Exploration of Factors affecting Severity in Acute Pancreatitis

Suzanne Elgammal

MBChB MRCS

A Thesis presented for the degree of Master of Science

(Medical Science)

to the University of Glasgow based on research conducted in the

Lister Department of Surgery at Glasgow Royal Infirmary.

October 2008

In the name of God, most merciful, most beneficient

Abstract of Thesis

Introduction

Acute Pancreatitis (AP) is associated with a significant mortality despite advances in critical care and surgical management of the disease. A significant subgroup of patients with AP will have complications including Multiple Organ Dysfunction Syndrome (MODS) and Infected Pancreatic Necrosis (IPN) which are associated with higher mortality rates. The pathophysiology of MODS is not yet fully understood but cytokines are known to play a role.

Three factors influencing mortality in patients with acute pancreatitis have been identified. Firstly, the host response to acute inflammation, which determines the pattern of cytokine response and may be subject to genetic control.

Secondly, the development of infected pancreatic necrosis and the influence of surgical strategies developed to deal with this. Thirdly, the nature of infecting organisms in patients with IPN.

Aims

The aims of this thesis are

1) To compare genetic polymorphisms in an interleukin-8 locus in a cohort of patients with AP and healthy controls with regard to severity and susceptibility

2) To examine a retrospective series of patients with infected pancreatic necrosis and compare open and percutaneous necrosectomy techniques with regard to organ dysfunction and mortality

3) To identify microbiological factors which may affect outcome in patients with infected pancreatic necrosis

1) Materials and Methods

Previously collected DNA samples from 106 patients with predicted severe AP were subjected to sequence specific PCR of 6 Interleukin-8 single nucleotide polymorphisms (SNPs) and compared with 100 healthy control DNA samples. SNP frequencies were compared in patients with mild and severe disease and between patients and controls.

Patients and Methods

2) Ninety-nine patients with infected pancreatic necrosis treated surgically at GRI between December 1989 and March 2002 were prospectively identified. Retrospective case note review was performed. Patients were divided into OPEN and PERC (percutaneous) groups for analysis. Outcome measures included mortality and ITU requirements.

3) The same IPN cohort was further analysed with respect to microbial spectrum, use of prophylactic antibiotics, development of resistant organisms and effect on mortality.

Results

1) No differences in Interleukin-8 SNP frequencies were noted between mild and severe patients or between patients and controls.

2) Fifty-six and 49 patients underwent open and percutaneous pancreatic necrosectomy respectively. Mortality rates were similar in both groups. Significantly fewer patients in the PERC group required ICU post-operatively indicating a reduction in post-operative MODS.

3) The spectrum of microbial infection varied slightly over the study period. Prophylactic antibiotic usage has been avoided latterly in this unit but overall prophylactic antibiotic use has not changed significantly.

Fungal infection was associated with higher mortality rates.

Conclusions

Several factors affecting severity have been identified in Acute Pancreatitis.

From this work, it has been demonstrated that:

1) Six Interleukin-8 polymorphisms do not seem to be related to disease severity or susceptibility in a cohort of AP patients

2) Minimally invasive pancreatic necrosectomy reduces post-operative MODS in patients with IPN and may improve mortality with continued improvements in technique.

3) Fungal infection is associated with higher mortality rates in patients with IPN.

Acknowledgements

This MSc thesis and the period of research leading up to its production is due in part to the immense support and guidance of several others. The initial ideas for the Interleukin-8 project were developed after discussion with Colin McKay, my supervisor, and Pete Chong on IL-6 and IL-8 microsatellites. Ross Carter, my advisor, has been a leading force in developing minimally invasive pancreatic necrosectomy and has given me considerable support and guidance in exploring further the effects of surgery on critically ill patients with acute pancreatitis. I would like to thank Anton Buter for his contribution of DNA samples and data from a cohort of patients with acute pancreatitis

Susan Evans offered sound advice on management. The laboratory work was achieved with advice from Jon Pete, my laboratory supervisor, who demonstrated the technical aspects of PCR and DNA extraction, density analysis and gel electrophoresis and assisted with some of the analysis. I wish to thank Seema Seetharam for her role in characterising some of the IL-8 control samples in addition to her support and friendship.

Throughout the period of study, Colin McKay and Ross Carter have supported me in my aim towards completion of this thesis. I would like to thank them for their help in accomplishing this.

I wish to thank the late Professor Timothy Cooke for arranging funding and for facilitating this research within the Lister Department of Surgery at Glasgow Royal Infirmary.

I am very fortunate in having had the opportunity to work with Professor Clem Imrie who has offered encouragement and wisdom throughout, for which I am extremely grateful.

I would also like to thank Dr Wilson Angerson for statistical advice, Debbie Clarkson, Frances Lyall and Diane Stewart for their administrational support and friendship. Thanks as well to the administrative staff in the microbiology, ITU, medical records and IT departments at Glasgow Royal Infirmary who helped with filling in the data gaps.

I also wish to thank my good friend Dr Ruth Gibson for her invaluable encouragement and support especially during the more demanding days of writing up. This work would not have been accomplished without support from my family; Aymann, Omar and Mariam. I would like to thank my parents for their life long guidance and encouragement, (thanks, mum for the late-night cups of coffee). I wish to give special thanks to my husband Innes for being patient throughout the whole process and providing valuable in-house IT support. My daughter Lena was considerate enough to delay her arrival into this world until the laboratory work was completed and the abstracts submitted. Her little sister Janna, however could not wait until the corrections were finished but encouraged me to finally complete this work. We can now look forward to having some more time and fun together.

Dedication

I would like to dedicate this work to Hamdy and Ellen, my parents.

Declaration

I do solemnly swear that the work contained within this thesis is my own except where other contributors have been acknowledged within the text. This thesis has not been presented for a degree elsewhere.

Suzanne Elgammal

List of Abbreviations

AP	**Acute Pancreatitis**
APC	**Activated Protein C**
AXR	**Abdominal X-Ray**
CT	**Computed Tomography**
CVP	**Central Venous Pressure**
CXR	**Chest X-Ray**
ERCP	**Endoscopic Retrograde Cholangio-Pancreatogram**
ES	**Endoscopic Sphincterotomy**
GRI	**Glasgow Royal Infirmary**
IL-1	**Interleukin-1**
IL-6	**Interleukin-6**
IL-8	**Interleukin-8**
IL-10	**Interleukin-10**
IPN	**Infected Pancreatic Necrosis**
MHC	**Major Histocompatibility Complex**
MODS	**Multiple Organ Dysfunction Syndrome**
NGT	**Naso-gastric tube**
NJ	**Naso-jejeunal**
SAP	**Severe Acute Pancreatitis**
SIRS	**Systemic Inflammatory Response Syndrome**
SNP	**Single Nucleotide Polymorphism**
TB	**Tuberculosis**
TNF	**Tumour Necrosis Factor**
TPN	**Total Parenteral Nutrition**

List of Tables

Page No

List of Figures

Contents **Page**

Chapter 3: Factors influencing mortality in pancreatic necrosis

Chapter 4 Effect of Microbiology on outcome in Infected Pancreatic Necrosis

Chapter 1

1.1 Acute Pancreatitis; General Introduction

1.1.1 Epidemiology of Acute Pancreatitis

Acute pancreatitis is a common cause of presentation to general surgical departments. The incidence of acute pancreatitis has increased markedly over the last 4 decades and appears to be rising (1).A retrospective analysis of all patients discharged from hospital with a diagnosis of acute pancreatitis in Scotland from 1961 to 1985 showed an eleven-fold increase in the number of men with the disease. In female patients this was less marked with only a four-fold increase in incidence(2). Despite this large increase, mainly in young and middle-aged males and elderly females, there was no associated rise in mortality rates. The apparent increase in incidence was attributed to improved diagnosis and earlier awareness of the disease.

A further study analysed the same stable Scottish population between 1984 and 1995. It demonstrated an increased incidence from 258 to 419 cases/million population with a similar trend in both men and women(3). The rise in younger females with the disease may be attributed to increased alcohol consumption.

The incidence in Scotland appears to be higher than in other UK regions. The highest incidence of first attacks of AP in the Bristol area between 1968 and 1979 was 111 cases/million compared with over double that figure in a smaller Scottish study 4 years later (4,5).

The proportion of alcohol related pancreatitis was also higher in Scotland. Alcohol accounted for 26% of attacks in Scottish men (15% overall) compared with 9% of men in the Bristol study. In a recent prospective multi-centre Italian study, only 8.5% of patients had acute pancreatitis in association with alcohol(6).

1.1.2 Mortality Rates in Acute Pancreatitis

Despite advances in the management of acute pancreatitis, mortality rates seem to have stabilised at approximately 8% of all cases. In a retrospective study in Southern England, standardised case mortality rates compared with the general population did not change since the 1970's, despite a doubling of hospital admission rates(1). In the latest Scottish national audit, the in-hospital mortality rate showed a slight reduction during the period 1984 to1995 from 9% to 7.5% of all cases(3). Interestingly, the proportion of early deaths did not change during this period and accounted for up to 60% of deaths.

1.1.3 Clinical features of Acute Pancreatitis

Most patients have a mild, self-limiting course and recover with simple supportive management. However approximately 20% of patients have a more protracted course associated with multiple organ dysfunction (MODS) and up to 50% of these patients die(3).

Patients classically present with a recent onset of upper abdominal pain, often radiating through to the back and frequently accompanied by vomiting. Epigastric or diffuse tenderness is common and is often mistaken as peritonitis. Very rarely, abdominal wall bruising may be seen in severe cases. Dehydration is commonly seen on admission as a result of significant fluid depletion secondary to fluid shifts from the intravascular component.

Patients often exhibit signs of fever, tachycardia and tachypnoea. In patients with organ dysfunction, hypotension, respiratory impairment, oliguria and mental impairment may be seen. The underlying pathophysiology effecting these changes will be discussed later.

1.1.4 Diagnosis of Acute Pancreatitis

The diagnosis is usually confirmed biochemically by the presence of a rise in serum amylase along with a compatible clinical picture. Hyperamylasaemia can be associated with other acute abdominal conditions; careful clinical evaluation is crucial. Serum amylase may be only mildly elevated in patients with a delayed presentation. Lipase has a longer half-life and is more specific in the diagnosis of AP, but is not always available in routine clinical practice. Where lipase can be measured, it is preferable to amylase for diagnosis(7).

Plain X–rays do not have a role in diagnosis, however Chest X-Rays provide an early indication of severity in the presence of a pleural effusion(8).

Computerised tomography (CT) is sometimes used when there is diagnostic uncertainty, particularly in atypical presentations, though it is more often used in determining extent of severity and evaluating complications.

1.1.5 Aetiology of Acute Pancreatitis

The two most common causes of acute pancreatitis are gallstones and alcohol. Together, these account for between 50 and 70% of cases (9,10) depending on local alcohol consumption.

Other less common causes include iatrogenic (most commonly after ERCP), drug induced (steroids, thiazide diuretics), trauma, viral (mumps, coxsackie), pancreatic malignancy, hyperlipidaemia, hypercalcaemia, hereditary and other rarer causes. Idiopathic cases should not be attributed to more than 20% of patients(11).

1.1.6 Initiation of Acute Pancreatitis

The mechanisms by which an aetiological stimulus initiates acute pancreatitis are not fully understood. Evidence suggests that AP begins within pancreatic acinar cells(12) rather than periductal cells, a hypothesis that was proposed previously(13). Activation of trypsinogen to the active trypsin is a key event. Trypsin is involved in the activation and deactivation of other inactive digestive pancreatic enzymes (zymogens) such as chymotrypsin, elastase, lipase and trypsinogen. In normal conditions this occurs in the duodenum. However, in acute pancreatitis, premature activation of trypsin followed by activation of zymogens may be an early initiating event, though this is controversial(14,15) and trypsin may be a protective factor(16).

Several theories have been proposed. One of these is the co-localisation hypothesis which suggests that intracellular zymogens come into inappropriate contact with lysosomal hydrolases (cathepsin B) resulting in intra-acinar zymogen activation(17). Alcohol may induce AP by either sensitising acinar cells to injury or stimulating the release of cholecystokinin(16). With gallstone pancreatitis, the initiating mechanism seems to be pancreatic ductal obstruction, possibly leading to pancreatic duct hypertension, reflux of bile or pancreatic secretions, or pancreatic duct hypertension. These can in turn result in pancreatic duct injury and premature zymogen activation(18).

The precise details of the mechanisms involved in the initiation of AP are unclear but are the subject of ongoing research.

1.1.7 Pathophysiology of Acute Pancreatitis; a summary

Whatever the initiating event, acute pancreatitis is the clinical outcome due to injury of the pancreatic parenchyma. As a result, pancreatic parenchyma is destroyed and an acute inflammatory response is initiated.

Polymorphonuclear leukocytes infiltrate perivascular pancreatic tissue, increased vascular permeability occurs, followed by margination and migration of neutrophils. Phagocytes and macrophages accumulate and further damage is caused by the production of free oxygen radicals. Ongoing inflammatory stimuli can lead to the development of enzyme-rich pancreatic ascites or the development of acute pancreatic collections. In more severe cases, necrosis of pancreatic parenchyma may occur.

The local inflammatory reaction precipitates a systemic inflammatory response by the involvement of a host of other cells including monocytes. These produce cytokines which have a key role in amplifying the inflammatory process and triggering a complex systemic inflammatory response. This involves a wide array of inflammatory mediators, and activates the complement, histamine, coagulation and bradykinin cascades(19).

As a result of excessive inflammatory activity, a systemic response is seen clinically and manifests as the Systemic Inflammatory Response Syndrome, evidenced by tachycardia, tachypnoea, pyrexia and a leukocytosis. If the inflammatory mediators driving this response dominate and are not down-regulated by anti-inflammatory mediators, this can result in organ dysfunction, MODS and in severe cases may lead to death(20).

The development of MODS and the involvement of cytokines are key factors affecting the severity of acute pancreatitis and are the focus of investigation in this

current work. These concepts are discussed in more detail in later sections of this chapter.

1.2 Clinical Management of Acute Pancreatitis

1.2.1 Supportive management

The initial management of acute pancreatitis is essentially supportive. In the majority of mild cases, symptoms and clinical parameters improve within 3-5 days; restoration of oral fluid intake followed by diet is guided by symptoms.

Aggressive initial fluid resuscitation followed by adequate intravenous fluid replacement is important in minimising hypovolaemia, particularly in patients developing organ dysfunction. Inadequate fluid resuscitation in the early phase of AP may be a factor in the development of necrosis(21). Central venous pressure (CVP) monitoring may be required to optimise fluid replacement.

Assessment of respiratory function by peripheral oxygen saturation monitoring and respiratory rate is beneficial and allows earlier detection of respiratory impairment. Oxygen saturation should be kept above 95%. Respiratory support consisting of supplementary inspired oxygen is required in patients with evidence of low oxygen saturation and/ or hypoxia on arterial blood sampling.

Adequate analgesia is prescribed according to the severity of pain.

The use of urinary catheterisation to monitor urine output, arterial lines for regular blood sampling, CVP measurement and NG tubes are frequent adjuncts in the management of these patients, particularly if there is any indication of a deterioration in the clinical condition.

In patients with MODS with evidence of renal and cardiac dysfunction, the addition of haemodialysis/haemofiltration and inotropic drugs to improve organ function may be required.

Although most patients do not present with evidence of organ failure, a significant proportion will develop this within the first week of admission. In a recent study in Glasgow Royal Infirmary, 44% of predicted severe (APACHE II >6) patients developed early organ dysfunction with a subsequent 20% mortality (22,23). Therefore, initial assessment of severity is important and regular review is vital to identify and treat any deterioration in the patient's condition as early as possible. The early recognition of complications including MODS allows timely use of High Dependency or Intensive Care units as appropriate. Referral to a specialist unit is indicated in patients with severe complications, particularly pancreatic necrosis of more than 30%(11).

1.2.2 Investigation of Acute Pancreatitis

Initial investigations include Serum amylase, FBC, U+Es, LFTs & CRP

Arterial Blood sampling is useful in identifying hypoxia and acidosis.

Abdominal Ultrasound (US) has been established in clinical practice to determine the aetiology indicated by the presence of gallstones and should be performed early in the course of the illness(24). Up to 30% of patients with acute pancreatitis have no apparent aetiology, limiting optimal management and prevention of recurrence. Endoscopic US (EUS) techniques have developed in recent years and can be useful in detecting microlithiasis in patients who have recurrent attacks(25).

Magnetic Retrograde Cholangio-Pancreatography (MRCP) has a role in identifying

ductal abnormalities and can be used to further investigate patients with idiopathic AP as a significant proportion of these may have undiagnosed gallstones(26).

Computerised Tomography (CT) is indicated on admission if there is diagnostic uncertainty. Dynamic contrast enhanced CT scanning (pancreatic protocol) is recommended in patients with ongoing symptoms and clinical evidence of sepsis a week after admission or in patients with deteriorating organ dysfunction(7).

It is also a useful modality in assessing disease severity and establishing the presence of local complications such as pancreatic necrosis and acute fluid collections. In addition, CT findings can give some indication of prognosis(27).

Pancreatic gas seen on CT scanning in association with necrosis is indicative of infection and requires definitive debridement.

CT guided Fine Needle Aspiration (FNA) is occasionally performed to exclude infection in patients with necrosis who have ongoing signs of sepsis, but should be used cautiously to avoid infection and other complications(7,28)

1.2.3 Nutrition

Maintenance of nutrition is an important aspect of management but remains controversial. Previously, it was considered essential to fast patients with AP in order to avoid further stimulation of the exocrine pancreas. Subsequently, the importance of maintaining nutrition was recognised and parenteral feeding became an important aspect of managing patients with severe AP.

Unfortunately the use of TPN in AP has been associated with infective and metabolic complications, possibly due to associated immune suppression and glucose intolerance(29). More recently, the role of the gut as a source of, and barrier to infection in patients with sepsis was recognised. Increased intestinal

permeability occurs early and has been linked to bacterial translocation(30). In addition parenteral nutrition has been identified as contributing to gut mucosal failure(31).When compared with parenteral nutrition in 2 randomised trials, enteral feeding was found to be safer, cheaper and associated with less septic complications (32,33). Another study found enteral feeding associated with less organ failure, pancreatic necrosis and mortality than TPN(34). A Cochrane review in 2003 found inconclusive evidence of benefit associated with enteral feeding based on two randomised trials(35). However, a more recent meta-analysis of six randomised studies showed reduced infection rates and hospital stay and recommended enteral feeding in AP(36).

Nasojejeunal feeding was initially recommended however more recent evidence suggests that naso-gastric feeding is as safe as nasojejeunal feeding and can be used in the majority of patients with AP(37,38). The latter is more technically demanding and requires additional endoscopic resources but is useful in managing patients with a degree of gastric outlet obstruction due to duodenal compression from pancreatic inflammation, collections or abscesses.

1.2.4 Management of Biliary pancreatitis

Gallstones account for up to 50% of cases of acute pancreatitis. US will detect the presence of gallstones in the majority. MRCP and EUS can be used to diagnose biliary pancreatitis if gallstones are not detected on initial US. In patients with evidence of obstructive jaundice or cholangitis, ERCP and endoscopic sphincterotomy (ES) is recommended within 48 hours of admission or 72 hours from pain onset(7,39). In cases of gallstone pancreatitis without biliary sepsis or jaundice, the evidence regarding the benefit of early ERCP is contradictory. Two

randomised studies have been performed comparing early ERCP with conventional management. Two of these showed a reduction in complications and in one a reduced hospital stay(40,41) and recommend ERCP in patients with severe or predicted severe biliary pancreatitis. However a later study did not show any benefit in early ERCP with increased complications in the patients who had ERCP(42). ERCP is routinely performed in patients with evidence of biliary sepsis and/ or obstructive jaundice but the use of ERCP outwith these indications depends on local practice. In elderly and unfit patients, there is a role for ERCP and prophylactic ES to avoid recurrent biliary pancreatitis where cholecystectomy is deemed high-risk(39). Cholecystectomy (open or laparoscopic with intra-operative cholangiogram) is recommended in patients with gallstone aetiology(43). In those with mild AP, this should ideally be performed within 2 weeks of discharge and preferably, within the same admission period. Patients with severe AP associated with MODS and / or necrosis should have delayed cholecystectomy to allow local and systemic inflammation to resolve(7).

1.2.5 Management of complications

Further management depends on the natural course. Acute peri-pancreatic fluid collections occur in 30-50% of cases (44,45). These are often self-limiting and do not require intervention unless associated with obstruction or sepsis. In severe cases, local complications such as pancreatic necrosis are associated with higher mortality rates in association with MODS. The diagnosis of necrosis is based on correlation between deteriorating clinical condition and contrast-enhanced CT changes in pancreatic perfusion. CT is indicated if there is deterioration in the patient's condition or evidence of sepsis. MODS is more likely in the presence of infection(46). Typically, infected pancreatic necrosis occurs in the 2nd or 3rd week

of illness. If infection is suspected, from clinical signs of sepsis and radiological evidence of gas or a necrotic collection, formal drainage by surgical, endoscopic or radiological methods is indicated(39).

Beyond the 5th week, pancreatic abscesses can develop. These are usually peri-pancreatic infected collections. They may develop in areas of necrosis or previous fluid collections. Compared with IPN, there is much less of an inflammatory response with fewer patients exhibiting MODS(47). Consequently, the mortality rate is significantly lower. Percutaneous or endoscopic drainage is often effective in their management(48).

1.2.6 Infected Pancreatic Necrosis

Infected pancreatic necrosis occurs in up to10% of patients with acute pancreatitis. It accounts for approximately half of deaths from acute pancreatitis in the UK and is associated with mortality rates between 10 and 80%(49,50). IPN is often heralded by a deterioration in the patient's clinical condition, and may be suspected in patients who develop recurrent or worsening organ dysfunction or SIRS. Blood cultures can be helpful in identifying bacteraemia but are not diagnostic of IPN. CT may be helpful as 20 to 55% of patients with IPN have gas noted on CT indicative of gas forming organisms (47,51). FNA may be performed in patients without CT evidence of infection in the face of continuing clinical suspicion of IPN. If FNA bacteriology is negative, it is usual to continue with conservative management. Consensus guidelines state that proven infected pancreatic necrosis in association with sepsis as a result of pancreatic infection is an indication for surgical treatment. High mortality rates for those with OD in association with IPN who are managed conservatively have been reported(20). Surgical management should include

debridement or necrosectomy, and optimal post-operative removal of debris and exudates from the retroperitoneum(39). In those without MODS, some centres report success with conservative measures with either antibiotics or simple drainage as the only intervention(52). Other specialist centres have had success with CT guided drainage and continuous catheter lavage as the main method of controlling and treating the resulting sepsis(20). Endoscopic transgastric drainage has also been described but all of these studies report results in carefully selected patients, most of whom have little or no evidence of MODS.

Conventional surgical debridement has comprised laparotomy and open pancreatic necrosectomy. Debridement was previously characteristically aggressive in keeping with basic surgical principles, multiple laparotomies were often required with associated high mortality rates. Open packing was developed in the 1980's with improved results(53). Intra and post-operative lavage have been added to open debridement procedures with variable results(54).

Patients undergoing laparotomy for IPN are often critically ill with significant organ dysfunction and do not make ideal surgical candidates. Post-operative ITU care is usually required due to the severity of their clinical condition. Though mortality is high, it is generally recognised that to neglect to debride the necrotic tissue is to invite a much higher risk of death.

There has been a move in recent years towards less invasive techniques for drainage of necrotic material and pancreatic debridement.

Variation in techniques for surgical drainage of IPN will be discussed in a later chapter (Chapter 3).

1.3 Role of Infection in Acute Pancreatitis

The optimal management of IPN includes surgical debridement or drainage of necrotic material as discussed above. In addition patients are commenced on intravenous antibiotics appropriate to the infecting organism and known sensitivities. Whilst the use of antibiotics in patients with IPN is an important adjunct in controlling sepsis, their use in patients without evidence of infection is controversial. Prophylactic antibiotics are often commenced in patients with MODS and with sterile necrosis. With increasing use of prophylactic antibiotics, the microbial spectrum of organisms found has shifted from predominantly gram-negative bacteria to gram-positive bacteria and fungi with an increased prevalence of antibiotic resistance in subsequently infected pancreatic tissue(55). Patients with resistant organisms had longer in-patient stays but mortality rates were not adversely affected. More recently, fungal infection has been associated with an increased mortality rate(56). The role of infection and the use of antibiotics in patients with IPN is discussed in greater depth in Chapter 4.

1.4 MODS and Acute Pancreatitis

1.4.1 Definition of MODS

The term Multiple Organ Dysfunction Syndrome (MODS) was proposed in 1992 at the American College of Chest Physicians and Society of Critical Care Consensus Conference(20) in order to better reflect the dynamic characteristics of this condition arising from various insults to the body. MODS was defined as the "presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention"

Previously, the term "organ failure" was used widely but the definitions used varied considerably and made comparison between studies difficult.

1.4.2 SIRS

This conference also clarified the differences between the terms "septic syndrome" and "sepsis" which were often used interchangeably and incorrectly in non-infectious inflammatory conditions. A new term "Systemic Inflammatory Response Syndrome" (SIRS) was recommended to reflect this response to a variety of clinical insults. This develops in patients with unregulated effects of pro-inflammatory mediators, clinically manifesting as tachycardia, hypothermia or pyrexia, tachyopnoea and a leukocytosis. These parameters can be objectively noted and are used to identify the presence of SIRS (Table 1.1). At this stage, either homeostasis can occur with improvement in the patient's condition or further deterioration resulting in widespread organ dysfunction.

Table 1.1: Definition of SIRS

Clinical Parameter	Criteria
Cardiac	Pulse > 90/ min
Respiratory	Respiratory rate> 20 or pCO2 >4.3kPA
Temperature	<36°c or > 38°c
Inflammatory markers	White Cell Count <40 or >120 ($x10^3$mm)

1.4.3 SIRS, MODS, CARS and MARS

Bone described the underlying pathophysiological mechanisms underpinning the progression from SIRS to MODS in his hallmark paper in 1996(57).

He proposed that the interaction between inflammatory and anti-inflammatory mediators resulted in several variations in response to a severe insult upon the body. In some patients, this response is down-regulated and although some organ dysfunction may manifest, this resolves quickly and the patient recovers. In these patients, pro-inflammatory mediators help to recruit other mediators such as neutrophils to the site of injury. These factors then stimulate anti-inflammatory mediators which oppose further pro-inflammatory activity and eventually restore homeostasis. In contrast, patients who have an overwhelming anti-inflammatory response continue to deteriorate and often this results in a fatal outcome. These patients initially demonstrate SIRS and progress clinically towards MODS. A compensatory anti-inflammatory response is initiated (CARS) which can be as extreme as the pro-inflammatory response. CARS is characterised by a period of anergy and marked immunosuppression. During this stage, patients are at increased risk of infection, which if occurs, acts as a further insult on an already weakened system and provokes a second anti-inflammatory response. A mixed inflammatory response (MARS) is seen where neither SIRS nor CARS predominate. Persistent inflammation, persistent immunosuppression or a combination of both responses results in "immunologic dissonance" which has been described by Bone as "a pathophysiologic response that is out of balance and inappropriate for the patient's biological needs". Organ failure eventually occurs followed soon after by death unless homeostasis can be restored.

1.4.4 SIRS and MODS in the development of Acute Pancreatitis

Although this pathophysiological description relates to MODS occurring from a wide variety of insults such as trauma, burns and severe infection, it is highly applicable to MODS occurring in association with acute pancreatitis (Figure1.1).

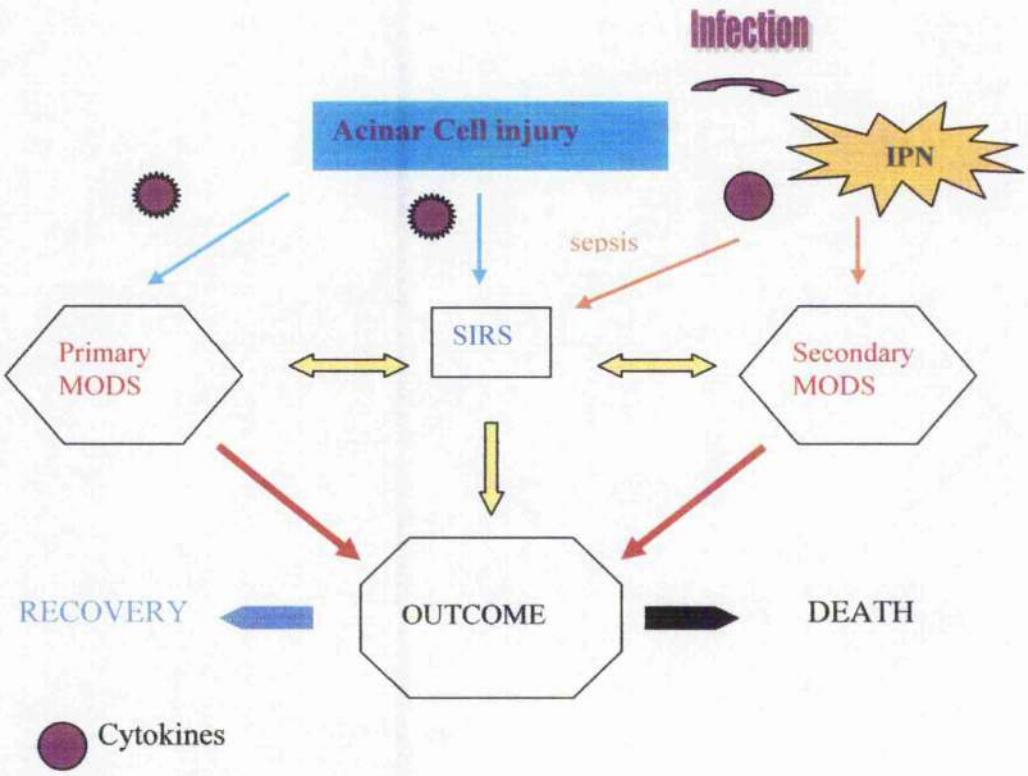


Figure 1.1: SIRS To MODS (adapted from (20))

Several possible clinical manifestations occur as a result of a combination of local and systemic inflammatory responses.

1) Pro-inflammatory mediators are balanced locally and homeostasis is restored, this is seen in mild cases of AP.

2) SIRS develops but is balanced by CARS and homeostasis is restored, in patients with mild AP but with SIRS noted initially
3) SIRS predominates and progresses to MODS. This may be transient due to subsequent CARS and resolves within a short period of time.
4) SIRS predominates progressing to persistent MODS that is not down-regulated by CARS. Immunologic dissonance and death may follow. This may be seen in patients with early mortality secondary to MODS
5) MARS can occur and lead to local cell death (apoptosis) without much evidence of inflammation. This may be seen in patients with sterile necrosis and no MODS. Inflammation is balanced and these patients have a good prognosis.
6) MARS occurring with persistent MODS in addition to CARS results in immunosuppression and in the presence of pancreatic apoptosis predisposes to infection in the form of infected pancreatic necrosis. These patients have a much higher risk of death if MODS persists
7) MARS resulting in initial MODS followed by IPN. Surgical stress either stimulates a further episode of MODS leading to death, or helps to down-regulate the SIRS response leading to resolution and recovery.

1.4.5 MODS and Mortality in Acute Pancreatitis

Early Deteriorating MODS

The Atlanta Symposium in 1992 defined severe AP in terms of patients with evidence of organ failure and / or local complications such as necrosis, pseudocyst and abscess formation(58).

A recent prospective study analysed the relationship between early MODS and mortality in patients with predicted severe AP(23). It showed that patients with deteriorating early organ dysfunction had an associated mortality rate of 55%, compared with almost zero mortality in patients with early resolving MODS ($p<0.001$). Based on these findings, the study concluded that it is patients with deteriorating MODS that should be characterised as having severe AP, as patients with transient MODS usually recover and have low mortality rates. This contradicts the Atlanta definition which was based on the presence of MODS rather than the dynamics of the condition. Further studies have confirmed this observation and shown persisting organ failure to be the important adverse factor(59,60).

Those with transient organ dysfunction which improves within 48 hours, although satisfying the Atlanta criteria for severe AP are not at increased risk of dying and should not be considered as having severe AP. This proposal was recently incorporated within UK guidelines on the management of AP(7).

Biphasic pattern and mortality rates

Acute Pancreatitis is associated with an overall mortality of 5-10%(3,61). This tends to occur in a biphasic pattern. Early mortality in the first week is due to overwhelming Multiple Organ Dysfunction Syndrome (3). Those with early MODS who survive the first week either recover or continue to have organ failure. Septic complications occurring during or after the second week can trigger a further episode of MODS in those who have recovered, or worsening MODS in those who continue to have organ failure and is known as the "second hit". This may be fatal and is responsible for the majority of deaths seen in the third week and beyond (22). Patients with MODS have a much higher mortality rate than those who have no organ dysfunction throughout their illness. In patients who die, the proportion

attributed to early MODS has been shown in several earlier studies to be as high as 60% (3).

This is in contrast to a prospective London audit which attributed only 32% of overall mortality to early MODS(61). More recently, it has been proposed that mortality rates from early MODS may be decreasing, possibly due to advances in supportive therapy in the initial period of hospitalisation(62), though wide variations in early mortality rates from between 4-85% of all deaths are reported in different centres globally(50,63). This apparently large variation may be due to differences in patient populations and medical care provision.

Several studies have shown that the presence of organ dysfunction alone is a major determinant of mortality. A retrospective analysis of 267 consecutive patients in the Netherlands with acute pancreatitis showed an overall mortality of 19% with 96% of deaths attributable to multiple organ failure(64). This relationship has been demonstrated in several studies since. A Chinese retrospective analysis of 74 patients with SAP showed a significant association between organ failure and mortality with a greater association between multiple organ failure and mortality(65). Other studies have confirmed a significant correlation between organ dysfunction and death(66).

At presentation, it can be difficult to predict clinically which patients fall into the high-risk group that will develop MODS. Several scoring systems have been developed in an attempt to stratify patients into mild and severe groups and these are discussed below.

1.4.6 Assessment and Prediction of MODS

Multiple factor scoring systems

It is important to be able to stratify patients into those likely to develop severe AP and the majority of AP patients who will have a mild, self-limiting illness(67). This enables early identification of patients who would benefit from specialist unit input and early intervention. In addition, it allows comparison of results in different units and identifies higher risk patients who may be suitable for recruitment into clinical studies. Over the last 3 decades, several multiple-factor scoring systems have been developed. The Ranson and modified Glasgow scores were developed in the 1970's and early 1980's and could predict severity with a sensitivity of up to 60% within 48 hours of admission(68,69). (Appendix 1&2)

APACHE II Score

More recently, the APACHE II score was developed and though used initially for ICU patients to predict outcome, found widespread use in research settings in the 1990's for patients with AP to predict those more likely to have a severe illness. It was first described in 1985 and uses multiple physiological and laboratory parameters to provide a numerical score relating to severity of illness(79). Although the APACHE II score can be more complex to calculate, it can be used within the first 24 hours of admission. When compared with Ranson and modified Glasgow scores, it was found to be more accurate in a number of prospective studies(71-73). The cut-off score can be altered with a subsequent change in sensitivity and positive-predictive value (though a value of 6 can easily be achieved in the elderly with little physiological derangement). APACHE II scores with a minimum cut-off of 6-10 points have shown a positive correlation with a severe outcome(74).

Other Predictive Factors

Simple clinical factors such as greater age (over 70) and obesity are now well recognised as being important risk factors for severe AP. A recent modification of the APACHE-II score to include obesity was proposed as it provides greater accuracy (85%) when compared to APACHE II alone(75).

C-Reactive Protein (CRP) has been most widely accepted for clinical practice. Its production by the liver is stimulated by Interleukin-6 and peak levels occur 46-96 hours after onset of symptoms. It is not specific to acute pancreatitis but is simple and cheap to assess and has been adopted widely as an adjunct in assessing severity, it can indicate resolution of inflammation as well as indicating further inflammation and/ or necrosis.

Several other factors for assessing severity have been used in research settings and are not available widely. These will not be discussed further here but include Trypsinogen Activation Peptide (TAP), Amyloid A, TNFa, Interleukin-6, Interleukin-8 and Procalcitonin(76).

1.4.7 Quantification of MODS

Marshall Score

The Marshall organ dysfunction score was initially described in 1995 for critical care patients and allocates a numerical value to the level of dysfunction noted in cardiovascular, renal, respiratory, neurological, hepatic and haematological systems(77). It was further modified by Bernard to exclude hepatic failure(78). This scoring system has been used extensively in more recent years in clinical trials of patients with AP. A modified Marshall score of 2 or more in any organ system

correlates very closely to the definitions for organ failure as described at the Atlanta symposium (Table1.2 and Figure 1.2).

Persistent or deteriorating Marshall scores of >2 in any one organ system is significantly associated with a higher mortality rate in patients with AP(23).

Table1.2:Marshall Score of Organ Dysfunction

	0	1	2	3	4
Respiratory PO2/ FiO2 ratio	>300	226-300	151-225	76-150	<76
Renal Serum Creatinine	<100	101-200	201-350	351-500	>500
Hepatic Serum Bilirubin	<20	21-60	61-120	121-240	>240
Cardiovascular Pressure adjusted heart rate	<10	10.1-15	15.1-20	20.1-30	>30
Haematological Platelets	>120	81-120	51-80	21-50	<20
Neurological GCS	15	13-14	10-12	7-9	<6

1) Three or more Ranson criteria
2) Eight or more APACHE II points
3) Organ failure defined as follows; Shock: Systolic BP <90mmHg
   Pulmonary Insufficiency:PaO2<60mmHg
   Renal Failure: Creatinine level> 170umol/L
   GI Bleeding: >500ml/24 hours
   DIC: platelets<100(1x10mm6)/ Fib <1g/L
   Severe metabolic disturbance e.g. hypocalcaemia

Figure 1.2 Atlanta criteria for Severe AP

1.5 The Role of Cytokines

1.5.1 General introduction to Cytokines

Cytokines are soluble chemical messengers of low molecular weight which are produced to facilitate cellular communication, including interaction between APC, T-helper cells and B cells. Chemokines are a subgroup of cytokines which have 4 characteristic cysteine residues within their structure(79.

The most common sources of cytokines include monocytes, macrophages, T-Helper cells, granulocytes and endothelium. Cytokines are continually being discovered and despite having distinct protein structures and sometimes opposing biological activity profiles, they share a number of properties. Cytokines exhibit pleiotropism whereby they can each act upon different types of target cells. Conversely, different cytokines can exert similar biological effects through their interaction with specific target cell surface receptors. This in turn activates intracellular synthesis of new mRNA followed by new protein production(80). By this method, cytokines are able to induce increased self-production as well as increasing production of other cytokines. This process is termed amplification and leads to the development of a cascade of cytokine-driven effects. Many have both autocrine and paracrine functions. Cytokines play an important role in regulation and activation of the immune and inflammatory responses by controlling humeral and cellular activity(81). They are produced rapidly by their cell of origin upon recognition of antigenic substances such as bacterial endotoxin or tissue injury. Each cytokine is produced specifically in response to a particular stimulus. They are involved in a wide range of acute and chronic inflammatory conditions (82) and have also been implicated in the development of neoplastic processes.

1.5.2 Cytokines and the Inflammatory Response

Cytokines are some of the principle mediators of the pathophysiological processes underlying SIRS and the development of MODS. These have been discussed previously in terms of their clinical manifestations. On a cellular level, cytokines are released as part of the body's normal response to infection and/or injury. The inflammatory cascade aims to eradicate any invading organisms and repair any tissue damage that has occurred. This is achieved by a variety of cellular mechanisms including the division of stem cells and activation of lymphocytes, macrophages and phagocytes. T cells, B cells and platelets are recruited. Migration and margination of neutrophils occurs along with extravasation of monocytes. These in turn, produce more pro-inflammatory cytokines, principally TNF, IL-1 and IL-6. Fluid exudation occurs as a result of increased vascular permeability. Activation of the complement, coagulation, histamine and bradykinin systems occurs which further amplify the inflammatory response(83).

There is accumulating evidence to suggest that it is the local production of inflammatory mediators in response to a specific insult, whether it be infection, trauma, burns, ischaemia or organ damage, that results in the systemic effects (Table 1.3)(84). If this inflammatory cascade, mediated by cytokines, is not well controlled, it results in a massive inflammatory reaction and leads to organ dysfunction(85;86). This has been discussed previously with respect to acute pancreatitis in terms of SIRS, MODS, CARS and MARS.

Table 1.3 : Pathophysiological processes underlying SIRS (adapted from (87))

- Progressive endothelial dysfunction, increased microvascular permeability
- Platelet sludging blocks microcirculation, blood flow misdistribution +/- ischaemia
- Ischaemia may cause reperfusion injury
- Induction of heat shock proteins
- Coagulation system activation, impaired Protein C-Protein S pathway

Pro-inflammatory and anti-inflammatory mediators

At the local site of injury or infection, the initial appearance of pro- and anti-inflammatory mediators in the circulation are beneficial. Restoration of homeostasis is the ultimate aim of these responses. If the balance between pro- and anti-inflammatory activity is lost, then these mediators become harmful.

This delicate balance between opposing natural forces was recognised almost 20 years ago and likened to the traditional Chinese philosophy of "Yin and Yang" (88). This description remains valid for the interaction between pro- and anti-inflammatory cytokines.

Cytokines such as TNFα, interleukin-1, interleukin-6, interleukin-8 and PAF are pro-inflammatory, resulting in priming and activation of neutrophils, increased macrophage activity and recruitment of B & T cells.

They are opposed by anti-inflammatory cytokines such as interleukin-1 receptor antagonist (Il-RA) and interleukin-10 (Il-10) which inhibit T helper cell cytokine production and modify the inflammatory response.

Other cytokines are included below (Table 1.4)

Table 1.4: Pro-inflammatory and Anti-inflammatory Cytokines adapted from (87)

Pro-inflammatory Cytokines	**Anti-inflammatory cytokines**
Tumour Necrosis Factor alpha (TNFα)	Interleukin-1 receptor antagonist
Interleukin-1b	Interleukin -4
Interleukin -2	Interleukin -10
Interleukin -6	Interleukin -13
Interleukin -8	
Interleukin -15	
Interleukin-18	
Interferon gamma (IFN-y)	
Platelet Activating Factor (PAF)	

1.5.3 Role of Cytokines in Acute Pancreatitis.

Acute Pancreatitis progressing from localised pancreatic inflammation to a more generalised systemic disease in the form of SIRS and MODS is now recognised as being mediated by cytokines(89;90). Increasing evidence suggests that MODS in acute pancreatitis is associated with excessive pro-inflammatory cytokine action(91-93). The exact pathways by which inflammatory mediators are triggered

in acute pancreatitis and exert their remote effects are not yet completely understood but several cytokines have been implicated.

Tumour Necrosis Factor (TNF)

TNF was one of the first cytokines to be described. It is produced by several cells including macrophages, monocytes, mast cells and T cells and acts by increasing endothelial permeability and inducing other inflammatory mediators such as neutrophils, macrophages, interleukin-1 and Platelet Activating Factor (PAF). TNFα (pro-inflammatory) and TNFβ (anti-inflammatory) are both involved in modulating the inflammatory response. TNFα is secreted primarily by monocytes and is involved in the initial stages of pancreatic inflammation. It initiates production of cytokines including IL-6 and IL-8. In an experimental model of AP, pancreatic acinar cells have been found to produce, release and respond to TNFα. Furthermore, neutralisation of TNFα resulted in reduced acinar cell apoptosis(94). In clinical studies, monocyte production of TNFα, IL-6 and IL-8 were significantly increased in AP patients with systemic complications compared to patients with a milder course(95), further implicating these cytokines as early mediators of the systemic effects of AP. Previous studies of TNFα plasma levels in AP patients had been inconclusive(96). This may be related to the short half-life of TNFa and its intermittent secretion, which together with infrequent sampling, make assessment of plasma levels difficult to interpret (97). Instead soluble TNF receptors can be measured to reflect TNF activity.

The association between TNFα, and organ failure in acute pancreatitis was further demonstrated by significantly increased serum levels of soluble TNF receptors ($sTNF_{55}$ and $sTNF_{75}$) (98). Activated pancreatic enzymes, including elastase, have been shown to induce macrophages to produce TNFα through specific

transmembrane receptors suggesting the mechanism by which TNF is produced in response to pancreatic damage(99). Pancreatic ascites has also been shown to induce TNFα and IL-1β production, though the exact mechanism for this is unclear(100). TNFα blockade has been successful in significantly reducing the mortality rate associated with severe AP in an animal model of AP using a recombinant form of soluble Type 1TNFα receptor. This effect was achieved by a reduction in inflammatory cytokine production. In addition, attenuation of the severity of the disease was more effective when TNFα antagonism was delayed until circulating cytokine levels were high(101). No studies involving TNFα inhibition in humans have been carried out to date. The potential of anti-TNFα treatment is worth considering as a subject for future study.

Interleukin-1

This cytokine is similar to TNFα in its properties and wide range of action. It is an early inducer of the acute inflammatory response. Like TNF, it is difficult to measure in serum. It is antagonised by IL-1 receptor antagonist (IL-1ra), measuring IL-1ra reflects IL-1 activity more accurately.

Interleukin-6

This cytokine is involved in the acute phase response; it is a T cell derived cytokine and activates B cell differentiation. Raised plasma IL-6 has also been found in patients with severe disease and organ failure (98). IL-6 is a good marker of disease severity and elevated levels precede CRP elevation in the first 48 hours (102).

Interleukin-8

The main role of IL-8 is in the recruitment and activation of neutrophils. In an early study of IL-8 and pancreatitis, plasma levels were higher in patients with complicated pancreatitis and correlated closely with neutrophil elastase (a marker

of neutrophil activation) (89). Increased monocyte secretion of IL-8 has also been correlated positively with increased disease severity (92) adding further weight to IL-8's role in the development of systemic complications in acute pancreatitis. IL-8 & IL-6 plasma levels were found to reflect severity of AP in the first 24 hours with greater sensitivity than CRP, indicating their involvement in the early inflammatory response(103).

Anti-inflammatory Cytokines

Several anti-inflammatory cytokines have also been implicated in the development of severe acute pancreatitis.

Interleukin-10

Interleukin-10 is produced by T helper cells, B cells, macrophages and keratinocytes. It is the principle anti-inflammatory cytokine and down regulates the production of TNF, IL-1, IL-6 and IL-8 at a transcriptional level by inhibition of macrophages and T1-helper cells. In patients with severe AP, higher IL-10/ IL-6 and IL-10/ IL-8 ratios were associated with improved clinical outcome despite elevated IL-10 plasma levels in these patients, suggesting that both pro- and anti-inflammatory cytokine activity is increased in severe AP (104). Further evidence of the role of IL-10 in modification of the inflammatory response comes from a study of ERCP patients. Exogenous IL-10 given prior to ERCP reduced the incidence of post-ERCP pancreatitis(105). In an animal model of SAP, human IL-10 gene administration resulted in significantly reduced mortality rates, histological changes and reduced tissue TNF levels (106).

IL-1RA, another anti-inflammatory cytokine, was administered to SAP induced mice with an associated reduction in mortality (101). In humans, cytokine inhibition in the form of Lexipafant, a potent inhibitor of PAF has been investigated

in patients with predicted severe pancreatitis. PAF is released by activated monocytes, platelets and endothelial cells. It is involved in the pathophysiology of AP by activating platelets, monocytes and neutrophils in addition to increasing vascular permeability. Initial studies with Lexipafant showed some promise in reducing AP associated organ failure, though no change in mortality was noted (107). A later phase III multi-centre double-blinded trial did not confirm these findings in a larger group of patients (108).

1.5.4 The Role of Cytokine Genetics

There is significant individual variation in the cytokine response seen in a number of inflammatory and immune mediated conditions. There is increasing evidence that polymorphisms in cytokine gene loci are important determining factors affecting cytokine gene function with subsequent effects on disease susceptibility and severity.

A single Medline search for the term "cytokine gene polymorphisms" elicited 2397 articles over the last 23 years. Obviously, not all of these have positive disease associations but the large number of studies indicates the interest in this area. Evidence suggests that variations in the genetic make-up of cytokines can affect their function (109). This could account for the variation seen in the regulation of the inflammatory response in individuals (110). There is increasing evidence to suggest that TNF polymorphisms are involved in susceptibility and severity in patients with sepsis. The TNFα –308A allele in particular has been implicated. It is associated with increased TNFα gene transcription, monocyte secretion and blood concentration levels (111). An association between TNFα –308 and mortality has been demonstrated in 98 British children with meningococcal sepsis (112). Further

evidence of a relationship to sepsis is demonstrated in a French study of 89 ICU patients with septic shock, with a greater susceptibility to sepsis and mortality in patients with the –308A allele (113). This study did not stratify ethnicity which can be a confounding factor in genetic studies. However a similar association was seen in postoperative Asian patients with septic shock. Non-survivors were more likely to possess the –308A allelic variant (114). No association was noted in a German study comparing 80 post-operative patients with ethnicity-matched normal controls. They proposed that positive associations with sepsis and TNFα –308A may be due to linkage between this locus and another Major Histocompatibility (MHC) locus (115). A more recent study found that the TNFα-308A variant as well as a Toll-like receptor (TLR4) SNP were both associated with an increased risk of sepsis following burn trauma but not mortality(116). Although the evidence for the importance of the TNFα-308A allele is mounting, some studies have been inconclusive. This could be due to a number of reasons including study design deficiencies, and difficulty in interpretation as a result of background genetic variability (117). In acute pancreatitis, few cytokine gene polymorphisms have been studied with varying associations between polymorphic gene frequencies and the disease. In the TNF promoter region, 5 microsatellites (TNFa-e) and 2 SNP sites (-308, -238) have previously been identified (118). Allele2 at the TNFa microsatellite has been associated with increased TNF production (119). Three of these TNF microsatellites (TNFa,b,c) and a SNP (-308 A/G) were typed in a population of AP patients and compared with normal controls. No differences in allelic frequencies were noted between mild and severe patients or patients and controls (120). However, in a series of 72 patients with AP, TNFα-308A was more than twice as frequent in patients with severe AP compared to those without (121).

In another study, TNF –308 SNPs and TNFB microsatellite, IL-1b and IL-1RA were determined in 190 AP patients and compared with controls. No difference was noted in the TNF genotype frequencies, however, an imbalance between IL-b and IL-1ra secretion appears to exist in patients with severe AP. The genetic basis for this could not be explained. In a separate study by the same group, the interleukin-1 gene cluster was compared in patients and controls. Allele 1 in the IL-1RN polymorphism (IL-1 receptor antagonist gene) was more frequent in patients with severe disease and more frequent in patients compared to controls. Allele 2 corresponds to high IL-1ra secretion; the authors proposed that Allele1 therefore is associated with lower IL-1ra secretion. This would support the hypothesis that by a reduction in IL-1ra, there would be less anti-inflammatory activity and a subsequent increase in severity (122).

1.5.5 Investigation of an Interleukin-8 Microsatellite

A microsatellite close to the interleukin-8 gene has been identified and is associated with susceptibility to panbronchiolitis (123). This microsatellite had not been investigated previously in patients with acute pancreatitis until recently. A study conducted at Glasgow Royal Infirmary explored this polymorphic microsatellite in the interleukin-8 genome in a population of 86 patients with acute pancreatitis (124) and compared allelic frequencies in patients and controls. Allele 9 was significantly over-represented in patients with no organ dysfunction compared to those with early OD ($p=0.006$). Patients with allele 9 were found to be three times less likely to develop MODS during the course of acute pancreatitis when compared with other acute pancreatitis patients. These results thus proposed a protective element associated with allele 9. It is not fully known what the significance of this

microsatellite is in relation to the functional expression of Interleukin-8 but as it located in the promoter region, it is possible that it exerts an influence on gene transcription/translation and thereby affects Interleukin-8 production levels. No evidence exists however to demonstrate the mechanism by which this microsatellite influences Interleukin-8 function or production. A functional haplotype containing six SNPs has been described and is associated with increased Interleukin-8 transcription (125,126). These SNPs have yet to be examined in patients with acute pancreatitis.

Why some individuals progress down a certain pathway of systemic inflammatory behaviour as a result of pancreatic inflammation is uncertain but may be due to inherent genetic variation. If individual cytokine gene variations could be mapped out in relation to their inflammatory behaviour, then this would lead to a greater understanding of the precise mechanisms by which cytokines are produced and their role in the development of MODS. Further studies are needed to investigate the role of cytokine gene polymorphisms in the development of severe AP.

1.6 Hypothesis

Acute Pancreatitis is a condition ranging in clinical severity from a mild uncomplicated illness to a severe, overwhelming disease involving multiple organ failure and in some, death. The difference in allelic frequency of an IL-8 microsatellite between patients with severe and mild AP is the first evidence for the role of interleukin-8 genetic polymorphisms in the development of acute pancreatitis. Several single nucleotide polymorphisms have been discovered in the interleukin-8 promoter gene locus (125). Their role in association with acute pancreatitis has not yet been explored. Patients frequently have MODS in association with infected pancreatic necrosis with a higher risk of mortality. Surgery for IPN can precipitate a second inflammatory response resulting in further MODS and death in a proportion of patients. With the development of minimally invasive necrosectomy techniques, this may be associated with reduced surgical stress and a reduction in MODS and/ or mortality. These techniques have yet to be compared to conventional open necrosectomy procedures. Bacteriological profiles are important determinants of survival. The widespread use of prophylactic antibiotics has led to a change from predominantly gram-negative to gram-positive and resistant organisms. The change in microbial spectrum may be associated with higher mortality rates.

Several factors have been recognised as having a role in the development of MODS and influencing outcome in acute pancreatitis. The work of this thesis will be based on the following hypotheses:

1) Cytokines are a key factor in mediating the local and systemic inflammatory responses seen after the initiation of acute pancreatitis. Interleukin-8 single

nucleotide polymorphisms are involved in the inter-individual variation in systemic response to acute pancreatitis

2) Minimally invasive pancreatic necrosectomy reduces the surgical stress response associated with infected pancreatic necrosis resulting in less ICU requirements and a lower mortality rate.
3) Increased use of prophylactic antibiotics in patients with MODS has changed the spectrum of microbial infection with increased fungal infection resulting in higher mortality rates.

1.7 AIMS

This thesis aims to focus on three aspects which seem to be important in the development of Severe Acute Pancreatitis

1) Interleukin-8 is a cytokine involved in mediating the inflammatory response in acute pancreatitis. Genetic polymorphisms in the IL-8 locus have yet to be explored in patients with AP. The role of interleukin-8 in acute pancreatitis will be explored by assessing IL-8 polymorphisms in patients with mild and severe pancreatitis and comparing them with a control population.
2) The influence of minimally invasive pancreatic necrosectomy on MODS in patients with infected pancreatic necrosis will be examined by comparing their outcomes with patients undergoing conventional open necrosectomy
3) The influence of prophylactic antibiotics in the development of resistant organisms and the effect on subsequent mortality will be explored further by assessing antibiotic use, infecting organisms and outcomes in a cohort of patients with IPN.

Chapter 2

Exploration of the influence of Interleukin-8 genetic polymorphisms on pancreatitis severity and susceptibility.

2.1 Introduction to Interleukin-8

2.1.1 Discovery of Interleukin-8

Interleukin-8 was first identified in 1987 as a novel type of neutrophil-activating chemokine and was known by a variety of terms such as "Human Monocyte-derived Neutrophil Chemotactic Factor (127) and was renamed "interleukin-8" in 1989 (128). It belongs to a family of small, structurally related pro-inflammatory chemokines similar to Platelet factor 4 (129). To date over 50 chemokines have been discovered and are broadly divided into CC and CXC subfamilies depending on the position of the first two cysteine residues in relation to each other(129;130). Chemokines play a major role in mobilising host defences by attracting and activating the cells of the immune system (131). Interleukin-8 is produced by T-helper cells, phagocytes and mesenchymal cells amongst others, in response to cellular injury from a variety of causes such as ischaemia, trauma and infection. The process of inflammation is the resulting host response and is characterised by the movement of neutrophil leukocytes to the site of injury in association with the extravasation of fluid. The main action of Interleukin-8 is to activate neutrophils and facilitate migration.

2.1.2 Interleukin-8 Structure and Function

The structure of Interleukin-8 varies from a precursor of 99amino-acids to several biologically active forms, the most predominant of which exists as a 72amino-acid

molecule containing 4 cystienes which form 2 disulphide bridges (129). Interleukin-8, along with most other chemokines, forms dimers, which dissociate upon dilution. The monomer constitutes the biologically active form (132). Interleukin-8 is one of the main tissue-derived chemoattractants for neutrophils and exerts its effects in three ways(129). Firstly by neutrophil shape-change and directional migration. This occurs after activation of the contractile system of neutrophils, allowing a change in cell body volume thereby enabling neutrophils to adhere to endothelial cells and to migrate. Secondly, exocytosis of specific granules and storage vesicles with subsequent enzyme release (e.g. elastase). Interleukin-8 dependant surface remodelling during exocytosis leads to the expression of adhesion molecules which enhance the neutrophil's ability to adhere to endothelial cells and the extracellular matrix. Thirdly, the "respiratory burst" involves the rapid and transient activation of hydrogen peroxide and superoxide radicals, a recognised characteristic of stimulated phagocytes. Interleukin-8 receptors exist in a variety of forms, with many acting as non-specific receptors. Interleukin-8 interacts with neutrophils by binding to two receptors on its surface, the chemokine receptors CXCR1 and CXCR2. A specific receptor (IL-8-RA) has been identified.

2.1.3 Interleukin-8 Genetics

Interleukin-8 is known to have a large variation in expression levels. It's production can be rapidly induced by a number of stimuli, including other pro-inflammatory cytokines such as TNF-1 and IL-1 which can result in secretion levels increasing up to 100-fold. Bacterial and viral products can induce up-regulation by 5-10 times (133). The gene for interleukin-8 production /secretion is located on Chromosme 4q. Genetic polymorphisms account for different traits being expressed in humans

and other organisms. Polymorphisms have also been associated with varied susceptibility to disease. A polymorphism is an allelic variant that occurs in >1% of the population, has a stable frequency and cannot be accounted for by mutation. They can either involve single nucleotide polymorphisms (SNP) or a varied sequence of nucleotides (microsatellite). SNPs tend to remain together within genes during meiosis. SNP pairs or units that are linked together in this way are known as haplotypes. SNPs are in sequence with non-variable parts of the genome and are linked with other SNPs in that region. This phenomenon is known as linkage disequilibrium. Therefore if a haplotype is associated with a differing disease outcome then it is not known which is the causal SNP and which is in linkage disequilibrium with the causal SNP. Polymorphisms can occur in the promoter or encoding parts of the gene and may alter the structure and therefore, the function of the resulting protein. Patients with various diseases have been investigated to establish the possible relationships between disease susceptibility or severity and genetic polymorphisms within particular genes. Several SNPs and a microsatellite have been found in the Interleukin-8 gene locus.

2.1.4 Interleukin-8 polymorphisms and Disease Association

Numerous studies have been carried out on interleukin-8 polymorphisms to investigate possible associations with disease severity and susceptibility in a wide range of inflammatory and neoplastic conditions. These studies are based primarily on the premise that interleukin-8 plays a key role in the pathophysiology of inflammation.

Several respiratory conditions have been studied and are associated with interleukin-8 polymorphisms. Diffuse panbronchiolitis is a chronic inflammatory

airway disease of unknown aetiology characterised by inflammatory lesions with infiltration of lymphocytes and foamy macrophages around bronchioles as well as marked neutrophil accumulation. It is more common in Asian populations. Elevated interleukin-8 concentrations are found in the airways of patients with the disease(134). In 1999 a Japanese group discovered an association between a microsatellite at the interleukin-8 locus and diffuse panbronchiolitis(123). Of 9 possible alleles, allele 2 was significantly over-represented in patients compared to controls suggesting increased susceptibility to the disease in these individuals. Interleukin-8 polymorphisms and susceptibility to respiratory infections was further demonstrated by a recent study into Tuberculosis (135). Interleukin-8, not surprisingly, is known to be involved in the pathogenesis of TB. Ma and colleagues compared the genotypes of the –251A/T SNP in patients with TB compared with controls and found that homozygosity for -251A was significantly more predominant in patients. They also found that the –251A allele was preferentially transmitted to TB-infected children. This study adds further weight to the evidence of a relationship between IL-8 polymorphisms and susceptibility to TB.

The role of interleukin-8 polymorphisms in susceptibility to disease has been explored in patients with non-small cell lung cancer. Inflammation may play a key role in the development of lung cancer. A French group recently studied a variety of inflammatory-gene polymorphisms including COX-2, interleukin-6 and interleukin-8 and found that an IL-8 promoter polymorphism was protective in female subjects against the development of lung cancer(136). Other cancers have been studied with respect to inflammatory gene polymorphisms. The –251A SNP in the interleukin-8 gene has been shown to be associated with a reduced risk of colorectal carcinoma. (137). Another gastro-intestinal condition has also been

linked with Interleukin-8 polymorphisms. Students travelling to Mexico for 5 weeks were studied for symptoms of enteroaggregative Escherichia coli (EAEC) diarrhoea. Faecal Interleukin-8 levels were measured and the genotypes of 5 SNPs in the Interleukin-8 gene were identified. (138). Susceptibility to EAEC was more common in those with A/A and A/T genotypes at the (–251) locus compared with homozygous (–251T). In addition, those with the AA genotype produced significantly greater quantities of faecal Interleukin-8. Three novel interleukin-8 polymorphisms have also been recently described in relation to severe systemic lupus erythematosus (SLE) nephritis (139). Allelic variations from the published sequence of a 1526 base pair segment of the Interleukin-8 5'-flanking region (between –57 and –1583 in relation to the translational start codon) were analysed in 162 healthy controls and 167 patients with SLE, of whom 120 had renal involvement. The novel SNPs are (-845C/T), (-738A/T) and (–353 A/T). This study found differences in the prevalence of SNP genotypes between healthy Caucasian and African-American individuals. The (-353A) allele was more dominant in African Americans. In addition, African American patients with severe SLE were found to have a significantly higher frequency of (–845C) compared to healthy African American individuals. The authors suggested that African-Americans with this polymorphism were more susceptible to severe SLE nephritis perhaps by an influence on IL-8 gene expression.

As discussed above, a Japanese group have previously described an over-representation of allele 2 in a microsatellite close to the IL-8 gene in patients with diffuse panbronchiolitis. They proposed that this polymorphism was associated with increased susceptibility to the disease(123). This microsatellite was investigated in patients with AP. Allele 9 was more frequent in patients without

organ dysfunction, suggesting a protective effect(124). However the role of this microsatellite in relation to the functional expression of Interleukin-8 is not known Interleukin-8 is also implicated in the pathogenesis of RSV bronchiolitis. It is produced by bronchial epithelial cells (140) after infection with RSV. Disease severity correlates with IL-8 mRNA levels in nasal secretions (141). Hull described six single nucleotide polymorphisms in the promoter region of the Interleukin-8 locus and found an association between increased susceptibility to RSV bronchiolitis and six SNPs (-1722delT/ -251A/ +396G/ +781T/ +1633T/ +2767T) (125). Furthermore these haplotypes were found to exist in almost perfect linkage disequilibrium. The SNPs described are found within the interleukin-8 promoter region and are titled according to the number of bases upstream from the start codon. Further work by this group suggests that the mechanism for disease susceptibility to RSV-induced bronchiolitis may be through a haplotype-specific increase in interleukin-8 transcription involving significant differential binding at the +781 T/C polymorphism (126). This functional Interleukin-8 haplotype has yet to be studied in patients with acute pancreatitis.

2.2 Hypothesis

Polymorphisms within the interleukin-8 gene locus have been associated with several infective, inflammatory and neoplastic diseases in terms of disease severity and susceptibility. A known microsatellite in the promoter region of the has been associated with the development of MODS in acute pancreatitis.

Other interleukin-8 polymorphisms seem to be related to the functional expression of interleukin-8 and may in turn have an influence on the development of MODS in patients with acute pancreatitis.

2.3 Aims

1) To investigate six known single nucleotide polymorphisms in the interleukin-8 locus in a cohort of patients with acute pancreatitis and relate polymorphism frequencies to severity of pancreatitis.
2) To compare these six SNP's frequencies in patients with acute pancreatitis with SNP frequency in a healthy control population to examine susceptibility.

2.4 Investigation of six Interleukin-8 polymorphisms in patients with acute pancreatitis

2.4.1 Introduction

Polymorphisms within the interleukin-8 locus have been associated with many diseases with an inflammatory component.

Several SNPs have been described in the IL-8 promoter region but have not been assessed in patients with acute pancreatitis. Investigation of six interleukin-8 single nucleotide polymorphisms in patients with acute pancreatitis may provide further insight into the mechanisms by which interleukin-8 functions. A greater understanding of the influence of genetic factors on cytokine function and outcome in acute pancreatitis may be reached. The interleukin-8 locus is represented showing the relative SNP positions within the locus (Figure 2.1).

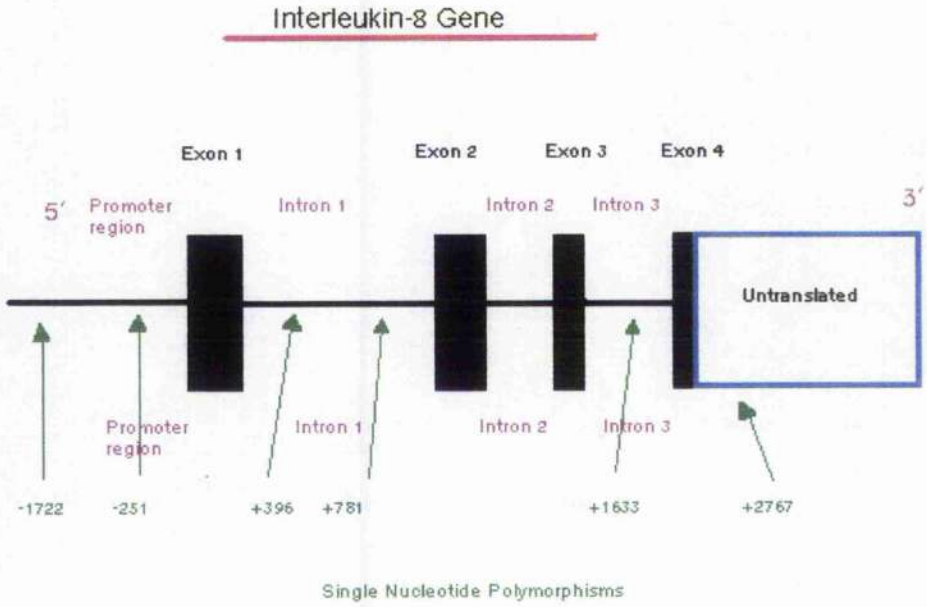


Figure 2.1 Interleukin 8 Gene Locus

2.4.2 Methods

A: Materials and Methods

Polymerase Chain Reaction (PCR)

This technique allows a specific sequence of DNA to be amplified. It was first described in 1974 (142). The basic principle of PCR involves two segments of short-stranded DNA (primers), which are complementary to opposing strands of the DNA segment of interest. Heating the reaction mixture allows denaturation of the template DNA as well as binding primers to their respective template DNA sequences (annealing), followed by the synthesis of a complementary strand (extension) in the 5' to 3' direction. One cycle includes one round of denaturation, annealing and extension and theoretically doubles the amount of template DNA. Several cycles of this process occur successively until an "amplification plateau" is reached after reaction reagents are depleted. The reaction temperature is extremely important and each step has an optimum temperature. This can vary for each stage of the PCR process (143). PCR is often performed in a thermal cycler which can automatically change the reaction temperature for specific lengths of time as well as the optimum number of cycles within each step. After 20 cycles, over a million copies of the template DNA are synthesised.

DNA Extraction

DNA can be extracted from a number of sources including peripheral blood, fresh tissue, hair, nail as well as archived material. Samples are incubated with Proteinase K for improved DNA quality. This is inactivated at the end of extraction by heat treatment. The DNA is purified using phenol chloroform in a number of extraction steps. The purified DNA is then suspended in a storage medium, which can either be a buffer or sterile distilled water.

Optimisation of Reaction Conditions

Before carrying out PCR analysis, it is advisable to assess the optimal conditions for each reaction. Primers should range from 15-30 bases in length and consist of 50-60% guanine and cytosine bases. Primer melting temperatures should be between 55-58 °C preferably. Annealing temperatures depend on the length of the primers as well as their composition and tend to be between 1-5°C lower than the lowest primer melting temperatures. If the annealing temperature is too low, then non-specific annealing and amplification can occur. If it is too high then PCR products are reduced.

Setting up PCR

The reaction mixture generally contains dNTPs (building blocks for DNA synthesis), test primers, control primers, Taq polymerase (catalytic DNA polymerase), as well as magnesium (stabilises the other components) and water (de-ionised) to make up the volume to either 50 or 100µl. The reagents are placed in 0.5ml tubes and placed in a thermal cycler programmed with the appropriate temperatures as well as the required number of cycles. The initial step involves an extended denaturation period to allow template DNA strand separation. At the end of the reaction there is a final extension step at 72 °C degrees to ensure that all template DNA is double –stranded.

Validation of DNA

Included within the PCR reaction is another set of primers (controls) which amplify a known sequence within the genome. This ensures that the PCR reaction process has been successful and negative test results can be counted as valid and not as a result of poor quality DNA.

Detection of PCR Products

The PCR products can then be viewed by gel electrophoresis. This process separates out differently sized DNA fragments. Gel electrophoresis is performed using an agarose gel (1-2%) usually containing Ethidium Bromide.

To determine polymorphic genotypes, 2 reactions are set up for each DNA sample, containing a different upstream primer. If the alleles are present then this will be viewed on the gel, if not, absent bands denote absence of that particular allele, when the SNP will be homozygotic

B: Patients and Methods

Patients with a diagnosis of acute pancreatitis were recruited on the basis of clinical presentation and hyperamylasaemia. In order to obtain a cohort with a higher risk of organ dysfunction, APACHE II scores were calculated on admission. Those with a score of 6 or more were included. Organ dysfunction was recorded daily as well as outcome. Patients were divided into two groups depending on severity of the illness. Those in the severe group had persisting MODS with a Marshall score of 2 or over in one or more organ/system for 48 hours or more. All other patients were categorised as having mild acute pancreatitis. Peripheral venous blood was obtained from patients at the time of initial recruitment and refrigerated at 4°C. Ethical committee approval was obtained. DNA from 100 healthy donors from the regional tissue-typing laboratory was obtained.

Patient DNA

DNA was extracted from peripheral venous blood samples taken from patients at the time of initial recruitment to the study

DNA samples were subjected to optical density analysis using a spectrophotometer in order to make up DNA samples to a standard concentration of 50ng/μl.

Control DNA

DNA taken from 100 West of Scotland healthy controls was used. These were selected randomly from a bank of samples maintained by the tissue-typing laboratory at Glasgow Royal Infirmary. DNA samples involved extraction from peripheral blood. The samples were from the same Caucasian population as the patients with acute pancreatitis. The samples were subjected to spectrophotometry to analyse optical density in order to confirm a DNA concentration of 50ng/μl. These DNA samples were novel to this work and had not been used in previous acute pancreatitis studies.

C: PCR reactions

Primers

The oligonucleotide primers for the interleukin-8 single nucleotide polymorphisms were described by Hull. Six of the polymorphisms described were analysed; -1722, -251, 396, 781, 1633, 2767 (Figure 1). Primers for these polymorphisms and control primers were synthesised by Holle & Hunter, UK (Figure 2.2).

Figure 2.2: SNP segments and control primers.

Primer Name	Primer Sequence
-1722 del T	5' GTA AAA TAC AGT GAT GAG TGT TAC GAT AC 3'
-1722 ins T	5' GTA AAA TAC AGT GAT GAG TGT TAC AAC AA 3'
-1722 consensus	5' GTT GTG TCC ATA TGA GAA TGT GTC 3'
-251 A	5' CCA CAA TTT GGT GAA TTA TCA AT 3'
-251 T	5' CCA CAA TTT GGT GAA TTA TCA AA 3'
-251 consensus	5' TGC CCC TTC ACT CTG TTA AC 3'
396 G	5' TTT ACG TTA AAT ATA TGC ATG TTA CC 3'
396 T	5' TTT ACG TTA AAT ATA TGC ATG CTA CA 3'
396 consensus	5' AAC ATG ACT TCC AAG CTG GC 3'
781 C	5' TCA TAA CTG ACA ACA TTG AAC G 3'
781 T	5' AGT CAT AAC TGA CAA CAT TGA ACA 3'
781 consensus	5' TGA GTT GAG CAA GGT AAC TCA G 3'
1633 C	5' TAT GTA TGG TCT TTC TGG TCG TG 3'
1633 T	5' AAC TAT GTA TGG TCT TTC TGG TCG TA 3'
1633 consensus	5' GGA CTT AGA CTT TAT GCC TGA CTT AAG 3'
2767 A	5' CCC AGT TAA ATT TTC ATT TCA GAT AT 3'
2767 T	5' CCC AGT TAA ATT TTC ATT TCA GAT AA 3'
2767 consensus	5' GAC AAA CAC TTG ATT ACT TTG ACA ACA 3'
Control 63	5' TGC CAA GTG GAG CAC CCA A 3'
Control 64	5' GCA TCT TGC TCT GTG GAG AT 3'

Optimisation of Sequence-specific SNP PCR Reactions.

Each SNP PCR reaction had to be optimised under different conditions including reagent concentration and annealing temperatures. Unless otherwise stated, 25 annealing cycles were usually performed.

The table below shows the different conditions that were explored for each SNP reaction (Table 2.1).

Table 2.1: Optimisation of SNP reactions

$MgCl_2$ concentration (mM)	Temp 56°C	Temp 58°C	Temp 59°C	Temp 60°C	Temp 61°C	Temp 64°C
1						
2.5			-251weak	+781	1633C -251 -1722	
5	1633 T	+396weak		+396		
7.5						
10				+2767A	+2767T	

Allele-specific PCR analysis

Each Interleukin-8 SNP genotype was determined in all patients and controls using the sequence specific Bioline protocol (Table 2.2).

Each downstream sequence specific primer (e.g. –251A and –251T) binds to their respective SNP bases along with the consensus upstream primer in both reactions.

In order to have a positive control in each genotyping reaction, 2 other primers were designed which amplified a consensus region of the HLA-DRB1 gene.
All six SNP experiments were carried out following a similar protocol (Table 2.2) based on the standard Bioline sequence-specific protocol, however some reagent concentrations and reaction temperatures varied depending on the optimal conditions needed for each reaction.

Table 2.2 Reagents and volumes (standard Bioline PCR Protocol)

Reagent	Source	Volume for one reaction
$MgCl_2$ (2.5mM)	Abgene, Surrey, UK	5µl
Reaction Buffer IV x1	Abgene, Surrey, UK	5µl
dNTPs (200uM)	Abgene, Surrey, UK	5µl
Specific Primer 1 or 2 (200uM)	Holle & Hunter, UK	1µl
Consensus Primer (200uM)	Holle & Hunter, UK	1µl
Control Primer 63 (200uM)	Holle & Hunter, UK	1µl
Control Primer 64 (200uM)	Holle & Hunter, UK	1µl
Taq Enzyme	Abgene, Surrey, UK	0.25µl
Sterile H2O		28.75µl
Total Volume		50µl

Sequence Specific PCR

Initially 4μl was taken from each sample of DNA and 2μl placed into 2 separate 1.5ml tubes.

One drop of paraffin and 48ul of master mix was added to each of the two tubes. The master mix was made up using a standard (Bioline PCR) protocol using the following; $MgCl_2$ (2.5mM, Abgene), Buffer (Reaction Buffer IV x1, Abgene), DNTPs (200μM, (Abgene), specific primer 1/2(200μM), consensus primer (20μ0M), 63 control primer (200μM), 64 control primer (200μM), Taq enzyme (Bioline) (1.25) μ and 28.75lμof sterile water to make a total volume of μ48l. When added to the DNA in the reaction tubes, the total volume was 50μl (Table 2.2). Further specific controls using the three possible genotypes were included with each experiment. Ionised DNA free H2O was used as a negative control. The samples were placed in a thermal cycler (Peltier PTC-225, MJ Research) and subjected to the following PCR reaction temperatures (Table 2.3); 4 cycles of 96, 35 secs; Temp (A), 45 secs; 72, 35 secs; 25 cycles of 96 (except for 781 SNP reaction), 25 secs; Temp (B), 50 secs; 72, 40 secs; 8 cycles of 96, 35 secs; Temp (C), 60 secs; 72, 90 secs with a holding temperature of 10C following this.

Table 2.3: Reaction conditions for each sequence-specific PCR.

PCR	$MgCl_2$ (mM)	temp (A)	No. cycles	temp (B)	No. cycles	temp (°C)	No. cycles
-251	2.5	68	4	61	25	58	8
396	5	67	4	60	25	58	8
781 C	2.5	70	4	61	30	58	8
781 T	6	70	4	56	38	NA	NA
1633	5	67	4	60	25	58	8
-1722	5	68	4	61	25	58	8
2767 A	10	67	4	60	25	58	8
2767 T	10	70	4	64	25	58	8

Viewing PCR

The resulting PCR products were viewed using gel electrophoresis. A 2% agarose gel was made up using 2g of Agarose (Bioline), 100mls of TAE buffer x1 and 8µl of Ethidium Bromide and allowed to set for 30 minutes. One litre of Buffer solution was made up using 1 litre of TAE x1 and 80µl of Ethidium Bromide. Wells were loaded with 10µl of PCR products from each tube (2 per DNA sample) and 5µl of Orange G (company, made up) and run for 45 minutes at 100Volts. A 100base pair ladder (Hyperladder IV, Bioline) was placed in the last well of each row to size the resulting fragments approximately. The gel was then photographed (Sony Videographic printer, Genetic Research Instrumentation) under UV light (Transilluminator UVP, Germany) to identify the genotype bands (Figure 2.3) Each patient's genotype was then entered into a database (Microsoft Excel) for later analysis.

Unsuccessful samples

DNA samples that did not have genotype bands or had absent bands were subjected to 2 further PCR reactions in an attempt to try and characterise the DNA. DNA samples that remained unsuccessful were disregarded from the data collection and subsequent analysis.

Statistical Analysis

Patient genotype frequencies were compared with healthy controls using chi-square testing. Patients with mild and severe disease were also compared using chi-square. The two least prevalent genotypes were grouped together as dominant groups and were analysed using Fishers exact test.

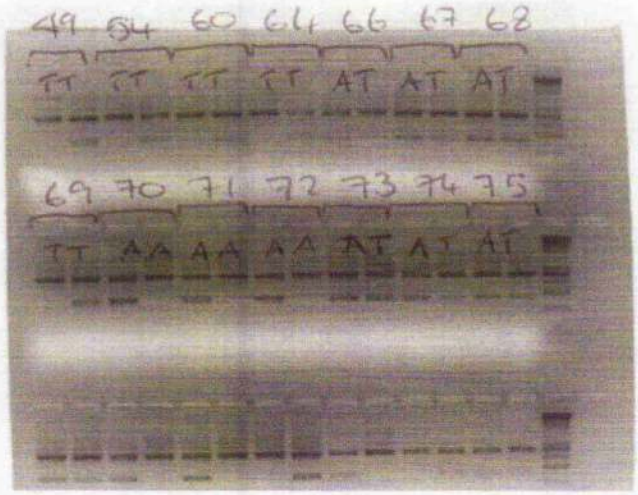


Figure 2.3 Gel Electrophoresis of –251 PCR

2.5 Results

106 patient samples were analysed, 2 of these had no successful genotypic bands with any of the six SNP assays.

Demographics

Table 2.4: Demographics of acute pancreatitis patients

Patients	Gender	Age median (SD)	APACHE II median (SD)	Mortality (n=)
Mild	45M/ 41F	60 (SD 15)	9 (SD 4.4)	2
Severe	12M/ 8F	65 (SD 17.9)	13.5 (SD 5.1)	13
All patients	57M/ 49F	60 (SD 15.6)	10 (SD 4.9)	15

Aetiology

Table 2.5: Aetiology in Acute Pancreatitis patients

Cause	Mild n (%)	Severe n (%)	Total n (%)
Gallstones	45 (52)	12 (60)	57 (53.7)
Alcohol	25 (29)	2 (10)	27 (25.4)
ERCP	4 (4.6)	0	4 (3.7)
Other	12 (14)	6 (30)	18 (16.9)

Although there is an observed preponderence of gallstones as the aetiology this approaches significance but does not reach it (p=0.1). If there were significantly more severe patients with gallstones as the aetiology, they would have to be investigated separately to avoid confounding factor bias.

Unsuccessful samples

There was a significantly greater number of unsuccessful PCR reactions in the patient DNA samples compared with the control samples (29% v 8%)(Table 2.6)

Table 2.6: Unsuccessful PCR samples

SNP	Controls	Mild	Severe	Total
-251	7	13	6	26
396	11	31	6	48
781	10	34	6	50
1633	4	35	5	44
1722	5	6	4	15
2767	9	29	7	54
Total	46	148	34	228

Mild and Severe

106 patients were analysed. Of these 20 patients had severe acute pancreatitis and 86 had mild disease. The 2 DNA samples with no successful results were in the severe group. Of the remaining 18 severe patients, some individual SNP PCR assays were unsuccessful. Each patient's results are shown with severe patients in **bold** type (Tables 2.7 & 2.8)

Patients and Controls

All six Interleukin-8 polymorphism assays were carried out on 106 patients and 100 controls. The individual results are shown (Tables 2.9 & 2.10)

Table 2.7: Interleukin-8 SNP genotypes in Acute Pancreatitis patients

Patient Sample	-1722	-251	396	781	1633	2767
3		TT				TT
4	No T					TT
5				CT	CC	AT
6	No T	AA		TT	TT	AA
8	No T	AA		TT		AA
9	No T	TT	TT			
12	No T	AT	GT			
13		TT		CT	CC	AT
14	No T	AT	GT		CT	AT
15	No T				CT	AT
17	No T					TT
18	No T	AA		TT	TT	AA
19	No T			CT	TT	AT
20	No T			CT	CT	AT
21	No T			CT	CT	AT
22	No T					
23	No T	AT				
24	No T	AA		TT	TT	AA
25	No T	TT		CT	CC	
27	No T	AA		TT	TT	
28	No T	AT		TT		TT
29	No T	AT		CT		AT
31	No T	AA	GG			
32	No T	AT	GT			
33	No T	AA	GG			
34	No T	AA				
35	No T	TT				
36	**No T**	**TT**	**TT**	**CT**	**CC**	
39	**No T**	**TT**		**CT**	**CC**	**AT**
40	No T	AT	GT	CT	CT	
42	No T	AA	GG	TT	TT	AT

43	No T	TT	GT	TT		
45	No T	TT		CT	CC	AT
46	No T					
47	No T	AA				
48	No T	TT	TT			TT
49	No T	AT	GT	CT	CT	AT
50	No T	AT	GT	CT	CT	AT
51	No T	AT	GT	TT	TT	AA
52	No T	AT	GT	CT	CT	AT
53	No T	TT	TT	CT	CC	AT
54	No T	AA			CT	AT
55	No T	AT	GT		TT	
56	No T	AA			TT	AA
57	No T	AT				
58	No T	AT	GT	CT	CT	AT
59	No T	AA	GG	TT	TT	AA
60	No T	TT	TT	CC	CC	AT
61	No T	TT			CC	AT
62	No T	TT				
63	No T	TT	TT	CT	CC	AT
64	No T	AT	GT	TT	CT	AT
65	No T	AT	GT	TT	CT	AT
66	No T	TT	TT	CT	CC	AT
67	No T	AT	GT	CT	CT	AT
68	No T	AA	GG	TT	TT	AA
69	No T	TT	TT	CT	CC	AT
70	No T	AT	GT		CT	AT
71	No T	AT	GT	TT	CT	AT
72	No T	AT	GT			
73	No T	TT	TT			
74	No T	TT	TT			
75	No T	TT	TT			
76	No T	AT	GT	TT		

77	No T	AA	GG	TT	CT	AT
78	No T	AT	GT	CT	CC	AT
79	No T	TT	TT	CT	CT	AT
80	No T	AT	GT	CT	CT	
81	No T	TT	TT	CT	CC	AT
82	No T	AT	GT	CT	CT	AT
83	**No T**	**AT**	**GT**	**CT**	**CT**	**AT**
84	**No T**	**AT**	**GT**	**TT**	**CT**	**AT**
85		**AT**				
86	**No T**			**TT**		**AA**
89	No T	TT	TT	CT	CC	AT
90	No T	AT	GT	CT	CT	AT
91	No T	TT	TT	CT	CC	AT
92	No T	AT	GT	CT	CT	AT
93	No T	AT	GT	TT	CT	AT
102	No T	AT	GT	CT	CT	AT
103						
104	No T	TT	TT			AT
105		AA	GG	TT		AA
106	No T					
107	No T		GG	TT		AA
108						
109	No T		TT			
114	No T	AA	GG	TT		AA
115	No T	AA	GG	CT	CT	AT
116	No T	AA	GG			
117	No T	AT	GT	TT	CT	AT
118			TT			
119	**No T**		**GG**	**TT**	**CC**	
120	**No T**	**AA**		**TT**	**TT**	**AA**
123	No T	AT	GT	CT	CT	AT
124	**No T**	**AT**	**GT**	**CT**	**CT**	**AT**
125	**No T**	**AT**	**GT**	**TT**		**AA**

127	**No T**		**GG**	**TT**	**TT**	**AA**
128	**No T**	**AA**	**GG**	**CT**	**CT**	**AT**
129	**No T**	**TT**	**GT**	**CT**	**CC**	**AT**
131	**No T**		**GT**	**CT**	**CT**	
132	**No T**	**TT**	**TT**	**CT**	**CC**	**AT**
135	**No T**	**AT**	**GG**		**CT**	**AA**
136		AT				
137	**No T**	**AT**	**GT**		**CT**	**AA**
138		**AT**	**GT**		**TT**	

Patients with persistent organ dysfunction are printed in **bold** type (severe)

Two patients were excluded from analysis (103 & 108) as no successful results were obtained.

Table 2.8:Interleukin-8 polymorphism frequencies in patients

SNP	Genotype	No OD	OD	Total patients	P value	Mortality
-1722	Delete T	80	16	96		12
	Insert T	0	0	0	0.1	NS
-251	AA	19	2	21		1
	AT	31	8	39		6
	TT	23	4	27	0.3	4
	AA+AT/TT	50/23	10/4	60/27	0.8	0.5
+396	GG	11	4	15		3
	GT	27	8	35		7
	TT	17	2	19	0.6	3
	GG+GT/TT	38/17	12/2	50/19	0.2	0.9
+781	CC	1	0	1		0
	CT	30	8	38		8
	TT	21	6	27	0.8	2
	CC+CT/TT	31/21	8/6	39/27	0.9	0.3
+1633	CC	14	5	19		4
	CT	26	7	33		6
	TT	11	3	14	0.2	2
	CT+TT/CC	37/14	10/5	47/19	0.65	0.9
+2767	AA	11	6	17		4
	AT	41	7	48		6
	TT	5	0	5	0.2	0
	AT+AA/TT	52/5	13/0	65/5	0.2	0.3

Table 2.9: Interleukin-8 SNP genotypes in controls

Controls	1722	251	396	781	1633	2767
1	NO T	AA	GG	CC	TT	
2	NO T	AT	GT	CT	CT	AT
3	NO T	AT	GT	CT	CT	AT
4	NO T	TT	GT	CT	CC	AT
5	NO T	AA	GG	CT	TT	AA
6	NO T	AT	GT	CT	CC	TT
7	NO T	TT	GT	CC	CT	AT
8	NO T	AT	GT	CT	CT	AT
9	NO T	AT	GT	CT	CT	TT
10	NO T	AT	GT	CT	CT	TT
11	NO T	AA	GG	CT	TT	AT
12	NO T		GT	CC	CT	AT
13	NO T	AT	GT	CT	CT	AT
14	NO T	AT	GT	CC		TT
15	NO T	TT	GT	CC	CT	TT
16						
17		AA	GT			
18	NO T	AA	GT	CC	CT	TT
19	NO T	TT	TT		CC	TT
20	NO T	TT	TT	CT	CC	AT
21	NO T	AT	GT	CC	CT	AT
22	NO T	AA	GT		CT	AT
23	NO T	AA	GG	CT	TT	
24	NO T	AT	GT		CT	TT
25	NO T	TT	GT	CC	CT	TT
26	NO T	AA	GT	CT	CT	AT
27	NO T	TT	TT	CT	CC	TT
28	NO T		TT	CT	CC	TT
29	NO T	TT	GT	CT	CC	TT
30	NO T	AA	GG	CC	TT	AT

31	NO T	AA	GT	CT	CT	TT
32	NO T	AA	GG	CT	CT	AA
33					CC	TT
34	NO T	AT	GT	CT	CT	TT
35	NO T	AA	GT	CT	CC	AA
36	NO T	AA	GG	CC	CC	TT
37	NO T	TT	TT	CC	CC	TT
38					TT	
39	NO T	TT	TT	CC	CT	AT
40	NO T	AT	GT	CC	CT	TT
41	NO T	AA	GG	TT	TT	AA
42	NO T	AT	GT		CT	TT
43	NO T	TT	GT	CT	CC	TT
44	NO T	AT	GT	CT	CT	TT
45	NO T	AT	GT	CC	CT	AT
46	NO T	AT	GT	CT	CT	AT
47	NO T		GG	CC	CT	TT
48	NO T	AA	GT	CT	TT	
49	NO T	AA	GT		TT	
50		AA	GT		TT	
51	NO T	TT	GT	CC	CC	TT
52	NO T	TT	TT	CT	CC	TT
53	NO T	AT	GT	CT	CT	TT
54	NO T	AT	TT	CC	CT	AT
55	NO T		GT	CC		TT
56	NO T	TT	GG	CT	CC	TT
57	NO T	AA	GG	TT	TT	AA
58	NO T	AA	GT	TT	TT	AA
59	NO T	TT	GG	CT	CC	AT
60	NO T	AT	GG	CT	CT	AT
61	NO T	AT	GT	CT	CT	AT
62	NO T	TT	TT	CT	CC	AT

63	NO T	AT	GT	CT	CT	AT
64	NO T	AT	GT	CT	CT	TT
65	NO T	TT	TT	CC	CC	AT
66	NO T	AA	GG	TT	TT	AA
67	NO T	AA	GG	CT	CT	AT
68	NO T	AT	GT	CT	CT	AT
69	NO T	TT	TT	TT	CC	AT
70	NO T	AA	GG	TT	TT	TT
71	NO T	AT	GT	CC	CT	AT
72	NO T	TT	TT	CC	CC	TT
73	NO T	AT	GT	CC	CT	TT
74	NO T	AT	GT	CT	CT	TT
75	NO T	AT	GT	CC	CC	AT
76	NO T	TT	TT	CC	CC	AT
77	NO T	AT	GT	CT	CT	TT
78	NO T	AT	GT	CC	CT	TT
79	NO T	AT	GT	CT	CT	TT
80	NO T	AA	GG	TT	CC	AA
81	NO T	TT	TT	CT	CT	TT
82	NO T	TT	TT	CT	CT	TT
83	NO T	AT	GT	CC	CC	AT
84	NO T	AT	GT	CT	CT	AT
85	NO T	TT	TT	CT	CC	AT
86	NO T	TT	TT	CT	CC	TT
87	NO T	AT	TT	CT	CC	TT
88	NO T	TT	GG	CT	CT	AT
89	NO T	TT	TT	CT	CC	TT
90	NO T	TT		CT	CC	AT
91	NO T	AT		CT	CC	AT
92	NO T	TT		CT	CT	TT
93	NO T	TT		CC	CC	AT
94	NO T	TT		CC	CC	TT

95	NO T	AT		CT	CT	TT
96	NO T	TT	TT	CT	CC	AT
97	NO T	AT	GT	CC	CT	TT
98	NO T	TT	TT	CC	CC	TT
99	NO T	AT		TT	TT	AA
100	NO T	TT		CC	CC	

Table 2.10 Interleukin-8 polymorphism frequencies; patients & controls

SNP	Genotype	Controls	Patients	Total number	P value
-1722	Delete T	95	96	191	
	Insert T	0	0	0	0.1
-251	AA	23	21	42	
	AT	37	39	74	
	TT	33	27	59	0.3
	AA+AT/TT	60/33	60/27	120/60	0.5
+396	GG	18	15	31	
	GT	50	35	87	
	TT	21	19	40	0.78
	GG+GT/TT	68/21	50/19	118/40	0.57
+781	CC	31	1	31	
	CT	51	38	85	
	TT	8	27	35	0.8
	CT+TT/CC	59/31	65/1	124/32	<0.0001
+1633	CC	35	19	53	
	CT	46	33	77	
	TT	15	14	27	0.2
	CT+TT/CC	61/35	47/19	108/54	0.3
+2767	AA	9	17	25	
	AT	37	48	82	
	TT	45	5	50	0.2
	AA+AT/TT	46/45	65/5	107/50	<0.0001

Severity of acute pancreatitis

The six SNP's (-1722, -251, +396, +781, +1633, +2767) did not exhibit differences in allelic frequency when comparing mild with severe patients. The –1722 polymorphism (deleteT/insert T) exhibited "delete T" in all patients.

No differences in allelic frequency were noted in survivors and non-survivors. There were 15 deaths in all but not all of these had complete results for each polymorphism.

Susceptibility to Acute Pancreatitis.

No differences were noted in allelic frequencies in any of the six SNPs studied in the IL-8 promoter region locus. When comparing genotypic dominant groups, 2 alleles at the +781C and +2767T loci were more frequent in controls ($p<0.001$). Significant results such as these can be seen when multiple statistical analyses are used but in isolation are not indicative of any association between the polymorphism and the disease.

2.6 Discussion

Interleukin-8 polymorphisms have been found to correlate significantly with clinical severity in diseases such as RSV bronchiolitis (125).

Interleukin-8 is a cytokine that is now known to be involved in the pathogenesis of acute pancreatitis but little is known about the genetic influences on Interleukin-8 production. This work attempted to characterise the allelic frequencies of six interleukin-8 single-nucleotide polymorphisms in a population of patients with severe and mild acute pancreatitis to explore the relationship between the genetic control of Interleukin-8 production and disease severity. No significant difference in allelic frequencies were noted between mild and severe patients or between patients and controls. This indicates that there is unlikely to be an association between these interleukin-8 polymorphisms and acute pancreatitis. Although a significant difference was noted on dominant genotype grouping, this analysis is performed to increase statistical power and in isolation is of no clinical relevance. It may be that there is no relationship between these six IL-8 polymorphisms and disease severity in pancreatitis, despite an association noted between the IL-8 microsatellite and organ dysfunction previously. It could be argued that the sample size that was used was small and perhaps more results could have been obtained if a larger group was studied. It should however be noted that not all patients with acute pancreatitis as the diagnosis on admission were eligible for recruitment into the study, only those with an APACHE II score of 6 or greater were approached. It is also important to remember that DNA quality diminishes with time and that was also a factor in determining the length of the recruitment period, which in this case was approximately 2 years.

However the complete absence of a trend in any of the six polymorphisms suggests that this sample was adequate at excluding a significant relationship. Another possibility is that other, as yet unidentified polymorphisms are responsible for differences in IL-8 gene expression and production. Until these are discovered, the role of IL-8 and other cytokines in the development of acute pancreatitis will not be fully understood.

Chapter 3

Factors influencing mortality in pancreatic necrosis

3.1 Introduction

3.1.1 Severe Acute Pancreatitis

Severe Acute Pancreatitis has been previously been defined as "associated with organ failure and/ or local complications such as necrosis, abscess or pseudocyst (144). It has been shown that various subgroups of patients exist within the umbrella heading of Severe Acute Pancreatitis and that outcomes for these subgroups can vary significantly. The qualitative difference between the presence of MODS and the characteristics of MODS (early resolving v persistent/deteriorating) with respect to mortality has already been discussed (23). Similarly, patients who develop local complications are not all alike with respect to morbidity and mortality. The mortality rate is up to twice as much for infected pancreatic necrosis compared with pancreatic abscess and three times as much compared with pseudocyst formation (145,146). The main determinant of mortality in patients with severe AP as defined above is the degree of organ dysfunction (66).

3.1.2 Management of MODS in Severe Acute Pancreatitis

Recent UK guidelines on the management of patients with severe AP suggest that the patient should be closely monitored, with maximal supportive therapy, including intravenous (IV) fluids, supplementary inspired oxygen and analgesia (7). The vast majority of patients with MODS have respiratory failure either alone or in combination with other organ failure. In one study, 90% of patients with AP who died from MODS did so as a result of respiratory failure (65). Consequently, most AP patients with MODS require ventilation and are managed in an intensive care

setting where renal replacement therapy and inotropic support can also be given if required (147).

3.1.3 Sepsis and Severe Acute Pancreatitis

The development of MODS in the early course of AP has already been discussed. Those who develop MODS in the second and third weeks do so predominately in association with infected pancreatic necrosis. (49,148) Infection of pancreatic necrosis tends to develop 2-3 weeks after symptoms start in approximately 40-70% of patients who develop necrosis(149). The risk of infection increases with the extent of necrosis. The involved organisms tend to be mostly gram-negative with an enteric origin (149,150) suggesting the gastro-intestinal tract as the source of infection. This is the basis of the hypothesis of failure of gut function as a barrier to infection(30). It has been shown that intestinal permeability is increased in patients with severe AP (151) and this, amongst other factors, can allow bacterial translocation (152). Other possible routes of infection have been suggested including haematogenous, lymphatic or via the pancreatic and common bile duct (30). Enteral nutrition has been shown to reduce bacterial translocation to mesenteric lymph nodes in an animal model of AP. Clinically enteral feeding has beneficial effects in patients with AP by reducing septic complications when compared with total parenteral nutrition (37).

3.1.4 Development of Pancreatic Necrosis

Pancreatic necrosis was defined at the 1992 Atlanta Consensus Conference as "diffuse or focal area(s) of non-viable pancreatic parenchyma which is typically associated with peri-pancreatic fat necrosis (58). It is important to distinguish this

from pancreatic abscess which may have some necrotic tissue within it (Figure 3.1). Previously, ambiguous terminology relating to inflammatory complications of AP has been used interchangeably, making it difficult to compare different reports in the literature.

> A pancreatic abscess is a circumscribed intra-abdominal collection of pus, usually in proximity to the pancreas, containing little or no pancreatic necrosis, which arises as a consequence of acute pancreatitis.

Figure 3.1: Atlanta Definition of Pancreatic Abscess (58)

Pancreatic abscess is associated with a much lower mortality rate than pancreatic necrosis (153) and tends to occur at a later point in the disease (146). Of those who develop necrosis, up to 70% are likely to become infected. The risk of infection increases with the extent of necrosis and duration of the disease (149). The management of sterile pancreatic necrosis has been controversial. Previously, patients with sterile necrosis were considered to be at risk of subsequent infection and of organ failure, therefore, surgical debridement was performed with the rational that by removing necrotic tissue, these risks are reduced. However, this approach can introduce infection into previously sterile necrosis and this has accounted for up to 30% of the total number of patients with IPN in the past. Over the last five to ten years, consensus opinion has shifted. More stringent indications for surgery in patients with sterile necrosis to include only those with severe AP unresponsive to maximal supportive therapy were suggested (154). However, the most recent guidelines suggest that surgical intervention should be reserved for patents with infected necrosis (7,39).

3.1.5 Diagnosis of Infected Pancreatic Necrosis

A high clinical suspicion of pancreatic necrosis is necessary to allow early diagnosis. Delayed diagnosis is associated with a worse outcome(155). Clinically, patients complain of worsening abdominal or back pain, and may develop signs of SIRS and/ or MODS as well as an elevated CRP. They may have an abdominal mass on examination. The best imaging technique for identifying pancreatic necrosis is CT scanning, with intravenous contrast enhancement (27). This can reveal the extent and site of necrosis as well as indicating extra-pancreatic disease. This modality however, cannot always identify infection, though air indicative of gas-forming organisms can be seen in 20-55% of patients with necrosis (47,51). Differentiation between sterile and infected pancreatic necrosis is crucial in planning further management. This can be achieved with Fine Needle Aspiration (FNA) and culture or gram staining of necrotic material. Radiologically (US or CT) guided FNA has been found to be a safe and reliable procedure with high rates (above 95%) of sensitivity and specificity in the diagnosis of IPN (156,157). Although this procedure has low complication rates, it should only be performed in patients with CT evidence of necrosis as well as systemic signs of sepsis (39) as the risk of introducing infection to sterile necrosis is not insignificant (150,157).

3.1.6 Mortality rates in patients with Infected Pancreatic Necrosis

Infected Pancreatic necrosis (IPN) has been recognised as one of the important clinical factors affecting both organ failure and mortality in acute pancreatitis. As discussed previously, it is known that the majority of patients who develop MODS after the first 2 weeks from the onset of symptoms do so in association with IPN. It was recognised over 15 years ago that patients who developed pancreatic necrosis

were more likely to die and have organ failure (158). The presence of necrosis in itself is a risk factor for MODS but this is less associated with mortality than the presence of MODS (150,159). It is now generally accepted that patients with infected rather than sterile pancreatic necrosis tend to have a worse outcome as they are more likely to have sepsis-related MODS (46). The mortality rate in patients who develop infected pancreatic necrosis varies widely between centres and studies (Table 3.1). This can range from 10% to as high as over 70%. Even in a relatively small area such as the UK, the mortality can vary from 14% in Southampton to 69% in Leeds, with Glasgow having a mortality rate of 28%.

Table 3.1 Mortality Rates in patients with PN

Study	Year	Patients n	Mortality (%)	Procedure
Allardyce(49)	1987	17	82	Open necrosectomy
Wilson (158)	1988	21	38	*Open necrosectomy/ pancreatic resection
Bassi (146)	1990	55	24	* Open necrosectomy & Lavage
Bradley(144)	1994	71	15	Open &- packing then open & post-op lavage
Rau (160)	1997	52	15	Open necrosectomy & Lavage
Farkas (161)	1996	142	6	Open necrosectomy & Lavage
Fernnadez-del Castillo (162)	1998	36	6	Open necrosectomy & Lavage
Freeny(163	1998	34	12	Percutaneous Catheter drainage
Buchler(28)	2000	29	24	Open necrosectomy & Lavage
Ashley (164)	2001	34	12	Necrosectomy / percutaneous drainage
Hungness (165)	2002	18	23	Open necrosectomy
Malangoni (66)	2004	13	15	Open necrosectomy & Lavage
Connor (166)	2005	88	28	*Minimally invasive & open pancreatic necrosectomy
Buchler (56)	2002	92	19	Open necrosectomy and lavage

*Includes patients without infected necrosis

It has been demonstrated that less aggressive techniques are associated with improved mortality rates. Previously, total pancreatectomy has been performed in cases of IPN with high mortality rates. The introduction by Beger of a post-operative closed lavage system in 1991 has contributed to reduced mortality rates(167).

3.1.7 Factors affecting mortality in IPN

Several risk factors have been found to affect mortality in patients with infected pancreatic necrosis (Table 3.2) and it likely that these factors operate to different extents in diverse patient populations.

Table 3.2 Factors affecting Mortality in patients with IPN

Risk Factor	Evidence	Potential Intervention
Presence of MODS	(66,168)	Limiting MODS by minimising "surgical hit"
Extent of Necrosis	(149,169	Limiting necrosis by debridement
Type of Infection	(28,170)	Avoidance of prophylactic antibiotics Treatment of Resistant organisms
Surgical Intervention	(169,171)	Minimally invasive Definitive debridement
Timing of Surgery	(162,165)	Delay until demarcation occurs 2 weeks after onset of AP. No benefit in delay beyond 4th week

Presence of MODS in patients with IPN

The presence of MODS in patients with an established diagnosis of IPN is associated with a negative outcome. Untreated, (i.e., no surgical intervention) the mortality rate may be as high as 100% in patients with MODS and IPN (172).

Type of Infection & Prophylactic Antibiotics

Recently, there have been increasing numbers of gram-positive organisms colonising necrotic pancreatic tissue. This is thought to be due to the widespread

use of prophylactic antibiotics. Randomised studies have shown conflicting evidence regarding prophylactic antibiotic usage and mortality (173,174). However, the incidence of antibiotic-resistant organisms has increased in recent years and is associated with higher mortality rates (175). Controversy remains on whether to use prophylactic antibiotics. This will be discussed further in the next chapter.

Referral patterns

It should be noted that due to various local and geographical factors, tertiary referral centres have wide variations in the referral practices of the surrounding district hospitals. This can also impact on outcome as it has been shown that late referral to a specialist unit is associated with a higher overall mortality (176).

3.1.8 Surgical Procedures for pancreatic necrosis

Laparotomy and Debridement

In 1984 Knol and his colleagues remarked that "*The ideal surgical treatment for necrotizing pancreatitis remains a matter of debate*" in their paper describing an open technique of pancreatic debridement in addition to "marsupialisation" (open packing) of the lesser sac, with repeated laparotomy at intervals of 2-5 days for further packing (177). Until then, the mainstay of surgical therapy for necrotizing pancreatitis had been laparotomy with debridement of devitalised pancreatic tissue and simple closed drainage of the pancreatic bed (158,178) though in some cases, partial or total pancreatic resection was performed which, in retrospect may have been a somewhat aggressive approach for patients without total pancreatic necrosis. Over twenty years later, the appropriate surgical management for necrotising pancreatitis remains a matter of debate but there have been several evolutions of

surgical treatment during this time. The technique of laparotomy, retroperitoneal exploration and simple drainage was widely adopted during the mid 1980's with slight variations but generally involving repeated second-look laparotomies and debridement of further necrosis with or without packing of the lesser sac. A new technique of open packing was developed (53) where the abdominal wound was not closed primarily and packs were left in the lesser sac. The wounds were allowed to heal by secondary intention. This was later modified by delayed closure of the wound whilst continuing with post-operative lavage(53). Continuous post-operative local lavage was widely adopted as an adjunct to initial open debridement allowing the removal of further debris and infected material via drainage tubes placed into the pancreatic bed and lesser sac during the initial (146,148). This removed the need for mandatory further laparotomy procedures in patients who were already critically ill and the associated increased risk of further morbidity and increased mortality. In a small prospective study of 24 patients, Nordback compared patients with necrosis post laparotomy with lesser sac lavage or simple drainage, no difference in outcome was noted. Septic complications were not improved with lavage (179).

The change to laparotomy plus continuous post-operative lavage rather than drainage was associated with a reduction in mortality rate and was adopted by many centres though no randomised trials have been performed looking at laparotomy with packing versus lavage. Several centres continued with open necrosectomy and closed packing with low mortality rates in patients with infected and sterile pancreatic necrosis (162). Overall, closed post-operative lavage is associated with lower mortality rates, fewer gastro-intestinal fistulae and bleeding complications when compared with open packing (180). The lavage is continued

for approximately 3-4 weeks until sepsis resolves. Open packing remains an option in patients with coagulopathy or difficult intra-operative bleeding.

Percutaneous & Endoscopic procedures

During the last decade, there has been a move towards developing less invasive procedures for managing pancreatic necrosis. Drainage of pancreatic abscess was described in 1981 using a percutaneous route (181) but this procedure was not suggested for pancreatic necrosis until more recently where it serves a useful role in temporising the situation until definitive surgery can be performed. The theoretical advantage of minimally invasive procedures, is a reduction in the inflammatory response associated with the trauma of surgery. It has been shown in several other conditions that laparoscopic procedures initiate a reduced systemic response compared to the equivalent open operation, even in elective patients (182). Baron in 1996 described a technique of endoscopic cyst-gastrostomy in combination with naso-cyst lavage to remove further debris with good results (183). However, this procedure was used in patients with pancreatic pseudocysts or abscesses and only 28% were infected at the time of intervention. Secondary infection however occurred in 38% of these patients, who required further intervention. They have since suggested that collections with solid debris of more than 1cm in size are not suitable for this technique(184). Following on from this, Freeny and colleagues, in 1998, described a series of patients with infected pancreatic necrosis who were primarily managed with percutaneous drainage using multiple catheters and lavage(163). This was achieved successfully with resolution of sepsis in 47% of patients. However the remaining 53% of patients required surgical intervention at some stage (26% as emergency procedures). The mortality

pancreatic necrosis who were primarily managed with percutaneous drainage using multiple catheters and lavage(163). This was achieved successfully with resolution of sepsis in 47% of patients. However the remaining 53% of patients required surgical intervention at some stage (26% as emergency procedures). The mortality rates in both of these studies are low but both studies were carried out in highly selected patients, usually with no significant organ dysfunction.

Further small series of patients undergoing minimally invasive procedures for IPN are described with reasonably good results in selected patients(185).

3.1.9 Pilot Study

In 2000, the Glasgow group described a percutaneous necrosectomy technique aimed at surgical debridement as well as simple drainage of necrotic abscess cavities (171). This method involves initial percutaneous, CT guided drainage of the area of necrosis and placement of a drain. The patient is then taken directly to theatre where the drain is changed over a guide-wire after administration of general anaesthesia. The area of necrosis is then visualised directly by using a modified rigid nephroscope after dilatation of the tract using a balloon dilator, allowing insertion of a 34French gauge Amplatz sheath. Lavage is performed until the return fluid is clear. The necrotic tissue is debrided piecemeal with continuous lavage. Retrieved material is sent for bacteriological culture. A drain (28F) is passed into the length of the cavity facilitating post-operative lavage.

This procedure can be repeated weekly until the devitalised pancreas is debrided adequately and sepsis settles. The patient is not subjected to open laparotomy routinely. Indications for proceeding to open laparotomy include haemorrhage, perforation of colon/ small bowel, mesenteric ischaemia and extensive peri-

pancreatic necrosis extending outwith the retroperitoneum into the paracolic and peri-nephric spaces.

3.2 Hypothesis and Aims

Hypothesis

Minimally invasive surgical debridement of infected pancreatic necrosis may reduce the incidence of MODS and the need for postoperative ICU thereby reducing mortality.

Aims

1) To compare and describe outcomes for patients undergoing percutaneous and open necrosectomy for infected pancreatic necrosis admitted to Glasgow Royal Infirmary over a 13 year period.

2) To examine other factors that may affect outcome in this population

3.3 Patients and Methods

Study Design.

This review was carried out retrospectively on patients admitted between Dec 1989 and March 2003 who underwent pancreatic necrosectomy for infected pancreatic necrosis. The data points collected for each patient are outlined below (Table 3.3).

Data collected
Gender
Age
Aetiology
Referring Hospital
Date of admission to hospital
Date of admission or transfer to GRI
Length of hospital stay
Index pancreatic necrosectomy procedure
Subsequent operative procedures
Surgical complications
Other invasive procedures
Intensive Care Unit stay
High Dependency Unit stay
Post-operative Complications
Outcome
Microbiology of pancreatic necrosis
Use of prophylactic antibiotics

Table 3.3 Data points for pancreatic necrosectomy patients

Patients were defined for the purposes of this study as having either open (OPEN) or percutaneous necrosectomy (PPN) depending on the index pancreatic necrosectomy procedure. In the event of conversion from percutaneous to open, the patients were allocated to the PPN group based on intention to treat.

Necrosectomy Procedures

Those in the OPEN group had an initial laparotomy and debridement of pancreatic necrosis. This was followed by either continuous postoperative lavage or open packing.

Patients in the PPN group underwent a percutaneous pancreatic necrosectomy as an index procedure. Any further percutaneous procedures were also recorded including sinus tract endoscopy.

MODS and ICU

Patients vary in their requirements for Intensive care (ICU). Physiological parameters were not available for all patients, precluding assessment of severity of MODS using a MODS scoring system. Admission to ICU in GRI is restricted to patients with respiratory failure requiring ventilatory support, with or without additional organ involvement. ICU admission was therefore used as a marker of significant MODS.

Microbiology

Microbiology sampling included CT-guided FNA of areas of pancreatic necrosis pre-operatively, pancreatic tissue or fluid taken during the index necrosectomy procedure. Data on antibiotic and anti-fungal usage was taken directly from patient's case-notes where available. Additional information was obtained from archived microbiology records.

Setting

The study took place in the Lister Department of Surgery at Glasgow Royal Infirmary in Glasgow. Patients studied were admitted to the general surgical wards,

the High Dependency Unit (HDU) or the Intensive Care Unit (ICU) within Glasgow Royal Infirmary depending on their clinical status. In some circumstances, due to limited bed availability in ICU, patients were transferred to other Intensive care units within the city.

Inclusion and Exclusion Criteria

All patients with pancreatic necrosis requiring necrosectomy were identified and their details recorded prospectively. Those with infected pancreatic tissue at the index necrosectomy procedure were included. Those with sterile necrosis were excluded for the purposes of this study.

Data Collection

Case records were requested for all patients included in the study. The medical records department assisted by permitting access to case notes.

Information from the case notes was recorded on a specifically designed database using Microsoft Excel. The database included a section for recording a range of clinical, biochemical and haematological parameters and calculating APACHE II and SIRS scores.

Additional information was obtained from other sources including microbiology results with the assistance of Dr Hood (Microbiology Lab). Microbiology results for patients admitted prior to March 1998 were stored in computerised files in the Information Technology department whose staff kindly assisted with accessing some of these results.

Additional information regarding ICU stay was obtained from the ICU secretary (Sheila Stewart).

Statistical Analysis

A computerised statistical programme (SPSS 9) was used to analyse the data. Mann-Whitney-U testing was used on non-parametric data. Two-sided Chi-square analysis was used on parametric values. Dr Wilson Angerson was consulted for advice on statistical methods.

A p value < 0.05 was considered significant.

Ethical Approval

This study was a retrospective audit of patients undergoing two differing approaches to pancreatic necrosectomy. All patient details were anonymised. Formal ethics approval was not required.

3.4 Pancreatic Necrosectomy Results

Between December 1989 and March 2003, 107 patients underwent pancreatic necrosectomy. Of these, 8 patients did not have evidence of infected necrotic tissue at the time of the initial procedure and were excluded from subsequent analysis. Patients studied had either open (OPEN) or percutaneous (PPN) pancreatic necrosectomy. Two patients were converted from percutaneous necrosectomy to open laparotomy and necrosectomy, during the index procedure for one patient and for a second, during their second percutaneous procedure. Both have been analysed within the PPN group based on pre-operative intention to treat. In total 46 patients in the PPN group and 53 in the OP group were included.

Patient Characteristics

The median age of all patients was 54 years (range 18-88 years) and did not differ significantly between both groups. The gender distribution was also similar (Table 3.4).

Patients managed in this specialist unit were frequently admitted initially to other hospitals in the West of Scotland or further and transferred to Glasgow Royal Infirmary (GRI) either after clinical deterioration or after a diagnosis of infected pancreatic necrosis was made.

Table 3.4: Patient Characteristics

	(OP)	(PPN)	Total	P value
Number of patients	53	46	99	
Median Age (years)	53 (39-67)	55 (39-71)	54 (39-69)	0.45
Sex Ratio (M:F)	36:17	33:13	70:30	0.72
Admitted to GRI	16	15	31	
Transferred from elsewhere	37	31	68	0.75

Sixty-nine percent of patients in this study were initially admitted to another hospital before transfer to GRI for definitive treatment. The median time to transfer was 16 days in all referred patients. The distribution of referred patients between both groups is similar.

Aetiology

The commonest cause was gallstones followed by alcohol. Iatrogenic and idiopathic causes were less common. There were 5 patients with AP secondary to ERCP in the open group with none having this aetiology in the PPN group, the only significant difference in the distribution of aetiology between the two groups (Table 3.5). Data regarding aetiology was unavailable for 4 patients.

Table 3.5 Aetiology of acute pancreatitis in patients with IPN

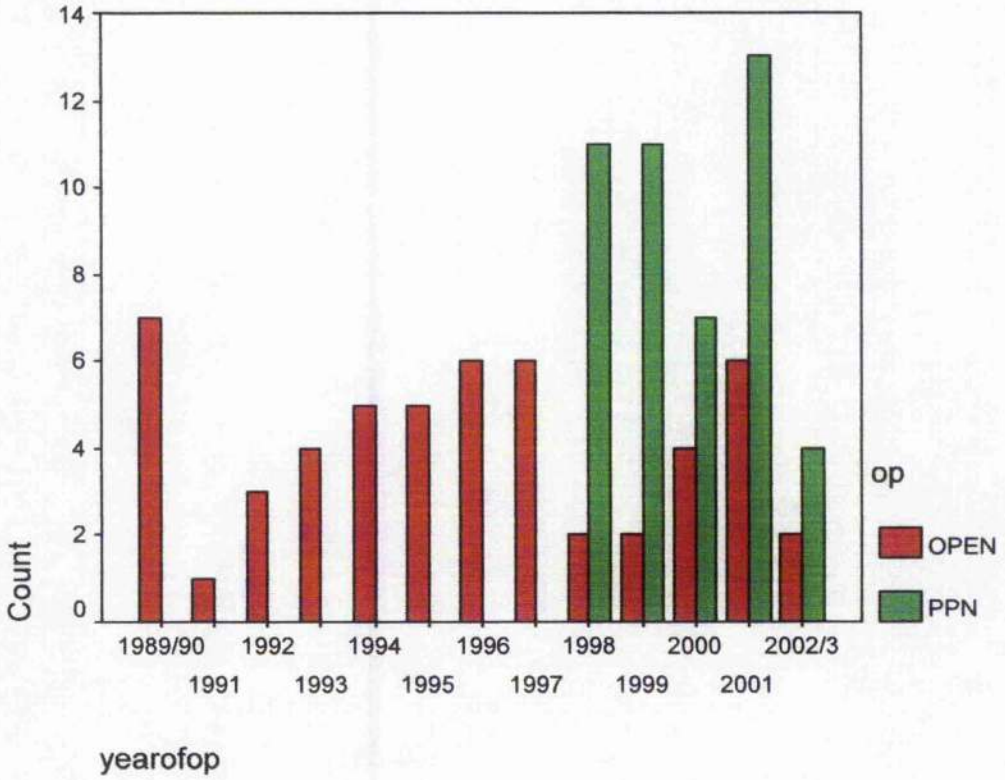
	(OP)	(PPN)	Total
Gallstones	33	30	63
Alcohol	10	9	19
Iatrogenic	5	0	5
Idiopathic/ unknown/ other	5	7	12

Procedures

Fifty-three patients underwent an initial open pancreatic necrosectomy. 46 patients had a percutaneous procedure (PPN). One of these patients was converted to an open laparotomy due to extensive retroperitoneal necrosis and mesenteric vessel bleeding. (This patient also required a splenectomy)

The number of patients requiring pancreatic necrosectomy increased over the study period and reached a peak of 19-patients/ year in 2001(Figure 3.4). The overall mean is 8-patients/ year over the total study period.

Figure 3.2: Procedures Dec1989- March 2003



Note: One patient in Dec 1989 and a patient in early 2003 were included with adjacent years.

Additional Procedures

In 38 patients, 30 in the OPEN and 8 in the PPN group, a single procedure was required. Thirty-eight PPN patients required further percutaneous debridement procedures and these were performed over the subsequent weeks (Figure 3.4). A median number of 3 PPN procedures were performed for each patient.

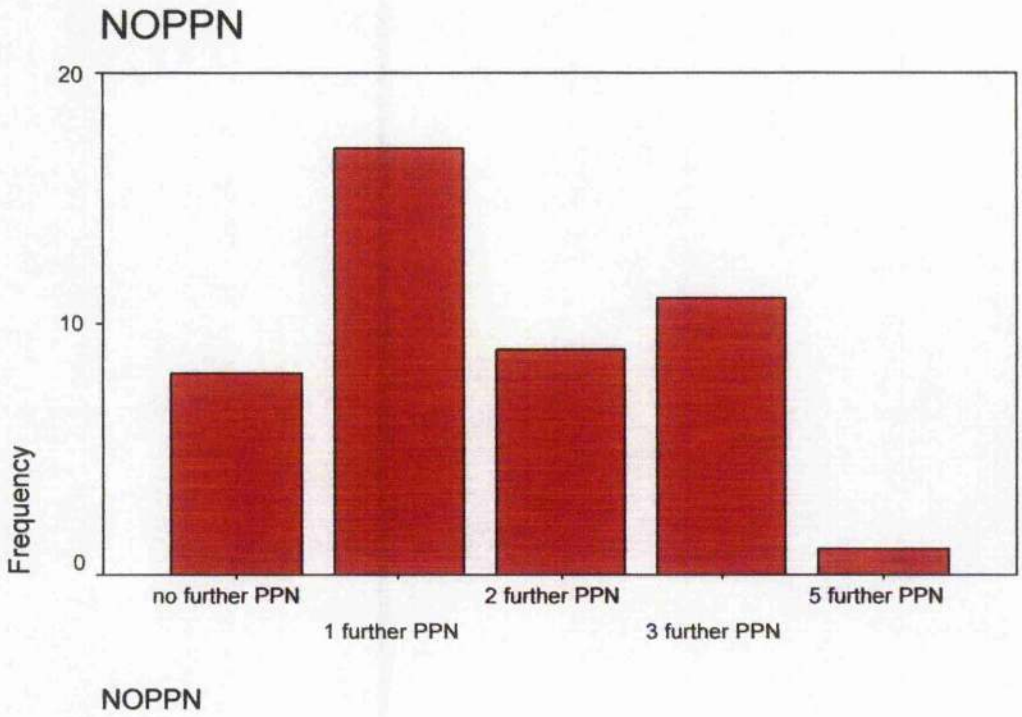


Figure 3.3: Number of Percutaneous (PPN) procedures

In addition to pancreatic necrosectomy and debridement at the index procedure, a number of other procedures were also performed.

In the OPEN group, several additional pancreatic resection or bypass procedures were performed including gastro-pancreatostomy, cholecystoduodenostomy, panctreatico-jejeunostomy roux-en-y and distal pancreatectomy. Four patients had a cystgastrostomy.

A proportion of patients had colonic complications associated with pancreatic necrosis. Nine patients in the OPEN group required colonic resection compared with 3 in the PPN group. (p=0.1)

Of these, 8 patients in the OPEN group had colonic resection at the same time as the initial necrosectomy. Seven of the OPEN patients had a right (3) or extended

right (4) hemicolectomy for poor colonic perfusion or necrosis of the colon. The remaining 2 OPEN patients had a transverse colectomy and a left hemicolectomy for extensive peripancreatic necrosis.

In the PPN group, one patient was converted to open during the 2nd PPN for perforated transverse colon requiring a right hemicolectomy. Two other PPN patients required laparotomy following their 3rd procedure for colonic ischaemia associated with extensive peri-pancreatic necrosis.

Further open procedures for persisting necrosis/ inflammation were performed in 8 patients in the open group, 2 with colonic complications, 4 with persistent necrosis and 2 with intra-abdominal/ pancreatic abscesses requiring debridement.

Four of the PPN patients required open laparotomy after an initial percutaneous procedure for ongoing necrosis/ pancreatic inflammation. Three of these have been discussed in relation to colonic complications. The fourth patient required open debridement of a persisting pancreatic abscess.

Cholecystectomy

Gallstones were identified as the main aetiology of acute pancreatitis in 63 patients, 33 in the OPEN and 30 in the PPN group. Thirty-seven patients in the OPEN group had a cholecystectomy performed at the index procedure.

Cholecystectomy was carried out during the same admission in a total of nine patients in the PPN group. Two underwent cholecystectomy at the index procedure; two patients had an open cholecystectomy during laparotomy at a later date. Five patients had a delayed laparoscopic cholecystectomy prior to discharge.

Haemorrhage

Bleeding was an indication for further surgery in 5 patients. In the open group, one patient had persistent problems, initially with a biliary leak and bleeding from the hepatic bed, followed by pancreatic vessel bleeding which required relaparotomy the following day. This was complicated by duodenal bleeding with a further laparotomy 10 days later. Excision of the first and second parts of the duodenum and oversewing of the duodenal stump was performed. He proceeded to have 2 embolisation procedures of the hepatic artery over the following 3 weeks and was eventually discharged 3 months later.

Another patient in the OPEN group bled from a superior Mesenteric artery pseudoaneurysm, this required laparotomy and oversewing after 2 unsuccessful embolisation attempts.

Three patients in the PPN group required laparotomy for bleeding complications. One of these patients also had persisting pancreatic and duodenal necrosis resulting in gastroduodenal arterial bleeding. This patient died intra-operatively.

One patient has already been mentioned above who required laparotomy for persisting pancreatic necrosis involving colon. Post-operatively, significant bleeding was noted and due to clinical deterioration, underwent re-laparotomy in ITU and subsequently died. The third patient had bleeding in association with colonic infarction necessitating laparotomy.

Five patients in the open group required further laparotomy after initial pancreatic necrosectomy for removal of packs. One patient required splenectomy, and another patient had ligation of the splenic artery performed.

Effect of Type of Procedure on Outcome in Patients with Infected Pancreatic Necrosis

Multiple Organ Dysfunction and ICU Admission

Patients in both groups had MODS to a variable extent and differed in their requirements for Intensive care (Table 3.6). More patients in the OPEN group than in the PPN group required ICU admission at some point during their total hospital stay (45/53 v 26/46 p=0.002).

	OPEN	**PPN**	*p-value*
No ICU admission	8	20	0.002
Pre-op ICU admission	15	18	0.2
Post-op ICU admission only	30	8	0.003
Total patients in ICU	45	26	0.002
Total	53	46	

Table 3.6 :ICU Admission

Pre-operative ICU admission

Pre-operative ICU admission was further subdivided into two groups as follows. Those with transient MODS requiring ICU care in the early phase of the illness (within two weeks of admission), which improved sufficiently to be discharged from ICU care before surgical intervention for necrosis. The other group consisted of patients who developed MODS pre-operatively but who had persistent MODS requiring ICU care up to and including the time of their index necrosectomy procedure (Table 3.7)

The time from initial hospital admission to ICU admission was similar in both groups regardless of the dynamics of MODS. Total ICU stay for all patients and survivors was significantly longer for PPN patients in the transient MODS group but not in those with persistent MODS. Mortality rates, though higher in those with persistent MODS compared with transient MODS, were similar when comparing both the OPEN and PPN groups.

Pre-Operative ICU		OPEN	PPN	Total	P value
Transient MODS	Number of patients	7	6	13	
	Time to ICU from hospital admission (days)	2 (1-7) +/- 2.5	2.5 (1-7) +/- 2.3	2 (-7) +/- 2.4	0.384
	Total ICU Stay (median days)	8 +/- 5.8	11.5 +/-9.2	8 +/- 8.4	0.036
	Mortality	2/7 (28.5%)	1/6 (16.7%)	3/13 (23%)	0.612
	Total ICU stay in survivors	2 (1-8) +/- 3.8	11 (8-33) +/-10.3	8 (1-33) +/- 9.2	0.015
Persistent Peri-operative MODS	Number of patients	8	12	20	
	Time to ICU from hospital admission	5 (1-19) +/- 6.2	4 (2-47) +/- 12.9	4 (1-47) +/-10.7	0.863
	Total ICU Stay	18.5 (5-32) +/- 10.1	28.5 (9-182) +/- 46.9	24 (5-182) +/- 38.4	0.053
	Mortality	5/8 (62.5%)	5/12 (41.6%)	10/20 (50%)	0.65
	Total ICU stay in survivors	5 (5-32) +/- 15	27 (19-182) +/- 58	27 (5-182) +/- 53	0.14

Table 3.7: Pre-operative ICU Admission

Post-operative ICU admission

In total 64 patients required ICU care post-operatively, this differed significantly between the two groups (42/53 v 22/46 p=0.001) (Table 3.8).

In patients who received pre-operative ICU care, a similar proportion in each group required additional post-operative ICU care (12/15 v 14/18, p=0.87).

In the OPEN group, more patients were admitted to ICU for the first time post-operatively. This is significantly higher than the proportion of patients in the PPN group (30/53 v 8/46 p=0.003).

The median time in days from initial admission to hospital and first admission to ICU was comparable for both groups. Mortality rates in patients with post-operative

ICU admission were not significantly different between the two groups (16/42 v 13/22, p=0.1). The median length of ICU stay in survivors ranged between 5.5 and 25 days in the OPEN and PPN groups respectively and was significantly greater in PPN patients (p=0.005).

Post-Operative ICU	OPEN	PPN	Total	P value
Pre- operative ICU and Post-operative ICU	12/15	14/18	26/33	0.87
Post-op ICU only	30	8	38	0.003
Total Post-op ICU	42	22	64	0.001
Time to ICU from hospital admission	17 (1-19) +/- 14	8.5 (1-99) +/- 30	15 (1-99) +/- 21	0.75
Total ICU Stay	8 (1-46) +/- 11.5	20 (1-182) +/- 38.6	10 (1-182) 25.5	0.009
Mortality	16/42 (38%)	13/22 (59%)	29/64 (45%)	0.1
Total ICU stay in survivors	5.5 (1-43) +/- 10.7	25 (1-182) +/- 55.6	8 (1-182) +/- 33	0.005
Post-op stay in survivors	5.5 (+/-6.5)	9.5 (+/-10.3)	6 (+/-7.3)	0.236

Table 3.8: Post-Operative ICU admission

Mortality

The overall mortality rate was almost identical in both groups (Table 3.9). Sixteen patients of 53 in the OPEN group died, compared with 14 of 46 patients in the PPN group (p=0.97). A similar proportion of patients in both groups had pre-operative MODS requiring ICU admission (7/16 v 6/14, p=0.96). Of the remaining 17 patients who died, 16 required post-operative ICU admission. One patient died intra-operatively with no pre-operative ICU care requirements.

	OPEN	PPN	P value
Mortality	16/53 (30.1%)	14/46 (30.4%)	0.97
Pre- Operative ICU Admission	7 Pre-op ICU 9 Post-op ICU	6 Pre-op ICU 7 Post-op ICU (1 no ICU*)	0.96
Hospital Stay	39.5 (18-98) +/- 23	70 (24-114) +/-29	0.051

Table 3.9: Mortality Rates for Pancreatic Necrosectomy Procedures

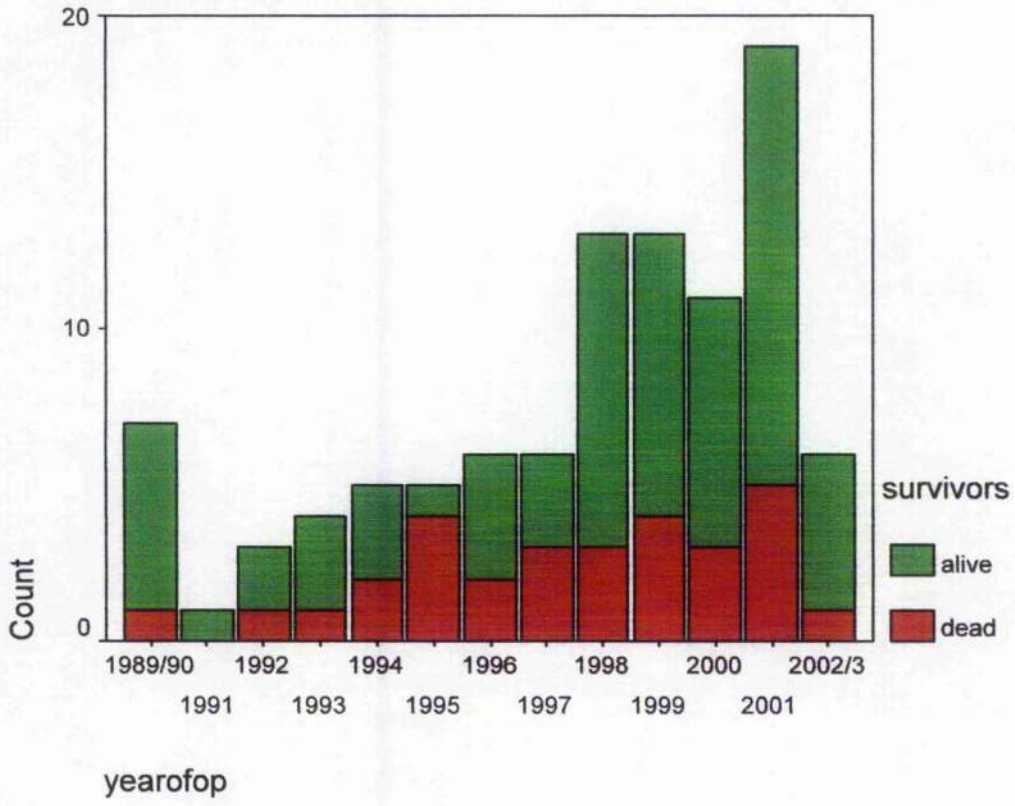
* Significant intra-operative gastroduodenal arterial haemorrhage.

Though median total hospital stay was greater in PPN patients who died, this just approaches significance (39.5 v 70 days, p=0.051). Hospital stay is similar in survivors when comparing OPEN and PPN patients (median 70 v 66 days, p=0.47)

Distribution of mortality

The proportion of deaths each year varied and the distribution throughout the study period is shown in Figure 3.4

Figure 3.4 Yearly Mortality rates



Mode of death

As reported previously, the mortality rates are similar in both groups. The mode of death, however, is different. The majority of patients had MODS as the main cause of death post-operatively (Table 3.10). However, more haemorrhagic complications were encountered in the PPN group (1/15 OPEN v 5/14 PPN, p=0.044) (Table 3.11).

	OPEN	**PPN**
Mortality	16	14
MODS alone	15	9
Bleeding alone	1	1
Bleeding and MODS	0	4*
MODS & Mesenteric ischaemia	2	1

Table 3.10: Mode of Death for Pancreatic Necrosectomy Procedures

* One patient had colonic ischaemia in addition to bleeding

Table 3.11: Bleeding Complications in PPN patients

Mesenteric venous bleeding post-operatively requiring laparotomy
Post-operative bleeding requiring laparotomy in ITU
Colonic ischaemia and post-op bleeding requiring laparotomy
Gastroduodenal Artery bleeding secondary to duodenal necrosis

Effect of pre-operative MODS on outcome in IPN

As noted previously, the proportion of patients with pre-operative MODS was similar in both the OPEN and PPN groups (15/52 v 18/46). In the total group, the mortality rate of those with any pre-operative MODS compared to those without was not significantly different (13/33 v 17/66, p=0.16) However when comparing those patients who had persistent pre-operative MODS to those without, the mortality rate was much higher (10/20 v 20/79, p=0.032)(Table 3.12). The type of procedure was not related to outcome in patients with persistent pre-operative MODS. Five patients in each of the PPN and OPEN groups had persistent pre-operative MODS and died. All patients with persistent pre-operative MODS required post-operative ICU care compared to only 57% of those without (20/20 v 45/79, p<0.001)

Table 3.12: Effect of persistent Pre-op MODS on outcome

	Persistent pre-op MODS	No persistent MODS	p-value
Number	20	79	
Mortality	10	20	0.032
Post-op ICU admission	20	44	<0.001
Percutaneous/ Open necrosectomy	12/ 8	34/45	NS 0.17
Mortality PPN/ OPEN	5 PPN/ 5 OP	9/11	NS 0.9
Total	53	46	

Other factors affecting mortality

Referral patterns

Thirty patients were admitted directly to the surgical unit at Glasgow Royal Infirmary at the onset of their symptoms. Sixty-nine patients were referred from other hospitals in the West of Scotland region upon diagnosis of pancreatic necrosis and/ or MODS. The mortality rate in patients referred from other hospitals compared to GRI-admitted patients varies with a slightly lower mortality in GRI-admitted patients (5/30 v 25/69, p= 0.05) (Figure3.5).

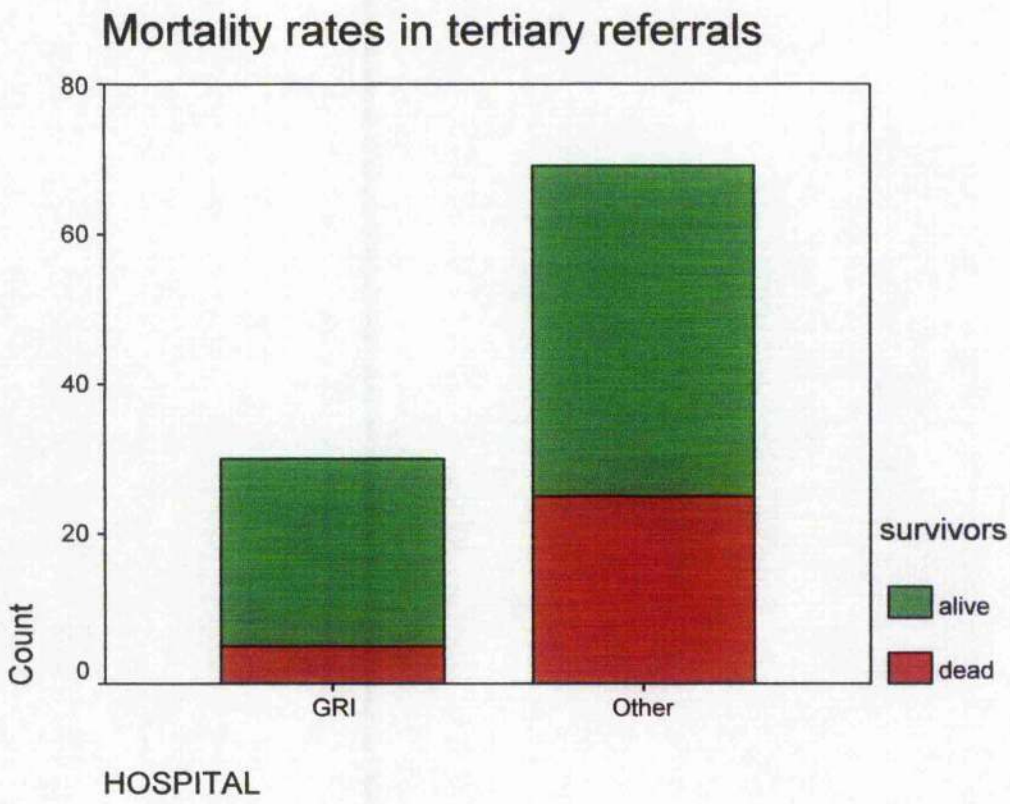


Figure 3.5: Mortality rates in tertiary referrals

Age related mortality
There is a significant association between older age and mortality ($p<0.001$)

Table 3.13: Age and mortality

Age Group	Survivors	Deaths	Total
15-30	9	0	9
30-45	22	3	25
45-60	19	12	31
>60	19	15	34
Totals	69	30	99

Multi-variate analysis of factors affecting outcome

Several factors in this group of 99 patients with infected pancreatic necrosis have contributed to a fatal outcome and post-operative MODS (Table 3.14). These factors have been investigated in isolation in the previous section. Using multi-

variate analysis these factors were assessed in relation to each other to avoid any confounding bias. Microbial factors have been included here and are discussed in greater detail in the next chapter (Chapter 4).

Table 3.14 : Factors affecting outcome in IPN

Factor affecting outcome	Mortality p value	Post-operative ICU p value
Increasing Age	**0.0003**	0.7
Tertiary Referral	**0.05**	0.2
Pre-operative ICU stay	0.1	0.5
Persistent pre-op MODS	**0.03**	**0.0003**
Prophylactic Antibiotics	0.8	0.3
PPN necrosectomy	0.9	**0.002**
Fungal infection	**0.003**	0.3

3.5 Discussion

Feasibility of percutaneous necrosectomy

Controversy remains regarding the optimal surgical procedure to combat infected pancreatic necrosis. Sepsis-related MODS pre-operatively combined with a significant surgical insult in the form of open pancreatic necrosectomy is associated with high mortality rates. Many new techniques have been explored to try and limit the surgical insult to these patients. Simple drainage catheters are ineffective in fully removing all necrotic debris from the abscess cavity and are deemed unsuitable for collections with solid components of >1cm in size (184). Several disadvantages of percutaneous drainage techniques have previously been suggested including the number of extra procedures needed, and the failure to adequately clear the necrotic area resulting in ongoing sepsis. A high proportion of patients who are initially managed percutaneously eventually require definitive open debridement to deal with the solid necrotic component. In high-risk patients with organ dysfunction, endoscopic and percutaneous drainage can be used to temporise a patient's condition until delayed necrosectomy can be performed. These have been described earlier in the chapter. Freeny et al reported that 47% of patients in his study avoided a laparotomy either for elective fistula repair or open necrosectomy for uncontrolled sepsis (163). The results shown above indicate that laparotomy was avoided in 37/46 patients (80%) in the percutaneous group, of which 29 (63%) were cured. This contrasts with 27/53 (51%) patients in the open group who required further surgical intervention. Twenty-four (45%) of these patients required laparotomy. The patients in the percutaneous group had a median of 2 (range 1-6) percutaneous procedures performed and although the total number of additional procedures is greater, the percutaneous operating time is much less. In

addition, total hospital stay, though expected to be longer in the percutaneous group due to the number of procedures required, was in fact similar. Post-operative stay was slightly longer in the percutaneous group but not significantly so.

Mortality Rates

Post-operative haemorrhage has been recognised as a major challenge in the surgical management of IPN with an incidence of approximately 26%. In this series, a significantly higher proportion of deaths in the percutaneous group were as a result of post-operative bleeding. It should be noted that the majority of the haemorrhagic complications occurred in the early stages of using the percutaneous technique. Since then, intra-operative and post-operative bleeding has been minimised by using a more cautious approach to debridement of devitalised pancreatic tissue, with improved results in those patients.

Post-operative ICU requirements

The observation from the initial study in this institution (171) that the percutaneous technique results in less organ dysfunction post-operatively has been verified. Significantly less patients required ICU care post-operatively in the percutaneous group in this series. Factors that may have contributed include reducing the surgical stress response, thereby influencing the severity of MODS, while simultaneously addressing the necrotic component and achieving adequate drainage, allowing sepsis to resolve. In addition, the detrimental effects on respiratory function and longer anaesthetic times associated with laparotomy are minimised with the percutaneous technique resulting in reduced respiratory impairment post-operatively.

Pre-operative MODS

In keeping with other studies we found an association between the presence of persisting MODS pre-operatively and a higher mortality rate (23) highlighting the difficulty in managing these severely ill patients. The type of procedure performed did not have any impact on outcome in this group. It should be noted that the numbers are small and this study did not set out to look at patients with pre-operative MODS and effect of procedure on outcome. Ideally this should be addressed by investigating a larger cohort of patients with pre-operative MODS.

Limitations of the study.

This retrospective review is a description of factors affecting outcome in patients undergoing two different procedures over a 13 year period. Although the patients were prospectively identified and data collected, they were not randomised to treatment and were not prospectively matched. In addition, various improvements in critical care such as the introduction of a High Dependency Unit may have affected patient outcome. Insufficient data regarding HDU stays was available for analysis. ITU stays were chosen as an indicator of MODS as it was more easily measurable in this group of patients and reflects severity of organ dysfunction appropriately. This study has shown that percutaneous necrosectomy is effective in the definitive surgical management of infected pancreatic necrosis. It facilitates drainage of necrotic fluid collections as well as effective debridement of solid necrotic tissue. It is less invasive than open necrosectomy which confers significant benefits in terms of minimising the inflammatory response to surgery, particularly in high-risk patients predisposed to MODS. In this series of patients, the mortality rate is similar to conventional open necrosectomy. It would be hoped that in future,

the reduction in bleeding complications would have a positive impact on reducing the mortality rate in patients undergoing percutaneous pancreatic necrosectomy. Further randomised clinical trials would be helpful in identifying the optimal procedure for an individual patient and may contribute to lowered mortality rates.

Chapter 4

Effect of Microbiology on outcome in Infected Pancreatic Necrosis

4.1 Introduction

4.1.1 Antibiotic treatment in Infected Pancreatic Necrosis

Current recommendations advise debridement and drainage as the optimal course of management of infected pancreas necrosis (39). The surgical management of infected pancreatic necrosis has been outlined previously. In addition to surgical drainage, patients are managed with additional anti-microbial therapy appropriate to the infecting organism(s) based on microbiology advice. The spectrum of antibiotic usage has changed in the last few decades for many infective conditions including infected pancreatic necrosis. Widespread usage of broad spectrum antibiotics for a wide variety of medical and surgical infections, both in hospitals and in the community, have altered their range of effectiveness and has been a factor in the development of resistant organisms (186, 187). In the last decade,methicillin-resistant Staph. Aureus (MRSA) has emerged as an aggressive, resistant gram-positive bacterium and is becoming increasingly prevalent, particularly in hospital patients who are immuno-compromised to some degree. The elderly post-operative patient is particularly at risk. The development of newer antimicrobial therapeutic agents in response to resistant infections allows more specific targeting of organisms. However some of these agents are more toxic than previous generations of drugs adding further complexity in patient management. The bacteriological spectrum of infected pancreatic necrosis is similar to intestinal flora(149.

Previously, broad spectrum antibiotics have been used to supplement surgical management of infected pancreatic necrosis with beneficial effects. Antibiotics penetrate the pancreas to varying extents and have differing efficacy against organisms involved in pancreatic infection. In a study of pancreatic antibiotic concentrations in necrotizing pancreatitis, Pefloxacin and metronidazole achieved good pancreatic excretion levels and exceeded the minimum inhibitory concentration (MIC) required for the majority of bacteria involved. Imipenem was slightly less effective(188). An efficacy factor has been applied to the majority of antibiotics used in pancreatic infections. This is based on type and frequency of infection, antibiotic tissue concentration and percentage of inhibited strains. A factor of 1 indicates complete inhibition of bacteria in infected pancreatic necrosis. (Table 4.1)

Table 4.1: Antibiotic efficacy factors (adapted from (189))

Antibiotic class	Antibiotic	Efficacy factor
Aminoglycosides	Netilmicin	0.14
	Tobramycin	0.12
Acylureidopenicillins	Mezlocillin	0.71
	Piperacillin	0.72
Cephalosporins	Cefotiam	0.75
	Ceftizoxime	0.76
	Cefotaxime	0.78
	Ceftriaxone	0.79
Quinolones	Ciprofloxacin	0.86
	Ofloxacin	0.87
Carbapenems	Imipenem	0.98

Other studies have shown that tobramycin, netilmicin and ampicillin are less effective in pancreatic infection as they do not penetrate well into the pancreas (188,190).

4.1.2 Selective Gut decontamination

Bacterial translocation from the gut has been proposed as a possible source of pancreatic infection. Increased intestinal permeability may be due to splanchnic hypoperfusion and reperfusion injury. An association between gut barrier failure and severity of illness has been demonstrated in AP patients (152). Selective decontamination reduces intestinal bacterial colonies by administration of non-absorbable antibiotics. Previous studies using selective decontamination in ICU

patients with sepsis have shown a reduction in Gram-negative septic complications. However, no clear reduction in mortality has been demonstrated (191). It has been proposed that by reducing pathogenic enteric bacteria, this may reduce microbial translocation and minimise pancreatic infection. Only one controlled trial has investigated prophylactic selective decontamination in acute pancreatitis. Although this trial demonstrated a reduction in pancreatic infection and mortality, a short dose of parenteral Cefuroxime was also administered making it difficult to interpret these outcomes in relation to selective gut decontamination alone (192). In an animal model of AP, selective decontamination of the gut by administration of early probiotic bacteria (Lactobacilli) reduced the incidence of pancreatic tissue and mesenteric lymph node colonisation with pathogenic bacteria (193).

4.1.3 Indications for antibiotic therapy

Patients with severe acute pancreatitis who have evidence of organ dysfunction but not of necrosis do not require antibiotic treatment. Conversely those with evidence of infected pancreatic necrosis will be commenced on antibiotic treatment for the duration of sepsis in addition to definitive surgical management. The antibiotics given will depend on the bacteriological spectrum and antibiotic resistance. Prolonged antibiotic therapy without surgical drainage has been attempted in a few selected patients with some success but they invariably tended to have a milder clinical course without multiple organ dysfunction (52). In those with organ dysfunction and pancreatic necrosis, not proven to be infected, the question of whether to give prophylactic antibiotics remains controversial.

4.1.4 Clinical Controversy: Use of prophylactic antibiotics

The issue of whether to give patients early prophylactic antibiotics has been the subject of discussion for many years and consensus is yet to be reached.

In the 1970's and early 1980's, several small studies were published which did not support the use of prophylaxis (194,195). Antibiotics were given early on in the course of illness to patients with mild acute pancreatitis. No obvious differences in mortality or infection rates were observed. In one study, Ampicillin was used and as mentioned previously, this does not penetrate the pancreas. In addition, it is now well recognised that patients with mild AP are unlikely to have septic complications or to die from their illness therefore limiting any useful information that can be obtained from these studies. New evidence recommending prophylaxis came from an animal study which found ciprofloxacin superior to imipenem in reducing mortality in rats with initiated acute pancreatitis (196).More recently, four small randomised prospective trials in the early 1990's were published with conflicting results. These focused on the use of prophylactic empirical antibiotics in high-risk patients and are discussed further below. In 1993, a multi-centre trial randomised 41 patients to receive Imipenem early in the course of AP, compared to 33 controls with no antibiotics (197). All patients had pancreatic necrosis. This trial showed a reduction in pancreatic and non-pancreatic infection rates but this was not reflected in mortality rates. In 1995, a series of 60 patients were studied with 50% receiving prophylactic Cefuroxime and the others no initial antibiotics (198). Twenty-three of these 30 patients however required antibiotic treatment for suspected infection at a later point. The patients in this study were recruited on the basis of either pancreatic necrosis and/ or a high CRP. Despite the heterogeneity of the patients, the mortality rates and septic complications were lower in the cefuroxime group. The

most common pathogen cultured was Staph. epidermidis from pancreatic necrotic tissue or central venous lines, despite negative blood cultures, suggesting contamination from skin commensals. Interestingly, no difference in pancreatic infection rates was observed. A study published in the same year compared prophylactic selective decontamination (norfloxacin , colistin and amphotericin as a gum paste with IV cefotaxime) in 50 patients, with 52 patients who received no additional treatment. Significant reductions in both late mortality and pancreatic infection were noted (192). A smaller study published a year later comparing ceftazidime, Amikacin and metronidazole with no prophylactic antibiotics in a total of 23 patients did not show any significant differences in mortality or pancreatic infection rates (174). These studies were reported as showing a decrease in infective complications. However as noted, on closer inspection, only two of these studies showed reduced *pancreatic* infection rates and only two showed a reduction in mortality rates. It is worth noting that the only study with a positive outcome for both mortality and pancreatic infection is the trial primarily aimed at selective decontamination of the gut as the main criterion of treatment rather than a specific prophylactic course of antibiotics. The combined number of patients in the antibiotic groups is 132. This number, considering the variety of agents used (including cefuroxime which doesn't attain adequate pancreatic tissue concentrations) and the various treatment protocols in different centres, is considered small. Despite this, the premise that prophylactic antibiotics were beneficial in preventing pancreatic infection, and in particular infected necrosis, led to widespread use of prophylactic antibiotics in patients with severe AP and in particular, those with sterile necrosis.

To further investigate the potential benefit of different prophylactic antibiotics, Bassi compared imipenem to pefloxacin in a randomised, controlled, multi-centre trial comprising 60 patients with pancreatic necrosis of over 50% of the gland (199). Imipenem was more effective in reducing the incidence of infected pancreatic necrosis compared to pefloxacin (10%v34%, $p<0.05$). No difference in mortality rates was observed. Interestingly, those given imipenem who subsequently developed infected necrosis all died, additionally, in those given pefloxacin, there was a higher incidence of fungal infection associated with a poorer outcome. This trial, as with the previously discussed randomised trials, was not blinded. In 2001, Nordback compared the timing of imipenem treatment in a randomised trial in patients with necrotising pancreatitis (200). Imipenem was given early to 25 patients. Late imipenem therapy was started upon reaching operative "criteria" (high CRP or WCC, positive pancreatic FNA) in 14 of 33 patients without prior early imipenem treatment. Only two patients in the early group fulfilled operative criteria. Of the 14 patients in the late group, 9 responded to imipenem and did not require surgery. Less organ dysfunction was noted in the early imipenem group. Mortality rates were similar (8%v15%, p=NS). The study reports a significantly reduced need for surgery in the early group. However, when those in the untreated group are given imipenem, the actual percentage of patients in each group who proceeded to surgery is similar (2/25 v 5/33, p=NS). This study therefore fails to show a reduction in the incidence of infected necrosis, mortality, or need for surgery.

Meropenem has been shown to have a similar profile to imipenem with regards to rates of pancreatic and extra-pancreatic infection, and outcome (201). Three meta-analyses were performed on the use of prophylactic antibiotics. (55,202,203). Based

on the evidence available up until then, they showed a reduction in mortality associated with the use of prophylactic antibiotics. Furthermore, the Cochrane database review showed a reduction in super-infection of necrosis.

As a result of these studies, several guidelines were issued promoting the use of prophylactic antibiotics resulting in their widespread use(7). This occurred not only in severe cases but also in mild AP where no benefit has been shown. A survey of UK and Irish surgeons a year after the UK guidelines were published showed that 88% prescribed prophylactic antibiotics, with 24% of respondents using prophylactic antibiotics in all cases of acute pancreatitis (204). More recently, the first double-blinded randomised control trial of prophylactic antibiotics in necrotising pancreatitis was performed (205). This compared ciprofloxacin and metronidazole in 56 patients versus placebo in 58 patients and did not show any differences in the development of infected pancreatic necrosis or mortality.

Recent IAP guidelines emphasise that no obvious reduction in mortality has been demonstrated by giving prophylactic antibiotics (39). Over the last decade, concern has been raised regarding the development of resistant organisms and an increase in fungal infection as a result of injudicious use of prophylactic antibiotics (206). It has been noted in several studies that patients with infected pancreatic necrosis have a worse outcome in the presence of candida infection as a result of prolonged antibiotic therapy (56). The routine use of prophylactic antibiotics remains controversial. In patients with sterile necrosis there may be a role for prophylactic antibiotics in reducing the risk of infection, though any additional benefit conferred in terms of a reduction in operation and mortality rates has not been consistently demonstrated. Balanced against this, however, are the consequences of frequent

prescribing of prophylactic antibiotics, as patients with resistant bacterial infection and fungal colonisation have significantly worse outcomes.

Microbiology of infection in IPN

Bacterial colonisation of devitalised pancreatic tissue occurs in 40-70% of patients who develop pancreatic necrosis (207) and can be mono-microbial in up to 80% of cases (149). The spectrum of pancreatic infection is very similar to that seen in gut flora, lending evidence to the bacterial translocation hypothesis. This spectrum has changed in recent years. Previously, gram-negative bacteria have been the most common pathogens isolated from necrotic pancreatic tissue, but there is increasing evidence to suggest that due to widespread use of prophylactic antibiotics in the 1990's, there has been a shift towards a higher prevalence of gram-positive bacteria and fungal organisms in pancreatic necrosis (50,208). Gram-positive infection has been associated with a higher mortality in one study of pancreatic necrosis (192). More recently, there have been several reports of an increased prevalence of antibiotic-resistant organisms.

4.1.5 Antibiotic resistant organisms

In 2004, De Waele et al found a high incidence of antibiotic-resistant organisms (52%) in IPN patients who had been given prophylactic antibiotics (55) but it should be noted that only 29% of these had primary infection of pancreatic necrosis with antibiotic-resistant organisms. Overall, those with resistant organisms required longer hospital stays and received longer antibiotic therapy. The mortality rate was slightly higher in these patients but not significantly so and no difference in MODS was noted.

4.1.6 Fungal infection in IPN

Fungal growth in pancreatic necrosis has increased from approximately 10% in the early 1990's up to 24% (50) in the new millennium. Studies which have described fungal pancreatic infection have had conflicting results in terms of the effect on mortality. Isenmann and Hoerauf both showed that fungal infection was associated with increased mortality (56,209). It should be noted however, that in Hoeraf's study, out of 7 patients with fungal infection who died, only 1 was treated with an appropriate anti-fungal.

Gloor did not demonstrate any difference in mortality in his study of 33 patients with IPN (50). Similarly, De Waele showed in a study of 46 IPN patients, that despite an incidence of 17% of primary and 22% of secondary fungal infection, there were no significant differences in mortality or severity of disease (210). Interestingly, there was a trend towards higher APACHE II scores in patients with fungal infection. The incidence of fungal infection seems to be rising and may be related to increasing prophylactic antibiotic use. Indiscriminate use of prophylactic antibiotics may predispose to fungal infection with an associated high mortality.

4.2 Aims

To examine microbiological factors in relation to mortality in patients with infected pancreatic necrosis

1) To identify microbiological spectrum in patients with infected pancreatic necrosis treated at Glasgow Royal Infirmary
2) To determine influence of prophylactic antibiotics on bacteriological spectrum and development of resistant organisms
3) To investigate relationship between resistant organisms and outcome.

4.3 Methods

Patient details were collected retrospectively as described in Chapter 3 with similar inclusion and exclusion criteria.

Microbiology

Microbiology sampling included CT-guided FNA of areas of pancreatic necrosis pre-operatively, pancreatic tissue or fluid taken during the index necrosectomy procedure. Data on antibiotic and anti-fungal usage was taken directly from patient's case-notes where available. Additional information was obtained from archived microbiology records.

Statistical analysis using SPSS9 was performed.

4.4 Results

All of the patients included in this study had evidence of infected pancreatic necrosis. The microbiology samples used included FNA of pancreatic necrosis pre-operatively or pancreatic tissue or fluid sampled intra-operatively.

4.4.1 Microbiology of Infected Pancreatic Necrosis

Ninety-nine patients with infected pancreatic necrosis treated surgically at Glasgow Royal Infirmary between Dec 1989 and March 2003 were included in this study. The incidence of individual infecting organisms is shown (Table 4.2). It outlines the relative proportion of gram-positive, gram-negative, anaerobic bacteria and fungi that were cultured in this group of patients.

Table 4.2: Incidence of organisms isolated (no of patients)

	Organism cultured	Total
Gram Negative	Coliforms	33
	Mixed faecal flora	4
	Klebsiella	5
	Pseudomonas	6
	Proteus	2
	Escherichia coli	4
	Bacteroides	4
	Clostridium	4
Gram Positive	Staphlycoccus aureus	24
	Streptococcus	16
	Enterococcus	20
Anaerobes		7
Fungi		21

Fifty-seven patients had monomicrobial infection, 42 patients grew polymicrobial flora.

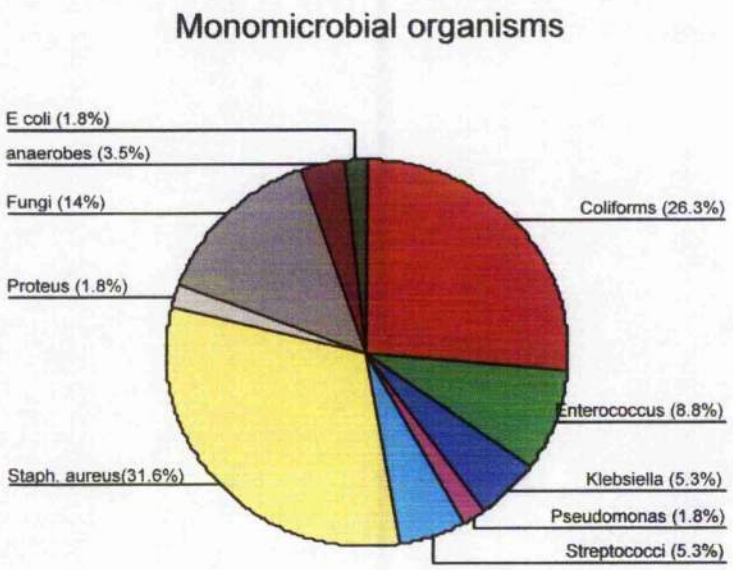


Figure 4.1: Monomicrobial infection

This pie chart shows that coliforms (26.3%)and staphlycoccus aureus (31.6%) are the most common organisms cultured in patients with initial monomicrobiaal infection (Figure 4.1). Fungi were identified as the sole primary infecting agent in 14% of patients.

4.4.2 Prophylactic Antibiotics

From 1989, some GRI patients had received prophylactic antibiotics prior to surgery for IPN. This was not conventional practice in the majority of referring hospitals until 1994. This practice has continued until the present day in many of the referring hospitals in the region. From 1998, early administration of prophylactic antibiotics to patients with severe AP has not been part of the standard treatment protocol for patients admitted directly to GRI with AP (Figure 4.2)

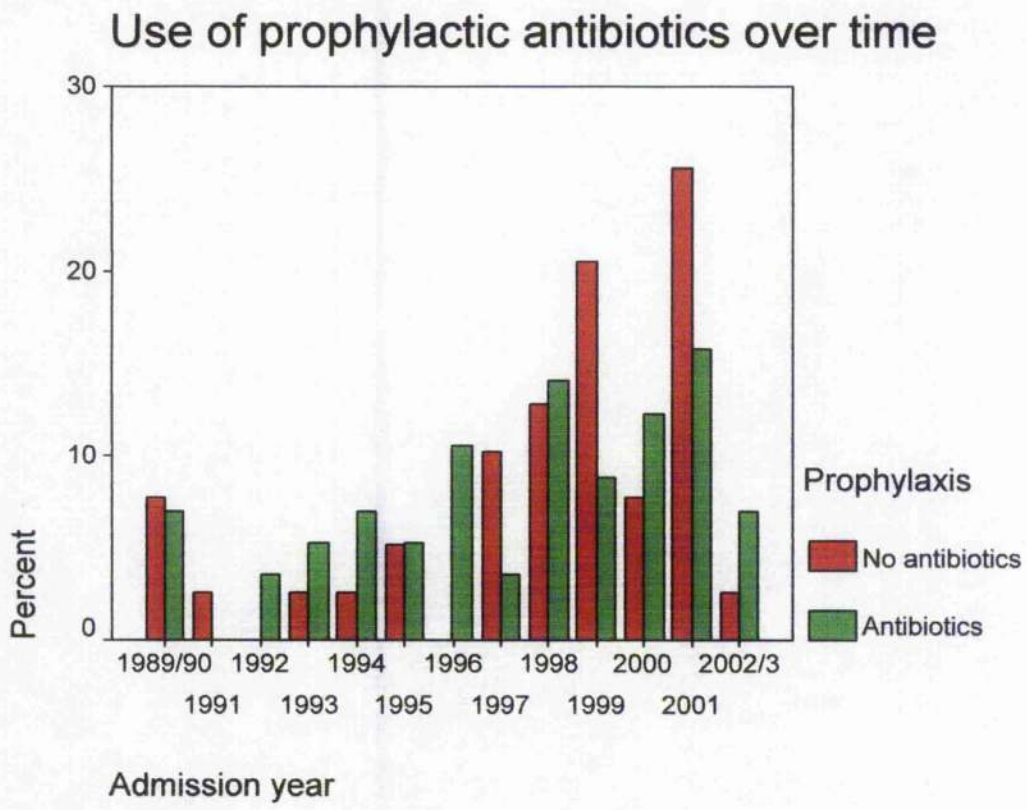


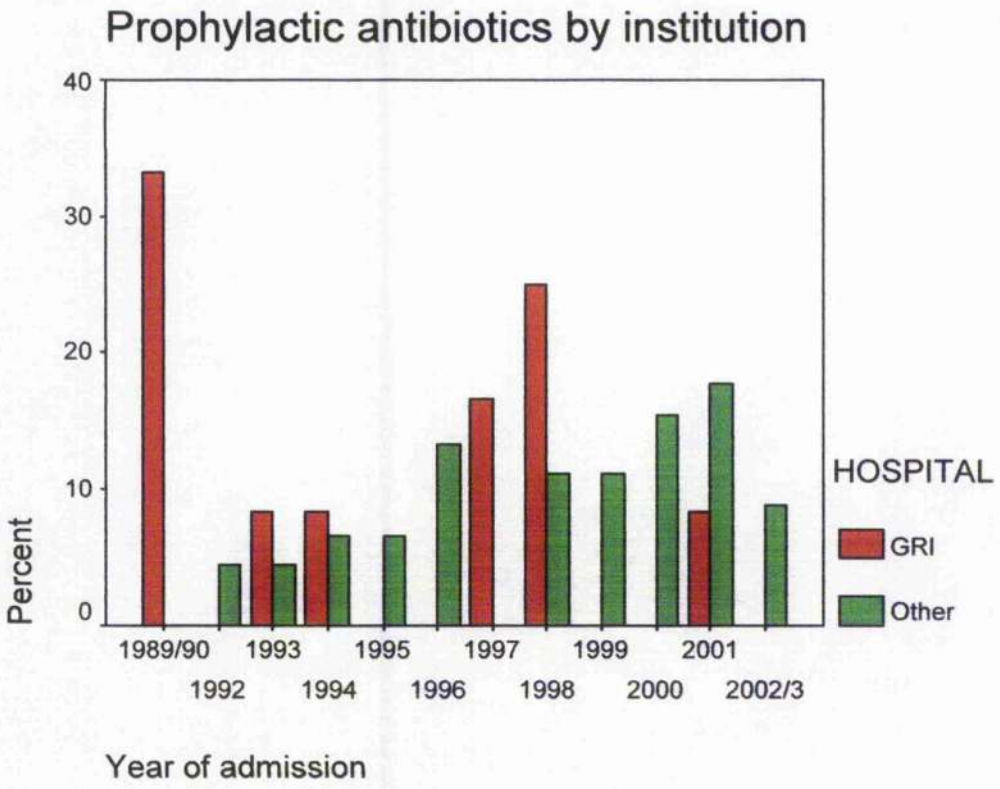
Figure 4.2 Use of prophylactic antibiotics over time

This bar chart demonstrates the distribution over time of patients given prophylactic antibiotics. In 3 patients, information regarding the use of prophylaxis was unavailable and they have been excluded from this section of analysis.

Prophylactic antibiotics and referring hospital.

Further analysis of patients receiving prophylactic antibiotics after 1998 reveals that the majority of these patients have been admitted initially to other hospitals and subsequently transferred to GRI (Figure 4.3). Overall, only 12 of 29 (41%) GRI patients received prophylactic antibiotics compared to 45 of 67 (67%) referred patients (p=0.018)

Figure 4.3: Prophylactic antibiotics and admitting hospital



Since 1998, few patients admitted initially to GRI received prophylactic antibiotics compared to those referred from other centres. Interestingly, significantly less GRI admitted patients developed resistant infection compared to patients transferred from referring hospitals (p=0.028) (Table 4.3)

Table 4.3: Resistant organisms and admitting hospital

Infection	GRI patients	Referred patients	Total
Bacteria alone	25	42	67
Fungi or MRSA	5	27	32
Total	30	69	99

Prophylactic Antibiotics and Microbial Spectrum

Table 4.4 Microbial organisms 1989-2002

	Admission period	**1989-1993**	**1994-1998**	**1998-2002**	**Total**	**p value**
	Patient numbers	**16**	**35**	**48**	**99**	
Organism cultured	**Monomicrobial (%)**	**50%**	**74%**	**52%**		
Gram Negative	Coliforms	2	9	22	33	0.005
	Mixed faecal flora	1	0	3	4	
	Klebsiella	0	5	0	5	
	Pseudomonas	3	1	2	6	
	Proteus	1	1	0	2	
	Escherichia coli	2	0	1	4	
	Bacteroides	1	1	2	4	
	Clostridium	0	1	3	4	
Gram Positive	Staphlycoccus aureus	5	6	13 *	24	
	Streptococcus	5	4	7	16	
	Enterococcus	0	4	16	20	0.006
Anaerobes		0	1	6	7	
Fungi		4	11	6	21	
* MRSA developed in 7 of these patients						
Secondary Fungal Infection		2	2	1	5	

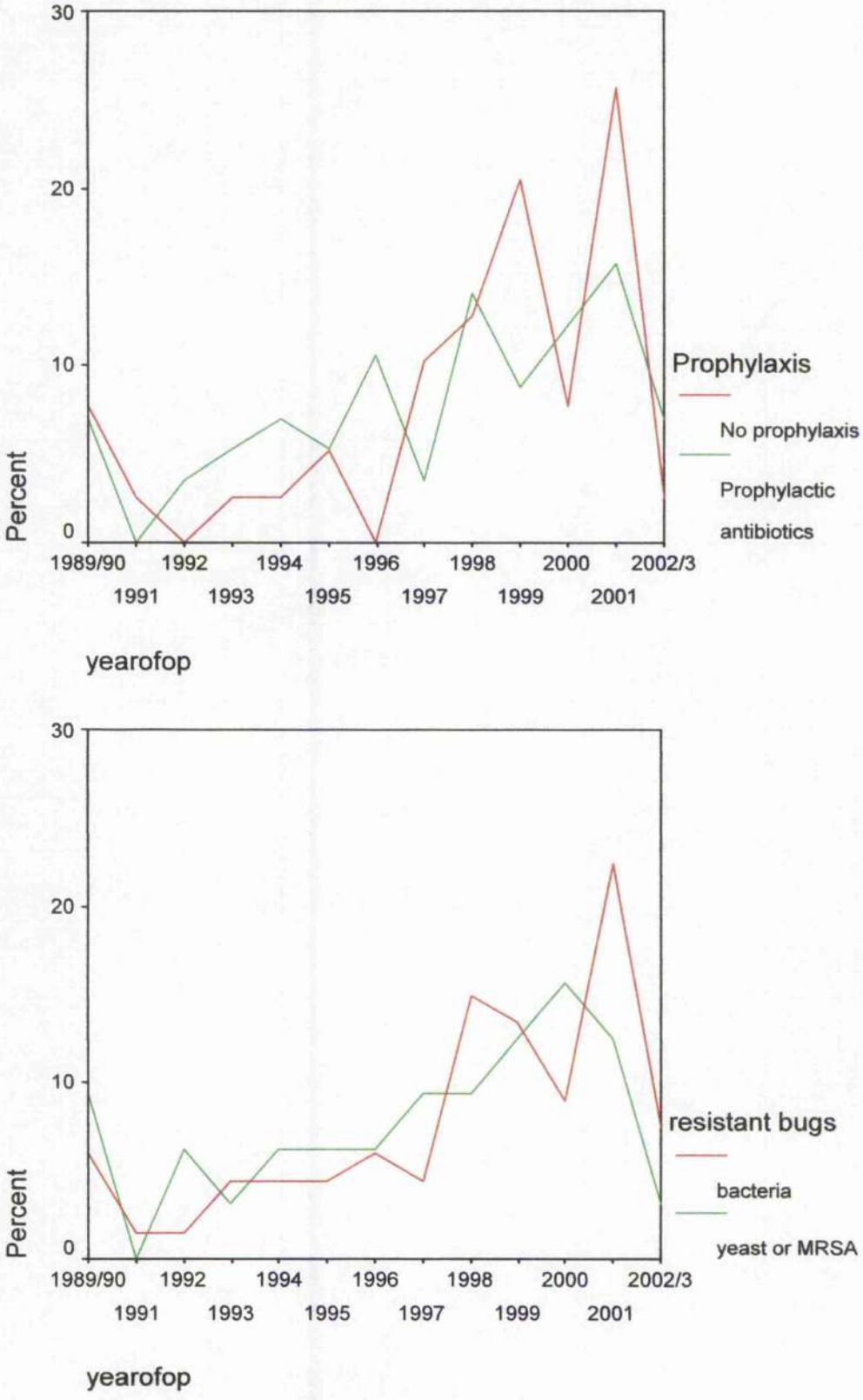


Figure 4.4: Prophylactic antibiotics and resistant organisms

Prophylactic Antibiotics and Microbial Spectrum

The study period was divided into 3 phases in relation to the use of prophylactic antibiotics. The microbial spectrum has changed slightly over the study period. Enterococci and coliforms became more prevalent in latter years. There was no significant difference in the prevalence of fungal infection with time. MRSA was recognised from 1998. (Table 4.4)

The development of resistant organisms increased slightly over the time period but not significantly so. Although there is a similar appearance in the distribution of prophylactic antibiotics and resistant organisms over time (Figure 4.4), this represents a trend toward significance but which is not reached. There was no association between the use of prophylactic antibiotics and the presence of resistant organisms in this group of patients. Data on the time from the start of prophylactic use until the procedure was available in 42 patients. In these patients there was no association between length of time from antibiotic commencement and resistant organisms.

Prophylaxis and Mortality

No association between antibiotic prophylaxis and mortality was demonstrated in this group of patients with infected pancreatic necrosis (Table 4.5).

Table 4.5: Mortality rates and antibiotic prophylaxis

n=patients	1989-1993	1994-1998	1999-2002	Total	Deceased
Antibiotics	9 (2)	23 (10)	25 (7)	57	19 (33%)
No antibiotics	5 (0)	12 (4)	22 (5)	39	9 (23%)

(Number of patient deaths in brackets)

4.4.3 Fungal infection and outcome

Both primary and secondary fungal infection were significantly associated with a higher mortality rate (Table 4.6). The presence of MRSA was not associated with mortality.

Table 4.6 Fungal infection and outcome in patients with IPN

Type of infection	Mortality		P value
	Survivors	Deceased	
Bacterial alone	57	17	
Primary fungi	9	11	0.007
Secondary fungi	3	2	0.006

4.5 Discussion

Microbial Spectrum

The microbial spectrum has changed a little over the 12 years of the study with an increase in enterococci and coliforms. A shift from predominantly gram-negative to gram-positive organisms has been seen more recently in the United States but is not demonstrated in this group of IPN patients (208). As with this study, there was no significant change in the proportion of fungal infection.

Prophylactic Antibiotics

The use of prophylactic antibiotic use in patients either with MODS or sterile necrosis remains controversial. No clear-cut evidence has demonstrated a reduction in procedures performed for IPN or a reduced mortality rate (39,205). In this institution, it was noted that there seemed to be a greater prevalence of antibiotic resistant organisms developing between 1994-98 (Table 4.4). The greatest use of prophylactic antibiotics occurred during this period, as a result, the unit policy changed and GRI-admitted patients thereafter were not prescribed prophylactic antibiotics. There was a reduction in the number of fungal infections seen after the change in policy, though when analysed in the context of total numbers, this is not significantly related to prophylactic antibiotic usage. Perhaps if this study was extended with larger patient numbers, the apparent trend towards significance in Figure 4.4 between use of prophylactic antibiotics and incidence of resistant organisms may be reached. It should be noted however, that the majority of referred patients still receive prophylactic antibiotics during the early course of the illness. In this group, the development of resistant organisms is significantly higher with an associated higher mortality rate. The duration of "prophylactic" antibiotic therapy is not clear from the data available for analysis but this may be a contributing factor to

the development of resistant infection in the referred patients. Howard used antibiotics for 2 weeks only with no increased rate of fungal infection (208). Howard's study also looked at the benefits of prophylactic fluconazole in patients with severe AP and while this was shown to reduce the incidence of Candida species infection, the emergence of resistant fungal strains is a significant drawback and as such, routine anti-fungal prophylaxis could not be recommended. Perhaps if patients do receive prophylaxis, it should be for a limited defined period unless there is evidence of infection. Future reduction of pathogenic flora by selective decontamination or probiotics may reduce bacterial translocation. However there may be a risk of further superinfection with multi-resistant organisms. This work has demonstrated a significant mortality rate in association with primary and secondary fungal contamination of IPN. This is a significant contributing factor to overall mortality and should be avoided if possible. This association is in keeping with previous studies which have demonstrated increased mortality rates in patients with fungal infection (56,209).This observation may simply reflect an abnormal immune response in some patients, increasing their likelihood of death. This aspect has not been assessed in this study or demonstrated in previous studies. Future work could include a comparision of the immune response in IPN patients with and without fungal infection.

Chapter 5 Discussion

5.1 Acute Pancreatitis: What we know now

Much evidence has accumulated in the last twenty years regarding the underlying processes in the development of acute pancreatitis. Progress in molecular biology has increased awareness of acinar cell function and communication. Novel cytokines continue to be discovered increasing the body of knowledge on the role of cytokines in the development of local and systemic inflammation. The discovery of genetic variability in cytokine genes and subsequent effect on cytokine production gave rise to the hope that cytokine gene therapy may down-regulate excessive inflammatory activity and improve outcomes. Experimental studies in animals have shown promise in this area, but to date, no specific gene therapy or anti-cytokine agent has yet been shown to improve outcomes in patients with acute pancreatitis. Improved imaging modalities and increased availability including contrast enhanced CT, MRI and more recently, EUS, have improved pancreatic imaging, allowing earlier diagnosis in uncertain cases. Using these modalities, local pancreatic complications can be detected and managed definitively. Enteral nutrition reduces septic complications. ERCP and sphincterotomy in patients with cholangitis and jaundice improve outcomes in biliary pancreatitis. The incorporation of same admission/ early cholecystectomy into the management of AP reduces the risk of further, more severe attacks. Increased collective global experience in the management of patients with sterile and infected pancreatic necrosis has shown that surgical intervention should be reserved for those with IPN in association with MODS. Consensus meetings have defined some of the terminology used in association with acute pancreatitis, facilitating comparison between clinical trials and studies. National and international guidelines have been

published, assisting clinicians in their management decisions based on recent evidence.

Despite these improvements, patients with MODS continue to die.

5.2 Why Do Patients Die?

The prime factor associated with mortality in acute pancreatitis is the development of organ dysfunction. Persistent MODS in early AP is associated with a mortality rate of over 50% and is a major determinant of survival (23). Up to 50% of all deaths occur within the first 1-2 weeks after admission, usually due to overwhelming MODS. The remainder of the deaths occur a few weeks after disease onset secondary to MODS, usually in association with infected pancreatic necrosis. The severity of the illness is dependant on multiple factors. Elderly and obese patients have a higher risk of death. This has been shown previously and in this series of IPN patients, is confirmed in elderly patients. The reason for this is probably related to pre-existing co-morbidity limiting resistance to the adverse effects of systemic inflammation resulting in MODS. From previous studies, the underlying aetiology does not seem to contribute to the development of MODS. However the development of local complications does influence outcome. Pancreatic necrosis is associated with higher mortality rates than other local complications, but this again is due to a greater association between necrosis and MODS. The greater the extent of necrosis, the higher the risk of organ failure (46). Infected necrosis is more likely to drive MODS as a result of undrained sepsis regardless of the extent of necrosis. As a result these patients have the highest mortality rates. In this series, IPN was associated with a mortality rate of 30%.

Although there seems to have been a relative reduction in mortality rates due to increasing incidence, the actual numbers of patients dying has changed little. Standardised mortality ratios have remained static for over 30 years (1). Deaths continue to occur despite maximal supportive therapy in specialist units. Although improvements in supportive therapy, surgical procedures and better clinical management may help in avoiding some deaths, it seems that a small selected group of patients are pre-determined to develop MODS and as a result, some of these will die. A reasonable explanation for this is that individual cytokine gene variation affects cytokine production and regulation of the inflammatory response. However the evidence so far is patchy and an exact understanding of the underlying mechanisms remains elusive.

5.3 Influence of cytokine gene polymorphisms

Cytokines are known to be amongst the principle mediators of local and systemic inflammation in acute pancreatitis (90). Polymorphisms in cytokine gene loci influence gene expression, transcription and production of various cytokines. Several gene polymorphisms have been associated with differential levels of cytokine production in patients with sepsis(211). The evidence regarding the relevance of cytokine gene polymorphisms affecting pancreatic disease severity is less clear. This study characterised interleukin-8 SNP frequencies in patients with acute pancreatitis in relation to severity and susceptibility. The results are in keeping with other studies of cytokine polymorphisms which did not show any positive associations between TNF and Il-1 gene polymorphisms and susceptibility or severity of AP (212) (120). All of these studies had patient numbers under 200, when these are divided into individual genotypic groups and severity of disease,

small individual group numbers are likely to occur. For the less frequent genotypes, these are often in single figures. These studies do not conclusively *exclude* an association between cytokine polymorphisms and acute pancreatitis, yet they provoke more questions. Further studies into cytokine polymorphisms should be performed. However, it is often difficult to recruit enough patients who fit the inclusion criteria within a relatively short period. Multi-centre recruitment is a possible option but inherent population differences may complicate the analysis and would need to be taken into account. With improvements in technology such as micro-array sequencing, more polymorphisms could be tested in a shorter time frame. Prospective analysis of samples would avoid the problems encountered with historic stored samples. There may come a time when an individual patient's unique genome is assessed on admission and treatment tailored to their individual genetic code, but that is still some time away.

5.4 Influence of Surgical Management

Infected pancreatic necrosis is a complication of AP associated with high mortality rates. Surgical debridement has been advocated previously in any patient with evidence of infected necrosis regardless of the presence of MODS (213). More recently, consensus guidelines recommend surgical treatment in patients with IPN associated with sepsis(39). Previously, radical resection procedures which removed healthy pancreatic tissue were associated with higher mortality rates (178) and are no longer recommended. There have been several modifications to surgical practice which have been associated with reduced mortality rates and fewer complications. The trend towards a less invasive approach continues. It is now recognised that a delay in surgery allows demarcation of necrotic areas, limits the amount of

debridement required and is associated with less bleeding complications. The optimal period for surgical debridement of necrosis is during the third or fourth week from onset, providing an opportunity for local inflammatory mediators to settle (93). In this study it has been shown that a minimally invasive necrosectomy procedure is associated with less post-operative MODS compared with the conventional open procedure. The percutaneous route was as successful in drainage and debridement of necrotic material when compared with the open procedure. However, the mortality rate was the same. There could be two reasons for this. Firstly, a greater number of haemorrhagic complications occurred with the percutaneous technique compared with the open. This was more prevalent in the earlier years and these deaths may obscure a real reduction in mortality associated with the newer procedure. Secondly, there may be no effect on mortality with the percutaneous procedure. Although the procedure is effective in minimising a "second hit" resulting from surgery, it is possible that these patients are on the downward spiral of immunological dissonance and that the percutaneous procedure has less of a hastening effect when compared with the open one. This would certainly be in keeping with the longer hospital stays seen in percutaneous patients who died. Another study of a minimally invasive necrosectomy technique performed in Liverpool and developed in conjunction with the Glasgow group showed a similar reduction in post-operative MODS but no significant reduction in mortality (214). Time will tell if a reduction in bleeding complications in association with the percutaneous procedure will impact positively on reducing mortality. Currently all patients are managed by the percutaneous route unless there is a contra-indication (no access, intra-abdominal complication). It has not been possible to determine which patients are likely to benefit most from the

percutaneous procedure. It may be that younger, fitter patients with less MODS may be suitable for a definitive open necrosectomy, avoiding frequent re-operation and a longer hospital stay. The benefits of the percutaneous procedure include drainage of septic foci due to necrosis, minimising complications associated with laparotomy in addition to definitive surgical management of necrosis.

5.5 Influence of bacteriology.

The emergence of antibiotic-resistant organisms in the last 10-15 years in infected pancreatic necrosis can be attributed to the widespread use of prophylactic antibiotics (55). Although previously published international guidelines have advocated their use in patients with severe acute pancreatitis (11,215), recent evidence in the form of a double-blind, randomised trial places this advice under question. The increase in fungal contamination of pancreatic necrosis with an associated higher mortality indicates that prophylactic antibiotics should be given with caution (199). This series of patients with infected pancreatic necrosis represents a high-risk subgroup of all AP patients treated in this institution including tertiary referrals from other centres within the West of Scotland.

The bacteriological spectrum of infected pancreatic necrosis in this series of high-risk AP patients has changed little over the study period. MRSA has developed since 1998 but the overall proportion of gram-positive and gram-negative organisms is similar. The lack of a significant change in microbial spectrum may be due to the fact that not all the patients received prophylaxis. However, a greater number of fungi were noted in referred patients who were also more likely to receive prophylaxis. The fact that they received prophylaxis is not surprising given the results of a UK wide survey indicating high levels of prescribing to patients

with severe AP (204). It has been the unit's policy in recent years not to indiscriminately prescribe antibiotics in the early course of the illness unless evidence of infection was obtained. The mortality rate in referred patients in this series tends toward significance, prophylactic antibiotic usage is higher and fungal infection more prevalent in this group, which could have been a factor influencing mortality. In this series, there was no association between receiving antibiotic prophylaxis and the development of resistant organisms except in the referred patients. This may be related more to the duration of antibiotic "prophylaxis" than whether or not patients received antibiotics. Prophylactic antibiotic use in isolation was not found to be significantly associated with death, however, those who developed resistant infection in the form of MRSA or yeasts were at increased risk of mortality. Fungal infection alone or mixed with bacteria, whether at primary surgery or subsequently, was found to be a factor in increased mortality. Fungal infection can be seen as an indicator of severe disease as it is likely to occur in patients who are immunocompromised. It is not the infecting organism per se that is aggressive, rather that the patient's host defence system is unable to sustain adequate protection. Other studies have shown an increased mortality rate in patients with fungal infection (56,170). Some authors have proposed prophylactic anti-fungal treatment (210) but this may cause further super selection of resistant organisms in vulnerable patients. The debate regarding the use of prophylactic antibiotics will continue. There is no evidence from this study to state that prophylaxis contributes to mortality, however an increased prevalence of fungal organisms in referred patients may be due to prolonged antibiotic therapy.

It is possible that the avoidance of prophylactic antibiotics in patients admitted directly to GRI with AP in recent years has resulted in a lower incidence of fungal

infection. It is clear that the prevalence of fungal infection is increasing in patients referred from centres outwith Glasgow Royal Infirmary since the routine introduction of prophylactic antibiotics. Based on the results in this series of patients with infected pancreatic necrosis, the policy of not prescribing prophylactic antibiotics is reasonable in order to minimise the development of resistant organisms with an associated higher mortality rate.

5.6 Conclusion

In conclusion, this thesis aimed to explore factors affecting MODS and mortality in patients with severe acute pancreatitis. The pathophysiology of acute pancreatitis is not fully understood but it is becoming apparent that cytokine gene polymorphisms contribute to the development of the systemic inflammatory response.

This work has not demonstrated a significant association between interleukin-8 polymorphisms and severity or susceptibility of AP. Less invasive surgical techniques reduce the incidence of MODS associated with surgical stress in patients with IPN but a subsequent beneficial effect on mortality has not yet been demonstrated. This may be due to pre-determined cytokine behaviour despite down-regulating the inflammatory response associated with IPN related sepsis.

In patients with IPN, the type of infection is important in determining outcome but this may reflect underlying immune/inflammatory activity rather than the virulence of a particular organism. Again this relates to an individual patient's response to infection and inflammation and may also be due to inherent genetic factors.

From these three different aspects of acute pancreatitis, it can be seen that the development of MODS is central to outcome in acute pancreatitis and this process is driven by cytokine production and regulation. By exploring further the role that cytokines play in the development of MODS and the genetic influence of cytokine polymorphisms, a closer understanding of the pathophysiology of acute pancreatitis may be reached.

5.7 Further Work

It is clear from this work that further investigation of cytokine gene polymorphisms should be carried out. Three further SNP's have been discovered in the IL-8 promoter region and these would make an ideal starting point. The continuing development of technology in parallel with the understanding of disease progression will allow faster analysis of a greater number of cytokines and potentially could occur during a patients admission.

Further work should include a randomised trial of minimally invasive and open necrosectomy techniques. The percutaneous procedure offers promising results with a potentially reduced mortality. If this is the case, it will then become important to identify which type of procedure is best suited to which patient.

Further trials are needed to investigate the role of prophylactic antibiotics in patients with severe AP. They may have a role in preventing infection in some patients but can be associated with resistant organisms and higher mortality. If they are found to be beneficial then the optimal duration of use and the ideal antibiotic agents need to be identified.

Appendix 1 RANSON Score

On admission to hospital
Serum Glucose >10mmol/l
WCC > 16x 10^9/l
AST or ALT > 250iu/l
or LDH > 350iu/l
Within 48 hours
Haematocrit decrease by >10%
Urea increase > 0.7mmol/l
Serum Ca^{2+} >2mmol/l
Fluid sequestration > 6 litres
PaO_2 <8KPa

Appendix 2 Modified GLASGOW Score

Within 48 hours
AST/ ALT >200iu/l
WCC > 15x 10^9/l
LDH > 600iu/l
Albumin <32g/l
Serum Glucose >10mmol/l
Serum Ca2+ < 2mmol/l
Urea >16mmol/l
PaO2 <60 mmHg (8kPa)

CRP >150

Reference List

(1) Goldacre MJ, Roberts SE. Hospital admission for acute pancreatitis in an English population, 1963-98: database study of incidence and mortality. BMJ 2004; 328(7454):1466-1469.

(2) Wilson C, Imrie CW. Changing patterns of incidence and mortality from acute pancreatitis in Scotland, 1961-1985. Br J Surg 1990; 77(7):731-734.

(3) McKay CJ, Evans S, Sinclair M, Carter CR, Imrie CW. High early mortality rate from acute pancreatitis in Scotland, 1984-1995. Br J Surg 1999; 86(10):1302-1305.

(4) Corfield AP, Cooper MJ, Williamson RC. Acute pancreatitis: a lethal disease of increasing incidence. Gut 1985; 26(7):724-729.

(5) Thomson SR, Hendry WS, McFarlane GA, Davidson AI. Epidemiology and outcome of acute pancreatitis. Br J Surg 1987; 74(5):398-401.

(6) Cavallini G, Frulloni L, Bassi C, Gabbrielli A, Castoldi L, Costamagna G et al. Prospective multicentre survey on acute pancreatitis in Italy (ProInf-AISP): results on 1005 patients. Dig Liver Dis 2004; 36(3):205-211.

(7) UK guidelines for the management of acute pancreatitis. Gut 2005; 54 Suppl 3:iii1-iii9.

(8) Pezzilli R, Billi P, Barakat B, Broccoli P, Morselli LA. The use of the peripheral leukocyte count and chest X-rays in early assessment of the severity of acute pancreatitis in comparison with the Ranson score system. Panminerva Med 1999; 41(1):39-42.

(9) Toh SK, Phillips S, Johnson CD. A prospective audit against national standards of the presentation and management of acute pancreatitis in the South of England. Gut 2000; 46(2):239-243.

(10) Bank S, Indaram A. Causes of acute and recurrent pancreatitis. Clinical considerations and clues to diagnosis. Gastroenterol Clin North Am 1999; 28(3):571-89, viii.

(11) United Kingdom guidelines for the management of acute pancreatitis. British Society of Gastroenterology. Gut 1998; 42 Suppl 2:S1-13.

(12) Lerch MM, Saluja AK, Dawra R, Ramarao P, Saluja M, Steer ML. Acute necrotizing pancreatitis in the opossum: earliest morphological changes involve acinar cells. Gastroenterology 1992; 103(1):205-213.

(13) Foulis AK. Histological evidence of initiating factors in acute necrotising pancreatitis in man. J Clin Pathol 1980; 33(12):1125-1131.

(14) Lerch MM, Gorelick FS. Early trypsinogen activation in acute pancreatitis. Med Clin North Am 2000; 84(3):549-63, viii.

(15) Halangk W, Kruger B, Ruthenburger M, Sturzebecher J, Albrecht E, Lippert H et al. Trypsin activity is not involved in premature, intrapancreatic trypsinogen activation. Am J Physiol Gastrointest Liver Physiol 2002; 282(2):G367-G374.

(16) Lerch MM, Albrecht E, Ruthenburger M, Mayerle J, Halangk W, Kruger B. Pathophysiology of alcohol-induced pancreatitis. Pancreas 2003; 27(4):291-296.

(17) Van Acker GJ, Saluja AK, Bhagat L, Singh VP, Song AM, Steer ML. Cathepsin B inhibition prevents trypsinogen activation and reduces pancreatitis severity. Am J Physiol Gastrointest Liver Physiol 2002; 283(3):G794-G800.

(18) Lightner AM, Kirkwood KS. Pathophysiology of gallstone pancreatitis. Front Biosci 2001; 6:E66-E76.

(19) Bentrem DJ, Joehl RJ. Pancreas: healing response in critical illness. Crit Care Med 2003; 31(8 Suppl):S582-S589.

(20) Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992; 101(6):1644-1655.

(21) Brown A, Baillargeon JD, Hughes MD, Banks PA. Can fluid resuscitation prevent pancreatic necrosis in severe acute pancreatitis? Pancreatology 2002; 2(2):104-107.

(22) McKay CJ, Buter A. Natural history of organ failure in acute pancreatitis. Pancreatology 2003; 3(2):111-114.

(23) Buter A, Imrie CW, Carter CR, Evans S, McKay CJ. Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. Br J Surg 2002; 89(3):298-302.

(24) McKay AJ, Imrie CW, O'Neill J, Duncan JG. Is an early ultrasound scan of value in acute pancreatitis? Br J Surg 1982; 69(7):369-372.

(25) Pellicano R, Repici A, Goss M, Carucci P, Rocca R, Bruno M et al. Role of endoscopic ultrasound in biliary and unexplained pancreatitis. Minerva Med 2007; 98(4):361-365.

(26) Levy MJ, Geenen JE. Idiopathic acute recurrent pancreatitis. Am J Gastroenterol 2001; 96(9):2540-2555.

(27) Balthazar EJ. Acute pancreatitis: assessment of severity with clinical and CT evaluation. Radiology 2002; 223(3):603-613.

(28) Buchler MW, Gloor B, Muller CA, Friess H, Seiler CA, Uhl W. Acute necrotizing pancreatitis: treatment strategy according to the status of infection. Ann Surg 2000; 232(5):619-626.

(29) Abou-Assi S, O'Keefe SJ. Nutrition support during acute pancreatitis. Nutrition 2002; 18(11-12):938-943.

(30) Ammori BJ. Role of the gut in the course of severe acute pancreatitis. Pancreas 2003; 26(2):122-129.

(31) Groos S, Hunefeld G, Luciano L. Parenteral versus enteral nutrition: morphological changes in human adult intestinal mucosa. J Submicrosc Cytol Pathol 1996; 28(1):61-74.

(32) Kalfarentzos F, Kehagias J, Mead N, Kokkinis K, Gogos CA. Enteral nutrition is superior to parenteral nutrition in severe acute pancreatitis: results of a randomized prospective trial. Br J Surg 1997; 84(12):1665-1669.

(33) Louie BE, Noseworthy T, Hailey D, Gramlich LM, Jacobs P, Warnock GL. 2004 MacLean-Mueller prize enteral or parenteral nutrition for severe pancreatitis: a randomized controlled trial and health technology assessment. Can J Surg 2005; 48(4):298-306.

(34) Targarona MJ, Barreda CL, Arroyo BC, Orellana VA, Portanova RM. Total Enteral Nutrition as Prophylactic Therapy for Pancreatic Necrosis Infection in Severe Acute Pancreatitis. Pancreatology 2005; 6(1-2):58-64.

(35) Al Omran M, Groof A, Wilke D. Enteral versus parenteral nutrition for acute pancreatitis. Cochrane Database Syst Rev 2003;(1):CD002837.

(36) Marik PE, Zaloga GP. Meta-analysis of parenteral nutrition versus enteral nutrition in patients with acute pancreatitis. BMJ 2004; 328(7453):1407.

(37) Guillou PJ. Enteral versus parenteral nutrition in acute pancreatitis. Baillieres Best Pract Res Clin Gastroenterol 1999; 13(2):345-355.

(38) Eatock FC, Chong P, Menezes N, Murray L, McKay CJ, Carter CR et al. A randomized study of early nasogastric versus nasojejunal feeding in severe acute pancreatitis. Am J Gastroenterol 2005; 100(2):432-439.

(39) Uhl W, Warshaw A, Imrie C, Bassi C, McKay CJ, Lankisch PG et al. IAP Guidelines for the Surgical Management of Acute Pancreatitis. Pancreatology 2002; 2(6):565-573.

(40) Fan ST, Lai EC, Mok FP, Lo CM, Zheng SS, Wong J. Early treatment of acute biliary pancreatitis by endoscopic papillotomy. N Engl J Med 1993; 328(4):228-232.

(41) Neoptolemos JP, Carr-Locke DL, London NJ, Bailey IA, James D, Fossard DP. Controlled trial of urgent endoscopic retrograde cholangiopancreatography and endoscopic sphincterotomy versus

conservative treatment for acute pancreatitis due to gallstones. Lancet 1988; 2(8618):979-983.

(42) Folsch UR, Nitsche R, Ludtke R, Hilgers RA, Creutzfeldt W. Early ERCP and papillotomy compared with conservative treatment for acute biliary pancreatitis. The German Study Group on Acute Biliary Pancreatitis. N Engl J Med 1997; 336(4):237-242.

(43) Johnson CD. Timing of intervention in acute pancreatitis. Postgrad Med J 1993; 69(813):509-515.

(44) Chatzicostas C, Roussomoustakaki M, Vardas E, Romanos J, Kouroumalis EA. Balthazar computed tomography severity index is superior to Ranson criteria and APACHE II and III scoring systems in predicting acute pancreatitis outcome. J Clin Gastroenterol 2003; 36(3):253-260.

(45) Balthazar EJ, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. Radiology 1990; 174(2):331-336.

(46) Isenmann R, Rau B, Beger HG. Bacterial infection and extent of necrosis are determinants of organ failure in patients with acute necrotizing pancreatitis. Br J Surg 1999; 86(8):1020-1024.

(47) Schoenberg MH, Rau B, Beger HG. [Diagnosis and therapy of primary pancreatic abscess]. Chirurg 1995; 66(6):588-596.

(48) Tsiotos GG, Sarr MG. Management of fluid collections and necrosis in acute pancreatitis. Curr Gastroenterol Rep 1999; 1(2):139-144.

(49) Allardyce DB. Incidence of necrotizing pancreatitis and factors related to mortality. Am J Surg 1987; 154(3):295-299.

(50) Gloor B, Muller CA, Worni M, Martignoni ME, Uhl W, Buchler MW. Late mortality in patients with severe acute pancreatitis. Br J Surg 2001; 88(7):975-979.

(51) Freeny PC. Incremental dynamic bolus computed tomography of acute pancreatitis. Int J Pancreatol 1993; 13(3):147-158.

(52) Adler DG, Chari ST, Dahl TJ, Farnell MB, Pearson RK. Conservative management of infected necrosis complicating severe acute pancreatitis. Am J Gastroenterol 2003; 98(1):98-103.

(53) Bradley EL, III. Management of infected pancreatic necrosis by open drainage. Ann Surg 1987; 206(4):542-550.

(54) Buchler M, Uhl W, Beger HG. Acute pancreatitis: when and how to operate. Dig Dis 1992; 10(6):354-362.

(55) De Waele JJ, Vogelaers D, Hoste E, Blot S, Colardyn F. Emergence of antibiotic resistance in infected pancreatic necrosis. Arch Surg 2004; 139(12):1371-1375.

(56) Isenmann R, Schwarz M, Rau B, Trautmann M, Schober W, Beger HG. Characteristics of infection with Candida species in patients with necrotizing pancreatitis. World J Surg 2002; 26(3):372-376.

(57) Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). Ann Intern Med 1996; 125(8):680-687.

(58) Bradley EL, III. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg 1993; 128(5):586-590.

(59) Isenmann R, Rau B, Beger HG. Early severe acute pancreatitis: characteristics of a new subgroup. Pancreas 2001; 22(3):274-278.

(60) Johnson CD, Abu-Hilal M. Persistent organ failure during the first week as a marker of fatal outcome in acute pancreatitis. Gut 2004; 53(9):1340-1344.

(61) Mann DV, Hershman MJ, Hittinger R, Glazer G. Multicentre audit of death from acute pancreatitis. Br J Surg 1994; 81(6):890-893.

(62) Werner J, Uhl W, Hartwig W, Hackert T, Muller C, Strobel O et al. Modern phase-specific management of acute pancreatitis. Dig Dis 2003; 21(1):38-45.

(63) Mutinga M, Rosenbluth A, Tenner SM, Odze RR, Sica GT, Banks PA. Does mortality occur early or late in acute pancreatitis? Int J Pancreatol 2000; 28(2):91-95.

(64) Tran DD, Cuesta MA, Schneider AJ, Wesdorp RI. Prevalence and prediction of multiple organ system failure and mortality in acute pancreatitis. J Crit Care 1993; 8(3):145-153.

(65) Zhu AJ, Shi JS, Sun XJ. Organ failure associated with severe acute pancreatitis. World J Gastroenterol 2003; 9(11):2570-2573.

(66) Malangoni MA, Martin AS. Outcome of severe acute pancreatitis. Am J Surg 2005; 189(3):273-277.

(67) McKay CJ, Imrie CW. Staging of acute pancreatitis. Is it important? Surg Clin North Am 1999; 79(4):733-743.

(68) Blamey SL, Imrie CW, O'Neill J, Gilmour WH, Carter DC. Prognostic factors in acute pancreatitis. Gut 1984; 25(12):1340-1346.

(69) Ranson JH, Rifkind KM, Turner JW. Prognostic signs and nonoperative peritoneal lavage in acute pancreatitis. Surg Gynecol Obstet 1976; 143(2):209-219.

(70) Knaus WA. APACHE II: a severity of disease classification system. Crit Care Med.1985 Oct;13[10], 818-829. 2006. Knaus WA, Draper EA Wagner DP Zimmerman JE.

(71) Wilson C, Heath DI, Imrie CW. Prediction of outcome in acute pancreatitis: a comparative study of APACHE II, clinical assessment and multiple factor scoring systems. Br J Surg 1990; 77(11):1260-1264.

(72) Larvin M, McMahon MJ. APACHE-II score for assessment and monitoring of acute pancreatitis. Lancet 1989; 2(8656):201-205.

(73) Paredes Cotore JP, Bustamante MM, Fernandez RE, Valeiras DE, Potel LJ. [Prognosis of acute pancreatitis: Ranson or APACHE II?]. Dig 1995; 87(2):121-126.

(74) Brisinda G, Maria G, Ferrante A, Civello IM. Evaluation of prognostic factors in patients with acute pancreatitis. Hepatogastroenterology 1999; 46(27):1990-1997.

(75) Johnson CD, Toh SK, Campbell MJ. Combination of APACHE-II score and an obesity score (APACHE-O) for the prediction of severe acute pancreatitis. Pancreatology 2004; 4(1):1-6.

(76) Werner J, Hartwig W, Uhl W, Muller C, Buchler MW. Useful markers for predicting severity and monitoring progression of acute pancreatitis. Pancreatology 2003; 3(2):115-127.

(77) Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. Crit Care Med 1995; 23(10):1638-1652.

(78) Bernard GR, Doig G. Quantification of organ failure for clinical trials and clinical practice. Am J Respir Crit Care Med 1995;(151):A323.

(79) Pease JE, Williams TJ. The attraction of chemokines as a target for specific anti-inflammatory therapy. Br J Pharmacol 2006; 147 Suppl 1:S212-S221.

(80) Aderem AA. How cytokines signal messages within cells. J Infect Dis 1993; 167 Suppl 1:S2-S7.

(81) Lowry SF. Cytokine mediators of immunity and inflammation. Arch Surg 1993; 128(11):1235-1241.

(82) Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation 2005; 12(5):255-269.

(83) Esmon CT. The interactions between inflammation and coagulation. Br J Haematol 2005; 131(4):417-430.

(84) Schwacha MG. Macrophages and post-burn immune dysfunction. Burns 2003; 29(1):1-14.

(85) Bone RC. Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med 1996; 24(1):163-172.

(86) Goris RJ, te Boekhorst TP, Nuytinck JK, Gimbrere JS. Multiple-organ failure. Generalized autodestructive inflammation? Arch Surg 1985; 120(10):1109-1115.

(87) Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. Chest 1997; 112(1):235-243.

(88) Pardee AB. The Yang and Yin of cell proliferation: an overview. J Cell Physiol Suppl 1987; Suppl 5:107-110.

(89) Gross V, Andreesen R, Leser HG, Ceska M, Liehl E, Lausen M et al. Interleukin-8 and neutrophil activation in acute pancreatitis. Eur J Clin Invest 1992; 22(3):200-203.

(90) Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. J Hepatobiliary Pancreat Surg 2002; 9(4):401-410.

(91) Exley AR, Leese T, Holliday MP, Swann RA, Cohen J. Endotoxaemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis. Gut 1992; 33(8):1126-1128.

(92) McKay C, Imrie CW, Baxter JN. Mononuclear phagocyte activation and acute pancreatitis. Scand J Gastroenterol Suppl 1996; 219:32-36.

(93) Norman JG. New approaches to acute pancreatitis: role of inflammatory mediators. Digestion 1999; 60 Suppl 1:57-60.

(94) Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S et al. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. J Clin Invest 1997; 100(7):1853-1862.

(95) McKay CJ, Gallagher G, Brooks B, Imrie CW, Baxter JN. Increased monocyte cytokine production in association with systemic complications in acute pancreatitis. Br J Surg 1996; 83(7):919-923.

(96) Banks RE, Evans SW, Alexander D, McMahon MJ, Whicher JT. Is fatal pancreatitis a consequence of excessive leukocyte stimulation? The role of tumor necrosis factor alpha. Cytokine 1991; 3(1):12-16.

(97) Dofferhoff AS, Bom VJ, Vries-Hospers HG, van Ingen J, vd MJ, Hazenberg BP et al. Patterns of cytokines, plasma endotoxin, plasminogen activator inhibitor, and acute-phase proteins during the treatment of severe sepsis in humans. Crit Care Med 1992; 20(2):185-192.

(98) de Beaux AC, Goldie AS, Ross JA, Carter DC, Fearon KC. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. Br J Surg 1996; 83(3):349-353.

(99) Jaffray C, Mendez C, Denham W, Carter G, Norman J. Specific pancreatic enzymes activate macrophages to produce tumor necrosis factor-alpha: role of nuclear factor kappa B and inhibitory kappa B proteins. J Gastrointest Surg 2000; 4(4):370-377.

(100) Denham W, Yang J, Fink G, Zervos EE, Carter G, Norman J. Pancreatic ascites as a powerful inducer of inflammatory cytokines. The role of known vs unknown factors. Arch Surg 1997; 132(11):1231-1236.

(101) Norman JG, Franz MG, Fink GS, Messina J, Fabri PJ, Gower WR et al. Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg 1995; 221(6):625-631.

(102) Heath DI, Cruickshank A, Gudgeon M, Jehanli A, Shenkin A, Imrie CW. Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. Gut 1993; 34(1):41-45.

(103) Pezzilli R, Billi P, Miniero R, Fiocchi M, Cappelletti O, Morselli-Labate AM et al. Serum interleukin-6, interleukin-8, and beta 2-microglobulin in early assessment of severity of acute pancreatitis. Comparison with serum C-reactive protein. Dig Dis Sci 1995; 40(11):2341-2348.

(104) Simovic MO, Bonham MJ, Abu-Zidan FM, Windsor JA. Anti-inflammatory cytokine response and clinical outcome in acute pancreatitis. Crit Care Med 1999; 27(12):2662-2665.

(105) Deviere J, Le Moine O, Van Laethem JL, Eisendrath P, Ghilain A, Severs N et al. Interleukin 10 reduces the incidence of pancreatitis after therapeutic endoscopic retrograde cholangiopancreatography. Gastroenterology 2001; 120(2):498-505.

(106) Zou WG, Wang DS, Lang MF, Jin DY, Xu DH, Zheng ZC et al. Human interleukin 10 gene therapy decreases the severity and mortality of lethal pancreatitis in rats. J Surg Res 2002; 103(1):121-126.

(107) McKay CJ, Curran F, Sharples C, Baxter JN, Imrie CW. Prospective placebo-controlled randomized trial of lexipafant in predicted severe acute pancreatitis. Br J Surg 1997; 84(9):1239-1243.

(108) Johnson CD, Kingsnorth AN, Imrie CW, McMahon MJ, Neoptolemos JP, McKay C et al. Double blind, randomised, placebo controlled study of a platelet activating factor antagonist, lexipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis. Gut 2001; 48(1):62-69.

(109) Haukim N, Bidwell JL, Smith AJ, Keen LJ, Gallagher G, Kimberly R et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 2. Genes Immun 2002; 3(6):313-330.

(110) Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis

factor-alpha concentrations and outcome of patients with severe sepsis. Crit Care Med 1996; 24(3):381-384.

(111) Holmes CL, Russell JA, Walley KR. Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. Chest 2003; 124(3):1103-1115.

(112) Nadel S, Newport MJ, Booy R, Levin M. Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. J Infect Dis 1996; 174(4):878-880.

(113) Mira JP, Cariou A, Grall F, Delclaux C, Losser MR, Heshmati F et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999; 282(6):561-568.

(114) Tang GJ, Huang SL, Yien HW, Chen WS, Chi CW, Wu CW et al. Tumor necrosis factor gene polymorphism and septic shock in surgical infection. Crit Care Med 2000; 28(8):2733-2736.

(115) Stuber F, Udalova IA, Book M, Drutskaya LN, Kuprash DV, Turetskaya RL et al. -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. J Inflamm 1995; 46(1):42-50.

(116) Barber RC, Aragaki CC, Rivera-Chavez FA, Purdue GF, Hunt JL, Horton JW. TLR4 and TNF-alpha polymorphisms are associated with an increased risk for severe sepsis following burn injury. J Med Genet 2004; 41(11):808-813.

(117) Imahara SD, O'Keefe GE. Genetic determinants of the inflammatory response. Curr Opin Crit Care 2004; 10(5):318-324.

(118) Pociot F, D'Alfonso S, Compasso S, Scorza R, Richiardi PM. Functional analysis of a new polymorphism in the human TNF alpha gene promoter. Scand J Immunol 1995; 42(4):501-504.

(119) Sandberg-Wollheim M, Ciusani E, Salmaggi A, Pociot F. An evaluation of tumor necrosis factor microsatellite alleles in genetic susceptibility to multiple sclerosis. Mult Scler 1995; 1(3):181-185.

(120) Jargen K, Demaine AG, Kingsnorth AN. Cytokine gene polymorphisms in acute pancreatitis. JOP 2000; 1(2):24-35.

(121) Zhang D, Li J, Jiang Z, Yu B, Tang X, Li W. The relationship between tumor necrosis factor-alpha gene polymorphisms and acute severe pancreatitis. Chin Med J (Engl) 2003; 116(11):1779-1781.

(122) Smithies AM, Sargen K, Demaine AG, Kingsnorth AN. Investigation of the interleukin 1 gene cluster and its association with acute pancreatitis. Pancreas 2000; 20(3):234-240.

(123) Emi M, Keicho N, Tokunaga K, Katsumata H, Souma S, Nakata K et al. Association of diffuse panbronchiolitis with microsatellite polymorphism of the human interleukin 8 (IL-8) gene. J Hum Genet 1999; 44(3):169-172.

(124) PS Chong et al. An Interleukin-8 Microsatellite Allele is associated with improved outcome
in predicted severe acute pancreatitis. Pancreatology 2001; 1(No. 2):118.

(125) Hull J, Ackerman H, Isles K, Usen S, Pinder M, Thomson A et al. Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. Am J Hum Genet 2001; 69(2):413-419.

(126) Hacking D, Knight JC, Rockett K, Brown H, Frampton J, Kwiatkowski DP et al. Increased in vivo transcription of an IL-8 haplotype associated with respiratory syncytial virus disease-susceptibility. Genes Immun 2004; 5(4):274-282.

(127) Mukaida N, Shiroo M, Matsushima K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 1989; 143(4):1366-1371.

(128) Balkwill FR, Burke F. The cytokine network. Immunol Today 1989; 10(9):299-304.

(129) Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. FEBS Lett 1992; 307(1):97-101.

(130) Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 1990; 347(6294):669-671.

(131) Horuk R. The interleukin-8-receptor family: from chemokines to malaria. Immunol Today 1994; 15(4):169-174.

(132) Baggiolini M, Dewald B, Moser B. Human chemokines: an update. Annu Rev Immunol 1997; 15:675-705.

(133) Ludwicka-Bradley A, Tourkina E, Suzuki S, Tyson E, Bonner M, Fenton JW et al. Thrombin upregulates interleukin-8 in lung fibroblasts via cleavage of proteolytically activated receptor-I and protein kinase C-gamma activation. Am J Respir Cell Mol Biol 2000; 22(2):235-243.

(134) Sakito O, Kadota J, Kohno S, Abe K, Shirai R, Hara K. Interleukin 1 beta, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. Respiration 1996; 63(1):42-48.

(135) Ma X, Reich RA, Wright JA, Tooker HR, Teeter LD, Musser JM et al. Association between interleukin-8 gene alleles and human susceptibility to tuberculosis disease. J Infect Dis 2003; 188(3):349-355.

(136) Campa D, Zienolddiny S, Maggini V, Skaug V, Haugen A, Canzian F. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. Carcinogenesis 2004; 25(2):229-235.

(137) Landi S, Moreno V, Gioia-Patricola L, Guino E, Navarro M, de Oca J et al. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. Cancer Res 2003; 63(13):3560-3566.

(138) Jiang ZD, Okhuysen PC, Guo DC, He R, King TM, DuPont HL et al. Genetic susceptibility to enteroaggregative Escherichia coli diarrhea: polymorphism in the interleukin-8 promotor region. J Infect Dis 2003; 188(4):506-511.

(139) Rovin BH, Lu L, Zhang X. A novel interleukin-8 polymorphism is associated with severe systemic lupus erythematosus nephritis. Kidney Int 2002; 62(1):261-265.

(140) Noah TL, Becker S. Respiratory syncytial virus-induced cytokine production by a human bronchial epithelial cell line. Am J Physiol 1993; 265(5 Pt 1):L472-L478.

(141) Smyth RL, Mobbs KJ, O'Hea U, Ashby D, Hart CA. Respiratory syncytial virus bronchiolitis: disease severity, interleukin-8, and virus genotype. Pediatr Pulmonol 2002; 33(5):339-346.

(142) Panet A, Khorana HG. Studies on polynucleotides. The linkage of deoxyribopolynucleotide templates to cellulose and its use in their replication. J Biol Chem 1974; 249(16):5213-5221.

(143) Baumforth KR, Nelson PN, Digby JE, O'Neil JD, Murray PG. Demystified ... the polymerase chain reaction. Mol Pathol 1999; 52(1):1-10.

(144) Bradley EL, III. A fifteen year experience with open drainage for infected pancreatic necrosis. Surg Gynecol Obstet 1993; 177(3):215-222.

(145) Bittner R, Block S, Buchler M, Beger HG. Pancreatic abscess and infected pancreatic necrosis. Different local septic complications in acute pancreatitis. Dig Dis Sci 1987; 32(10):1082-1087.

(146) Bassi C, Vesentini S, Nifosi F, Girelli R, Falconi M, Elio A et al. Pancreatic abscess and other pus-harboring collections related to pancreatitis: a review of 108 cases. World J Surg 1990; 14(4):505-511.

(147) Nathens AB, Curtis JR, Beale RJ, Cook DJ, Moreno RP, Romand JA et al. Management of the critically ill patient with severe acute pancreatitis. Crit Care Med 2004; 32(12):2524-2536.

(148) Beger HG, Buchler M, Bittner R, Block S, Nevalainen T, Roscher R. Necrosectomy and postoperative local lavage in necrotizing pancreatitis. Br J Surg 1988; 75(3):207-212.

(149) Beger HG, Bittner R, Block S, Buchler M. Bacterial contamination of pancreatic necrosis. A prospective clinical study. Gastroenterology 1986; 91(2):433-438.

(150) Gloor B, Muller CA, Worni M, Stahel PF, Redaelli C, Uhl W et al. Pancreatic infection in severe pancreatitis: the role of fungus and multiresistant organisms. Arch Surg 2001; 136(5):592-596.

(151) Ammori BJ, Leeder PC, King RF, Barclay GR, Martin IG, Larvin M et al. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 1999; 3(3):252-262.

(152) Rahman SH, Ammori BJ, Holmfield J, Larvin M, McMahon MJ. Intestinal hypoperfusion contributes to gut barrier failure in severe acute pancreatitis. J Gastrointest Surg 2003; 7(1):26-35.

(153) Howard TJ, Wiebke EA, Mogavero G, Kopecky K, Baer JC, Sherman S et al. Classification and treatment of local septic complications in acute pancreatitis. Am J Surg 1995; 170(1):44-50.

(154) Bradley EL, III. Operative vs. Nonoperative therapy in necrotizing pancreatitis. Digestion 1999; 60 Suppl 1:19-21.

(155) D'Egidio A, Schein M. Surgical strategies in the treatment of pancreatic necrosis and infection. Br J Surg 1991; 78(2):133-137.

(156) Banks PA, Gerzof SG, Langevin RE, Silverman SG, Sica GT, Hughes MD. CT-guided aspiration of suspected pancreatic infection: bacteriology and clinical outcome. Int J Pancreatol 1995; 18(3):265-270.

(157) Rau B, Pralle U, Mayer JM, Beger HG. Role of ultrasonographically guided fine-needle aspiration cytology in the diagnosis of infected pancreatic necrosis. Br J Surg 1998; 85(2):179-184.

(158) Wilson C, McArdle CS, Carter DC, Imrie CW. Surgical treatment of acute necrotizing pancreatitis. Br J Surg 1988; 75(11):1119-1123.

(159) Lankisch PG, Pflichthofer D, Lehnick D. No strict correlation between necrosis and organ failure in acute pancreatitis. Pancreas 2000; 20(3):319-322.

(160) Rau B, Uhl W, Buchler MW, Beger HG. Surgical treatment of infected necrosis. World J Surg 1997; 21(2):155-161.

(161) Farkas G, Marton J, Mandi Y, Szederkenyi E, Balogh A. Progress in the management and treatment of infected pancreatic necrosis. Scand J Gastroenterol Suppl 1998; 228:31-37.

(162) Fernandez-del Castillo C, Rattner DW, Makary MA, Mostafavi A, McGrath D, Warshaw AL. Debridement and closed packing for the treatment of necrotizing pancreatitis. Ann Surg 1998; 228(5):676-684.

(163) Freeny PC, Hauptmann E, Althaus SJ, Traverso LW, Sinanan M. Percutaneous CT-guided catheter drainage of infected acute necrotizing pancreatitis: techniques and results. AJR Am J Roentgenol 1998; 170(4):969-975.

(164) Ashley SW, Perez A, Pierce EA, Brooks DC, Moore FD, Jr., Whang EE et al. Necrotizing pancreatitis: contemporary analysis of 99 consecutive cases. Ann Surg 2001; 234(4):572-579.

(165) Hungness ES, Robb BW, Seeskin C, Hasselgren PO, Luchette FA. Early debridement for necrotizing pancreatitis: is it worthwhile? J Am Coll Surg 2002; 194(6):740-744.

(166) Connor S, Alexakis N, Raraty MG, Ghaneh P, Evans J, Hughes M et al. Early and late complications after pancreatic necrosectomy. Surgery 2005; 137(5):499-505.

(167) Beger HG. Operative management of necrotizing pancreatitis--necrosectomy and continuous closed postoperative lavage of the lesser sac. Hepatogastroenterology 1991; 38(2):129-133.

(168) Tenner S, Sica G, Hughes M, Noordhoek E, Feng S, Zinner M et al. Relationship of necrosis to organ failure in severe acute pancreatitis. Gastroenterology 1997; 113(3):899-903.

(169) Gotzinger P, Sautner T, Kriwanek S, Beckerhinn P, Barlan M, Armbruster C et al. Surgical treatment for severe acute pancreatitis: extent and surgical control of necrosis determine outcome. World J Surg 2002; 26(4):474-478.

(170) Connor S, Alexakis N, Neal T, Raraty M, Ghaneh P, Evans J et al. Fungal infection but not type of bacterial infection is associated with a high mortality in primary and secondary infected pancreatic necrosis. Dig Surg 2004; 21(4):297-304.

(171) Carter CR, McKay CJ, Imrie CW. Percutaneous necrosectomy and sinus tract endoscopy in the management of infected pancreatic necrosis: an initial experience. Ann Surg 2000; 232(2):175-180.

(172) Widdison AL, Karanjia ND. Pancreatic infection complicating acute pancreatitis. Br J Surg 1993; 80(2):148-154.

(173) Bassi C, Falconi M, Talamini G, Uomo G, Papaccio G, Dervenis C et al. Controlled clinical trial of pefloxacin versus imipenem in severe acute pancreatitis. Gastroenterology 1998; 115(6):1513-1517.

(174) Delcenserie R, Yzet T, Ducroix JP. Prophylactic antibiotics in treatment of severe acute alcoholic pancreatitis. Pancreas 1996; 13(2):198-201.

(175) Grewe M, Tsiotos GG, Luque de-Leon E, Sarr MG. Fungal infection in acute necrotizing pancreatitis. J Am Coll Surg 1999; 188(4):408-414.

(176) de Beaux AC, Palmer KR, Carter DC. Factors influencing morbidity and mortality in acute pancreatitis; an analysis of 279 cases. Gut 1995; 37(1):121-126.

(177) Knol JA, Eckhauser FE, Strodel WE. Surgical treatment of necrotizing pancreatitis by marsupialization. Am Surg 1984; 50(6):324-328.

(178) McCarthy MC, Dickerman RM. Surgical management of severe acute pancreatitis. Arch Surg 1982; 117(4):476-480.

(179) Teerenhovi O, Nordback I, Eskola J. High volume lesser sac lavage in acute necrotizing pancreatitis. Br J Surg 1989; 76(4):370-373.

(180) Nieuwenhuijs VB, Besselink MG, van Minnen LP, Gooszen HG. Surgical management of acute necrotizing pancreatitis: a 13-year experience and a systematic review. Scand J Gastroenterol Suppl 2003;(239):111-116.

(181) Gerzof SG. Percutaneous drainage of renal and perinephric abscess. Urol Radiol 1981; 2(3):171-179.

(182) Buunen M, Gholghesaei M, Veldkamp R, Meijer DW, Bonjer HJ, Bouvy ND. Stress response to laparoscopic surgery: a review. Surg Endosc 2004; 18(7):1022-1028.

(183) Baron TH, Thaggard WG, Morgan DE, Stanley RJ. Endoscopic therapy for organized pancreatic necrosis. Gastroenterology 1996; 111(3):755-764.

(184) Morgan DE, Baron TH, Smith JK, Robbin ML, Kenney PJ. Pancreatic fluid collections prior to intervention: evaluation with MR imaging compared with CT and US. Radiology 1997; 203(3):773-778.

(185) Ammori BJ. Laparoscopic transgastric pancreatic necrosectomy for infected pancreatic necrosis. Surg Endosc 2002; 16(9):1362.

(186) Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 2005; 36(6):697-705.

(187) Hanlon GW. The emergence of multidrug resistant Acinetobacter species: a major concern in the hospital setting. Lett Appl Microbiol 2005; 41(5):375-378.

(188) Bassi C, Pederzoli P, Vesentini S, Falconi M, Bonora A, Abbas H et al. Behavior of antibiotics during human necrotizing pancreatitis. Antimicrob Agents Chemother 1994; 38(4):830-836.

(189) Buchler M, Malfertheiner P, Friess H, Isenmann R, Vanek E, Grimm H et al. Human pancreatic tissue concentration of bactericidal antibiotics. Gastroenterology 1992; 103(6):1902-1908.

(190) Roberts EA, Williams RJ. Ampicillin concentrations in pancreatic fluid bile obtained at endoscopic retrograde cholangiopancreatography (ERCP). Scand J Gastroenterol 1979; 14(6):669-672.

(191) Cerra FB, Maddaus MA, Dunn DL, Wells CL, Konstantinides NN, Lehmann SL et al. Selective gut decontamination reduces nosocomial infections and length of stay but not mortality or organ failure in surgical intensive care unit patients. Arch Surg 1992; 127(2):163-167.

(192) Luiten EJ, Hop WC, Lange JF, Bruining HA. Controlled clinical trial of selective decontamination for the treatment of severe acute pancreatitis. Ann Surg 1995; 222(1):57-65.

(193) Mangiante G, Colucci G, Canepari P, Bassi C, Nicoli N, Casaril A et al. Lactobacillus plantarum reduces infection of pancreatic necrosis in experimental acute pancreatitis. Dig Surg 2001; 18(1):47-50.

(194) Trudel JL, Thompson AG, Brown RA. Prophylactic use of antibiotics in pancreatic sepsis: a 25-year reappraisal. Can J Surg 1984; 27(6):567-570.

(195) Howes R, Zuidema GD, Cameron JL. Evaluation of prophylactic antibiotics in acute pancreatitis. J Surg Res 1975; 18(2):197-200.

(196) Mithofer K, Fernandez-del Castillo C, Ferraro MJ, Lewandrowski K, Rattner DW, Warshaw AL. Antibiotic treatment improves survival in experimental acute necrotizing pancreatitis. Gastroenterology 1996; 110(1):232-240.

(197) Pederzoli P, Bassi C, Vesentini S, Campedelli A. A randomized multicenter clinical trial of antibiotic prophylaxis of septic complications in acute necrotizing pancreatitis with imipenem. Surg Gynecol Obstet 1993; 176(5):480-483.

(198) Sainio V, Kemppainen E, Puolakkainen P, Taavitsainen M, Kivisaari L, Valtonen V et al. Early antibiotic treatment in acute necrotising pancreatitis. Lancet 1995; 346(8976):663-667.

(199) Bassi C, Falconi M, Talamini G, Uomo G, Papaccio G, Dervenis C et al. Controlled clinical trial of pefloxacin versus imipenem in severe acute pancreatitis. Gastroenterology 1998; 115(6):1513-1517.

(200) Nordback I, Sand J, Saaristo R, Paajanen H. Early treatment with antibiotics reduces the need for surgery in acute necrotizing pancreatitis--a single-center randomized study. J Gastrointest Surg 2001; 5(2):113-118.

(201) Manes G, Rabitti PG, Menchise A, Riccio E, Balzano A, Uomo G. Prophylaxis with meropenem of septic complications in acute pancreatitis: a

randomized, controlled trial versus imipenem. Pancreas 2003; 27(4):e79-e83.

(202) Golub R, Siddiqi F, Pohl D. Role of antibiotics in acute pancreatitis: A meta-analysis. J Gastrointest Surg 1998; 2(6):496-503.

(203) Bassi C, Larvin M, Villatoro E. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis. Cochrane Database Syst Rev 2003;(4):CD002941.

(204) Powell JJ, Campbell E, Johnson CD, Siriwardena AK. Survey of antibiotic prophylaxis in acute pancreatitis in the UK and Ireland. Br J Surg 1999; 86(3):320-322.

(205) Isenmann R, Runzi M, Kron M, Kahl S, Kraus D, Jung N et al. Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo-controlled, double-blind trial. Gastroenterology 2004; 126(4):997-1004.

(206) Fraser VJ, Jones M, Dunkel J, Storfer S, Medoff G, Dunagan WC. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. Clin Infect Dis 1992; 15(3):414-421.

(207) Schmid SW, Uhl W, Friess H, Malfertheiner P, Buchler MW. The role of infection in acute pancreatitis. Gut 1999; 45(2):311-316.

(208) Howard TJ, Temple MB. Prophylactic antibiotics alter the bacteriology of infected necrosis in severe acute pancreatitis. J Am Coll Surg 2002; 195(6):759-767.

(209) Hoerauf A, Hammer S, Muller-Myhsok B, Rupprecht H. Intra-abdominal Candida infection during acute necrotizing pancreatitis has a high prevalence and is associated with increased mortality. Crit Care Med 1998; 26(12):2010-2015.

(210) De Waele JJ, Vogelaers D, Blot S, Colardyn F. Fungal infections in patients with severe acute pancreatitis and the use of prophylactic therapy. Clin Infect Dis 2003; 37(2):208-213.

(211) Arcaroli J, Fessler MB, Abraham E. Genetic polymorphisms and sepsis. Shock 2005; 24(4):300-312.

(212) Powell JJ, Fearon KC, Siriwardena AK, Ross JA. Evidence against a role for polymorphisms at tumor necrosis factor, interleukin-1 and interleukin-1 receptor antagonist gene loci in the regulation of disease severity in acute pancreatitis. Surgery 2001; 129(5):633-640.

(213) Buchler P, Reber HA. Surgical approach in patients with acute pancreatitis. Is infected or sterile necrosis an indication--in whom should this be done, when, and why? Gastroenterol Clin North Am 1999; 28(3):661-671.

(214) Connor S, Raraty MG, Howes N, Evans J, Ghaneh P, Sutton R et al. Surgery in the treatment of acute pancreatitis--minimal access pancreatic necrosectomy. Scand J Surg 2005; 94(2):135-142.

(215) Dervenis C, Johnson CD, Bassi C, Bradley E, Imrie CW, McMahon MJ et al. Diagnosis, objective assessment of severity, and management of acute pancreatitis. Santorini consensus conference. Int J Pancreatol 1999; 25(3):195-210.