0.083	0.078	0.078	0.079	0.078
2.256	2.257	0.08	0.079	0.087	0.088	0.081	0.081	0.079	0.083	0.084	0.082
1.201	1.210	0.078	0.076	0.098	0.088	0.082	0.078	0.08	0.081	0.082	0.084
0.641	0.643	0.086	0.077	0.075	0.077	0.08	0.082	0.087	0.082	0.081	0.082
0.332	0.331	0.101	0.098	0.077	0.074	0.079	0.075	0.113	0.119	0.076	0.075
0.201	0.207	0.083	0.084	0.076	0.078	0.076	0.076	0.112	0.11	0.077	0.081
0.114	0.103	0.087	0.085	0.079	0.081	0.09	0.08	0.305	0.307	0.083	0.079
0.058	0.060	0.105	0.099	0.086	0.083	0.083	0.103	0.111	0.102	0.081	0.084

Figure: 2.10: Optical density readings at 450nm for TNF-α



X-axis: Concentration of TNF- α in pg/ml. Y-axis: Absorption at 450 nm (optical density)

Figure 2.11: Standard curve for TNF- α

B	B	8.938	8.938	0.742	0.742	1.023	1.305	0.836	0.836	0.929	0.836
S1	S1	1.023	0.929	1.68	1.774	1.117	1.117	0.929	1.305	1.399	1.211
S2	S2	0.836	0.648	2.714	1.774	1.211	0.836	1.023	1.117	1.211	1.399
S3	S3	1.586	0.742	0.554	0.742	1.023	1.211	1.68	1.211	1.117	1.211
S4	S4	2.996	2.714	0.742	0.46	0.929	0.554	4.125	4.69	0.648	0.554
S5	S5	1.305	1.399	0.648	0.836	0.648	0.648	4.031	3.843	0.742	1.117
S6	S6	1.68	1.492	0.929	1.117	1.962	1.023	22.367	22.396	1.305	0.929
S7	S7	3.372	2.808	1.586	1.305	1.305	3.184	3.937	3.09	1.117	1.399

Figure: 2.12: Calculated Values of TNF-α in 1:10 pg/ml.

B. Blank

S1 to S7 Standards

1-40 test Samples calculated values of TNF-α in duplicate. (Mean of the two values represents concentration of TNF-α in pg/ml)

2.5.3: **Detection of Microbial DNA in peripheral blood of surgical patients by the Polymerase chain reaction (PCR):**

PCR is a relatively simple technique that amplifies a DNA template to produce specific DNA fragments in vitro. Traditional methods of cloning a DNA sequence in to a vector and replicating it in a living cell often requires days or weeks of work, but amplification of DNA sequence by PCR requires only hours. Basic PCR has become commonplace in many molecular biology labs where it is used to amplify DNA fragments and detect DNA or RNA sequences within a cell. However PCR has evolved far beyond simple amplification and detection, and many extensions of the original PCR method has been described.

The PCR process was originally developed to amplify short segments of a longer DNA molecule (Saiki et al. 1985). A typical amplification reaction includes the target DNA, a thermostable DNA polymerase, two oligonucleotide primers, deoxynucleotide triphosphates (dNTPs), reaction buffer and magnesium. Once all the reagents are assembled, the reaction is placed in a thermal cycler with preset temperatures and cycles. Series of temperature and time adjustments is referred to as one cycle of amplification. Each PCR cycle theoretically doubles the amount of targeted sequence in the reaction.

Identification of minute quantities of microbial-specific DNA has been made possible by the advent of PCR techniques. A study by Kane et al. In animals showed that PCR methods were more sensitive than standard cultures for detecting microbial products in the blood after thermal injury and attributed this to detection of bacterial translocation in peripheral blood.

This thesis aims to obtain proof of principle for gut origin of bacteria in peripheral blood by perfecting the technique of PCR to detect DNA fragments in peripheral blood and correlating these findings with results assessed by lymph node culture described above.

Sample collection: Four ml of peripheral venous blood was collected from consenting patients on the day of surgery and a further four ml of blood was collected immediately after

major abdominal surgery in recovery, to use patients as their own controls. These venous blood samples were decanted in to conventional EDTA tubes in duplicate and transported to the laboratory within 30 minutes to store at -80°C. These samples were transported on dry ice to The University of Hull laboratory in batches of fifteen samples.

DNA Extraction:

Frozen samples were defrosted for two hours prior to use. Whole blood samples were processed in aliquots of 400µl for DNA extraction. Blood was transferred from EDTA tubes to sterile 1.5ml Eppendorf tubes, red cells were lysed, and total DNA was extracted according to manufacturer's protocol described in the "Bacterial identification kit K5081100" Biochain Institute Inc, CA, USA. This kit detects the bacteria at concentrations of 0.2 or more cfu/PCR reaction. The specific primer used was for genomic DNA encoding 16s ribosomal RNA to detect gram positive and gram negative bacteria.The PCR was performed in an air Thermo-Cycler as recommended by the kit manufacturer. After two minutes of initial melting at 95°C, the mixture was amplified for a total of 37 cycles using a three step cycle process that begin with melting at 95°C for 30 seconds, followed by annealing at 56°C for 45 seconds, and followed by extension at 72°C for 30 seconds. The final cycle was followed by 10 minutes of soaking at 72°C. The amplified DNAs were separated by 1.5% agarose gel electrophoresis and then visualised by staining with ethidium bromide. This gel plate was red under UV light.

To validate the methodology described above and to assess the reproducibility of the results, in the first instance series of experiments were conducted. When the methodology advised by the manufacturer of commercial kit was followed to extract DNA from frozen samples no DNA product was identified in the first experiment. In the second experiment thinking the amplification was inadequate number of PCR cycles were increased in gradual fashion up to 50 cycles. This was met with limited success of identifying PCR products on agarose gel plate. When same experiment was repeated with same samples the results were

not reproducible. Based on further literature review and manufactures advise considering haemoglobin may be inhibiting the DNA extraction erythrolysis and specimen cleansing was undertaken in a third series, this has improved PCR yield, however when the experiments were repeated in similar conditions the results were not reproducible. In fourth series of experiments original sample quantity used was increased up to 3 ml instead of 1 ml gradually to address the possibility of less bacteria in the samples, this again has not improved the PCR yield or reproducibility. In the fifth series of experiments samples were spiked with known E-Coli live bacterial colonies, this has significantly improved the PCR yield, however throughout the series of experiments could not maintain negative controls as negatives and there was problem with control contamination. Further series of experiments were carried out using fresh whole blood samples within 15 minutes of venesection and also using buffy coat alone, these experiments again did not produce reliable or reproducible results to analyse test samples and report the results. In view of time and resource limitations attempts to develop own primers or to proceed with RT-PCR or southern blotting were felt to be outside the scope of this thesis.

2.6 Statistical analysis:

All data collected for this thesis was tabulated on XL spreadsheets (Microsoft Corporation, USA). All parametric data are expressed as means, whilst non-parametric data are expressed as medians. Statistical analysis was performed using the XLStatistics Excel Workbooks for Data Analysis software package or SPSS Windows version 17 (SPSS Inc., Chicago, USA). Non-parametric quantitative data were compared using the Wilcoxon Signed Rank test, Freidman test or Mann-Whitney U test as appropriate. Qualitative data were analysed using the Chi-squared test or Fisher's exact test for small numbers. A P value of 0.05 or less was taken to signify statistical significance.

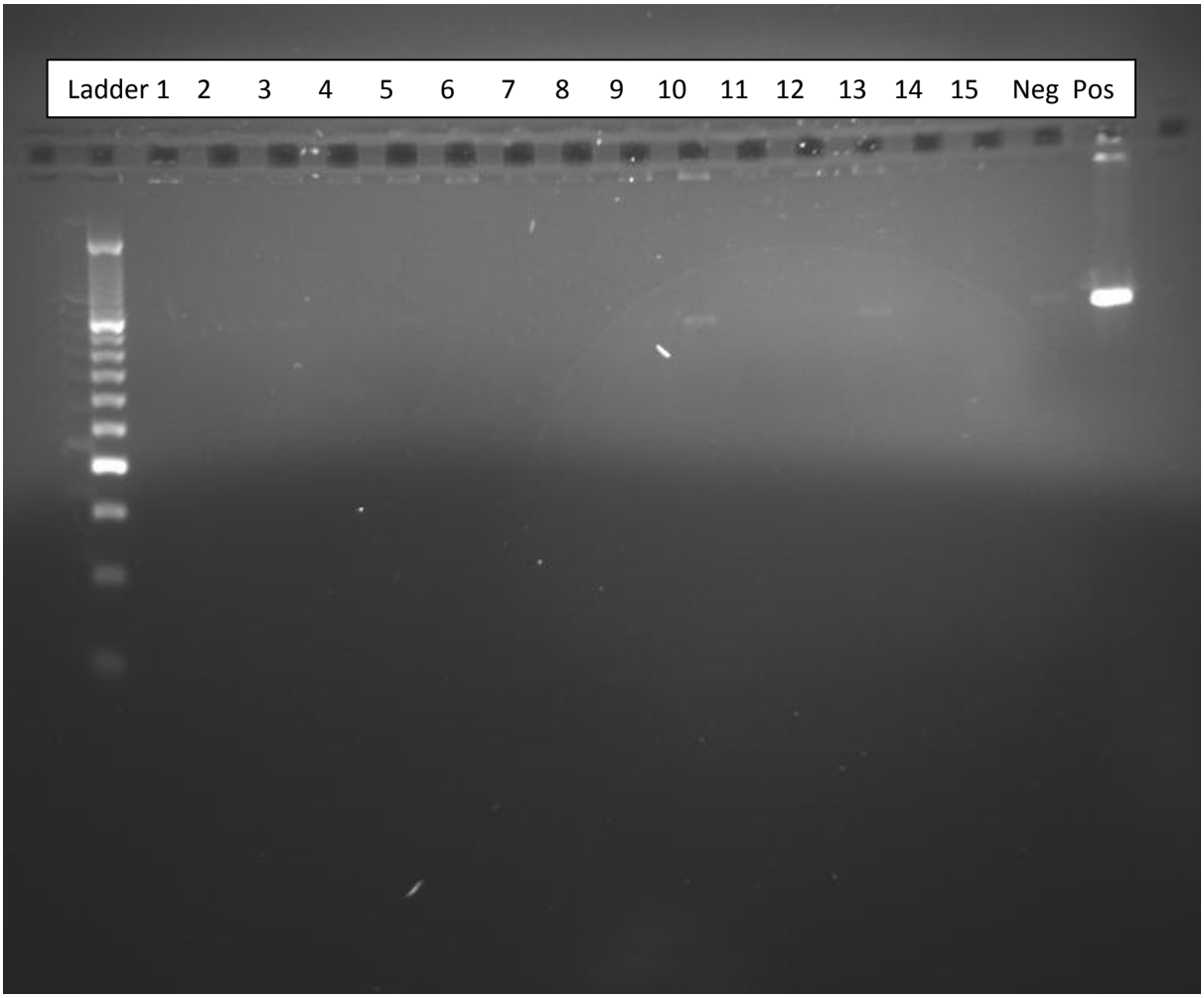


Figure: 2.13 Agarose gel electrophoresis of samples 1-15 run after the PCR. Ladder represents the template and 1-15 were the test samples. Negative and positive represent respective controls. (37 PCR cycles with standard samples)

Note the PCR product in samples 10 and 13.

Same protocol as described above was followed. Result from agarose gel electrophoresis of PCR products:

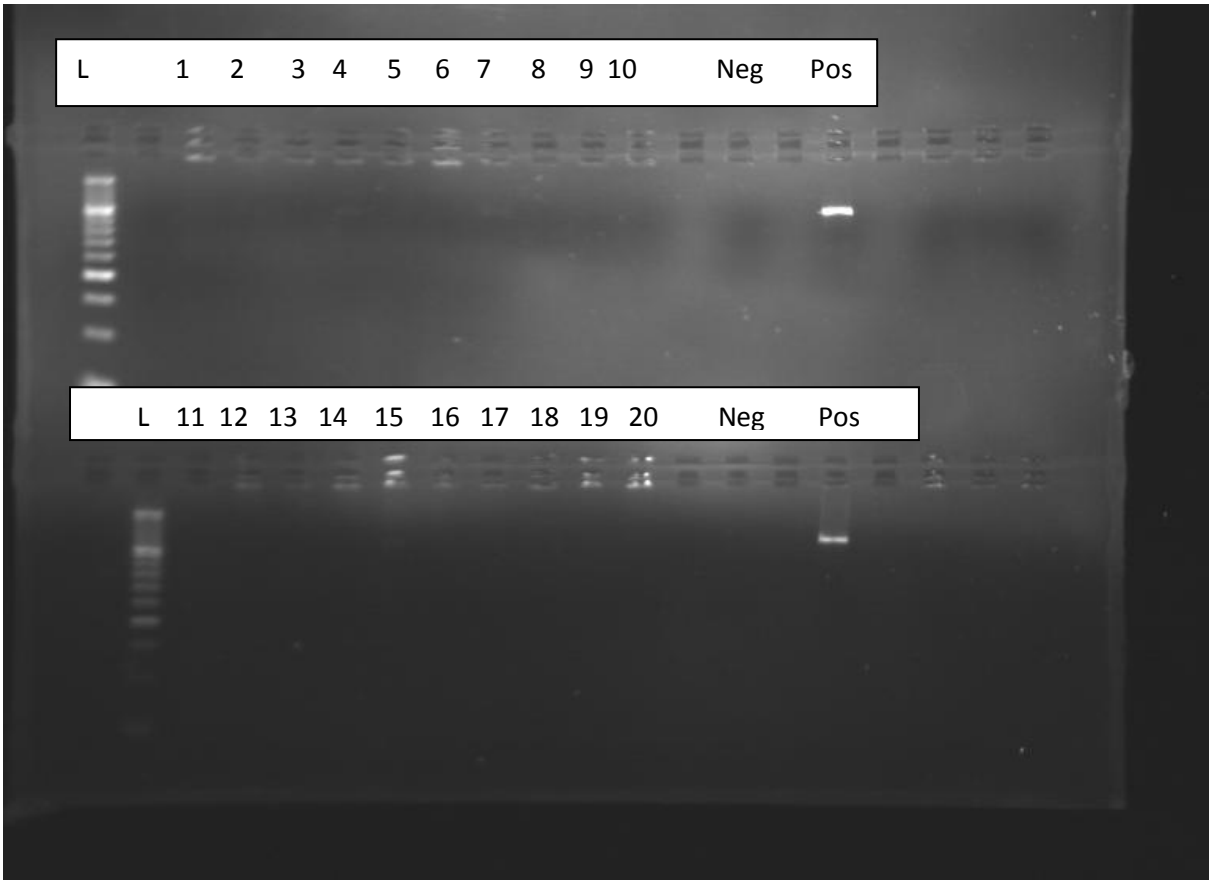


Figure: 2.14 L representing ladder 1-10 spiked samples 11-20 standard samples. In this experiment no PCR product could be detected in either spiked samples or standard test samples. (37 PCR cycles)

CHAPTER THREE

The effects of Gut function on systemic inflammatory response and clinical outcomes amongst patients with acute pancreatitis: A Prospective observational cohort study.

3.1 ABSTRACT:

Background: The outcome of acute pancreatitis has scarcely improved over last two decades. Further improvement will require new paradigms in pathophysiology and treatment in pancreatitis. There is mounting evidence to suggest that the intestine has a key role in the pathophysiology of pancreatitis. It may be possible that, inadequate gut function from ischaemia reperfusion injury to gut and loss of gut barrier may drive the motor of SIRS, sepsis, MODS and death resulting in poorer outcomes and gut directed therapies may improve the clinical outcomes.

Methods: This was a single centre prospective observational study including thirty patients with acute pancreatitis. Natural course of the treatment outcomes were followed during these patients hospital stay. Prospective data was collected with respect to the severity of illness, primary aetiology, state of gut function, prevalence of SIRS, septic complications, any organ dysfunction, length of hospital stay and overall clinical outcomes. This study aims to investigate the natural history of gut function in pancreatitis and clinical outcomes.

Results: A total of 30 (25 with mild and 5 with severe) patients with pancreatitis were studied. Transient gut failure was observed in 17 (57%) of these patients. Transient gut failure was associated with SIRS in 3 (17%) of patients. SIRS was observed in 3 (23%) of patients who were thought to have normally functioning gut. Persistent gut failure was observed in 5 (16%) of this group. Persistent gut failure was associated with SIRS and MODs in 4(80%) of this sub group which was statistically significant. There were 2 (7%) deaths in this group due to severe pancreatitis and pancreatic necrosis.

Conclusion: Persistent gut failure in pancreatitis is associated with increased prevalence of SIRS/MODS and poorer clinical outcomes. Transient gut failure is common in pancreatitis and this is not associated with any poor clinical outcomes.

3.2 BACKGROUND:

Acute pancreatitis accounts for 3% of all patients admitted with abdominal pain to hospital in the UK and represent a significant source of morbidity and mortality (B.S.G., 1998). Acute pancreatitis results from the premature activation of enzymes within the pancreatic acinar cells, which causes inflammation spreading into the surrounding tissues (Friedman *et al.*, 2006). Acute pancreatitis is a multi-system disease with an unpredictable clinical course and significant morbidity and mortality (Wilmer, 2004). Approximately 20% of patients develop multi-organ failure requiring management within a critical care environment (Dambrauskas et al., 2009). However much of the pathophysiology of the disease, particularly understanding why some patients develop life-threatening disease whilst others have a relatively benign course, remains unclear. As an inflammatory process, acute pancreatitis represents a spectrum of disease ranging from a mild, self-limiting course requiring only brief hospitalization to a rapidly progressive, fulminant illness resulting in the systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), Sepsis syndrome and extensive pancreatic necrosis. Necrotic infection is the most severe complication and has mortality rates in the range of 30% to 50% (Fenton-lee *et al.*, 1993). The causative organisms are usually gut-derived bacteria (Bassi *et al*, 2003, Garg *et al*, 2001, Isaji *et al*, 2003).

It is important to avoid the development of sepsis in the treatment of acute pancreatitis. Although prophylactic antibiotics with sufficient antimicrobial spectrum have always been used in the treatment of acute pancreatitis, this treatment does not completely satisfy the expectation of a decreased incidence of complications and mortality. The mechanism by which the necrotic pancreas becomes infected is unclear, but experimental and clinical data suggest that the gastrointestinal tract is the likely source of organisms because intestinal colonization by pathogens often precedes pancreatic infection (McNaught *et al.*, 2002).

Once considered a quiescent organ, the intestine is now seen to have a pivotal role in the development of the SIRS, sepsis and MODS in critical illness (Marshall *et al.,* 1993). There is now very little doubt that bacterial translocation occurs but uncertainty remains over the mechanism and how this relates to SIRS and MODS. Studies of haemorrhagic shock, gram-negative sepsis and trauma have provided an insight into the way that the intestine influences the course of clinical outcomes in critical illness. Similar mechanisms which are poorly understood may be operational in acute pancreatitis to produce those unpredictable clinical outcomes.

Many predictive scales have resulted from attempts to predict which patients are likely to develop severe disease (Glasgow, Atlanta, Imrie, Ranson, APACHE-II etc...). However none of these scoring systems actually correlate clinical findings with the pathophysiology of the disease process, making comprehension of the rationale for the prognostic value which these scales have been shown to have difficult.

Acute pancreatitis, therefore, may represent a model of systemic inflammation arising initially from a non-gastrointestinal source. In the "gut origin of sepsis" model, secondary gut injury and breakdown of the gut barrier may occur to result in the release of inflammatory mediators and colonic bacteria, perpetuating systemic inflammation and resulting in distant sepsis. There is therefore a sound theoretical basis for proposing that state of gut function may help to predict the clinical course and outcomes in acute pancreatitis. This hypothesis has been investigated in this prospective observational cohort study.

3.3 METHODS:

This was a prospective observational cohort study including thirty patients admitted to Scarborough Hospital with acute pancreatitis. Approval for this study was obtained from the locally organised Research Ethical Committee.

3.3.1 Inclusion and exclusion criterion:

All patients with acute pancreatitis who can provide consent were eligible for inclusion in the study. Patients under the age of eighteen years, patients with chronic pancreatitis, patients with pre existing sequel from previous pancreatitis and pre existing single or multiple organ failure on regular organ support were excluded from study to avoid bias.

3.3.2 Data collection:

Several data points were prospectively recorded for each patient. The data collected included patient demographics, the severity of illness and state of gut function, Systemic inflammatory response, length of hospital stay, and complications that developed during hospital stay. Several biochemical parameters including CRP, White blood cell count, and Serum albumin were measured.

3.3.3 Diagnosis of Pancreatitis and severity assessment:

Diagnosis was confirmed by a combination of suggestive history and serum amylase concentration of greater than three times the upper limit of normal and with the absence of any features of chronic pancreatitis (B.S.G., 2005).

Disease severity was assessed following admission using the modified Glasgow scoring system (Blamey, Imrie *et al*, 1984). Severity was re-evaluated at 48 hours after admission. Patients with at least one or more organ failure on admission as per Atlanta criterion were classified as severe. Any patients with severe pancreatitis based on modified Glasgow scoring system had CT evaluation of their pancreas at appropriate time. Pancreatic necrosis was

diagnosed when non-enhancing areas were discovered on a contrast enhanced CT scan of the pancreas.

3.3.4 State of Gut function, Gut failure or inadequate gut function:

In this study a pragmatic and clinically applicable approach was taken to define gut function based on its primary function that is nutrition. Ability to tolerate three light meals a day was defined as adequate function. If patient is on enteral nutritional support adequacy of gut function was defined as the tolerance of 80% or more of calculated nutritional requirements for a continuous period of at least 48 hours. Anything less than this was classified as inadequate gut function or gut failure. Gut failure was classified as transient if it lasted for less than forty eight hours and classified as persistent if it lasted beyond forty eight hours. This definition was adhered to analyse the results.

An extra sample of peripheral whole blood was collected on diagnosis and day three of admission to assess bacterial translocation as a marker of gut barrier function, using the novel PCR method described in the methodology chapter.

3.3.5 SIRS/ MODS/ Sepsis syndrome:

All these patients were closely monitored by the research fellow along side of the caring surgical team till the patient was discharged from the hospital. Cultures of blood, urine, or sputum were taken whenever deemed clinically appropriate. As a measure of clinical progress of the disease Serum CRP, WBC count, and Serum albumin levels were monitored. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee proposed definitions for sepsis and the clinical syndromes associated with it were used (American College 1992).

All complications during hospital stay were recorded prospectively. For purposes of this study, septic morbidity was defined as the presence of recognized pathogens in body tissues that are normally sterile, confirmed by the results of culture and supported by clinical, radiological, or haematological evidence of infection as described in chapter2.

Aetiological factors for pancreatitis, length of hospital stay, any intensive care stay, and other clinical outcomes were recorded prospectively and analysed.

3.3.6: Statistical analysis:

Patient data was collected prospectively during the admission and hospital stay. Statistical analysis was performed using the XL statistics software for Microsoft Excel. Data are expressed as medians with Interquartile ranges (IQR) in parenthesis. Nonparametric data was analysed using the Chi squared, Mann-whitney or Fishers exact test. A p value of 0.05 or less was taken to signify statistical significance.

3.4 RESULTS:

3.4.1: Patients:

Thirty patients with acute pancreatitis were entered in to this study. Twenty seven patients on admission had mild pancreatitis and the remaining three had severe pancreatitis. On review at 48 hours two further patients who initially had mild pancreatitis were found to have severe pancreatitis. Of these total thirty patients 17 (57%) were male patients and 13 (43%) were females with a median (IQR) age of 48 (23-91) years. Median (IQR) BMI for this group was 26.2 (21.7-51.3).

All the patients had appropriate investigations to identify the aetiological factor for pancreatitis. Out of these thirty patients 18 (60%) of the patients were found to have gall stones. Alcohol was found to be the cause in 3 (10%) of the patients. Two (7%) of these 30

patients developed Pancreatitis following ERCP for common bile duct stones. Anti epileptic drugs were identified as cause for pancreatitis in one patient and one patient was found to have hyper triglyceridemia as cause for pancreatitis. In 5 (17%) aetiology was unknown.

Patient demographics and the aetiological factors for pancreatitis in this cohort are summarised in the tables 3.1 and 3.2.

Demographic factor	**Total patients (n=30)**
Age Median (IQR)	48 (23 - 91)
Sex Male : Female ratio	17 : 13
BMI median (IQR)	26.2 (20.9 – 51.3)
Severity at diagnosis Mild : Severe	 27 (90%): 3 (10%)
Severity at 48 hours after admission Mild : Severe	 25 (83%): 5 (17%)

Table 3.1 Patient demographics

Aetiological factor for Pancreatitis	**Total patients (n-30)**
Gall stones	18 (60%)
Alcohol	3 (10%)
Post ERCP	2 (7%)
Drugs	1 (3%)
Hyper triglyceridemia	1 (3%)
Idiopathic (unknown cause)	5 (17%)

Table 3.2 Aetiological factors for Pancreatitis.

3.4.2: State of Gut function:

Out of total of 30 patients in the study 17 (57%) patients had transient gut failure during initial 48 hours. However all 3 patients with severe pancreatitis had inadequate gut function. One important caveat here will be the approach taken by the caring team of keeping nil by mouth as part of conventional management of Pancreatitis. Only Five patients (16%) in the group had persistent inadequate gut function lasting for more than 48 hours after diagnosis. All these five patients were managed with TPN started between days 4 and 7 after admission. This included four patients with severe pancreatitis and one patient with mild pancreatitis. Two further patients received enteral nutrition support initiated on Day 5 and Day 6 as their oral intake was poor and clinically felt that they have functioning gut and both these patients were established on enteral feed within 48 hours of initiation.

3.4.3: SIRS/MOF/Sepsis/Death:

On admission 4 (13%) patients have two positive criterion and 2 (7%) patients have three positive criteria for SIRS. At 48 hours 2(7%) patients in mild pancreatitis group and 3 (60%) patients in severe pancreatitis group qualified for SIRS. Out of five patients with severe pancreatitis three people went on to develop pancreatic necrosis and multi organ failure necessitating ITU admission and organ support. In this group there were all together Five septic events among Four patients, Two developed bacteraemia, two patients developed chest infection and One patient developed infective pancreatic necrosis. There were two (7%) deaths in the group.

3.4.4: Gut Function and SIRS:

Out of 17 patients who were found to have transient gut failure there were 3 (17%) patients with SIRS and there were 3 (23%) patients with SIRs who were thought to have functioning gut. Out of 5 patients with persistent gut failure 3 (60%) patients had SIRS from remaining

25 patients who were thought to have functioning gut 2 (8%) had SIRS This difference was statistically significant .

There were 2 (7%) deaths in the group. Both these patients were with persistent gut failure and multi organ failure.

3.4.5: Serum CRP and Albumin levels:

There were slightly higher levels of serum CRP in patients with severe pancreatitis when compared to mild group. There was no significant difference in Serum CRP and Albumin levels between mild and severe pancreatitis patients in the initial 48 hours.

3.4.6: Length of Hospital stay:

Length of hospital stay was prospectively recorded in days. Median (IQR) duration of hospital stay for this group was 5 (4-31) days.

3.5: DISCUSSION:

3.5.1: General:

From this small cohort of patients with pancreatitis there is no evidence to suggest that transient gut failure is associated with poorer outcomes. However there is strong positive correlation between persistent gut failure and prevalence of SIRS. There is increased morbidity and mortality associated with severe pancreatitis when compared to mild. Clinical significance of transient gut failure is unclear as it does not seem to have any impact on clinical outcomes.

3.5.2: Pancreatitis and Gut function:

Conventionally gut was thought to be quiescent organ; however emerging evidence is suggestive that the gut plays pivotal role in development of SIRS, MODS and critical illness.

Acute pancreatitis is characterised by retroperitoneal oedema, third space fluid loss, hypo-volaemia and circulatory shock. The physiological response is to prioritise blood flow to the vital organs at the expense of splanchnic circulation. Splanchnic tissues can adapt to low

perfusion states by extracting up to 90% of the oxygen from the blood. This protective effect is limited because prolonged extraction of oxygen can lead to regional ischaemia (Rowell, 1984). Resuscitation does not provide immediate relief, as the splanchnic region is the last to be reperfused due to complex neuro endocrine mechanisms involved.

Complex relation between intestinal ischaemia and SIRS/MODS has not been well established. It has been hypothesised that this may be related to 'gut starter' hypothesis or the 'gut motor' hypothesis.

Role of neutrophils is central for 'gut starter' hypothesis. Neutrophils are primed as they pass through mesenteric circulation during reperfusion phase and continue to circulate in the body in this primed but inactive phase until they are provoked by an insult such as exposure to endotoxins (Moore, 1994). Neutrophils primed in this way exhibit an increased oxidative burst and augmented release of cytokines and reduced apoptosis when they are activated. These activated neutrophils become potent mediators for distant organ dysfunction (Biffl, 1999).

The 'gut motor' hypothesis focuses on the role of gut barrier in development of SIRS and MODS. When gut barrier is disrupted the luminal organisms and endotoxins invade the portal venous and lymphatic system. This invasion activates immune cells associated with GALT. These activated immune cells release inflammatory mediators that drive SIRS and MODS even though a focus of infection may not be evident (Meakins 1986).

Bacterial translocation may happen in patients with acute pancreatitis due to loss of gut barrier and also from alteration in gut microflora due to bacterial overgrowth and reduced intestinal motility. Mechanism by which BT occur in pancreatitis is poorly understood.

As gut has been implicated in the progression of pancreatitis or sequel associated with pancreatitis it is reasonable to argue gut directed therapies may hold the key for improved clinical outcomes in acute pancreatitis.

3.5.3: Gut directed therapies:

Traditional management of acute pancreatitis included resting the pancreas by keeping the patients nil by mouth. It is now well recognised that the intestine require luminal nutrients for the maintenance of structure and function of enterocytes. Fasting leads to mucosal atrophy, increased rate of apoptosis, decreased glutamine transport, altered mucin composition resulting in loss of gut barrier and increased bacterial translocation and associated sequelae.

Acute pancreatitis is characterised by hypermetabolism and there is strong advocacy for TPN. This advocacy was based on the premise of 'gut rest' and avoidance of pancreatic stimulation. Human studies have demonstrated that there are more benefits than harm with early enteral nutrition. Clinical trials comparing PN with EN in pancreatitis have shown that EN is associated with reduced inflammatory response and disease severity (Windsor, 1999), reduced septic complications (Kalfarentzos, 1997)), reduced caecal bacterial overgrowth (Alverdy, 1988) and reduced permeability (Illig, 1992). As a result enteral nutrition has become preferred mode of nutritional support for patients with acute pancreatitis. One has to remember to assess the state of gut function and if the gut function is inadequate alternative route should be preferred to meet nutritional requirements. Several alternative approaches have been described by using combination of EN and PN with a view to meeting nutritional requirements through parenteral route and low residue micronutrients via enteral route to protect the intestinal mucosa (Omura, 2000).

One other potential gut directed therapy to improve outcomes in pancreatitis is glutamine. Glutamine, a conditionally essential amino acid, is one of the major fuel sources for enterocytes (Hall *et al*, 1996). Glutamine has been shown to improve outcome in patients receiving intravenous nutrition (Griffiths *et al*, 1997, Houdijk *et al*, 1998). Glutamine appears to exert a beneficial effect on a number of organ systems, and its exact mechanisms of action remain unclear. There is, however, a mounting body of evidence to suggest that one of the

main therapeutic actions of glutamine is a beneficial effect on the gut barrier. Several animal studies have demonstrated glutamine to be protective against TPN-induced gut atrophy (Chen *et al.*, 1994, O'Dwyer *et al.*, 1989, Platell *et al.*, 1993). In addition, glutamine has been demonstrated to protect against bacterial translocation across epithelial cell monolayers (Clark *et al*, 2003).

A series of studies have demonstrated that significant oxidative stress occurs in pancreatitis. Source for reactive oxygen species could be either activated neutrophils or intestinal ischaemia and reperfusion injury. Several anti oxidants have been investigated in animal models of pancreatitis showing benefit; however there is no convincing evidence yet from human studies. This highlights the need for more robust human trials using anti oxidants in humans as there is convincing theoretical argument for possible benefit.

The complex cytokine interactions and inflammatory pathways which mediate SIRS/MODS in acute pancreatitis are being unravelled and may provide potential targets for novel therapies. TNF-α and IL-1 are important mediators in SIRS and probably play a role in the early stages of pancreatitis. Almost all the experimental studies examining the effects of anti-cytokine therapies in acute pancreatitis have neglected the effect on the intestine and intestine derived cytokine response (Denham, 1999).

The wider recognition of the role of the intestine in the pathophysiology of acute pancreatitis provides the impetus to identify markers of intestinal integrity and function that will have clinical and research utility. Intestinal mucosal acidosis by PH assessment techniques, for instance indicates ongoing splanchnic hypo-perfusion, and has been used to predict organ failure and death in critically ill patients (Marik, 1993).

Evolution of PCR and DNA fingerprinting techniques to identify bacterial DNA fragments in the circulation may yet provide a useful marker for gut barrier function. At present this

approach is too sensitive and may be representing live invading organisms, dead pre-absorbed organisms and the aftermath of bacterial phagocytosis.

3.6: Conclusion:

There is good evidence from this small cohort study to suggest persistent gut failure in pancreatitis may be associated with increased prevalence of SIRS and poorer clinical outcomes. There is no evidence from this study to suggest, that transient gut failure in patients with pancreatitis has any influence on SIRS/MODS and clinical outcomes. These observations merit further investigation in a larger trial.

CHAPTER: FOUR

The effect of gut failure on SIRS/MODS and clinical outcomes in critically ill patients.

4.1 ABSTRACT:

Background: Multiple organ dysfunction syndrome (MODS) is the leading cause of death in critically ill patients. The role of dysfunction of the gastrointestinal tract in the pathogenesis of systemic inflammatory response syndrome and multiple organ dysfunction syndrome complicating the course of critically ill patients has been suspected for more than 50 years. However, several hypotheses have been proposed and sometimes refuted to establish a link between state of gut function and clinical outcomes in critical illness. The aim of this study is to investigate the relationship between the functional state of gut and clinical outcomes in critically ill patients.

Methods: This was a prospective observational audit at a single centre. Patients admitted to intensive care unit at Scarborough hospital were recruited in to the study. Natural course of their illness and clinical outcomes were followed and prospectively recorded. Several data points relating to the illness, state of gut function, SIRS, MODS, septic complications, length of ITU stay and clinical outcomes were recorded and analysed to assess the relationship between state of gut function and clinical outcomes.

Results: A total of 40 patients were studied as part of this prospective audit. Out of these 40 patients twenty patients had gut failure or inadequate gut function and received parenteral nutrition and twenty patients had functioning gut and received enteral nutrition. There was no significant difference between the groups with respect to prevalence of SIRS at various time points. There was no significant difference with respect to acute phase response or overall clinical outcomes.

Conclusion: The results of this prospective observational study do not show any significant increased prevalence of SIRS amongst critically ill patients with gut failure when compared with critically ill patients having adequate gut function.

4.2 BACKGROUND:

Patients with multiple organ failure (MOF) are the most challenging group of patients in modern intensive care. The gastrointestinal system is one of the clinically important systems in MOF, with strong evidence of bacterial translocation in ICU patients supporting the concept of the gut being a motor of MOF (Galley et al., 2002; Wiest et al., 2003). On one hand, it has been suggested that nosocomial infection caused by enteric bacteria is directly related to the development of MOF in some patients. On the other hand, this may be a secondary phenomenon and a symptom rather than a cause of MOF (Nieuwenhuijzen et al., 1996). The functional status of the gastrointestinal tract has not been studied in full depth and consensus criteria for diagnosis of gastrointestinal failure (GIF) have not been established. Some authors describe GIF as "gastroparesis and intestinal ileus" (Marino, 1998), while others define it as gastrointestinal haemorrhage (Baue, 2000).

There are several scoring systems for organ dysfunction in day to day clinical practice. None of these scoring systems for organ dysfunction and severity of illness widely used today take the function of the gastrointestinal tract into account. The multiple organ failure (MOF) score, one of the first attempts to quantify the severity of organ dysfunction and failure, originally included gastrointestinal tract when it was developed in 1985 (Goris et al., 1985). Revision of the score 15 years later however, concluded that GIF should not be considered in the assessment of the MOF score due to problems in definition and reliability (Lefering et al., 2002). The SOFA score is another well-established score for assessing organ dysfunction or organ failure over time, and has proven useful for evaluating morbidity and predicting ICU mortality (Vincent et al., 1998; Levy et al., 2005). The six organ systems used in calculating this score do not include GIF. It has not been proven whether an assessment of the gastrointestinal system would add predictive power to SOFA estimations of ICU survival.

Gut dysfunction occurs frequently among critically ill patients. Little attention has been given to the status of the gastrointestinal tract in the critically ill patient historically. This is mainly due to the fact that traditional teachings in critical illness have promoted the dogma that the gut is dormant, metabolically inactive, and of little physiologic and pathologic significance.

In spite of several human studies suggesting that the gastrointestinal tract contributes significantly to morbidity and mortality in critically ill ICU patients, data on risk factors, time-course, prognostic importance, and suggestions for clinical evaluation of gastrointestinal function are surprisingly poor and inconsistent in existing literature.

The gastrointestinal (GI) tract and its associated mesenteric lymphatics present the body's largest absorptive surface that is exposed to foreign antigens, microbes, and other potentially injurious elements. The dual functions of selective absorption and constant protection make the GI tract both critical to the body's functioning and vulnerable to infection in situations of physiologic stress such as critical illness. Evidence supporting the role of intestinal barrier dysfunction in the gut-origin of sepsis hypothesis and subsequent SIRS, MODS and sepsis syndrome has been well documented in studies relating to major surgery, trauma, burns and critical illness.

Gut mucosal damage occurring as a result of ischemia and subsequent reperfusion or malnutrition thus enables intestinal flora in association with gut associated lymphoid tissue to activate a systemic inflammatory response that overwhelms the critically ill patient leading to development of SIRS, MODS and sepsis syndrome (Rotstein et al., 2000). Approximately one-third of bacteraemic patients who die of MODS do not have causative septic foci of infection identified. This observation suggests that bacteria and endotoxin translocation from the gut initiates and exacerbates SIRS, which promotes the development of MODS.

Various mechanisms mediated via Toll-like receptor 4 (TLR4), Imbalanced production of cytokines from gut associated lymphoid tissue have been described in the literature to explain gut playing central role in development of SIRS, MODS and sepsis syndrome.

From the perspective of day to day clinical management of critically ill patients it is important to recognise the state of gut function and address the shortfalls early to improve the clinical outcomes.

The aim of this observational audit is to identify the correlation between the state of gut function and its influence on SIRS and clinical outcomes amongst critically ill patients.

4.3 METHODS:

4.3.1 Patients

This was a prospective observational audit including 40 patients admitted to intensive care unit at Scarborough Hospital. Out of these forty patients 20 patients had inadequate gut function and were fed parenterally by means of TPN to meet calorie and nitrogen requirement. Remaining twenty patients had adequate gut function and were receiving enteral nutrition to meet calorie and nitrogen requirement. Patients in this group were treated primarily by the caring physicians and intensivists as per the standard of care appropriate for individual patients. Several of these patients received antibiotics, organ support and inotropes as considered appropriate for the clinical condition. This patient group included both medical and surgical patients with wide variety of clinical conditions. Several data points relating to these patients was collected prospectively and analysed relating state of gut function to prevalence of SIRS, MODS, Sepsis and other clinical outcomes. Data points collected has been summarised in table 2.1.

Variables recorded (Table 4.1)

Demographic data

- Age
- Sex
- BMI
- Diagnosis
- Any surgical intervention
- Reason for admission to ITU
- Length of ITU and hospital stay

State of gut function and nutritional support

- Clinical assessment
- Tolerance to enteral feed
- Episodes of feed intolerance
- Feed related complications
- Length of TPN
- Time taken to establish feeding

Severity of Illness

- APACHE-II score
- SOFA score
- SIRS/Sepsis syndrome
- Any other organ failure

Complications and clinical outcomes

- Septic complications
- Readmission to ITU
- Mortality

4.3.2 State of gut function:

A pragmatic approach was taken in defining gut function based on primary function of gut that is nutrition. If patient is on enteral nutritional support adequacy of gut function was defined as the tolerance of 80% or more of calculated nutritional requirements for a continuous period of at least 48 hours. Anything less than this was classified as inadequate gut function or gut failure. Time taken to establish enteral tolerance in those who are on enteral feed was prospectively recorded. Patients who could not tolerate enteral feed as required were on parenteral nutritional support and were classified as having inadequate gut function.

4.3.3 SIRS, Sepsis and clinical outcomes

Data relating to incidence and prevalence of SIRS, any organ failure sepsis and clinical outcomes were prospectively recorded. Cultures of blood, urine, or sputum were taken whenever deemed clinically appropriate. As a measure of clinical progress of the disease Serum CRP, WBC count, and Serum albumin levels were monitored.

American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee proposed definitions for sepsis and the clinical syndromes associated with it were used (American College 1992). These definitions were described in chapter 2 methods section of this thesis.

All complications during hospital stay were recorded prospectively. For purposes of this study, septic morbidity was defined as the presence of recognized pathogens in body tissues that are normally sterile, confirmed by the results of culture and supported by clinical, radiological, or haematological evidence of infection.

Data relating to acute inflammatory response was collected by recording serum levels of C-reactive protein, Total white blood cell count, neutrophils count and serum albumin.

4.3.4 Statistical analysis

All the prospectively collected audit data was stored on Microsoft Excel spreadsheet. Statistical analysis was carried out using SPSS software, version 19 (SPSS inc., Chicago, USA) Qualitative data was analysed using Chi-square test or fishers' exact test for smaller samples. Quantitative data were expressed as medians and inter quartile ranges (IQR). Difference between the medians was evaluated using the Mann-Whitney U test or Friedman's test. A p-value of 0.05 or less was taken to signify statistical significance.

4.4 RESULTS

4.4.1 Patients

A total of 40 patients admitted to intensive care unit with various medical and surgical primary pathologies and were receiving adjuvant nutritional support as were unable to eat normally. Out of these 20 patients had inadequate gut function and were fed parenterally, remaining 20 patients had adequate gut function and were fed enterally. All the patients in enteral feed group were fed via an NG tube placed at the bed side. Both the groups were comparable with respect to their age, sex, BMI, APACHE II score, POSSUM at the time of entry in to the study. Demographics are summarised in Table 4.2.

4.4.2 Gut function and feeding:

All 40 patients were assessed by the clinicians and dietetic team to decide on need for adjuvant nutritional support, state of gut function and route of feed administration. At the time of recruitment in to the audit patients were either established on enteral feed or parenteral feed. A total of 20 patients were receiving TPN as their gut function was inadequate due to feed intolerance. Out of these 20 patients 4 were receiving small volume stimulant enteral feed at the rate of 10-20 ml per hour. Remaining 20 patients were established on enteral feeding at the goal rate as they have functioning gut. Patients on enteral feed took a median (IQR) of 3 (1-4) days to establish on feeding. Median length of feeding was 7 (4-13) days in patients with adequate gut function and 9 (5-17) days in patients with inadequate gut function. There were all together 7 episodes of enteral feed intolerance in group with adequate gut function due to high aspirates. In the inadequate gut function group 5 patients further went on to receive enteral nutrition once they have had their gut function returned.

Demographics	Functioning gut (n=20)	Gut failure (n=20)	p value
Age	69 (58-77)	71 (60-83)	ns
Sex ratio (Male : Female)	13 : 7	11 : 9	ns
BMI	23.7 (18.3-33.7)	25.3 (19.6-34.3)	ns
APACHE II	10 (9-14)	11 (10-15)	ns
POSSUM	35 (30-40)	36 (32-40)	ns
Surgical intervention	12	9	ns
Length of ITU stay	7 (4-10)	6 (4-14)	ns
Total length of hospital stay	13 (8-21)	17 (10-29)	ns
Primary diagnosis:			
Intestinal obstruction	3	5	
Pancreatitis	2	3	
Post ‘AAA’ repair	2	1	
Gastro intestinal bleeding	1	2	
Post operative complication	5	6	
Pneumonia	4	2	
others	3	1	

TABLE 4.2 Baseline patient characteristics of the study population.

4.4.3: Prevalence of SIRS, organ failure and septic complications:

Data relating to prevalence of SIRS is shown in Figure 4.1. There were 9/20 (45%) patients in adequate gut function group with SIRS on day1 when compared to 11/20 (55%) in gut failure group this was not statistically significant. On day 3 there were 6/20 (30%) patients in functioning gut group when compared to 8/20 (40%) of patients in gut failure group. On day 5 there were 5/20 (25%) patients were found to have SIRS in functioning gut group when compared to 7/20 (35%) patients. On Day 7 there were 3/20 (15%) patients with SIRS when compared to 5/20 (25%) patients with gut failure. Even though there is slight increased prevalence of SIRS in gut failure group when compared to functioning gut group this difference was not statistically significant.

There were no recordable differences between the two groups with regards to the presence of other single organ failures: 14/20 (70%) in functioning gut group when compared to 16/20 (80%) in gut failure group. This may look very high percentage of organ failure, but these are critically ill patients with organ failure on intensive care receiving appropriate organ support. The time patients required organ support with inotropes, ventilators or dialysis and the length of ICU stay was not statistically significant.

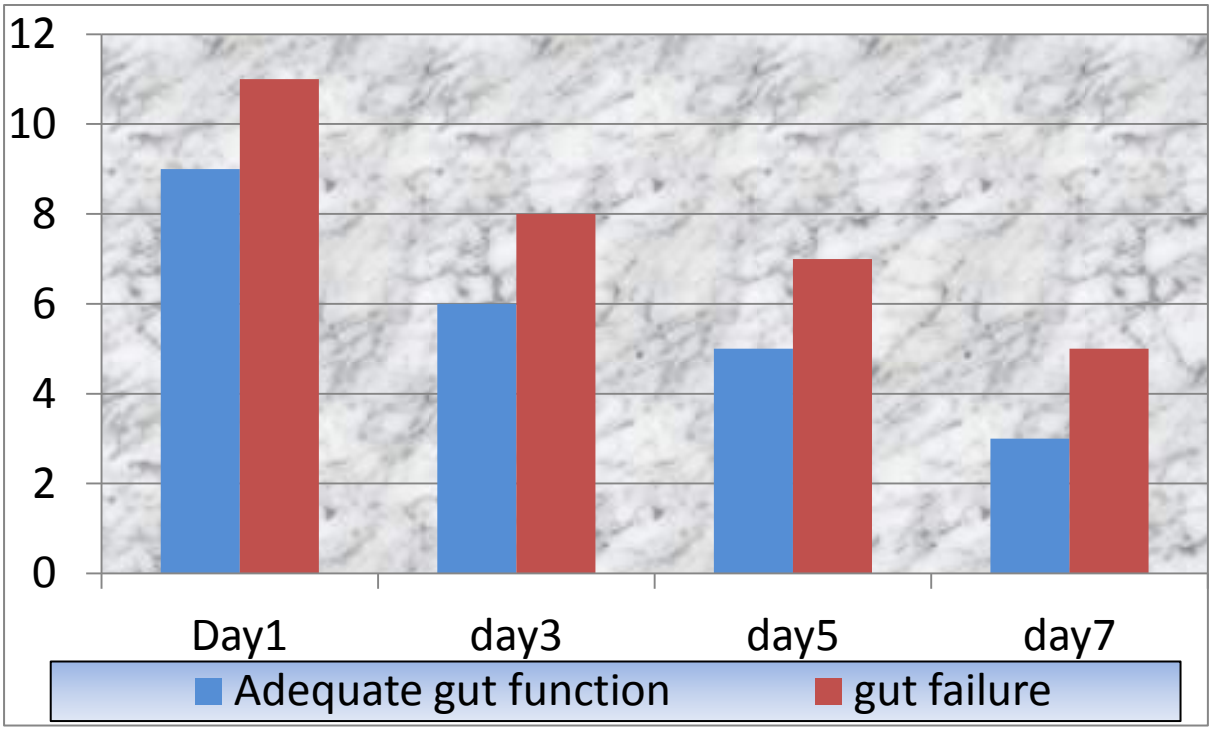
All together there were 24 septic episodes in the study population during study period 10/20 (50%) in functioning gut group when compared to 14/20 (70%) in gut failure group. This difference ($p=0.12$) was not significant. Chest was the commonest site of septic complication in both groups 4 in functioning gut group and 6 in gut failure group, followed by wound infection, urinary tract infection, intra abdominal sepsis and central line sepsis.

There were all together 12 deaths amongst 40 patients 5/20 (25%) in functioning gut group when compared to 7/20 (35%) in gut failure group this again was not statistically significant.

4.4.4 Acute phase response:

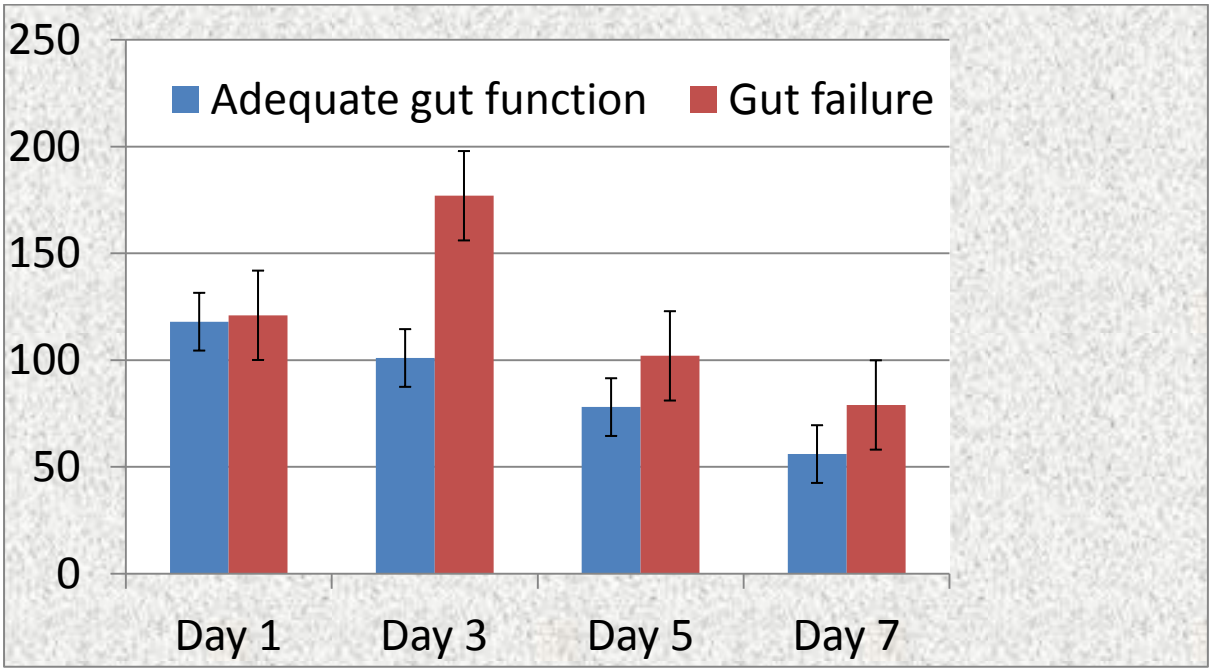
Acute phase response was measured by Serum CRP, Total white cell count and serum Albumin on Days1, 3, 5 and 7 and the results are expressed as Medians in figures 4.2, 4.3 and 4.4. There was no significant difference between the groups.

Prevalence of SIRS: on days 1, 3, 5, and 7 in between groups.



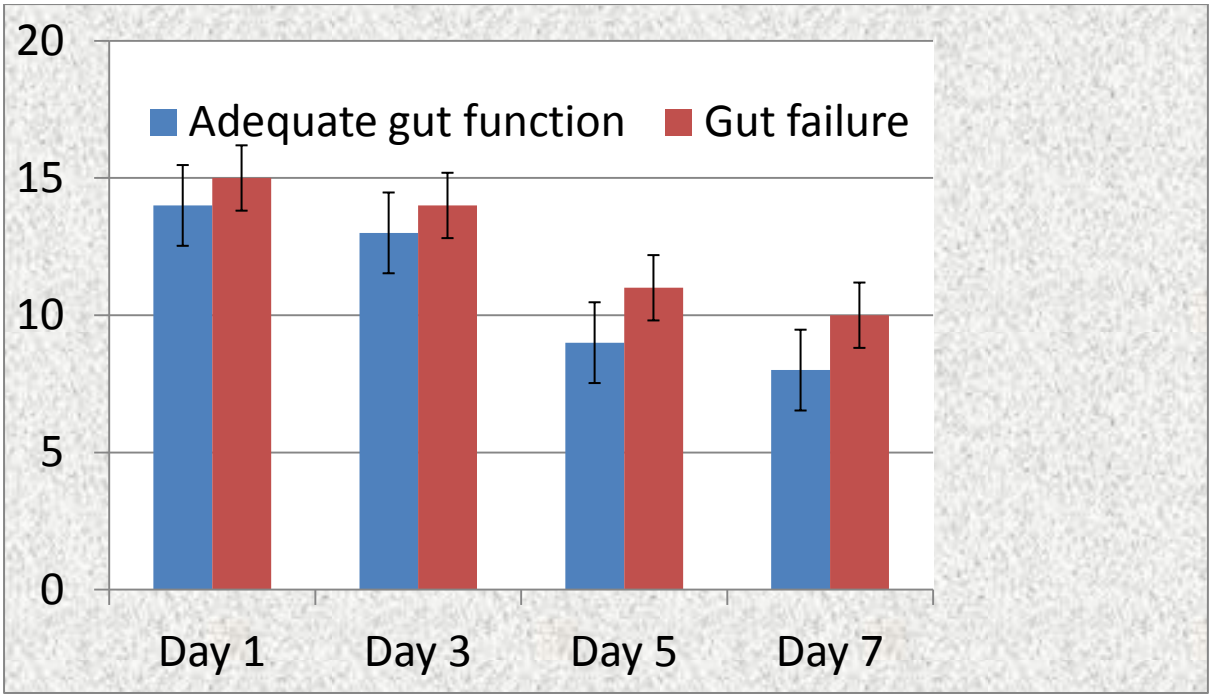
X-axis: Time scale in days Y-axis: Prevalence of SIRS

Figure 4.1: Prevalence of SIRS between the groups at various time points.



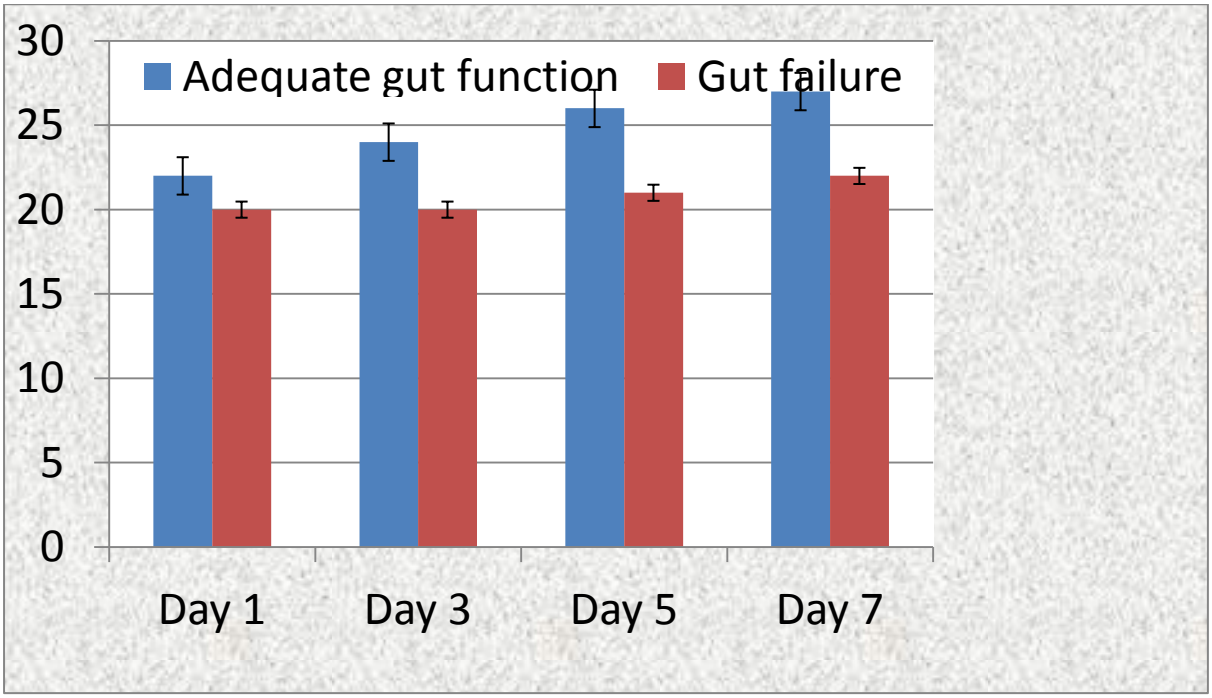
X-axis: Time scale in days, Y-axis: Median CRP level

Figure 4.2: Serum CRP levels on day1, 3, 5 and seven expressed as mg/L



X-axis: Time scale in days, Y-axis: median WBC count($X10^9$/L)

Figure 4.3: Total WBC count on day1, 3, 5 and 7 ($X10^9$/L)



X-axis: Time scale in days, Y-axis median serum albumin level (gm/L)

Figure 4.4 Serum Albumin level on day1, 3, 5 and 7 (gm/L)

4.5 DISCUSSION:

Results from this observational study amongst critically ill patients suggest SIRS is common in critically patients as a group. Even though this study has not shown any statistically significant difference with respect to prevalence of SIRS between group with gut failure and the group with adequate gut function, there is a tendency towards increased prevalence of SIRS in patients with gut failure.

Although the pathogenesis of SIRS/ MODS and sepsis in critically ill remains unclear, it is generally agreed that the gut plays a central role in pathogenesis. There is good evidence to suggest that changes in the GI microflora associated with the use of antibiotics, immunosuppression or change in the intestinal permeability may disrupt the normal ecological balance and alter gut barrier function (Stechmiller et al., 1997). This altered gut barrier function may predispose to the translocation of bacteria and endotoxins from the intestinal lumen resulting in SIRS and septic complications (Marshall et al., 1999; Botterill et al., 2000). Several interventions have been proposed aiming at gut protection in critically ill, they include maintenance of splanchnic perfusion and the prevention of mucosal ischemic/reperfusion injury (Landow 1994). Gut specific nutrients like glutamine have been proposed aiming at improved enterocyte function to prevent gut barrier function (Conejero et al., 2002) One other potential target proposed was synbiotics aimed at altering gut microflora in critically ill.

4.5.1: Gut function measurement and monitoring:

Due to the lack of objective, uniform definition of gut dysfunction, monitoring of gut function must be based on indirect indicators. Tolerance to enteral feeding is probably the most commonly used indicator in the clinical setting. Its relevance can be improved when performed in the context of a predefined feeding protocol. However interpretation of this in clinical context needs further evaluation. Absorption tests and markers of increased

permeability assess relevant aspects of gut function, and they can be relatively easily applied for research purposes. With the current methodology, absorption and permeability tests are not suitable for day to day clinical monitoring of gut function in the intensive care setting.

Gastrointestinal tonometry has been described to assess the splanchnic perfusion. This does not reflect the gut function directly.

4.5.2: Critically ill and potential gut directed therapies

Several strategies have been proposed to prevent gut dysfunction and to treat existing functional abnormalities, they can broadly be categorised in to:

A. Methods to improve tissue perfusion in order to balance tissue oxygen supply and blood flow to the metabolic Demands.
B. Methods of modifying GI microflora ranging from prevent bacterial overgrowth to selective decontamination.
C. Gut specific nutrients aimed at supporting structural and functional intestinal integrity.

Distribution of blood flow within the intestinal wall is heterogeneous, and attempts to improve perfusion may have variable effects in the different layers of the gut. The mucosa, with its high metabolic demands and villous micro vascular anatomy, is particularly susceptible to inadequate perfusion. Hypovolemia reduces gastrointestinal blood flow and splanchnic vasoconstriction is prolonged after correction of hypovolemia. Accordingly, prevention and aggressive treatment of hypovolemia is the cornerstone of supporting gut perfusion. Splanchnic blood flow responses may markedly vary among individuals who are critically ill. In general, therapeutic interventions that increase cardiac output are likely to increase total splanchnic blood flow in patients with relatively intact vasoregulation. Attempts to improve perfusion may also result in metabolic alterations and modify the balance between oxygen supply and demand for the intestinal mucosa.

Loss of bacterial homeostasis is a characteristic feature of gut dysfunction in the critically ill. Selective decontamination of the gastrointestinal tract reduces nosocomial infections. Several regimens and combination treatments have been tested in critically ill setting with very limited success. Theoretically there is sound argument for selective decontamination to favourably alter GI microflora in combination with synbiotics.

The most important stimulus for intestinal epithelial growth and function is the presence of nutrients within the gut lumen. The direct presence of nutrients provides local substrate for cellular oxidation and creates a mechanical stimulus for increased proliferation of enterocytes. The indirect trophic effects of enteral nutrients are mediated through the increased production of trophic gastrointestinal hormones which act via autocrine, paracrine, and endocrine pathways.

Several gut specific immune enhancing substrates have been studied they include glutamine, arginine, fish oils, and nucleotides. Diminution of glutamine availability leads to increased intestinal permeability, which can be counteracted by enriching parenteral nutritional regimens with glutamine dipeptides in patients with inadequate enteral tolerance. The depleted gut is characterized by an inflammatory response in which villus atrophy coincides with increased crypt cell proliferation. Glutamine appears to have an anti-inflammatory effect in this setting.

4.6 CONCLUSION:

The results of this prospective observational study do not show any significant increased prevalence of SIRS amongst critically ill patients with gut failure when compared with critically ill patients having adequate gut function. These findings needs to be evaluated in robust clinical studies to identify mechanisms involved in development of SIRS and MODS and role of gut.

CHAPTER FIVE

GUT FUNCTION, SIRS AND CLINICAL OUTCOMES IN ELECTIVE SURGICAL PATIENTS

5.1 ABSTRACT

Background: Surgical stress response is part of the systemic reaction to every surgical procedure and encompasses a wide range of endocrinological, immunological and haematological effects. Although the magnitude and duration of the stress response are proportional to the surgical injury many factors may alter the extent and intensity of the human body reaction. Gut is one of the pivotal organs which has multitude of functions and non functioning gut is associated with poorer surgical outcomes. The aim of this study was to assess effect of gut function in response to surgery on clinical outcomes amongst elective surgical patients undergoing Breast and GI surgery.

Methods: This was a prospective observational audit including 20 patients undergoing mastectomy with level II axillary clearance for breast carcinoma and comparing these patients with 20 patients undergoing hemi colectomy for colonic carcinoma and assessing state of gut function in the post operative period and its influence on SIRS and clinical outcomes.

Results: In this study there were 2/20 (10%) of patients with Post operative gut dysfunction who underwent breast surgery on day one post operatively when compared to 9/20 (45%) of patients who underwent colectomy. This difference was statistically significant. Post operative gut failure was transient and lasted for less than 48 hours across the group. There was associated relative increase in prevalence of post operative SIRS amongst patients undergoing colectomy 5/20 (25%) when compared to 2/20 (10%) amongst colectomy patients.

Conclusion: Transient gut failure in the post operative period is common amongst GI surgical patients when compared to breast surgical patients. This was associated with increased prevalence of SIRS and post operative septic morbidity.

5.2: Background:

Interest in the importance of the gut after injury or operation has waxed and waned over five decades. The gastrointestinal (GI) tract is now considered an important organ in contributing to systemic toxicity, SIRS, sepsis, and single or multiple organ failure (MOF) in patients after injury or operation and has been associated with overall clinical outcomes. Mechanisms involved in these outcomes are still very poorly understood.

The reaction of the human body to various noxious stimuli is termed as 'stress response' (Aosasa et al.,2000). In particular, surgical procedures lead to a variety of profound physiological alterations characterised by changes in haemodynamics, endocrine and immune functions. Under normal circumstances, the human's regulator systems respond to surgical stress by mounting complex neuro endocrine and inflammatory response. This complex stress response has been implicated in gut dysfunction in surgical patients; however there exists no direct linkage.

Tolerance of an enteral diet is one of the fundamental components of postoperative wellness, along with the ability to mobilize freely without supplemental oxygen and a readiness to be discharged home as soon as possible. Post operative gut dysfunction is common and is most common cause for delayed discharge from hospital following surgery.

The pathophysiology of postoperative GI tract dysfunction can be ischemic, metabolic, toxic, neurogenic, myogenic, pharmacologic, and mechanical or combination of any. It is important to recognize that in many cases no single factor explains the whole story behind postsurgical GI tract dysfunction. Mechanical pathogenesis refers to any manipulation of the gut that causes an inflammatory response in the guts various layers, resulting in injury (Schwarz et al., 2002). However, GI tract dysfunction commonly occurs after operations involving non GI

tract procedures like hip operations, cardio thoracic operations and pulmonary surgery to name a few. In these procedures gut was not handled at all.

A common mechanism that is often implicated in post operative gut dysfunction is perioperative gut ischemia resulting in low-grade injury. Low-grade hypovolemia and hypotension in peri-operative period is common irrespective of surgery, this in turn can cause loss of perfusion to the tip of the microvillus, triggering apoptosis and potentially necrosis, contributing to post operative gut failure and associated sequel.

After understanding that the gut dysfunction is common in post operative period, the aim of this prospective observational study was to assess the prevalence of gut dysfunction or failure in patients undergoing mastectomy with level II axillary clearance for breast carcinoma and hemi colectomy for colonic carcinoma, and its influence on clinical outcomes.

5.3 Methods:

5.3.1 Patients:

This was a prospective observational audit including 20 female patients undergoing elective mastectomy with level II axillary clearance for breast carcinoma with curative intent and comparing these patients with 20 female patients undergoing hemicolectomy for colonic carcinoma with curative intent. All the patients were prospectively observed in the peri-operative period to follow the natural course without any interventions. Several data points were recorded in the peri operative period including age, BMI, ASA grade, blood loss, state of gut function, prevalence of SIRS, Sepsis, length of hospital stay, episodes of peri-operative hypotension and overall clinical outcomes for final analysis.

5.3.2 Gut function:

For the purpose of this study gut function was defined based on primary function of gut IE nutrition. If patient can tolerate three light meals a day they were classified as having adequate gut function and if not they were classified as inadequate gut function. Inadequate gut function was further classified as transient it lasted for less than 48 hours and persistent if it is more than 48 hours in the post-operative period.

5.3.3 Clinical outcomes:

Data relating to prevalence of SIRS, and associated syndromes were recorded as per the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee proposed definitions (American College 1992). Intra operative fluid balance and hypotensive episodes were prospectively recorded. Data relating to post operative length of hospital stay, inflammatory response measured by CRP and WBC count was collected.

5.3.4 Statistical analysis

All the prospectively collected audit data was stored on Microsoft Excel spreadsheet. Statistical analysis was carried out using SPSS software, version 19 (SPSS inc., Chicago, USA) Qualitative data was analysed using Chi-square test or fishers' exact test for smaller samples. Quantitative data were expressed as medians and inter quartile ranges (IQR). Difference between the medians was evaluated using the Mann-Whitney U test or Friedman's test. A p-value of 0.05 or less was taken to signify statistical significance.

5.4 RESULTS:

5.4.1 Patients:

All together 40 female patients who underwent elective surgery for either breast or colonic carcinoma were audited prospectively. Out of the 40 patients 20 patients underwent mastectomy with level II axillary clearance for breast carcinoma with curative intent, remaining 20 patients underwent right hemi colectomy for colonic carcinoma with curative intent. None of these patients have any metastatic disease. To avoid the sex bias only female patients were selected. These patients were comparable with respect to their age, BMI, duration of surgery, and ASA grade. Basic demographics for these patients are summarised in table 5.1. All the patients who underwent hemi colectomy were managed as per enhanced recovery after surgery protocol. All 20 patients who underwent breast surgery had 14-Fr vacuum drain applied to axilla as standard operative protocol and the drain was removed when the drainage volume was less than 50 ml in 24 hours prior to their discharge from the hospital. Only one out of 20 patients who underwent hemi colectomy had a single non suction drain placed in to peritoneal cavity and was removed on the first post operative day.

5.4.2 State of gut function:

Out of 20 patients who underwent mastectomy 18 (90%) patients were able to tolerate three light meals on first post operative day when compared to 11 (55%) patients who underwent colectomy. On day two 19 (95%) of patients who underwent breast surgery were able to take three light meals a day when compared to 13 (65%) patients who underwent hemi colectomy this again was statistically significant (p=0.0436). On day 3 all 20 (100%) patients who underwent breast surgery were able to tolerate three light meals a day when compared to 16(80%) of patients who underwent hemi colectomy this was statistically not significant (p=0.106). Transient gut failure or inadequate gut function was observed in both groups however this was more common in colectomy patients when compared to patients who

underwent breast surgery. None of the patients who underwent breast surgery had persistent gut failure beyond 48 hours when compared to 4 patients who underwent colonic surgery.

5.4.3 SIRS and clinical outcomes:

Out of 20 patients who underwent breast surgery 2 (10%) patients were found to have 2 criterion for SIRS present on fist post operative day when compared to 5 (25%) patients who underwent colectomy this was statistically significant (p=0.040). Out of 2 patients with SIRS in breast surgery group one had normal enteral tolerance and one patient had inadequate enteral tolerance. Out of five patients in colectomy group with SIRS 3 had inadequate oral diet and two had adequate oral diet. On day 2 none of the patients who had breast surgery had SIRS when compared to 3 patients with SIRS in colectomy group this was not statistically significant (p=0.23).

Median (IQR) duration of hospital stay for mastectomy group was 2 (2-4) days when compared to colectomy group 4(4-6) days. This was statistically significant.

5.4.4 Septic complications and mortality:

There was only one septic complication in breast surgery group and this was post operative wound infection after discharge and this superficial infection was treated with oral antibiotics. In colectomy group there were all together 4 septic complications during the hospital stay. One patient developed chest infection and two patients had wound infection. One patient had urinary tract infection. Further 2 patients were found to have wound infection and treated in the community with oral antibiotics and one patient had urinary tract infection and was again treated in the community with oral antibiotics. None of the patients required readmission within 30 days. There were no deaths in either of the groups within 30 days.

5.4.5 Inflammatory response measured with CRP:

As standard peri operative follow up 17 out of 20 patients who underwent breast surgery had serum CRP levels measured on first post operative day with a median (IQR) value of 67(32-141) mg/L and 19 out of 20 patients had their serum CRP levels measured in colectomy group with a median (IQR) range of 78(41-167). Serum CRP levels were markedly elevated in both groups on first post-post operative day. On second post operative day 8 out of 20 patients in breast surgery group with a median (IQR) of 72(55-156) mg/L and 9 out of 20 patients in colectomy group with median (IQR) of 81(53-202) had their CRP levels measured. This was not statistically significant between the groups.

5.4.6 Intra-operative hypotension and blood transfusion:

There were two patients in breast surgery group and three patients in the colectomy group with recorded brief period of intra operative hypotension. By the time of this audit Oesophageal Doppler or goal directed fluid therapy was not a standard operative protocol in our unit. One patient who underwent hemi colectomy had pre operative three unit blood transfusion due to anaemia. None of the remaining patients received any perioperative blood transfusion in either of the groups. These numbers are too small to make any meaningful analysis or draw a conclusion.

Patient demographics	Breast surgery group (n=20)	Colectomy group (n=20)
Age median (IQR)	59 (47-79)	62 (53-81)
Sex (Female: Male)	20 : 0	20 : 0
BMI	27 (22-31)	24 (20-31)
ASA	2 (1-3)	ASA 2 (2-3)
Duration of Surgery in min	80 (60-110)	90 (70-120)
Operative blood loss in ml	110 (70- 330)	100 (80-3000

Table: 5.1: Patient demographics between the groups.

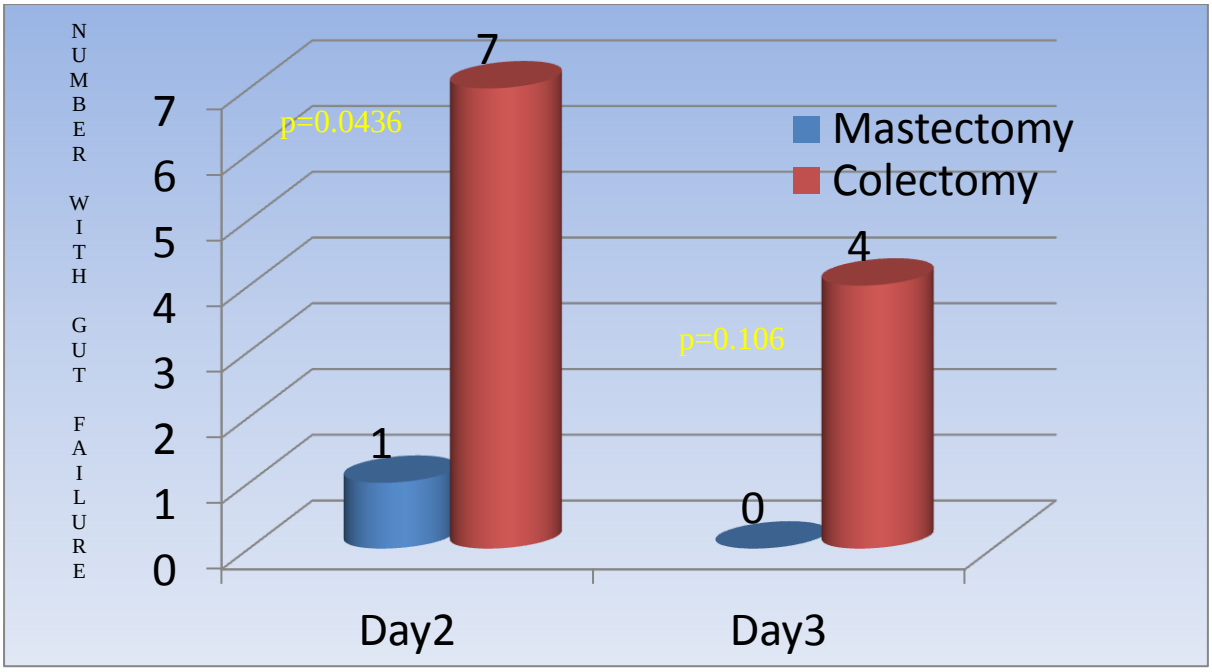


Figure 5.1 Patients with inadequate gut function in post operative period

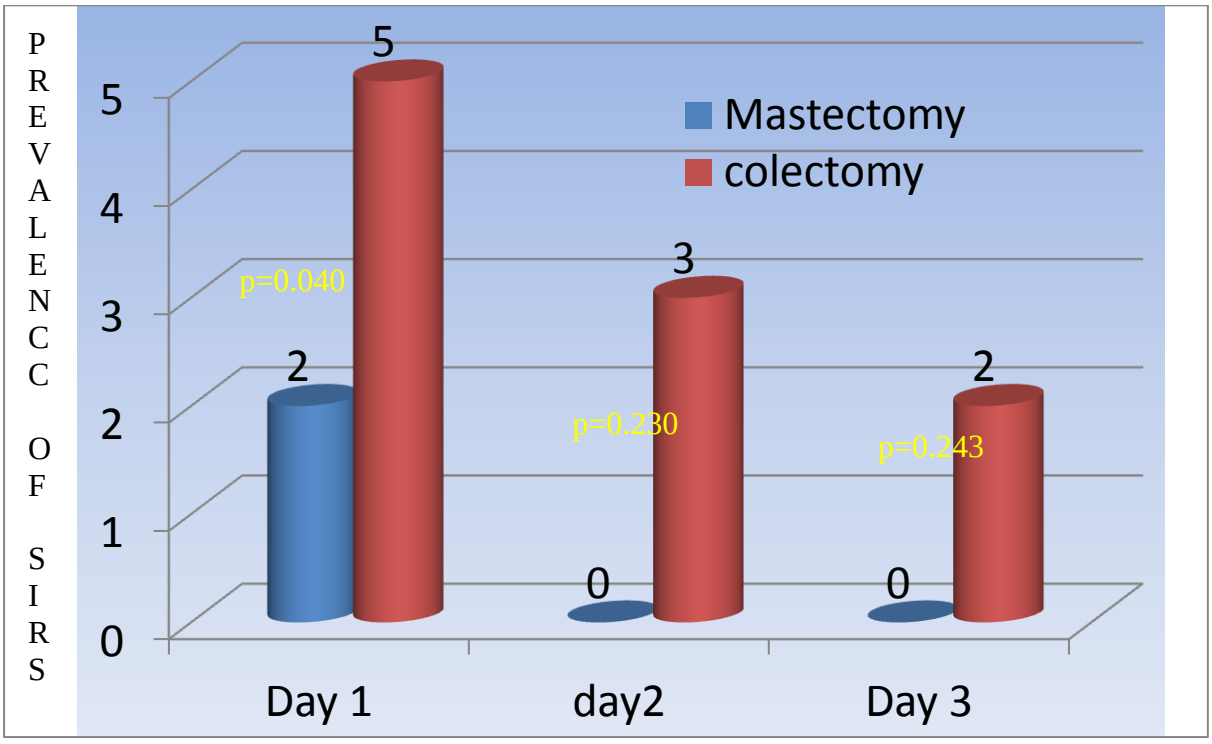


Table: 5.2 Prevalence of SIRS in the post operative period between the groups.

5.5 DISCUSSION:

From this study it is apparent that inadequate gut function is common in post operative period in elective surgical setting. This is more common in elective GI surgery when compared to breast surgery. Inadequate gut function was primarily transient lasting for less than 48 hours in post operative period. Prolonged or persistent gastro intestinal dysfunction is uncommon in breast surgery. This transient gut failure after elective surgery was associated with increased prevalence of SIRS in the post operative period amongst patients who underwent elective colectomy; however this was not the case in elective mastectomy. Both groups of patients have shown increased inflammatory response in the immediate post operative period. There was relative increase in septic morbidity in the post operative period amongst patients undergoing GI surgery when compared to breast surgery.

One possible mechanism which could be postulated for increased gut failure amongst patients undergoing GI surgery when compared to patients undergoing breast surgery could be via local trauma to the bowel serosal surface from mechanical handling of bowel leading to local inflammatory response in turn gut mounting generalised systemic inflammatory response from gut associated lymphoid tissue. Alternative possibility could be due to ischaemia reperfusion injury to the gut mucosal surface due to temporary disruption to microcirculation from mechanical bowel handling.

The pathophysiology of postoperative GI tract dysfunction can be attributed to ischemic events in the peri-operative period, metabolic factors, toxic influence of pharmacological agents, neurogenic, myogenic, or mechanical factors. Out of all these possible events mechanical and ischaemic factors have been investigated in the setting of GI surgery.

Postsurgical ileus, signifying the temporary impairment of coordinated propulsive intestinal peristalsis leading to inadequate gut function, remains a well-documented and virtually inevitable consequence of open abdominal surgery. Despite its frequency and impact,

accounting for prolonged hospital stays and patient discomfort, little is known of the underlying cellular mechanisms of this surgical conundrum. Several theories on the mechanism of postsurgical ileus have been proposed. The activation of inhibitory spinal and inhibitory sympathetic reflexes, anesthesia and related hypovolaemia and poor end organ perfusion, humoral agents, and inflammation has been implicated as causes for this phenomenon. Several remedies have been suggested for improved clinical outcomes.

A targeted increase of intravascular volume and global blood flow perioperatively has been shown to improve surgical outcome. In clinical trials, the most common intervention to achieve the predetermined hemo dynamic goal has been fluid loading. This benefit in improved post operative outcome could be due to maintenance of end-organ perfusion thereby avoiding ischaemia re-perfusion injury associated loss of gut barrier and gut function. Use of esophageal Doppler ultrasonography to guide fluid administration intraoperatively is fairly common practice during surgery. Doppler-guided small boluses of colloid increased stroke volume, cardiac output, and oxygen delivery compared with conventional fluid management. This approach will help to maintain end-organ perfusion and avoid tissue ischaemia and ischaemia re-perfusion injury related gut failure.

One other therapy investigated was use of laxatives in the immediate post operative period to enhance recovery of gut function and this has not proven beneficial. Several studies have shown that postoperative chewing of gum was associated with early return of gut function and shorter hospital stay; however the mechanism involved in this benefit is unclear.

From this study it is clear that post operative gut dysfunction is more common following abdominal surgery. In spite of optimal fluid management and practice of multi modal optimisation gut failure associated morbidity is common. Theoretically there is sound argument for investigating further gut directed therapies to improve clinical outcomes following abdominal surgery. Elective GI surgical patients are unique group of patients who

are expected to have gut dysfunction in the post operative period. After reviewing the literature designed the next RCT using combination of interventions to pre optimise gut function and assess the benefit of such intervention in clinical setting.

5.6 CONCLUSION:

Gut dysfunction is common following GI surgery when compared to breast surgery and this is associated with increased prevalence of SIRS and post operative septic complications. Underlying mechanisms involved are unclear and may be multi factorial.

CHAPTER SIX:

Can the systemic inflammatory response to surgery be attenuated by optimisation of intestinal function? Prospective randomized clinical trial.

Abstract:

Aim: Inadequate gut function (IGF) is common following major gastrointestinal (GI) surgery. This attenuated GI function adversely and independently affects patient outcome. The use of gut specific nutrients (GSN) and selective gut decontamination (SGD) have separately been shown to curtail IGF and preserve gut barrier function respectively. In this study, both these strategies have been used together in an attempt to 'optimize GI function'. The primary aim of this study was to assess the combined effects of both GSN and SGD administration on clinical outcomes in patients undergoing major GI surgery.

Methods: One hundred consecutive patients undergoing elective gastrointestinal surgery were randomised to optimised gut function group or control group. Optimised gut function group received a cocktail of gut specific nutrients including glutamine, synbiotic and antioxidants, 5 days prior to surgery and selective bowel decontamination. Control group received standard peri-operative care. End points included assessment of gut barrier function by culture of mesenteric lymph nodes, measurement of SIRS and septic morbidity.

Results: Five patients were excluded owing to protocol violation, leaving 47 patients in control group and 48 patients in optimised gut function group. The groups were comparable with respect to age, sex, pathology, operation type, ASA and POSSUM physiologic score. Optimisation was associated with a significant reduction in bacterial translocation (33/47 versus 15/48, $p< 0.001$), a reduced incidence of SIRS on post-operative days 2 to 5 and fewer septic complications (21/47 versus 12/48, $p = 0.044$).

Conclusion: In this study optimisation of intestinal function by combining GSN and SGD preserved the gut barrier, reduced the incidence of SIRS and lowered septic morbidity. Before recommending changes in practice, further clinical trials are needed to better understand the mechanisms involved.

6.1 BACKGROUND:

Colonic carcinoma is the third commonest malignancy in adults in the UK affecting approximately 40,000 patients each year. The majority of these patients will undergo surgical resection of their tumours. The average duration of hospital stay following such surgery is of the order of 10-14 days. There is now good evidence to show that "enhanced recovery protocols" reduce lengths of inpatient stay without compromising patient safety. Common to all enhanced recovery programmes is an attempt to attenuate the surgical stress response, accelerate recovery, decrease complications, shorten hospitalisation and reduce health costs, all without compromising patient safety. The mechanisms by which such improvements are brought about are not well characterized, but it has been suggested that they may partly be a consequence of an enhanced recovery of gut function (Gatt *et al.*, 2005; Wakeling *et al.*, 2005).These protocols include a number of interventions which have been summarised elsewhere in this chapter. There is increasing evidence to suggest that preservation of gut function equates with improved outcomes possibly mediated by an attenuation of the systemic inflammatory response.

The gut is not just an organ of digestion. It is the largest immunological organ in the body, has an essential antigen recognition role, maintains a stable ecoflora which is essential to our health and well being and maintains the critically important intestinal barrier which prevents escape from the lumen of the gut of potentially pathogenic enteric bacteria. The mechanism by which standard ERAS protocols modify gut function is unclear. Most probably it is multifactorial. Interventions such as epidural anaesthesia and preoperative carbohydrate loading attenuate the stress response to surgery which indirectly effects gut function by

reducing splanchnic hypoperfusion and ischemia reperfusion injury. Avoidance of opiates and early reintroduction of oral feeding probably have a direct effect on gut function by stimulating gut motility.

In recent years there has been increasing interest in the role of treatments which are specifically designed to influence different aspects of gastrointestinal functions. Perhaps the most well documented gut directed therapy is glutamine. Glutamine is a conditionally essential amino acid which is the preferred fuel source for enterocytes. Previous research has demonstrated that glutamine can promote enterocyte growth, augment gut barrier function and reduce sepsis in critically ill patients. It has also recently been shown to favourably alter the cytokine response in surgical patients during the postoperative period. Other "gut specific" nutrients include fish oils, arginine and the use of anti-oxidants. Conveniently, anti-oxidants together with glutamine are now available as commercial preparation called "Glutamine plus".

One further target for improving gut function is the gastrointestinal microflora. It is now recognized that the gastrointestinal microflora are a fundamental component of the gut barrier. Not surprisingly, many previous studies have attempted to modify the gastro intestinal microflora using a variety of techniques of selective bowel decontamination. Many of these have demonstrated reductions in post operative septic morbidity but a consensus as to which combination of antibiotics or bowel preparation and pre and pro biotics are optimal remains unclear. Our unit has recently reported a study using a combination of synbiotics, mechanical bowel preparation and neomycin in which a significant reduction in the

prevalence of bacterial translocation suggesting a modulation in gut barrier function was found in elective colorectal surgical patients (Reddy *et al.*, 2008).

There is therefore a sound theoretical basis for proposing gut directed therapy in the form of gut specific nutrients and selective gut decontamination to optimise gut function with a view to improved clinical outcomes in this group of elective surgical patients undergoing elective colorectal surgery for colonic carcinoma.

In summary, therefore, the aim of this study was to optimise intestinal function in surgical patients undergoing elective colorectal surgery and determine whether or not this is associated with a reduction in the systemic inflammatory response. The hypothesis which underpins this study is that the use of specific gut directed interventions will attenuate the systemic inflammatory response with concomitant clinical benefits to the patient.

6.2: Aims:

1) The primary aim of this study was to assess whether optimisation of intestinal function is associated with attenuation of the systemic inflammatory response.

2) Secondary end points were length of hospital stay, time to return of gut function, prevalence of bacterial translocation, septic morbidity, and mortality.

6.3: Methods:

Approval for the study was obtained from both North Yorkshire Alliance R&D committee (ref: SNE-A0814) and York Research Ethics Committee (ref: 06/Q1108/118). Subjects were selected by the investigators from a pre-assessment clinic at least one week prior to their planned date of surgery. All these patients were provided with study information and details regarding study medication. Patients who expressed willingness to take part in this study have signed a study consent form.

All the patients in this study receive prophylactic antibiotics on induction of anaesthesia and two doses thereafter. Prophylactic antibiotics comprise a third generation cephalosporin and Metronidazole.

6.3.1: Randomisation:

Those patients who have been recruited in to the study were randomised to either control or study groups. Randomization was carried out by a series of sealed envelopes generated by a random computer-generated number sequence.

6.3.2: Exclusion criterion:

- Failure to obtain informed consent.
- Patients with pre-existing infections.
- Pregnant women and children under the age of 18 years will be excluded from the study.
- Patients on antibiotics in the previous 2 weeks before recruitment in to the study.
- Patients who were found to have intra abdominal sepsis at laparotomy were excluded from the data analysis to avoid bias.
- Patients with reported allergy to any study medication were excluded.

6.3.3: Study groups:

A) Control group:

This group was managed using standard enhanced recovery package (see Table 1). This is the current, accepted surgical practice in this unit.

B) Study group (optimisation of intestinal function):

1) Gut specific nutrients using "Glutamine plus®" as oral solution one sachet dissolved in 200ml of water three times a day for five days pre operatively and at the same dose in the post operative period, until gut function returns to normal.

2) Selective gut decontamination using combination of:

- Synbiotics: Travis® capsules 1 capsule three times a day along with Oligofructose powder 15gm per day for five days prior to surgery.
- Mechanical bowel preparation: using two sachets of Picolax® on the day before surgery.
- Oral Neomycin: for one day (1gm x 3doses) the day before surgery.

6.3.4: Data Collection:

All the patients enrolled in the study had:

1. NG tube passed under anaesthesia to obtain gastric aspirate. First 10ml of aspirate was discarded and next 5ml of NG aspirate was stored in a sterile container and transported to the microbiology laboratory for conventional microbiological culture and the methodological aspects of this has been described in detail in chapter2 This was to assess the gastric colonisation which is a surrogate marker for gut barrier function.

2. Sample of mesenteric lymph node was taken after mobilization of the colon and before ligating the vascular pedicle (intact blood supply). This sample of lymph node was transported to the microbiology laboratory in a sterile container in normal saline. This lymph node was homogenized with 0.5 to 1 ml of peptone water in a stomacher. This homogenate was subjected to conventional microbiological culture. Methodology used in bacteriological culture has been described in detail in chapter2.

   Isolates grown from the lymph nodes and NG aspirate were identified using standard microbiological techniques.

3. Four extra blood samples were collected from the patients for the purpose of this study, on days 0, 1, 3 and 5. These samples were alequeted and stored at -80^{o}c these samples were used to measure plasma levels of cytokines IL-6, TNF-α, IL-10 by ELISA.

4. The patients were seen by the principal investigator on every post operative day during the patients hospital stay. Numerous clinical and biochemical parameters were prospectively recorded. Clinical parameters were recorded on a patient data sheet. The data includes SIRS criteria, clinical recovery parameters, enteral tolerance, WBC count, and Haemoglobin, serum albumin and CRP levels.

5. Post operative septic morbidity and clinical outcomes: All postoperative septic morbidity was recorded prospectively for thirty days. Septic morbidity is defined as the presence of pathogens in the body tissues that are normally sterile, confirmed by result of culture and supported by clinical, radiological or haematological evidence.

6.3.5: Statistical analysis:

All patient data was prospectively collected for 30 days in post operative period. Results were tabulated on an Excel® spreadsheet (Excel for Windows®, Microsoft Corporation, Redmond, Washington, USA) and then analysed using SPSS® for Windows® version 17.0 (SPSS®, Chicago, Illinois, USA). Results for non-parametric data were expressed as medians and interquartile ranges (IQR). Relationships between groups were assessed using Chi squared test for binary outcomes or Fischer's exact test for small cohorts as appropriate. Continuous variables were compared with the Mann-Whitney *U*-test. Changes over time within groups were analysed with Friedman's test. Statistical significance was considered at the 5 percent level. A sample size calculation based on the published prevalence of bacterial translocation demonstrated 45 patients would be required in each group to show a significant decrease in the prevalence of bacterial translocation at a 5% significance level with a power of 80%. Owing to possible protocol violation and dropout rates of 10% in this kind of clinical trials intended to recruit one hundred patients with fifty patients in each group.

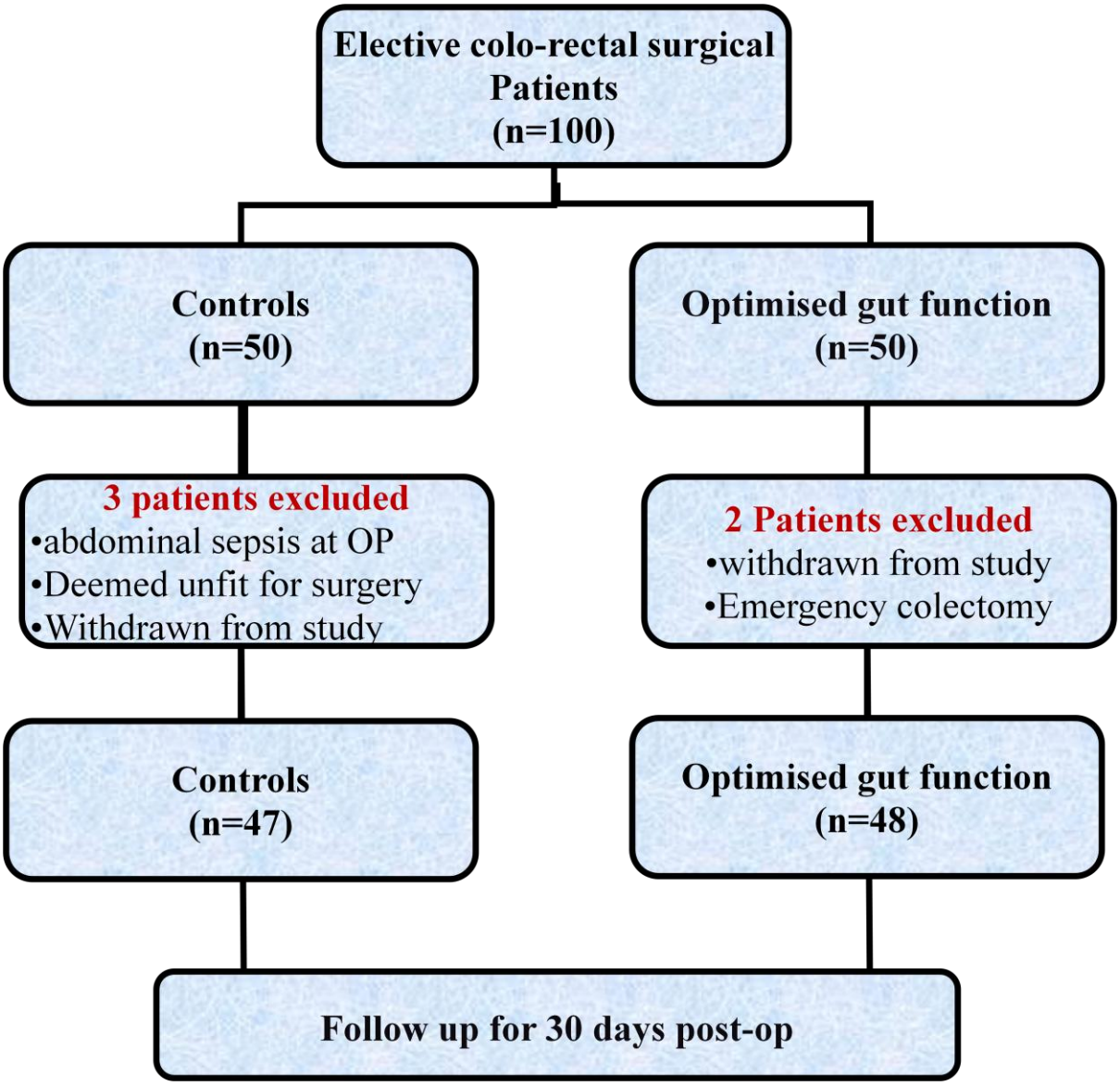


Figure: 6.1: Consort flow chart showing study design.

Control group	Study group
Pre operative: • Written information • No mechanical bowel prep • No Synbiotics • No neomycin • No gut specific nutrients • Carbohydrate loading & 3h fast	**Pre operative**: • Written information • Mechanical bowel preparation. • Synbiotics • Neomycin • Gut specific nutrients • Carbohydrate loading & 3h fast
Per operative: • High inspired O2 (80%) • No drains	**Per operative:** • High inspired O2 (80%) • No drains
Post operative: • Early diet and fluid reintroduction • Epidural analgesia and no opiates • Structured mobilisation plan	**Post operative:** • Early diet and fluid reintroduction • Epidural analgesia and no opiates • Structured mobilisation plan • Gut specific nutrients

Table:6.1 Comparitive table for peri-operative care between the groups.

6.4: RESULTS:

6.4.1: Patients:

A total of one hundred patients were recruited in to the study, 50 in the control group and 50 in the study group. Five patients, two from the intervention group and three from the control group were excluded from the study owing to protocol violation for the reasons as shown in the figure 1. Data from these patients were excluded from final analysis. This left 47 patients in the control group and 48 patients in the study group for final analysis.

There was no statistically significant difference between two groups with respect to their age, sex ratio, BMI, ASA grade and POSSUM at the time of entry in to the study (Table 2). Groups were comparable with respect to the surgical interventions.

	Controls (n=47)	Intervention (n=48)
Age	67 (29-94)	69 (27-91)
Sex ratio M:F	25:22	23:25
BMI	27 (21-33)	26 (19-34)
Malignancy	47	48
ASA	2 (2-3)	2 (2-3)
POSSUM	32 (20-36)	31 (24-34)

TABLE 6.2: Patient demographic details

Operation	Control group (n=47)	Study group (n=48)
Hemicolectomy	31	30
Hartmann's	3	4
Ant/AP resection	13	14

TABLE: 6.3: Types of operation carried out.

6.4.2: Intake of study medication:

All patients involved in the study were recruited at least five days prior to the planned surgery date and the study group was provided with gut specific nutrients and synbiotics. Compliance with medication was variable and median (IQR) duration of intake of glutamine plus was 4 (3-5) days in the pre operative period. Median (IQR) duration of intake of glutamine plus was 2 (1-4) days in the post operative period. This calculation was based on the amount of study medication returned to the investigator at admission. Forty four out of forty eight patients received oral neomycin three doses the day before surgery. Four patients received only one dose of oral neomycin due to late hospital admission. Forty two out of forty eight patients received two sachets' of Sodium picosulphate for mechanical bowel preparation day before surgery. The median (IQR) duration of intake of synbiotic was 4 (3-5) days in the pre operative period.

6.4.3: Gastric colonisation:

Nasogastric aspirates were obtained from 42/47 (89%) patients in the control group and 44/48 (91%) in the study group. At least one organism was isolated from NG aspirate of 23/42 (54%) in the control group and 20/44 (45%) in the intervention group. This was not statistically significant. *Candida* was the most frequently cultured species. 13/23 (56%) in the control group and 12/20 (60%) in the study group. *Enterobacteriaceae* group of organisms were isolated from 6/23 (26%) in the control group and 4/20 (20%) in the study group. This difference was statistically not significant. More than one organism was isolated from 11/23 (47%) in control group and 9/20 (45%) in the study group. These results are shown in table 4 in the appendix.

	Control group (n=47)	Study group (n=48)	p value
NG aspirate obtained	42 (89%)	44 (91%)	
Positive NGA	23/42 (54%)	20/44 (45%)	0.51
Candida	13/23 (56%)	12/20 (60%)	1.00
Enterobacteriaceae	6/23 (26%)	4/20 (20%)	0.72
Multiple organisms	11/23 (47%)	9/20 (45%)	1.00

TABLE 6.4: Spectrum of organisms in Nasogastric aspirates obtained at laparotomy

6.4.4: Systemic Inflammatory Response:

The American college of chest physicians and the society of critical care medicine defined criterion for SIRS were used (table 5) to record the true prevalence of SIRS between the groups in the post operative period (Figure 2)**.** Study group showed reduction of prevalence of SIRS from day one onwards when compared to control group (36/47, 76% versus 33/48, 68%, p = 0.391). This reduction in SIRS was statistically significant on days 3 (22/47, 46% versus 13/48, 27%, p = 0.046) and 4 (15/47, 31% versus 6/48, 12%, p = 0.022). By day five most of the patients in this study were discharged from hospital and the numbers remaining were too small to carry out any meaningful analysis.

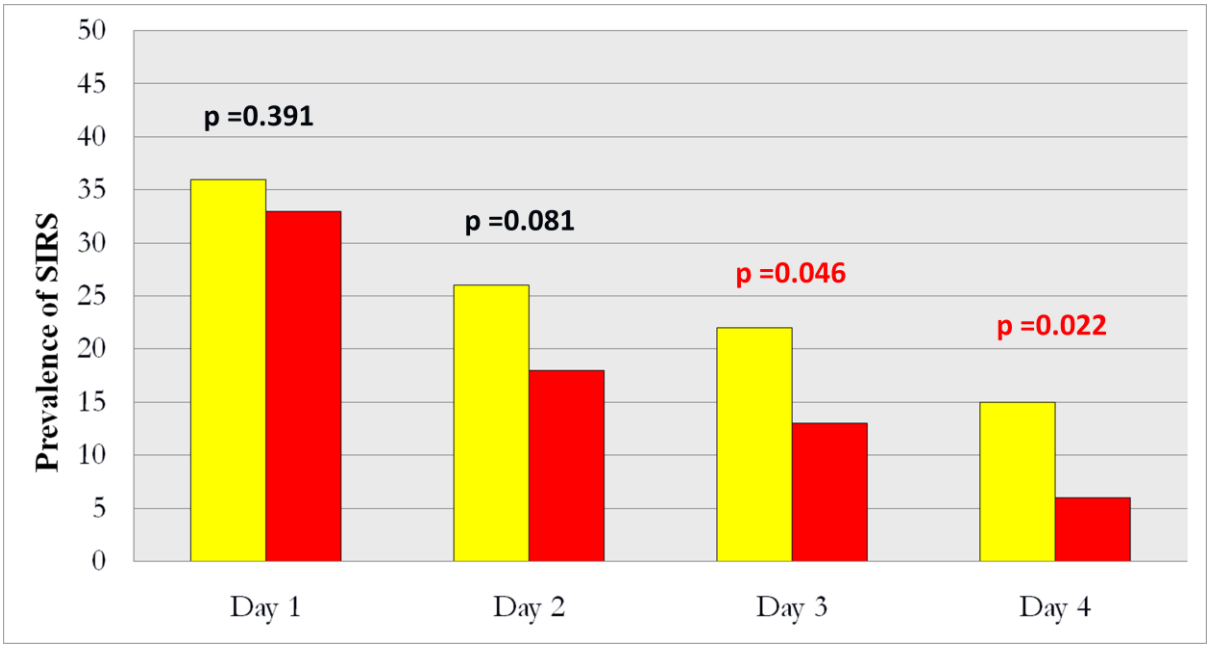
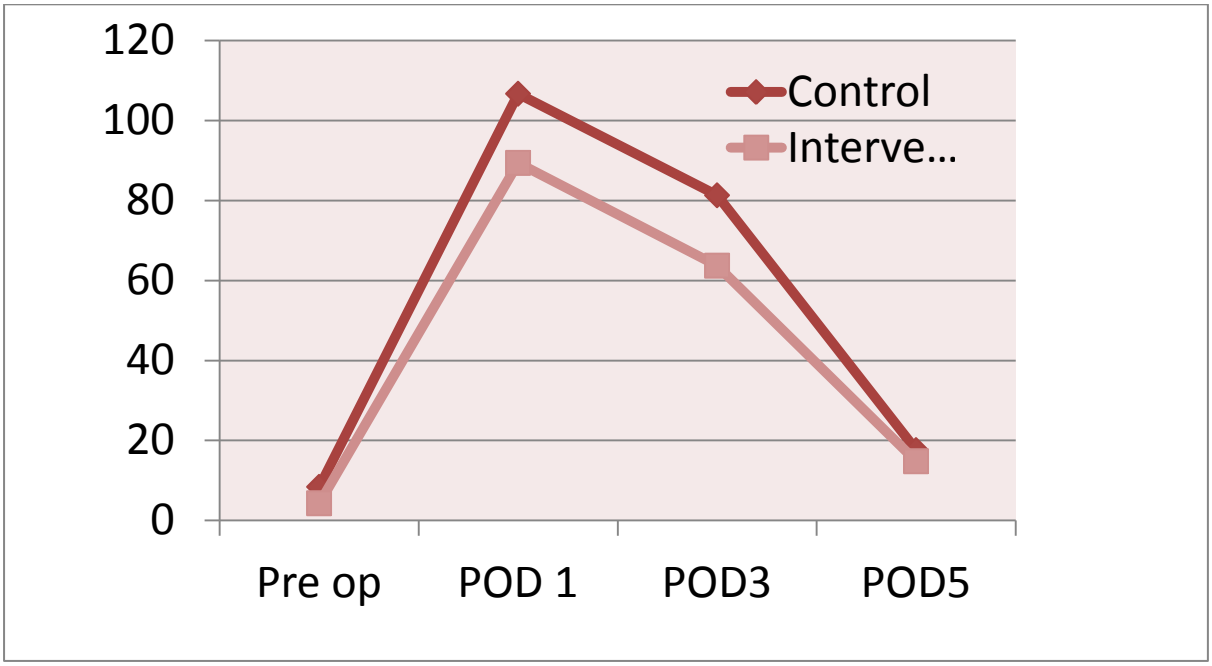


FIGURE 6.2: Prevalence of SIRS in the post operative period. Control group shown in yellow and the study group in red.

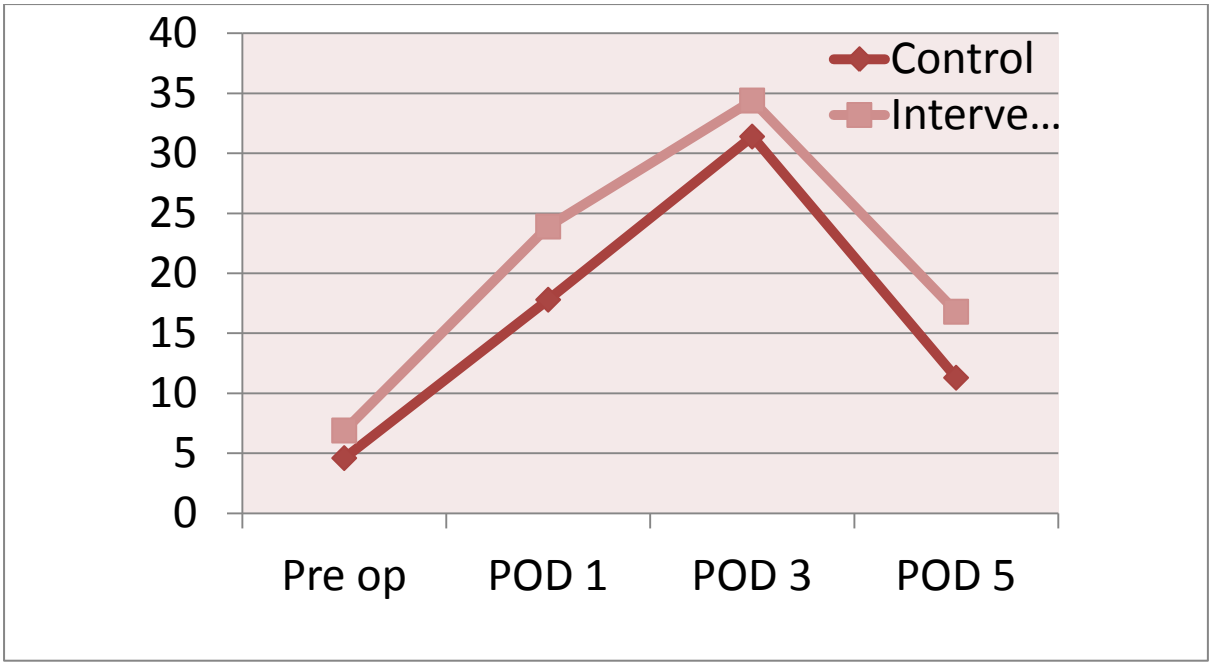
6.4.5: Plasma cytokine response:

Plasma levels of IL-6, TNF-α, IL-10 were measured in all the 95 patients pre-operatively and on post operative day one (control n = 47, study n = 48) and in 77 patients on day three (control n =37, study n = 40) and 44 patients on day five (control n = 24, study n = 20). There was no significant difference between the groups at any time point (Figures 3, 4 and 5). As expected plasma cytokine levels were much higher in the post operative period when compared to pre-operative levels.



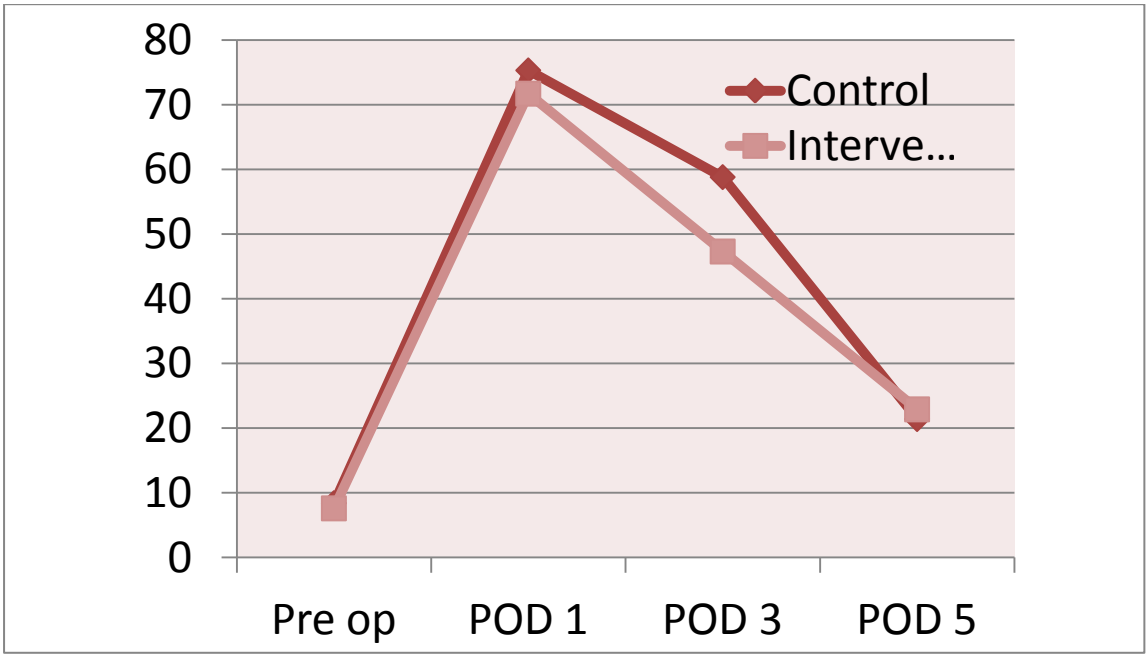
X-axis: Time scale in days, Y-axis: median IL-6 levels in pg/ml

FIGURE 6.3: Plasma IL- 6 levels in the pre and post operative period between the groups measured in picograms per ml.



X-axis: time scale in days: Y-axis: median IL-10 levels in pg/ml.

FIGURE 6.4: Plasma IL-10 levels in the pre and post operative period between the groups measured in picograms per ml.



X-axis: time scale in days, Y-axis: median TNF-α level in pg/ml.

FIGURE 6.5: Plasma TNF-α level in the pre and post operative period between the groups measured in picograms per ml.

6.4.6: Bacterial translocation:

Gut barrier function was assessed by measuring bacterial translocation to the mesenteric lymph node in this study. A mesenteric lymph node was harvested in all 95 patients after mobilisation of colon and before ligation of vascular pedicle. Forty eight patients had bacteria isolated from these mesenteric lymph nodes, 33/47 (70%) in the control group and 15/48 (31%) in the study group. The overall prevalence of BT was 48/95 (46%). The incidence of translocation to the mesenteric lymph node was significantly lower in the study group when compared to control group (15/48, 31% versus 33/47, 70%, $p < 0.001$). Total number of organisms isolated was 47 in control group and 18 in study group. Seven patients had more than one organism cultured from mesenteric lymph node in control group when compared to three patients in the study group.

The commonest isolated organisms were coagulase negative staphylococci (15/33, 45% versus 7/15, 46%) followed by Klebsiella species (10/33, 30% versus 3/15 20%). These results are shown in Figure 1.6 and table1.6.

Organism	Control group (n = 47)	Study group (n = 48)
MLN obtained	47	48
MLN positive	33	15
Organisms cultured	42	18
Multiple organisms	7	3
CNS	15	7
Escherichia coli	7	3
Klebsiella species	10	3
Pseudomonas	3	1
others	7	4

TABLE 6.6: Spectrum of translocating organisms to mesenteric lymphnodes.

CNS: Coagulase negative staphylococci.

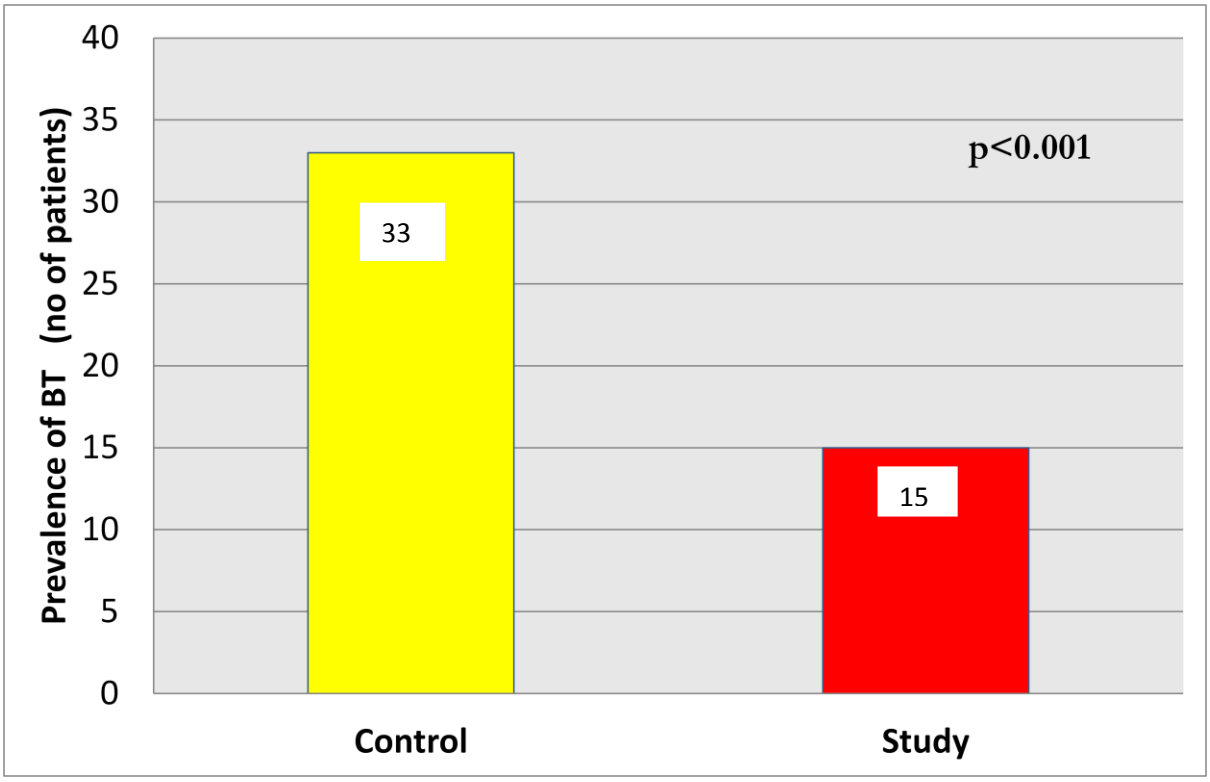
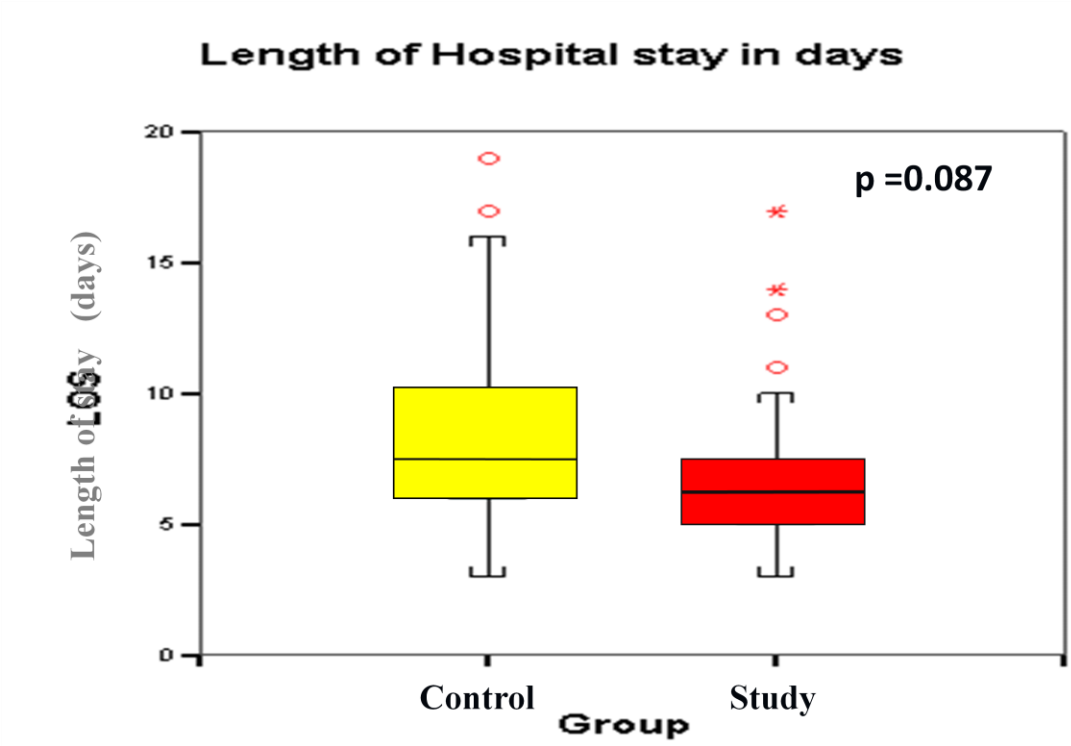


FIGURE 6.6: Prevalence of bacterial translocation between the groups.

6.4.7: Length of Hospital stay:

The results for length of hospital stay are shown in figure 1.7. Patients in the control group stayed in the hospital for a median (IQR) duration of 6(5-10) days when compared to 5(4-7) days in the study group. This difference between the groups was statistically not significant.



Y-axis: median (IQR) length of stay in days

FIGURE 6.7: Length of hospital stay between the groups.

6.4.8: Septic morbidity and mortality:

A total of 33 septic complications occurred in 95 patients in the study period of thirty days in the post operative period. There were 21 septic episodes amongst 47 (44%) patients in the control group when compared to 12 septic episodes amongst 48 (25%) patients in the study group, this difference was statistically significant (p = 0.044). Most commonly urinary tract infections (n = 13), wound infections (n = 8), chest infections (n = 5), Intra abdominal abscess (n = 4) and septicaemia (n = 3) were noted. These results are shown in figure 1.8 and table 1.7.

The overall mortality in this study was (5/95, 5%). There were all together five deaths in the thirty day study period, Three deaths were in the control group (3/47, 6%) and two in the study group (2/48, 4%). This difference was not statistically significant (p = 0.67).

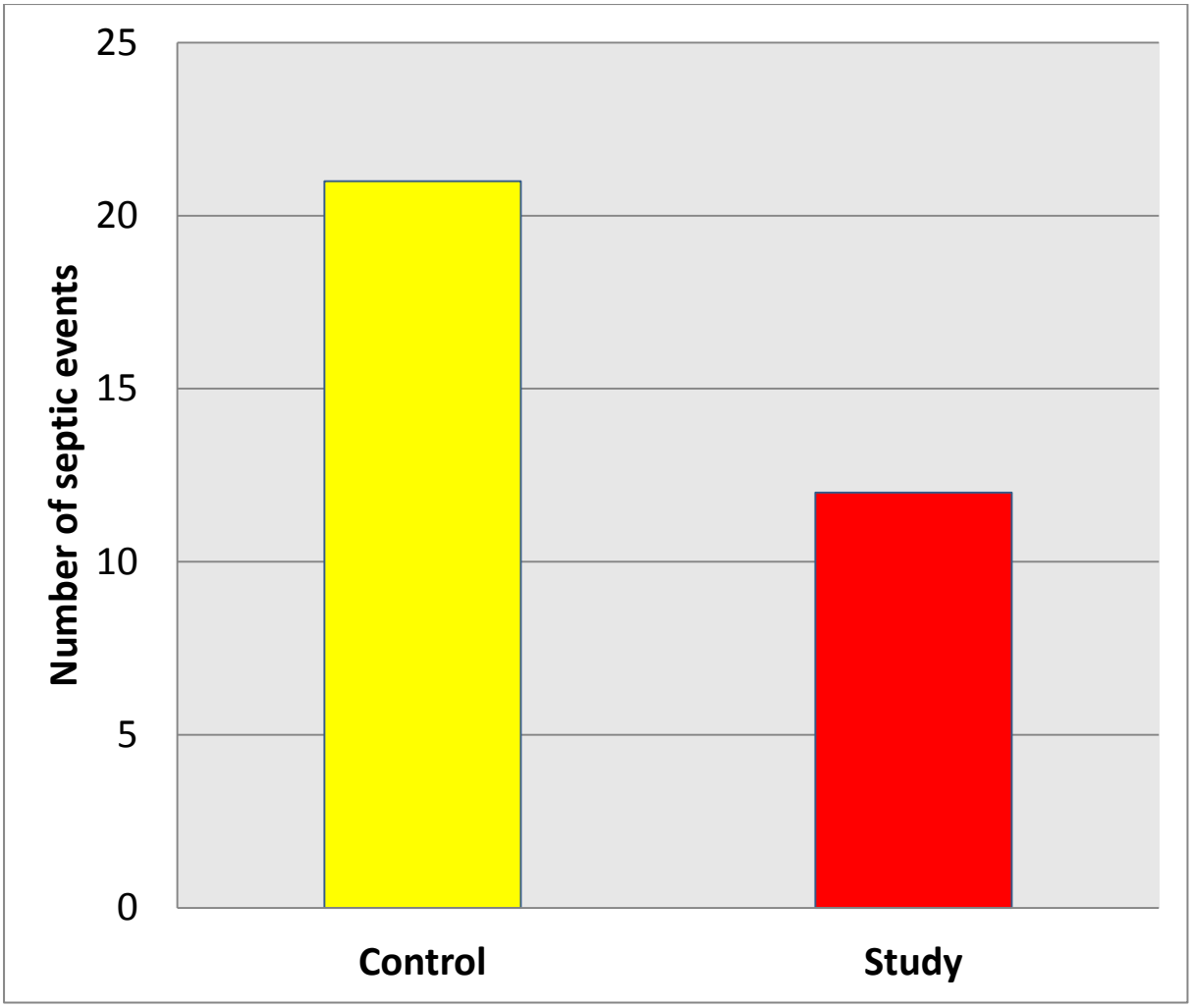


FIGURE 6.8: septic morbidity between the groups.

	Control	Study
UTI	9	4
Wound infection	5	3
Pneumonia	3	2
Abdominal sepsis	2	2
Bacteraemia	2	1

TABLE 6.7: Septic complications between the groups.

6.5:Discussion:

The results of this prospective randomised, controlled clinical trial suggest that the systemic inflammatory response to surgery can be attenuated by optimisation of intestinal function amongst elective gastro intestinal surgical patients. This method of optimisation of intestinal function was also associated with significant reduction in bacterial translocation and also significant reduction in post operative septic morbidity. However this has not resulted in any measurable difference in plasma cytokine response or length of hospital stay.

The interventions used in this study were selected on the basis of existing evidence.

We recognise a number of limitations to this study. Firstly, this prospective randomised controlled study could not be blinded as it involved the use of mechanical bowel preparation. However, wherever possible the operating surgeon, microbiological and biomedical staff involved in the patient care and analysis of the specimens from this study, were uninformed of the randomisation. Secondly only could argue that it would have been ideal to have a placebo control for study medications, but due to limitations with respect to availability and some parts of interventions like mechanical bowel preparation could not be blinded.

There is increasing recognition of the "gut barrier" in the health and disease. Failure of the gut barrier has been associated with significant increase in both morbidity and mortality. Gastrointestinal epithelial cells and gut associated lymphoid tissue (GALT) are an integral part of gut mucosal barrier. These epithelial cells and GALT are dependent on both luminal and bloodstream sources for nutrition. Certain nutrients, collectively known as gut specific nutrients (GSN), have been shown to exert specific effects on mucosal integrity and gut immunology; these effects are separate and distinct to their role as nutrients.

Numerous substances have been shown to have gut specific effects and include glutamine, arginine, fish oils, short-chain fatty acids, and anti-oxidants. However, despite the fact that enormous research has been undertaken to assess the effects of these substances, their role in clinical practice remains uncertain. Conveniently Glutamine with anti-oxidants is commercially available as "glutamine plus" at the onset of this trial. Having established safety of a similar product in the post operative period in our previous study and this product having desired constituents, and properties necessary to fit the study design, we have selected this product.

One further target for optimising gut function is the gastrointestinal microflora. It is now well recognised that the gastrointestinal microflora are a fundamental component of gut barrier. Many previous studies have attempted to modify these microfloras using a variety of techniques popularised as selective gut decontamination. Many of these studies have shown reduction in post operative septic morbidity. However, a consensus as to which combination of antibiotics or bowel preparation is optimal remains unclear.

Controversy surrounds the use of mechanical bowel preparation but the fact that it remains in widespread use and is considered by many surgeons as being important in reducing bacterial loads justified its adoption as an intervention in this study (*Ram, et al.* 2005). Neomycin was chosen on the basis of antibiotic sensitivity studies done on translocating organisms obtained from our previous studies (data not shown). This aminoglycoside antibiotic was commonly used in the past for bowel cleansing before elective colorectal surgery and is still prescribed in cirrhotic patients to prevent hepatic encephalopathy (*Strauss, et al.* 1992). We encountered no adverse reactions to neomycin in this study.

We have recently reported a study using a combination of synbiotics, neomycin and mechanical bowel preparation in which a significant reduction in the prevalence of bacterial

translocation suggesting a modulation in gastrointestinal microflora in elective colorectal surgical patients.

The results of this study suggests that the combination of gut specific nutrients and selective gut decontamination might optimise the intestinal function amongst elective gastrointestinal surgical patients and there by observed clinical benefits.

6.6: Conclusion:

In conclusion, results of this study demonstrates optimisation of intestinal function by combining gut specific nutrients and selective gut decontamination was associated with reduced post operative systemic inflammatory response, reduced prevalence of bacterial translocation and also reduced post operative septic morbidity. Prior to recommending any change in clinical practice it is desirable to conduct further multi-centre clinical trials to understand the mechanisms involved in these clinical findings.

CHAPTER SEVEN

Influence of intestinal function on SIRS and clinical outcomes in surgical patients:

Summary and final conclusions

7.1 Thesis Hypothesis and rationale:

Gut failure is associated with SIRS, MODS and poorer clinical outcomes of patients. As a result, gut directed therapies that optimise gut function by attenuating the gut failure could possibly improve patient outcomes.

Aims of this thesis were to investigate the importance of gut function in clinical practice and influence of gut failure on clinical outcome in surgical patients, subsequently device a method to optimise the gut function and assess the influence of this optimised gut function on clinical outcomes.

A thorough review of relevant literature found that supporting evidence for the role of the gut in clinical practice exists in various forms. Gut failure as the motor which drives SIRS, MODS and death in disease also exists in literature. This corroborative evidence can be drawn from a variety of seemingly unrelated sources in the medical and surgical literature. Studies investigating the 'gut origin of sepsis hypothesis' and BT provided strong support for the role of gut failure as motor driving poor clinical outcomes, additional evidence could be drawn from trials assessing enhanced recovery programmes after surgery as well as from papers relating to various aspects of human nutrition and metabolism. There exists, no standard definition for gut failure due to complexity and multitude of functions gut has and this made it difficult to standardise the state of gut and directly relate gut failure to clinical outcomes in day to day clinical practice. Several attempts by various authors to define gut function were unsuccessful due to shortcomings in developing an ideal definition which relates to its primary function and can be adapted in day to day clinical practice.

After reviewing the literature in chapter one it is apparent that there exists no direct correlation to suggest gut failure may be associated with poorer clinical outcomes like other single organ failures. If gut failure is associated with poorer outcomes would it be possible to develop a method or a package to attenuate gut failure to improve clinical outcomes?

This thesis is set out to investigate these shortcomings in clinical setting. This chapter summarises the conclusions of clinical studies conducted by the author.

7.2 Summary of research findings:

To achieve the goal set out four clinical trials were conducted and these trials were presented in chapters 3, 4, 5 and 6.

The first trial was a prospective observational study described in chapter 3, investigating the state of gut function and its influence on SIRS, MODS, sepsis and clinical outcomes in patients with pancreatitis.

Acute pancreatitis is a multi-system disease with an unpredictable clinical course and significant morbidity and mortality. Many of these patients with acute pancreatitis develop multi-organ failure requiring management within a critical care environment. However much of the pathophysiology of the disease, particularly understanding why some patients develop life-threatening disease whilst others have a relatively benign course, remains unclear. Once considered a quiescent organ, the intestine is now seen to have a pivotal role in the development of the SIRS, sepsis and MODS. Acute pancreatitis, therefore, may represent a model of systemic inflammation arising initially from a non-gastrointestinal source. In the "gut origin of sepsis" model, secondary gut injury and breakdown of the gut barrier may occur to result in the release of inflammatory mediators and colonic bacteria, perpetuating systemic inflammation and resulting in SIRS, MODS and sepsis.

From this study it is apparent that the gut failure is common amongst patients with acute pancreatitis. Transient gut failure may not be associated with poorer overall clinical outcomes but persistent gut failure is associated with increased prevalence of SIRS, sepsis and poorer clinical outcomes. Early initiation of gut directed therapies may attenuate persistent gut

failure thereby curtailing the SIRS and sepsis facilitating improved clinical outcomes. Findings from this small cohort study needs to be evaluated in larger multicentre trials.

The second study was a prospective observational audit amongst critically ill patients described in chapter4. Multiple organ dysfunction syndrome (MODS) is the leading cause of death in critically ill patients. The role of dysfunction of the gastrointestinal tract in the pathogenesis of systemic inflammatory response syndrome and multiple organ dysfunction syndrome complicating the course of critically ill patients has been suspected for many years. However, none of the modern day scoring systems used in critical care take in to account state of gut function to predict the outcomes unlike other single organs. The aim of this study was to investigate the relationship between the functional state of gut and clinical outcomes in critically ill patients. This prospective study has compared the outcomes between the patients with gut failure and functioning gut. Even though there was a trend towards increased prevalence of SIRS amongst critically ill patients with gut failure when compared to patients with inadequate gut function this difference was not statistically significant. One reason could be that this is a type 2 error due to small sample size. Findings from this study needs to be evaluated in a larger trial possibly using multivariate logistic regression to assess direct relation between the gut failure and clinical outcomes.

The third study described in chapter 5, was a prospective observational study investigating the gut function in elective surgical patients undergoing GI surgery and non GI surgery and its influence on SIRS and clinical outcomes. Gut mucosal hypo-perfusion has been hypothesized as the motor for development of SIRS and multiple organ failure. During any surgical procedure under general anaesthesia episodes of hypotension are common and goal directed fluid therapy using oesophageal Doppler has been proposed to prevent these

hypotensive episodes to prevent mucosal hypo-perfusion and improve clinical outcomes. There are several other complex physiological changes occur as a response to surgical stress. These mechanisms are poorly understood and have effect on various organ systems including gut function. There was increased prevalence of inadequate gut function amongst patients undergoing gastro intestinal surgery when compared to patients undergoing non gastro intestinal surgery and this increased gut failure in patients undergoing GI surgery is associated with increased prevalence of SIRS and septic complications. From this study it is evident that inadequate gut function is associated with poorer clinical outcomes amongst elective surgical patients.

After investigating the role of gut function and its influence on clinical outcomes in these three clinical studies it is apparent that gut function is important. The final study in this series of clinical trials is described in chapter 6. This study addressed the issue of optimisation of gut function and attenuating gut failure. The basic argument behind this study is that if one accepts that gut function is indeed independently associated with outcome, then might it be possible to optimise gut function and attenuate gut failure, would this, in turn, be associated with demonstrable clinical improvements?

It was unclear whether gut function could be optimised amongst patients who are likely to develop gut failure in the post operative period, For this reason, a gut-directed therapy consisting of a number of interventions was put together by the author and investigated. This package was chosen based on the available literature evidence for individual factors having a favourable influence on various factors of gut function. The setting was that of a randomised controlled clinical trial amongst patients undergoing elective colorectal surgery for cancer. This group was selected for a number of reasons. First and foremost that these patients are going to undergo severe surgical stress and this has a direct bearing on each and every single organ. Secondly there is good evidence to suggest that these patients have increased

prevalence of inadequate gut function in the immediate post operative period and thirdly this gives an ideal setting to optimise their gut function before the expected insult happens and test whether this optimisation has any influence on overall clinical outcomes.

The results from this study demonstrated that the pre operative optimisation of gut function using chosen package was associated with an earlier return of gut function, and improved outcomes amongst patients undergoing elective colo-rectal surgery for cancer. Patients receiving this gut optimisation package were noted to have a reduced bacterial translocation diminished chance of developing SIRS and septic complications in the post operative period. They also demonstrated an attenuation of the acute phase response. Whether these beneficial outcomes came about as a result of the enhanced recovery of gut function or whether they were only associated with a curtailed period of gut failure remains unclear.

This study concluded that, optimisation of intestinal function using the chosen package of combined gut directed therapies was associated with attenuated gut failure preserving gut barrier function there by reducing bacterial translocation, prevalence of SIRS and septic complications benefiting the overall outcome of these patients. These findings merit further evaluation in a large randomised clinical trial before recommending any change of clinical practice.

Taken together, the findings from the literature review and subsequent four clinical studies presented in this work fulfil the aims of the thesis. The importance of gut function on clinical outcomes is emphasised, and the deficiencies in the relevant medical literature are highlighted. Relation between state of gut function or failure and its influence on SIRS, sepsis and clinical outcomes was studied. Finally, the question of whether gut function could be optimised and whether this was to the advantage of patients was considered. A package combining various gut directed therapies including gut specific nutrients and selective gut decontamination was developed and using this package in a clinical setting, able to

demonstrate that this intervention is associated with attenuation of gut failure and that this in turn was associated with direct benefits to clinical outcomes. In this logical sequence full filled the thesis aim.

7.3 Future research:

Findings from these four clinical studies have highlighted several areas where there is need for further research.

First of all, all the studies presented in this thesis were single centre small studies, some with equivocal findings hence this work needs to be validated in possibly large multicentre clinical trials for the purpose of scientific validity and robustness.

Even though a definition using primary function of gut was adapted to define the state of gut function pragmatically to apply in the clinical practice this definition has a shortcoming with respect to defining overall state of gut, due to multitude of complex functions and their direct and indirect effects on various patho-physiological mechanisms influencing clinical outcomes. There is need for further research to develop a robust definition for the state of gut function.

Current methods for assessing bacterial translocation as a marker for gut barrier function have limitation as is applicable only to patients undergoing laparotomy. Even though there are PCR methods described in the literature to identify DNA fragments in peripheral blood, to assess gut barrier function they are still in laboratory research stage with very limited validity. There is scope for developing a PCR technique to assess bacterial translocation first in the setting of surgical patients to appropriately validate, once validated this can have wide variety of clinical applications in day to day clinical practice to assess gut barrier function. As part of this thesis methodology we have attempted to develop such method without success.

The method used in this thesis to optimise gut function was selected based on effects of individual components on gut function based on available literature and studied in a single centre and selected group of elective surgical patients. This package needs further evaluation in larger clinical trial and also in other clinical conditions. Similar to the management of other organ failures, a wide range of therapies aimed at curtailing gut failure and enhancing the return of normal gut function need to be developed and validated. There are several other individual gut directed therapies which needs evaluation in combination to assess combined effect of these interventions on overall clinical outcome.

APPENDICES:

1. **ASA Grading**

ASA Grade	Definition
I	Normal healthy individual
II	Mild systemic disease that does not limit activity
III	Severe systemic disease that limits activity but is not incapacitating
IV	Incapacitating systemic disease which is constantly life-threatening
V	Moribund, not expected to survive 24 hours with or without surgery

2. **SIRS criteria:**

Temperature	>38^{o} C or <36^{o}C
Heart rate	>90
Respiratory rate	>20 or $PaCO_2$ <32 mm Hg
WBC count	>12K or <4K or >10% immature forms

3. POSSUM Physiological Score

	Score			
	1	2	4	8
Age (years)	<=60	61 – 70	>=71	
Cardiac signs	Normal	Drugs	Oedema/Warfarin/ Cardiomegally	JVP Cardiomegally
ECG	Normal		AF Controlled Rate 60-90	MI Abnormal ECG
Respiratory	Normal	SOBOE mild COPD	SOB stairs Mod COPD	SOB rest RR 30 Fibrosis
Blood pressure (systolic)	110-130	100-109 131-170	90-99 >170	<90
Heart rate	50-80	81-100 40-49	101-120	>=121 <40
Glasgow coma score	15	12-14	9-11	<=8
Haemoglobin (g/100ml)	13-16	11.5-12.9 16-17	10.0-11.4 17.1-18	<10 >18
White cell count ($x10^{12}$/l)	4.1-10	10.1-20.0 3.1-4	>20 <3	
Urea (mmol/l)	<7.6	7.6 – 10	10.1-15	>15
Sodium(mmol/l)	>135	131-135	126-130	<126
Potassium(mmol/l)	3.5-5.0	3.2-3.4 5.1-5.3	2.9-3.1 5.4-5.9	<=2.8 >=6.0

4. POSSUM Operative Severity Score:

	Score			
	1	2	4	8
Case	Minor	Moderate	Major	Major+
Previous ops	0 or 1		2	>2
Blood loss (surg)	<=100	101-500	501-999	>1000
Peritoneal soiling	None	Minor (serous)	Local pus	Free pus/Blood/Bowel contents
Malignancy	None	Primary	Nodal mets	Distant mets
Timing	Elective		<24 > 2 hrs Emergency resus.	< 2hrs to theatre No resus

5. APACHE II SCORING SYSTEM:

	Acute physiology score								
	High normal range					*Low normal range*			
Variable	*+4*	*+3*	*+2*	*+1*	*0*	*+1*	*+2*	*+3*	*+4*
Temperature (°C)	>41	39–40.9		38.5–38.4	36–38.4	34–35.9	32–33.9	30–31.9	<29.9
Mean arterial pressure (mm Hg)	>160	130–159	110–129		70–109		50–69		<49
Heart rate (ventricular; beats/min)	>180	140–179	110–139		70–109		55–69	40–54	<39
Respiratory rate	>50	35–49		25–34	12–24	10–11	6–9		<5
Oxygenation (mm Hg)									
A_{aDO_2} when F_{iO_2} >0.5	>500	350–499	200–349		<200				
P_{aO_2} when F_{iO_2} <0.5					PO_2 >70	PO_2 61–70		PO_2 55–60	PO_2 <55
Arterial pH	>7.7	7.6–7.69		7.5–7.59	7.33–7.49		7.25–7.32	7.15–7.24	<7.15
Serum Na (mmol/l)	>180	160–179	155–159	150–154	130–149		120–129	11–119	<110
Serum K (mmol/l)	>7	6–6.9		5.5–5.9	3.5–5.4	3–3.4	2.5–2.9		<2.5
Serum creatinine (mg/100 ml)	>3.5	2–3.4	1.5–1.9		0.6–1.4		<0.6		
Double score for ARF									
Packed cell volume (%)	>60		50–59.9	46–49.9	30–45.9		20–29.9		<20
While blood cell count ($\times 10^3$/mm^3)	>40		20–39.9	15–19.9	3–14.9		1–2.9		<1
Glasgow coma scale*									

*Score = 15 − actual Glasgow coma scale.

The APACHE II score is given by the sum of the acute physiology score, the age (in years) points, and the chronic health points. Age points are assigned as follows: 0, <44; 2, 45–54; 3, 55–64; 5, 65–74; and 6, >75. Chronic health points are assigned if the patient has a history of severe organ system insufficiency or is immunocompromised, as follows: 5, non-operative or emergency postoperative patients; 2, elective postoperative patients. Organ insufficiency or an immunocompromised state must have been evident before admission to hospital and must conform to the following criteria: *liver*, biopsy confirmed cirrhosis and documented portal hypertension, episodes of past upper gastrointestinal bleeding attributed to portal hypertension, or prior episodes of hepatic failure/encephalopathy/coma; *cardiovascular*, New York Heart Association Class IV (that is, symptoms of angina or cardiac insufficiency at rest or during minimal exertion); *respiratory*, chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction—that is, unable to climb stairs or perform household duties, or documented chronic hypoxia, hypercapnia, secondary polycythaemia, severe pulmonary hypertension (>40 mm Hg), or respirator dependency; *renal*, receiving chronic dialysis; and *immunocompromised*, the patient has received treatment that suppresses resistance to infection—for example, immunosuppression, chemotherapy, radiotherapy, long term, high dose steroids, or has a disease that is sufficiently advanced to suppress resistance to infection, such as leukaemia, lymphoma, AIDS. A_{aDO_2}, alveolar–arterial oxygen difference; P_{aO_2}, arterial partial pressure of oxygen; F_{iO_2}, fraction of inspired oxygen; ARF, acute renal failure.

6. **Contents of Glutamine Plus:**

Average content	per 22.4 g sachet	
Caloric value	333*/325** (= 80*/78**	kJ kcal)
Protein	10	g
of which glutamine	10	g
of which nitrogen	1.9	g
Carbohydrates	10*/9.4**	g
of which sugars	0.36*/2.1**	g
of which lactose	0	g
Fat	0	g
Fibre	1.34*/1**	g
Osmolarity	235*/329**	mosmol/l
Osmolality Ready to use product (1 sachet + 200 ml water)	250*/350**	mosmol/kg H_2O
Vitamins		
ß-Carotene	1.6	mg
Vit. E	83	mg
Vit. C	250	mg
Minerals and trace elements		
Sodium	3*/6**	mg
Potassium	10*/55**	mg
Zinc	3.3	mg
Selenium	50	µg
Other minerals, trace elements and vitamins are present in amounts clinically not relevant.		
Caloric distribution (energy %)		
Protein 50*/52* %, fat 0%, carbohydrates 50*/48** %		

PUBLISHED PAPERS:

1) "Bacterial translocation may influence the long-term survival in colorectal cancer patients". K.F.Chin, R.Kallam, C.O'Boyle, Prof. J.MacFie.

   DCR 2007:50(3): 323-30.

2) "Role of multimodal optimisation in patients undergoing elective surgery for colorectal cancer".
   MM Rao, R Kallam, K Mainprize, J Macfie: *Cancer action 2007:25:3*

3) Impact of oedema on recovery after major abdominal surgery and potential value of multi frequency bio- impedance measurements.
   M Rao, M Gatt, R Kallam, J MacFie BJS 2006:93(6): 769-70

4) Compliance with enhanced recovery programmes in elective colorectal surgery
   J. Ahmed, S. Khan, M. Gatt, R. Kallam, J. MacFie *British Journal of Surgery* 2010; **97**: 754–758

Published Abstracts:

1) Gut function is an independent prognostic indicator and can be modulated to benefit patient outcome: proof of principle.
   M. Gatt, J. MacFie, L. McNaughton, C. Ramsey, A. Coppack, M. M. Rao, R. Kallam, S. McKenzie
   British Journal of Surgery 2007; 94(S2):3

2) Gut specific nutrients in patients undergoing elective surgery: A prospective randomised trial.
   M. M. Rao, M. Gatt, R. Kallam, J. MacFie

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3) Gastric colonisation in surgical patients.
   R. R. Kallam, B. S. Reddy, M. Rao, M. Gatt, C. J. Mitchell, J. MacFie

   British Journal of Surgery 2007; 94(S2):154

4) Gastric colonisation in surgical patients: An indicator of altered gut barrier function
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5) Gut-specific nutrients may be used to enhance the recovery of gut function in the critically ill: Results of a double-blind, placebo-controlled, randomised clinical trial.
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6) Predicting the return of gut function using gastric emptying studies.
M. M. Rao, R. Kallam, I. Flindall, M. Gatt, J. MacFie
Clinical Nutrition 2007:2(S2):26

7) Specific identification of live probiotic organism *Bifidobacterium animalis* subspecies *lactis* from faecal samples of surgical patients by fluorescent colony hybridisation assay. R. R. Kallam, B. S. Reddy, M.M. Rao, M. Gatt, J. MacFie
Clinical Nutrition 2007:2(S2):45

8) Gut specific nutrients in patients undergoing elective surgery: A prospective randomised trial.
M. M. Rao, M. Gatt, R. Kallam, I. Flindall, J. MacFie
Clinical Nutrition 2007:2(S2):60

9) Gut function is an independent indicator of patient outcome: Proof of principle.
M. Gatt, J. MacFie, L. McNaughton, A. Coppack, N. Yassin, M. M. Rao, R. Kallam.
Clinical Nutrition 2007:2(S2):108

10) Use of Bioelectrical Impedance Spectroscopy in patients undergoing elective laparotomy.
M. M. Rao, R. Kallam, I. Flindall, J. MacFie
Clinical Nutrition 2007:2(S2):113

11) Changes in whole gut permeability following elective Colorectal resections.
M. M. Rao, R. Kallam, I. Flindall, M. Gatt, J. MacFie
Clinical Nutrition 2007:2(S2):122

12) The role of peri-operative fluid balance on post-operative outcomes after elective Colonic resection in the setting of multimodal optimization.
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Presentations to learned societies:

Oral Presentations:

1) Can the systemic inflammatory response to surgery be attenuated by optimisation of intestinal function? A randomised clinical study ASGBI 2009 Moynihan Prize Paper
   R Kallam, M Rao, R Arsalanizadeh, M Gatt, J MacFie

2) Gut function is an independent prognostic indicator and can be modulated to benefit patient outcome: proof of principle. ASGBI 2007 Moynihan Prize Paper
   M. Gatt, J. MacFie, L. McNaughton, C. Ramsey, A. Coppack, M. M. Rao, R. Kallam, S. McKenzie:

3) Gastric colonisation in surgical patients: An indicator of altered gut barrier function ESPEN 2007
   R. R. Kallam, B. S. Reddy, M. Rao, M. Gatt, C. J. Mitchell, J. MacFie

4) Gut-specific nutrients may be used to enhance the recovery of gut function in the critically ill: Results of a double-blind, placebo-controlled, randomised clinical trial. ESPEN 2007
   M. Gatt, J. MacFie, L. McNaughton, A. Coppack, N. Yassin, M. M. Rao, R. Kallam.

5) Bacterial translocation may influence the long-term survival in colorectal cancer patients. SARS 2004
   K.F.Chin, R.Kallam, C.O'Boyle, J.MacFie.

6) Bacterial translocation may influence the long-term survival in colorectal cancer patients: Leeds regional surgical club, November 2003
   K.F.Chin, R.Kallam, C.O'Boyle, J.MacFie.

Poster presentations:

1) Gut specific nutrients in patients undergoing elective surgery: A prospective randomised trial: ASGBI 2007
   M. M. Rao, M. Gatt, R. Kallam, J. MacFie

2) Gastric colonisation in surgical patients: ASGBI 2007
R. R. Kallam, B. S. Reddy, M. Rao, M. Gatt, C. J. Mitchell, J. MacFie

3) Predicting the return of gut function using gastric emptying studies: ESPEN 2007
M. M. Rao, R. Kallam, I. Flindall, M. Gatt, J. MacFie

4) Specific identification of live probiotic organism *Bifidobacterium animalis* subspecies *lactis* from faecal samples of surgical patients by fluorescent
colony hybridisation assay: ESPEN 2007
R. R. Kallam, B. S. Reddy, M.M. Rao, M. Gatt, J. MacFie

5) Gut specific nutrients in patients undergoing elective surgery: A prospective randomised trial: ESPEN 2007
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6) Gut function is an independent indicator of patient outcome: Proof of principle: ESPEN 2007
M. Gatt, J. MacFie, L. McNaughton, A. Coppack, N. Yassin, M. M. Rao, R. Kallam.

7) Use of Bioelectrical Impedance Spectroscopy in patients undergoing elective laparotomy: ESPEN 2007
M. M. Rao, R. Kallam, I. Flindall, J. MacFie

8) Changes in whole gut permeability following elective Colorectal resections: ESPEN 2007
M. M. Rao, R. Kallam, I. Flindall, M. Gatt, J. MacFie

9) The role of peri-operative fluid balance on post-operative outcomes after elective Colonic resection in the setting of multimodal optimization: ESPEN 2007
M. M. Rao, R. Kallam, I. Flindall, M. Gatt, J. MacFie

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