NOVEL APPROACHES TO THE DIAGNOSIS AND MANAGEMENT OF SEVERE

ACUTE PANCREATITIS.

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Charles Joseph Miranda

The School of Medical Sciences

Contents

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Abbreviations

ACP	- Abdominal compartment pressure
ACS	- Abdominal compartment syndrome
AE	- Adverse event
APACHE II	- Acute physiology and chronic health evaluation score, version 2
AP	- Acute pancreatitis
APP	- Abdominal perfusion pressure
CECT	- Contrast enhanced computed tomography
CNI1493	- Semapimod (a synthetic inhibitor of signal transduction, which inhibits phosphorylation of P38 MAPK)
CVP	- Central venous pressure
ERK 1/2	- Extracellular signal-regulated Kinases
EWS	- Early warning score
i.v.	- Intravenous
IAH	- Intra-abdominal compartment hypertension
IAP	- Intra-abdominal pressure
IL-6	- Interleukin 6;
JNK	- c-Jun N-terminal Kinases
LODS	- Logistic organ dysfunction score
MAP	- Mild acute pancreatitis
MAPK	- mitogen-activated protein kinases.
mmHg	- Millimetres of mercury
mmol	- Millimoles
MOD	- Marshall organ dysfunction score
MODS	- Multiple organ dysfunction syndrome
MPO	- Myeloperoxidase

mRNA   - messenger ribonucleic acid

MSAP   - Moderately severe acute pancreatitis

PaO2   - Partial pressure of oxygen in blood

pH   - Power of Hydrogen

rhAPC   - Recombinant human activated protein C

SAE   - Severe adverse event

SAP   - Severe acute pancreatitis

SIRS   - Systemic Inflammatory response syndrome

SOFA   - Sepsis-related organ failure assessment score

TNF-α   - Tumor necrosis factor alpha

Abstract

University of Manchester   Charles Joseph Miranda   Doctor of Medicine

THESIS TITLE: Novel approaches to the diagnosis and management of severe acute pancreatitis.

DATE : January 2016

INTRODUCTION: Severe Acute Pancreatitis (SAP) is the rapid onset of inflammation within the pancreatic organ. Unlike the milder form of this illness, SAP is associated with a high mortality and morbidity. No significant reduction in the outcomes of this disease has been made since the implementation of organ supportive management over two decades ago. This is due to difficulties in distinguishing between the milder form of the disease in the early period of the onset of symptoms when clinical intervention is most likely to prevent complications and death. Clinical equipoise exists in the management of one of these complications, namely Abdominal Compartment Syndrome (ACS) as the conventional management of surgery runs contrary to published evidence showing early abdominal surgery deteriorates clinical outcomes.

AIMS: Validation of the potential use of the Early Warning Score (EWS) as a predictor of SAP. Evaluation of the evidence for recombinant human protein C (Xigris™) in the early treatment of SAP. Determination of the safety profile of Xigris™ when given early in SAP. To determine if surgical management of ACS in SAP is of significant benefit compared to conventional management alone.

METHODS: Four studies were performed: A prospective observational study assessing the median EWS of patients admitted with acute pancreatitis; a systematic review of published evidence reporting the use of Xigris™ in SAP; a prospective cohort study using a 24 hour infusion of Xigris™ early in patients diagnosed with SAP and a pilot randomized controlled trial of targeted decompression in patients with ACS complicating SAP.

RESULTS: The highest EWS values for 130 patients with acute pancreatitis within the first 3 days of admission were not shown to have significant sensitivity and specificity in predicting an unfavourable outcome. A review of the published literature between from January 1985 to January 2011 supported the further investigation of Xigris™ as a treatment for SAP. No significant adverse events or differences in outcomes were evident in 19 patients who received a 24-hour infusion of Xigris™ early in SAP compared to matched historical controls. 22 patients were screened for the development of ACS. No patient developed ACS and consequently no randomization to either treatment arm was possible.

CONCLUSION: With the recent advent of an updated classification system for the severity of acute pancreatitis, further prospective evaluation of the use of EWS in clinical practice is warranted. The results of the Phase 1 clinical trial of Xigris™ did not reveal significant safety issues that might preclude the further investigation of Xigris™ as a specific therapy early in the onset of SAP. The absence of ACS in patients with SAP lends support to a theory that ACS may be an epiphenomenon in the course of SAP.

Declaration

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Publications arising from this work

The following research articles have been published from Chapter 3 (1) and Chapter 4 (2) of this thesis.

1. *Original article:* Twenty-four hour infusion of human recombinant activated protein C (Xigris™) early in severe acute pancreatitis: The XIG-AP 1 trial. <u>Charles J Miranda</u>, James M Mason, Benoy I Babu, *et al.* Pancreatology 2015 Nov;15 (6):635-41.

2. *Original article:* Recombinant human activated protein C as a disease modifier in severe acute pancreatitis: Systematic review of current evidence. <u>Charles J Miranda</u>, Benoy I Babu, Ajith K Siriwardena. Pancreatology 2012 Mar;12 (2):119-23.

3. *Comment:* Intra-Abdominal Pressure and Abdominal Perfusion Pressure Early in Severe Acute Pancreatitis Misses the Forest for the Trees. <u>Charles J Miranda</u>. J Gastrointest Surg 2011 Sep.

The author

I graduated from St. John's Medical College, University of Bangalore, India in 2001. Upon the completion of mandatory house officer training in India, I began training in general surgery in the United Kingdom. This culminated in membership of the Royal College of Surgeons of Edinburgh in 2007.

Dedication

This work is dedicated to my wife, Richa Miranda, for her support, and my son,

Calvin Miranda.

CHAPTER 1:

Background

1.1 Disease definition and classification

1.1.1 *The human pancreas*

The human pancreas is a gland with dual endocrine and exocrine roles that lies in the retro-peritoneum between the second part of the duodenum and hilum of the spleen. It secretes approximately 1500 – 3000 ml of iso-osmotic fluid per day, which is alkaline in nature (pH >8) and comprises of a mixture of around 20 enzymes. This pancreatic fluid is responsible for the function of an effective digestive system by performing the dual roles of providing digestive enzymes necessary for the breakdown of ingested food into smaller molecules for absorption and in creating the optimal pH for the function of these enzymes.

1.1.2 *Acute pancreatitis*

Acute pancreatitis is inflammation of the pancreas that typically manifests with epigastric pain of sudden onset, which often radiates to the back. Vomiting, nausea, increased sweating and fever may accompany this pain. Conditions such as choledocholithiasis, cholecystitis; ischemic bowel; perforated viscus, myocardial infarction and bowel obstruction also present with similar symptoms and need to be excluded as their management is different. In order to diagnose acute pancreatitis it is necessary for at least two of the following to be present (3):

- Serum levels of amylase or lipase to be thrice the normal upper limit
- Typical upper abdominal pain as described.

- Cross-sectional imaging and analysis to exclude other conditions, if clinically warranted.

1.1.3 Incidence of acute pancreatitis

The incidence and global distribution of acute pancreatitis is heterogeneous due to the self-limiting nature of the mild version of the disease and variations in healthcare systems across different countries. Variance in pre-disposing factors such as the availability and ingestion of alcohol due to cultural factors may also contribute to this variation. In the United Kingdom, a figure of 30 per 100,000 population was reported in 2013 (4), an increase from a previous report of 22.4 per 100,000 population in 2008 (5). The reasons for this increase may be due to increased awareness, changes in socio-economic conditions, improved awareness and routine testing of pancreatic enzymes in patients presenting with acute abdominal pain (6, 7).

Despite improvements in the diagnosis of acute pancreatitis, a significant reduction in mortality has not occurred. Overall mortality in patients admitted with acute pancreatitis has been reported as ranging between 2-22% (7-9). Mortality was previously considered to be bimodal with death occurring early, due to multi-organ dysfunction, or later, from complications linked to the development of sepsis following pancreatic necrosis as a result of the inflammation. This concept has since been revised following a retrospective analysis of patients in a North American population by Mutinga *et al* (8) who concluded "Approximately half of deaths in acute pancreatitis occur within the first 14 days owing to organ failure and the remainder of deaths occur later because of complications associated with

necrotising pancreatitis. Reducing the mortality in the future will require innovative approaches to counteract early organ failure and late complications of necrotising pancreatitis."

1.1.4 Causes of pancreatitis

Alcohol and gallstones constitute the primary causes of acute pancreatitis in many parts of the world (10, 11). Other causes are pancreatic ductal obstruction, trauma, drugs, infectious agents and hypertriglyceridemia. About 10-25% of acute pancreatitis cases appear to have no discernible cause but often turn out to be caused by microlithiasis (12, 13), genetic mutations in the trypsinogen gene (14) or the cystic fibrosis gene (15).

Aetiology	Salient Features
Cholelithiasis	Gallstone obstruction of ampulla results in reflux of bile. Oedema during the passage of stone is another potential inciting event. Variable incidence (35-60%)
Alcohol	Second most common cause. Alcohol lowers threshold for trypsin activation and sensitizes the pancreas to injury via various mechanisms such as calcium signalling, zymogen secretion, unfolded protein response and by altering mitochondrial membrane integrity.
Smoking	Independent dose dependent risk factor. Synergistic effect with alcohol.
Post ERCP	Due to mechanical trauma to the papilla leading to obstruction to the outflow of pancreatic juice and administration of radiological contrast media.
Hypertriglyceridemia	Most commonly found in association with gestational pancreatitis.
Hypercalcemia	Very rare. Usually a diagnosis of exclusion.
Autoimmune	Steroid responsive and commonly associated with chronic pancreatitis.
Hereditary	Autosomal dominant with 80% penetrance. Mutation of Serine protease 1 gene (PRSS1) on chromosome 7q35 which encodes trypsinogen.
Congenital malformation	E.g. Pancreatic divisum.
Drug induced	6-mercaptopurine, sulfanilamide, sulphonamides, diuretics, valproic acid, tetracycline, azathioprine, oestrogen and corticosteroids have been mooted as causative factors for pancreatitis in the absence of the other causes listed.
Traumatic	Blunt and penetrating trauma. Rare due to retroperitoneal location.
Infectious	Various bacteria, fungi, virus and parasites have been implicated as potential etiological factors for pancreatitis.
Miscellaneous	Haemorrhagic shock, Systemic lupus erythematosus, polyarteritis nodosa, Thromboembolism. Pregnancy

Table 1.1: Aetiology of pancreatitis (16, 17). Conditions that are associated with an increase the likelihood of developing acute pancreatitis.

1.1.5 The pathophysiology of pancreatitis

The mechanisms, by which pancreatic inflammation is triggered before either resolving or progressing to necrosis, are still unclear. Current consensus holds that the initial event is an insult to acinar cell component of the pancreatic parenchyma, which in turn initiates a local inflammatory response (18-20). A better understanding of the processes behind the development and progression of the disease and its sequela would offer insights into new avenues of management.

1.1.5.1 Pancreatic parenchymal inflammation

Acinar cell injury occurs due to zymogen activation (conversion of the pro-enzyme form of trypsinogen to its active form) which can be triggered by a variety of mechanisms such as Cathepsin B (21) or the free cytosolic calcium ion mediated pathways (22). Once acinar cell injury has been initiated, cascades of other enzymes are also released into the surrounding parenchyma and adjacent tissues. These enzyme groups predominantly consist of peptidases and elastases resulting in the breakdown of cellular membranes and loss of connective tissue responsible for the maintenance of the organ's integrity and the vascular network responsible for oxygenation, nutrition and removal of respiratory end-products. There is evidence from both experimental models (23) and clinical studies (24), that microvascular thrombosis in the pancreatic vascular bed is a mediator of pancreatic parenchymal necrosis and is also involved in the endothelium-inflammatory cell interplay.

1.1.5.2 Systemic response to pancreatic inflammation.

The products of cellular breakdown arising from the acinar cell injury and enzymatic auto-digestion of the gland and surrounding tissues, activates a systemic response to the inflammation via inflammatory cytokines. Inflammatory mediators that have been demonstrated to play a role in the pathogenesis of a systemic inflammatory response syndrome (SIRS) (25) following the advent of pancreatic inflammation include: pro-inflammatory cytokines (tumor necrosis factor-a (TNF-α), interleukin-1b (IL-1b), interleukin-6 (IL-6) and Interleukin 8), arachidonic acid metabolites (such as platelet activating factor (PAF), prostaglandins and leukotrienes), intercellular adhesion molecule-1 (ICAM-1), complement component C5a, substance P, heat shock proteins, cyclo-oxygenase and hydrogen sulfide (16, 17, 19, 20, 26).

Excessive activation of the systemic inflammatory response cascade leads to multiple organ failure (27). The inflammatory mediators described, lead to end-organ endothelial cell activation which enhances permeability (28). This enhanced permeability in microvascular system leads to third space fluid loss and systemic hypo-perfusion due to the lost intravascular volume. When coupled with vasodilatation, this results in the clinical symptoms of shock and hypotension. Due to the collection of inflammatory cells within tissues, activation of the coagulation cascade and the resultant microvascular thrombosis leads to a system wide oxygen deficit within metabolically active tissues. This initially manifests clinically as Systemic Inflammatory Response Syndrome (SIRS).

SIRS (25) is identified by the presence of two or more of the following criteria:

- Temperature > 38° Celsius or < 36° Celsius

- Respiration >20 beats/min

- Pulse >90 beats/min

- White blood cell count > 12,000 cells/mm^3 or < 4000 cells/mm^3 or more than 10% immature forms.

Detecting the presence of SIRS gives critical prognostic data. 25-60% of patients present with SIRS on admission (29, 30), but this usually resolves within 24 hours for more than 50% of these patients upon adequate fluid resuscitation (29). A persistent SIRS within the first 24 hours following admission and fluid resuscitation increases the risk of persistent organ failure and necrosis thereby increasing the risk of death (30). The chances of mortality are 11-25% in patients with persistent SIRS that lasts over 48 hours (30, 31).

1.1.5.3. Organ failure in acute pancreatitis.

Multiple organ dysfunction syndrome (MODS) is the usual cause of death in SAP. The currently accepted definition of MODS is that proposed by the 1991 Consensus Conference of the American College of Chest Physicians and the Society of Critical Care Medicine as 'the presence of altered organ functions in an acutely ill patient such that homeostasis cannot be maintained without intervention' (32). The typically affected organ systems and their supportive therapy are (33):

- Respiratory: requiring artificial ventilation

- Cardiovascular : requiring ionotropic support

- Renal : Haemodialysis or haemofiltration.

The exact mechanisms by which a generalised systemic inflammatory response, characterised by SIRS, progresses towards organ specific impairment have not been conclusively determined. Nonetheless more than 50% of patients with pancreatitis display symptoms of organ dysfunction at admission (34). MODS usually develops within the first four days following admission (35). The mortality associated with SAP occurs in over 50% of patients that manifest MODS within the first week of onset of disease (36).

1.1.5.4. Pancreatic microvascular dysfunction in acute pancreatitis

As previously described, the vascular endothelium of the pancreas and other organ systems sustains damage from the released digestive enzymes into the general circulation (37-39). Experimental models in animal studies have demonstrated microcirculatory changes, ranging from mild vasoconstriction to frank ischemia. In animal studies injection of various microspheres led to the development of acute pancreatitis due to end artery occlusion; a similar effect was not observed in the obstruction of larger vessels due to the development of collateral circulation. The microcirculatory changes usually amplify the pancreatic insult. Pancreatic ischemia is known to cause glandular oedema with elevated serum amylase; but the acinar cell injury leading to the activation of digestive zymogens, remains unanswered. This impairment of microcirculation during acute pancreatitis certainly plays a role in the progression of the disease. Altered vascular permeability, mediated by bradykinin, and free oxygen radicals has been

demonstrated in in-vivo models (40, 41). Increased permeability of the endothelium to large molecules such as albumin is a hallmark of endothelial inflammation and is often observed in acute pancreatitis. Microcirculatory changes and consequent ischemia-reperfusion injuries, if not a causative factor, could certainly play a role to aggravate the disease process leading to necrosis of the pancreatic parenchyma.

1.2   Diagnosis

1.2.1   Signs and symptoms of acute pancreatitis

The most common symptom is pain that the patient perceives to originate from within the abdomen. This is seen in close to 95% of patients with acute pancreatitis. It is of sudden onset, persistent, boring and deep in nature. The pain is often associated with nausea and vomiting. The site for the pain appears to arise over epigastric and periumblical region before radiating to back, chest, flank or lower abdomen. A typical sign associated with acute pancreatitis is the tendency of patients to be restless and bend forward (Knee Chest position) in an effort to relieve the pain (42).

The physical findings of the patient are extremely variable and cannot be used to grade the severity of the disease. Abdominal tenderness may or may not be accompanied by guarding. Other abdominal findings include hypoactive bowel sound which may progress to ileus, epigastric distension (42).

Radiographically, a prominent gas filled '*sentinel loop'* in the small intestine; due to spread of inflammation from peripancreatic region, may be seen. Likewise the '*Colon cut off sign*' may be produced due to the spread of the same to the transverse colon(43).

Systemic findings may include a low grade fever, respiratory insufficiency, pleural effusion and hypotension due to fluid sequestration in the peri-pancreatic region. Some patients may present with mild jaundice due to choledochal obstruction,

hematemesis and or melena. Hypocalcemia may be evident as muscular spasm. Physical examination may reveal a patient who is listless, diaphoretic with variable degree of hemodynamic disturbances.

Rare physical signs include 'Cullen sign' which refers to the bluish discoloration at periumblical region because of underlying hemoperitoneum and the "Grey-Turner sign" which comprises of a reddish brown discoloration along the flanks due to retroperitoneal blood dissecting along tissue plane (44).

1.2.2   Classification of acute pancreatitis

Given the importance of MODS in the presence of acute pancreatitis as the main cause of death, efforts to determine those individuals at highest risk of mortality and morbidity have focused on the early identification of those with the highest risk of developing MODS using organ failure scoring systems.

1.2.1.1 The 1992 classification of acute pancreatitis

The need to identify individuals with acute pancreatitis with an increased risk of high mortality and morbidity was addressed by a symposium on acute pancreatitis held at Atlanta, USA in 1992. The consensus from that symposium was published the following year (45) and gave definitions for a mild and severe form of acute pancreatitis:

Mild acute pancreatitis (MAP) – acute inflammation of the pancreas associated with a threefold rise in serum amylase/lipase without features associated with SAP.

Severe acute pancreatitis (SAP) – features of MAP in addition to:

- 3 or more Ranson (46) criteria or 8 or more APACHE II (Acute physiology and chronic health evaluation) points.

- Organ failure (defined as a systolic blood pressure < 90 mmHg, pulmonary insufficiency with a PaO_2 < 60 mmHg, renal failure with a creatinine level > 177 μmol/L (2 mg/dL) after rehydration, or gastrointestinal bleeding > 500 mL/24 hours).

- Systemic complications such as disseminated intravascular coagulopathies due to platelets < 100,000/mm^3; fibrin split products of > 80 μg/mL; or calcium levels < 1.87 mmol/L (7.5 mg/dL) may also be present.

Subsequent analysis of the definitions revealed shortcomings in their practical application, namely the presence of a group of patients meeting the criteria for SAP but with a significantly lower mortality than expected (47). This is understandable in light of the fact that the classification system was meant to be *post hoc*. Consequently there was the potential for significant variability between the outcomes of patients admitted with severe acute pancreatitis across units and geographical areas.

1.2.1.2 The 2013 classification of acute pancreatitis.

A review of the evidence was undertaken and a revised classification system was published in 2013 (48). The current classification of pancreatitis recognizes an early and late phase of the disease. It also seeks to describe two states of the disease, namely:

Interstitial Oedematous Pancreatitis – is characterised by inflammatory oedema of the pancreatic parenchyma which leads to the enlargement of the pancreas, either diffusely or within a local area. The illness is self-limiting, with imaging showing homogeneous enhancement in pancreas with/without peripancreatic fluid collection.

Necrotising Pancreatitis – occurs in approximately 10% of patients presenting with acute pancreatitis. Necrosis of both the pancreatic parenchyma and peri-pancreatic tissue is present. The ischemic insult and consequent pancreatic necrosis usually develop over a period of days, leading to underestimation by abdominal imaging in the initial period. These patients have a significantly higher mortality compared to those with interstitial oedematous pancreatitis.

The new consensus report (48) recommends that patients admitted with acute pancreatitis be classified into one of 3 categories in order to better understand the progress of the disease and evaluate research aimed at restricting the morbidity and mortality associated with it. These categories are:

- *Mild acute pancreatitis (MAP)*: no organ failure, local or systemic complications and usually resolves in the first week.

- *Moderately severe acute pancreatitis (MSAP)*: the presence of transient organ failure, local complications or exacerbation of co-morbid disease.

- *Severe acute pancreatitis (SAP)*: persistent organ failure >48 hours and associated with local complications such as peripancreatic fluid collections, pancreatic and peripancreatic necrosis (sterile or infected), pseudocyst and walled-off necrosis (sterile or infected).

Mild disease does not result in organ failure, local or systemic complications. Pancreatic imaging analysis is not necessary in MAP and discharge usually takes place three to five days after onset of disease. MSAP is characterised by transient organ failure, as well as local or systemic complications (49, 50). Transient organ failure typically lasts for 48 hours and patients with MSAP usually need a prolonged hospital stay but have a better prognosis than SAP. SAP manifests with persistent organ failure that is, that which is present for over 48 hours. Typically, patients with persistent organ failure are at a high risk of developing pancreatic necrosis with a concomitant mortality rate of 30% (51). Of note is a move away from previous reliance on the APACHE II, Ranson (46) and SOFA (52) (Sepsis-related Organ Failure Assessment) scores as determinants of organ failure. The current system recommends the use of the Marshall Organ Dysfunction (53) (MOD) score as an indicator of organ dysfunction. Within the context of acute pancreatitis, the 2013 classification uses the following definitions (Table 2):

Term	Definition
Interstitial oedematous pancreatitis	Acute inflammation of pancreas without any evidence of necrosis, with Contrast Enhanced Computed Tomography (CECT) showing pancreatic parenchymal enhancement by intravenous contrast agent.
Necrotising pancreatitis	Inflammation combined with pancreatic or peripancreatic necrosis.
Acute peripancreatic fluid collection	Peripancreatic fluid collection associated with interstitial oedematous pancreatitis without any evidence of necrosis. It refers to collection within the first 4 weeks after the onset of oedematous pancreatitis and without any features suggestive of pancreatic pseudocyst. CECT in these patients is characterised by homogeneous collections with fluid density lying adjacent to pancreas without any intrapancreatic extension and confined within peripancreatic fascial planes.
Pancreatic pseudocyst	Encapsulated fluid confined by an inflammatory wall lying outside the pancreas with minimal or no evidence of necrosis. Usually occurs more than 4 weeks after symptom onset. CECT image is characterised by a well circumscribed, encapsulated cavity with no non-liquid component and well defined capsule.
Acute necrotic collection	A collection commonly associated with the necrotic form of pancreatitis. It contains a variable amount of fluid and necrotic pancreas or peripancreatic tissue. The CECT picture is characterised by heterogeneous and non-liquid density of varying degree in different locations.
Walled off necrosis	A mature collection of either pancreatic or peripancreatic necrotic tissue, bounded by a well-defined inflammatory wall. Usually occurs after 4 weeks of the onset of necrotising pancreatitis.

Table 1.2: Current definitions in the 2013 classification of acute pancreatitis (Banks *et al*, 2013).

The introduction of the revised classification of acute pancreatitis (48) was after the design and execution of the individual studies that comprise this thesis, hence the terms *mild acute pancreatitis* and *severe acute pancreatitis*, will refer to the pre-existing 1993 classification system in use at the time the research was conducted (45).

1.2.3 Organ failure scoring systems in acute pancreatitis

Clinical organ failure is assessed by means of organ failure scoring systems. In the context of acute pancreatitis, 'The Acute Physiology and Chronic Health Evaluation II score'(APACHE II) (54), Marshall Organ Dysfunction (MOD) score (53); Logistic Organ Dysfunction System (LODS) (55) and Sepsis-related Organ Failure Assessment (SOFA) (52) are the most commonly used organ failure scoring systems.

APACHE II was initially created to contend with patients with critical morbidity in intensive care units (56). Twelve physiologic measures are used to determine the score as well as extra points stemming from the age of the patient and whether the disease is chronic. Among the scoring systems associated with acute pancreatitis, APACHE II is probably the most widely studied (57-59). It suffers from the limitation that in comparison to other scoring systems, it requires an extensive set of values before it can be calculated. Although it has been used for daily assessment of organ failure, this use has not been prospectively validated in acute pancreatitis. Pancreatitis specific prognostic indicators also include the Ranson criteria (46) as well as the Imrie score (60) (also known as Glasgow system). It takes 48 hours to complete the collection of data for Imrie and Ranson scoring

systems, although they are the most commonly used in clinical practice (46, 61) compared to the intricate APACHE II scoring system. Although no explicit reason for this has been determined, it is likely due to the simplicity in the calculation of these scores in comparison to the unwieldy APACHE II system. In a prospective study of 181 patients, Mason *et al* (62) showed that LODS, MOD, SOFA and APACHE II performed similarly at 24 hours (Pearson r range, 0.48-0.78; P < 0.01) and at 48 hours (Pearson r range, 0.48-0.67; P < 0.01) in all comparisons for sensitivity and specificity.

The organ dysfunction scores described categorise deteriorating organ function through the use of an ordinal scale or graded scores. The cumulative score gives the level of severity for a specific organ system as well as the general severity of organ failure. The interpretation of this cumulative score enables mortality to be predicted on the basis of the mortality rates seen in the research patients who used to formulate the original scoring system (63). When used as part of a classification system, it assumes that patients with the highest risk of mortality when admitted with acute pancreatitis must therefore have the severe form of the disease. The caveat is that some individuals may be assessed as of higher risk of mortality due to chronic health conditions and physiological age. These cannot be varied during the course of an acute admission. Therefore an older individual with chronic disease and mild acute pancreatitis *may* achieve the same score as a younger person with severe disease and significant organ dysfunction. Although the risk of mortality is the same, the management required to prevent an adverse outcome is likely to be dissimilar.

1.2.4 Systemic scoring systems in acute pancreatitis.

Given the complexity of the organ failure scoring systems described in the previous section, the use of non-organ specific physiological scoring systems in determining the severity of acute pancreatitis has been investigated. Two such systems are the Early Warning Score (EWS) (64) and the SIRS score (25). EWS is a simple physiological scoring system, measured at hourly interval and derived from simple parameters like blood pressure, urine output, respiratory rate, pulse rate and the conscious level with a number assigned to each derangement, the total of which gives a final score.

The use of EWS in acute pancreatitis has been investigated by Garcea *et al* (65, 66). Using a retrospective set of patient data, the EWS scores for 110 patients admitted with acute pancreatitis were compared with APACHE II, Imrie, CT grading scores and Ranson criteria. In this study, EWS emerged as the best predictor of adverse outcome within 24 hours of admission. A EWS of more than 3 in the first 24 hours was shown to predict a possible adverse outcome with a sensitivity and specificity of 70.0% and 79.1% respectively. It was also the most accurate predictor of mortality on day 3 of admission with a negative predictive value of 94.3% and 92.0% respectively. The failure of EWS to respond to treatment or deterioration in values from admission up till day 3 was also associated with an increased mortality.

A similar retrospective study using a SIRS score in lieu of EWS was recently reported by Kumar *et al* (67). The outcomes of 117 patients admitted with acute pancreatitis were assessed on the basis of patients categorised into 2 groups on the day of admission. These were negative SIRS group (less than 2 SIRS criteria)

and a positive SIRS group (2 or more criteria). In contrast to preceding investigations into SIRS, daily values for a period of 14 days following admission were available. The group concluded that the sensitivity and the negative predictive values of SIRS used to assign patients into the positive SIRS group on day of admission were very high, ranging from 73% to 100%, when predicting the adverse events associated with SAP. The specificity ranged from 55% to 62%. However the positive predictive value was low, ranging between 5 to 36% for the same adverse outcomes of SAP.

Despite the promising results it should be noted that both studies had multiple values available throughout the day. The decision in both methodologies to use the highest values contributed to a reduction the specificity and made analysis subject to outlying values. Another potential disadvantage of physiological scoring systems is that their use remains restricted to a non-critical care setting. The institution of supportive therapies such as mechanical ventilation and ionotropic support alters the descriptive nature of the observations. Further prospective evaluation of physiological scoring systems in different centres is required, to rule out local variations in management of acute pancreatitis and the effect of local critical care admission policies.

1.3 Summary

In conclusion, despite more than two decades of investigation and research there remains significant scope for improvement in the outcomes following the development of SAP in humans. It is anticipated that such benefits will derive from:

1. Improved classification of disease severity in the early stages of the disease. This is the first step to the successful management of any disease. Although the presence of acute pancreatitis is easily confirmed, the need exists for clinical validation of a scoring system for the early diagnosis of SAP from the mild version of pancreatitis, which is simple, yet robust, to use in a busy clinical setting. Once validated, this system would permit the foundation for further research into targeted management and assessment of outcomes.

2. Identification of a potential therapeutic agent via means of a systematic review of published literature.

3. Following the critical assessment of the scientific evidence and provided that sufficient justification exists, a Phase 1 clinical trial is required to assess the safety of the therapeutic agent and identify potential adverse effects.

4. Despite optimal conventional treatment, complications arise during the management of any disease. Hence investigation into the optimal management of any of the complications arising from SAP will also contribute to the improvement of clinical outcomes.

CHAPTER 2:

The Value of the Early-Warning Score and Determination of Mortality in Acute Pancreatitis.

2.1 Abstract

Background: The early and accurate determination of the severity and predicted clinical outcome of patients admitted with acute pancreatitis would greatly aid in the reduction of mortality and morbidity. Current clinical methods are complex and reliant upon invasive procedures and laboratory assays. A simple physiological assessment known as the Early Warning Score (EWS) was proposed a good indicator of mortality in acute pancreatitis but had not been validated for use in prospective patient cohort.

Methods: A pilot prospective observational study. EWS was compared with the Acute Physiological and Chronic Health Evaluation II (APACHE II) scores used in the Atlanta 1992 criteria for determining the severity of acute pancreatitis. The sensitivity and specificity of EWS was assessed using Receiver Operator Characteristic Area Under the Curve (AUC) for the end points of mortality and severity.

Results: Data was collected on 130 patients admitted with acute pancreatitis between July 2010 and February 2012. APACHE II showed an AUC of 0.980 ($P<0.05$) compared to AUCs of 0.586, 0.608 and 0.500 for days 1 to 3 using the highest EWS score in predicting mortality.

Conclusion: Despite the easier implementation of EWS, it was not shown to be as sensitive and specific in predicting mortality from acute pancreatitis compared to APACHE II scores.

2.2   Introduction

Patients admitted to hospital for pain caused by inflammation of their pancreas can vary in their presentation. Most will have a self-limiting disease but up to one third of patients with acute pancreatitis have the severe form of the disease characterized by pancreatic and peri-pancreatic tissue necrosis, an intense systemic inflammatory response and multiple organ failure. The severe form of the disease carries a 10% risk of mortality (31). Mofidi *et al* (31) demonstrated that the development of systemic inflammatory response (SIRS) within the first 48 hours of admission was associated with raised cumulative Marshall scores and reduced survival. This was subsequently corroborated by Kumar et al with a retrospective review of patients admitted with acute pancreatitis and assessed by means of a SIRS score. Early identification of patients with SIRS allows for the allocation of scarce intensive care facilities and aggressive resuscitation. The differentiation of patients with mild self-limiting pancreatitis or severe pancreatitis, with a higher risk of mortality, using organ dysfunction scores allows for the evaluation of therapeutic approaches targeting the latter.

The most popular organ dysfunction system, the Acute Physiology and Chronic Health Evaluation II (68) allows for the evaluation of interventions in the alteration of mortality across various diseases and is well validated. It requires the recording of 12 different physiological measurements, is calculated at admission within the first 24 hours of admission. Once calculated no further validated score can be computed. The calculation of the score is unwieldy, requiring the use of a table or calculator. As is the case with many other organ dysfunction scores, it requires data from an arterial blood gas investigation, haematological and biochemical investigations. The procedures for obtaining these results (venepuncture and

arterial blood aspiration) are usually outside the clinical remit of nursing staff responsible for documenting patient physiological observations. Furthermore, it is impractical to conduct these haematological investigations on an hourly basis, which precludes the early detection of organ function deterioration, outside of an intensive care setting.

The Early Warning Score (EWS) is based upon that initially proposed by Morgan *et al* (69)and consists of 6 basic parameters: blood pressure; heart rate; urine output; temperature, respiratory rate and Glasgow Coma Score.

Garcea et al (70) explored the utility of EWS in the prediction of survival for patients admitted with acute pancreatitis. The initial retrospective analysis of a 110 patient case notes showed a EWS score of ≥ 3 as the best predictor of adverse outcome in the first 24 hours of admission (AUC= 0.768) in comparison to APACHE II, Imrie, Ranson and CRP. A follow-up study examined the progression of scores within the 48 to 72 hour period following admission. It was seen that a trend towards a EWS score of ≥ 3 was associated with an increased risk of mortality (n=7, p<0.001, sensitivity 50%, specificity 100%) even if the initial EWS score was < 3 in the 24 hours following admission. The study reported a low number of non-survivors (n=7). Radiological findings and the presence or absence of infection was not elaborated upon. It also appeared that statistical analysis of the EWS trend was categorical, separating patients into groups with a median EWS score of < 3 or above.

It should be noted that the development of EWS parameters was based on arbitrary definitions of physiological parameters with a specified score (i.e. a systolic blood pressure range of 71-80 mmHg was arbitrarily allocated a value of 2 as opposed to 3 or any other value). When the original EWS system was used to

trigger clinical activity, it was found to be of little benefit (71). The SOCCER study by Jacques *et al (72)*, suggested that the physiological observations are delayed responses to deterioration in the underlying disease process.

Having defined a model and validated it on retrospective data, Garcea et al (66) concluded that a prospective study of the value of EWS as a predictor of severity and survival in patients admitted with AP was required. The use of the EWS in a different hospital setting to the original study would aid in determining if the system is robust for use across a wider geographical setting with possible variations in patient demographics and disease aetiology. This would also address the issues inherent in a retrospective analysis of data (such as missed cases and observer bias) and also adjust for different admission policies to critical care units across hospitals.

2.3   Methods

2.3.1   Study design.

A pilot prospective observational study to validate the hypothesis that Early Warning Score (EWS) is of value as an early predictor of severity and survival in patients admitted with acute pancreatitis.

2.3.2   Setting.

This study was carried out in the hepatopancreatobiliary unit of the Dept. of surgery at the Manchester Royal Infirmary. Data was prospectively gathered on all patients meeting the inclusion criteria, between July 2010 to February 2012.

2.3.3   Inclusion and exclusion criteria.

Inclusion criteria

Patients with the following criteria will be included:

1. Acute pancreatitis – defined as acute abdominal pain with a threefold elevation of serum amylase or a twofold elevation of serum lipase.

   OR

   Radiological confirmed acute pancreatitis – diagnosis confirmed by computed tomography (CT).

2. Not pregnant.

3. Over 18 years of age.

4. Patients able to give informed consent (or complying with current United Kingdom criteria for consent in critical care unit trials).

Exclusion criteria

1. Patients who were under the age of 18 years.

2. Patients who were unable to give informed consent.

3. Pancreatitis diagnosed at laparotomy.

4. Patients with an underlying diagnosis of malignancy.

5. Patients with pre-episode chronic renal failure or chronic liver failure with ascites.

Classification according to the 1992 system of grading patients into mild or severe categories of pancreatitis (45) within the first 24 hours of admission. Patient age, aetiology of pancreatitis, gender, total hospital stay, total critical care stay and sequential EWS for the first 7 days of in hospital stay and APACHE II score following 24 hours from admission was collected.

2.3.4 Early warning score measurement

The hourly EWS warning score was calculated using the following parameters:

Physiological parameter	Score						
	3	2	1	0	1	2	3
Systolic Blood pressure (mmHg)	<70	71–80	81–100	101–199		≥200	
Heart rate (bpm)		<40	41–50	51–100	101–110	111–129	≥130
Respiratory rate (bpm)		<9		9–14	15–20	21–29	≥30
Temperature (°C)		<35		35–38.4		≥38.5	
AVPU score				Alert	Reacting to Voice	Reacting to Pain	Unresponsive

Table 2.1: Early Warning Score system

2.3.5 Ethics committee approval

Patient data was collected under North West Regional Ethics Committee reference 10/H1010/43.

2.4   Results

Between July 2010 and February 2012, 132 patients admitted to the Manchester Royal Infirmary, met the eligibility criteria. 2 patients were excluded as they were transfers to intensive care under the joint management of the regional hepatopancreatobiliary unit. Patient demographics for this cohort are detailed in Table 2.2

Statistical analysis was calculated using the 2-tailed student t test using SPSS for Windows version 20.

Variable	N=130
Age (years)	49 (16-94)
Gender (M/F ratio)	7:6
Aetiology	
Gallstones	60 (46.2%)
Alcohol	58 (44.6%)
Drugs	1 (0.8%)
Viral	3 (2.3%)
Unknown	7 (5.4%)
Tumour	1 (0.8%)
APACHE II on admission (Modal)	6 (0-23)
Inpatient stay (days)	12 (1-200)
Critical stay (days)	0 (0-16)
Severity	
Mild	107 (82.3%)
Severe	23 (17.7%)
Inhospital death	6 (4.6%)

Table 2.2: Demographics for patients admitted with acute pancreatitis between July 2010 to February 2012. Data presented as median with range or count and percentage unless otherwise indicated. APACHE II – Acute Physiology and Chronic Health Evaluation II.

A significant difference was seen in the age of non-survivors, with the latter being within an older age range compared to survivors (Table 2.3). As with Garcea et al (66), a greater proportion of patients in this study had gallstones identified as the aetiology for acute pancreatitis. In addition, the study population also showed alcohol –induced pancreatitis within the non-survivor group. Within the UK, alcohol induced pancreatitis is usually the leading cause and their absence from the non-survivor group in Garcea et al's study (66) was noted by the authors. This was attributed to the retrospective nature of the study and paucity of patient data in the historical casenotes, leading to a large number of exclusions (n = 119). The patient demographics in this study are therefore more in keeping with the outcomes of admissions for acute pancreatitis within the UK.

			Survivors (n = 124)	Non-survivors (n = 6)	Significance	Mild Pancreatitis (n=107)	Severe Pancreatitis (n= 23)	Significance
Age (years)	Median		48	68.5	P<0.05	45	69	P<0.05
	Range		16-94	60-75		16-87	32-94	
Gender	Male		67	3	NS	58	12	NS
	Female		57	3	NS	49	11	NS
Aetiology	Gallstones	Value	60		NS	52	8	NS
		%	48.4			48.6	34.8	
	Alcohol	Value	53	5	NS	44	14	NS
		%	42.7	83.3		41.1	60.9	
	Drug induced	Value	1		NS	1		
		%	0.8			0.9		
	Unknown	Value	6	1	NS	6	1	NS
		%	4.8	16.7		5.6	4.3	
	Viral	Value	3		NS	3		
		%	2.4			2.8		
	Tumour	Value	1		NS	1		
		%	0.8			0.9		

Table 2.3: Demographic Data from patients with acute pancreatitis categorised as Survivors and Non-survivors and according to severity. Significance calculated using the 2 tailed T test. NS – Not Significant.

		Mild Pancreatitis		Severe Pancreatitis		
		Median	Range	Median	Range	Significance
Day 1	EWS	0	0-8	2	0-6	P<0.05
	APACHE II	4	0-7	12	9-23	P<0.05
Day 2		0	0-4	1	0-3	NS
Day 3		0	0-3	0	0-5	NS

Table 2.3: Median and Range of values for the highest EWS in 24hrs and APACHE II scores between Mild and Severe Acute Pancreatitis classified using the Atlanta 1992 criteria.

Median ranges of EWS between the two groups classified according to severity (Table 2.3) are markedly lower than the retrospective cohort in the validation study (66). Garcea et al's data showed a median EWS of 1 (range 0-9) on day 1 for the Mild Pancreatitis group and a median EWS of 4 (range 2-8) on day, median EWS of 5 (range 2-8) and a median EWS of 2 (range 2-8) on day 3 for the Severe Pancreatitis group. In every instance their study showed a significant difference between EWS on day 1 to 3, between the Mild and Severe Pancreatitis groups. In contrast, patients in this prospective study population did not show a significant difference in EWS between groups classified according to severity using the Atlanta 1992 criteria.

		Survivor		Non Survivor		
		Median	Range	Median	Range	Significance
Day 1	EWS	0	0-8	2	0-6	P<0.05
	APACHE II	5	0-23	12	8-20	P<0.05
Day 2		0	0-4	1	0-2	NS
Day 3		0	0-3	0	0-5	NS

Table 2.4: Median and Range of values for the highest EWS in 24hrs and APACHE II scores between Mild and Severe Acute Pancreatitis

No significance was seen in subsequent comparisons over the 7 days from admission.

A similar trend is seen in the EWS and APACHE II scores for survivors and non-survivors (table 2.4), with significant differences between the EWS for the two groups only present in the first 24 hours.
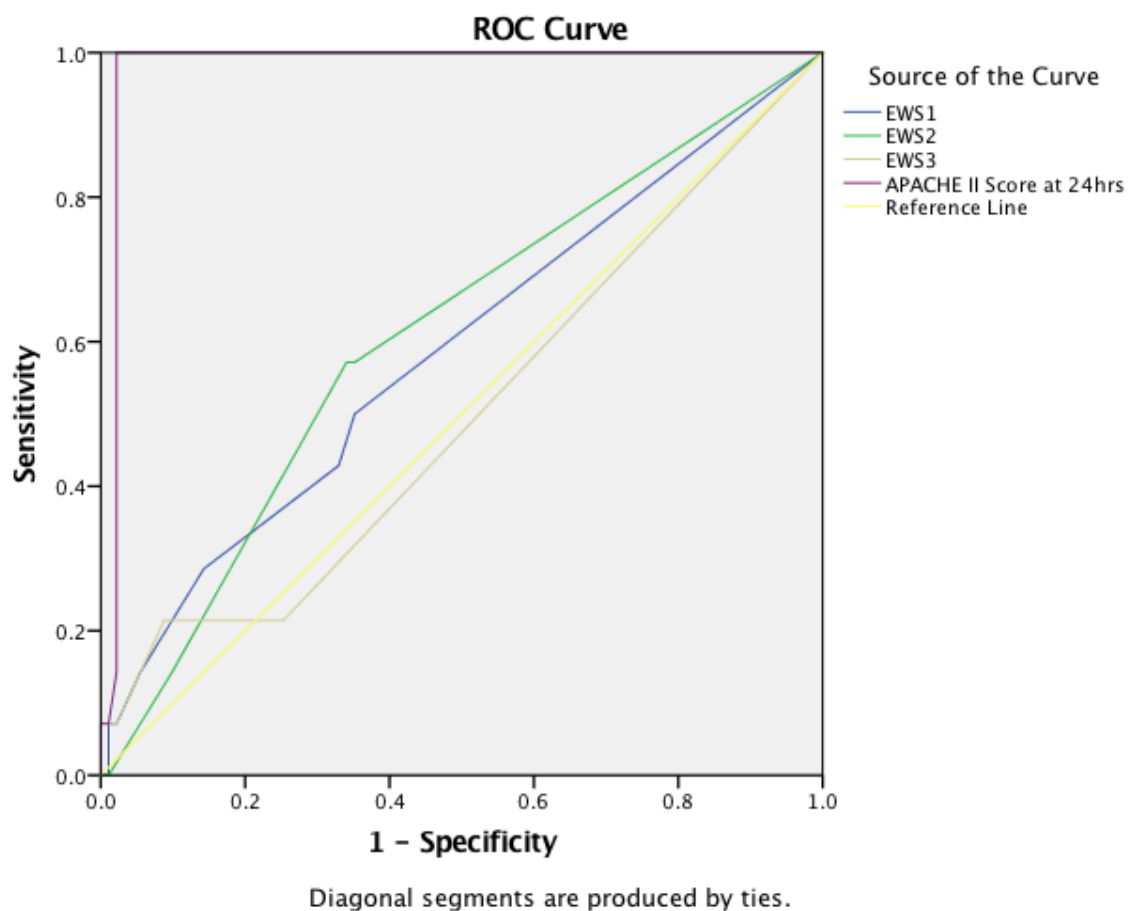
Figure 2.1: ROC curve for the highest EWS on the first 3 days and APACHE II in as a predictor of Severe Pancreatitis.

In Figure 2.1, APACHE II shows an Area Under the Curve (AUC) of 0.980 and P<0.05 compared to AUCs of 0.586, 0.608 and 0.500 for days 1 to 3 using the highest EWS score. The high sensitivity and specificity of APACHE II is due to its use as the determinant for Mild and Severe Pancreatitis (as per the Atlanta 1992 classification) in this study.
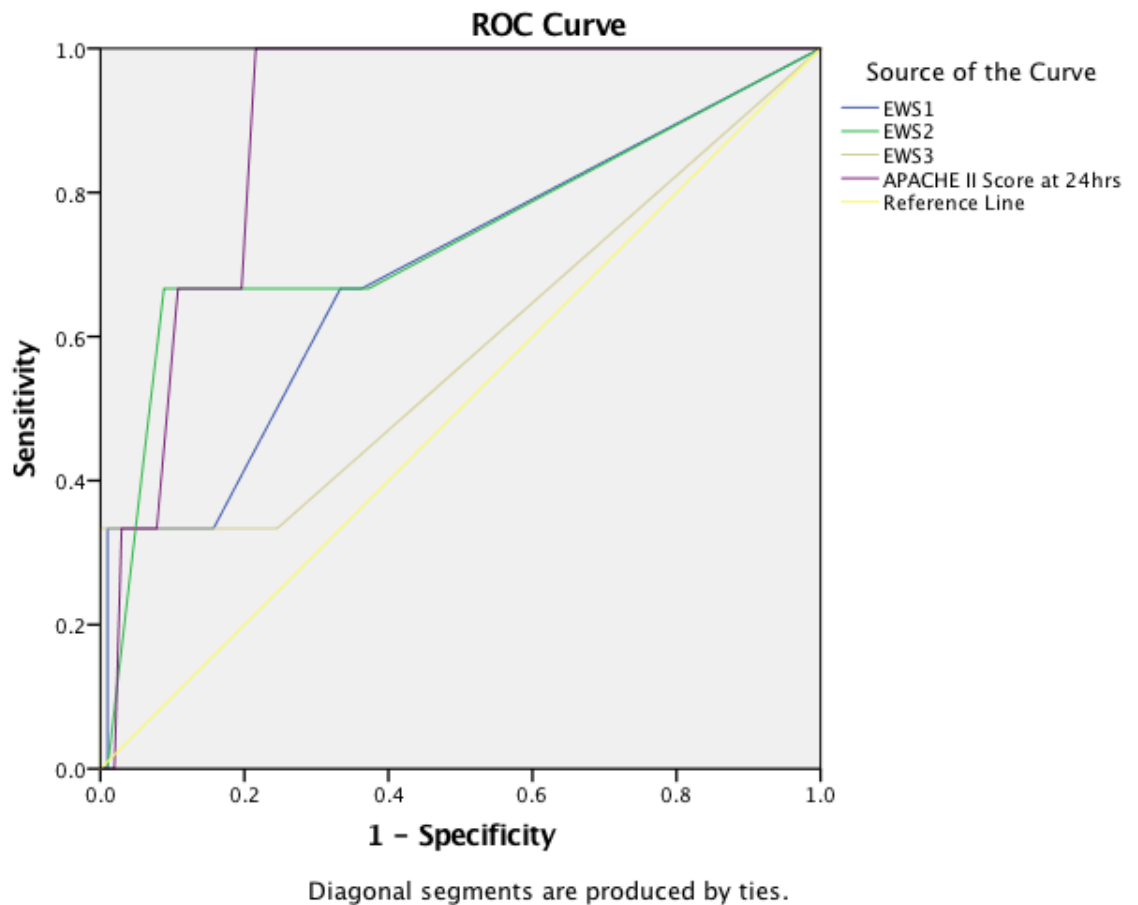
Figure 2.2: ROC curve for the highest EWS on the first 3 days and APACHE II in as a predictor of death in all cases admitted with acute pancreatitis.

In Figure 2.2, the highest EWS on each of the first 3 days of admission had AUCs of 0.688, 0.739 and 0.585 respectively, compared to an AUC of 0.892 for APACHE II on admission. Although the AUCs for the first 2 days are reasonably high, they were not statistically significant.

Garcea et al (66) described a significant difference between the survivors of patients admitted with alcohol induced pancreatitis compared to other aetiologies. The authors observed that because of the nature of the retrospective study, there were a high number of exclusions, due to lack of data, for their sample population resulting in a bias. This is corroborated by the binomial logistic regression analysis in this study, which showed no significance for the increased proportion of patients with acute pancreatitis secondary to gallstones when compared to alcohol-induced pancreatitis or other aetiologies..

2.5   Discussion

This study compared the ability of a simple and universal clinical scoring system (EWS) used in the United Kingdom's National Health Service, to stratify patients admitted with acute pancreatitis, into mild and severe categories, with the accepted method in use at the time the study was conducted (Atlanta 1992 criteria). The results reported do not show a sufficiently high specificity and sensitivity for the use of a patient's highest EWS result as a predictor of severity or mortality.

Reassessment and reclassification of disease severity on day 2 and 3 may have changed the allotment of a patient to either the mild or severe group. Ethical permission provided for the study did not permit the invasive procedures such as venepuncture or arterial blood gas assessment needed for calculation of APACHE II, Multiple Organ Dysfunction (MODS) or Systemic Organ Function Assessment (SOFA) scores, on subsequent days, unless indicated by the clinical team. Any estimation would therefore require the use of null data and be prone to Type II observer error.

It is also likely that management patterns between the two sites and time periods have affected patient outcomes and severity. The original study (65) analysed patient record from 2002 to 2006 for 110 patients. A subsequent retrospective validation (66) used a population of 300 patients between 1999 to 2010. Critical care outreach teams consisting of staff from intensive care were in place at the time of this study. Their role was to identify and institute care packages for patients who might need eventual admission to intensive care while still resident on a general ward. This has the effect of reducing the recording critical care occupancy

and mediating the systemic inflammatory response syndrome (SIRS) responsible for high organ dysfunction scores and severity of acute pancreatitis.

The use of predictive modelling in investigating which early warning scores from this data set of 130 patients could be used as a marker of severity was considered. Predictive modelling in medicine has known limitations. In large samples all p-values are statistically significant; this limits the use of regression in interpretation of cut-off values for outcomes, as the effect size is practically zero. One approach to compensate for this would be to divide the data set into two groups. This might be on the basis of time period e.g. first year of the study or even groups e.g. every alternate patient or first half of the patients in chronological order and is not feasible in the relatively small sample size of 130 patients. Using an entire data set to derive a model (known as the classifier) and then applying it to the same data results in false estimation of the model's prediction's sensitivity and specificity as it is uniquely adapted to the data set used to derive it and is why this study is a logical progression from the original work by Garcea et al.

Even if such analysis was possible and used to investigate if additional factors within the dataset were able to predict of future medium term outcomes such as mortality, prolonged length of stay and critical care admission, etc. any results derived using underlying physiological data or other factors, would only produce results that could be applied to an outmoded binary classification of disease severity. Clinical application of the results would need to be in context of the reclassification of acute pancreatitis into mild, moderate and severe categories(48) since 2012. Reclassification of existing data according to the new system would introduce observer bias into the subsequent analysis.

On the basis of results and subsequent change in the classification of acute pancreatitis severity, further evaluation into the use of EWS as a predicator of severity or outcomes, is warranted before it can be used to aid patient management.

CHAPTER 3:

Recombinant Human Activated Protein C as a Disease Modifier in Severe

Acute Pancreatitis: Systematic Review of Current Evidence

3.1 Abstract

Background: The severity of organ failure caused by acute pancreatitis (AP) is the most important determinant of mortality in the disease. Recombinant human activated protein C (Drotrecogin Alfa; Xigris™, APC, rhAPC) is the first drug to show a decrease in all-cause mortality due to multiple organ failure caused by sepsis. As the systemic inflammatory response syndrome (SIRS) that causes organ failure in early AP is similar to that caused by severe sepsis, the use of rhAPC in the management of AP has been investigated in experimental and clinical studies which are collated in this review.

Methods: A literature review of published material identified from MEDLINE and EMBASE databases, for the period from January 1985 to January 2011, reporting rhAPC usage in AP.

Results: 3 of 4 experimental studies reported an improvement in outcome in animals with AP given rhAPC. The clinical randomized trial showed no improvement in outcome in the treatment arm.

Conclusion: The experimental evidence of disease amelioration in AP following intervention with rhAPC has not translated to the small clinical RCT. Given that there were only 16 patients in the treatment arm, further clinical evaluation is justified.

3.2   Introduction

Severe acute pancreatitis (SAP) is characterized by the co-existence of local peri-pancreatic complications with sustained organ failure (45). The treatment remains essentially supportive and comprises careful and adequate fluid resuscitation together with support for cardiovascular, respiratory and renal systems in addition to appropriate analgesia (73, 74). Prophylactic antibiotic therapy (75-77) early enteral nutrition (78, 79) and early endoscopic sphincterotomy (80) for patients with biliary acute pancreatitis and features of bile duct obstruction are the only specific interventions for which there is supportive randomized trial evidence. However, none of these interventions are universally accepted as genuinely ameliorating the disease course and in the case of antibiotics in particular, current guidance does not favour prophylaxis.

Given that the early stage of severe acute pancreatitis does not require surgical intervention (81, 82), this phase of the disease is ideally and logically suited to pharmacological intervention aimed at disease modification. Many drugs have been evaluated as specific pharmacological treatments for severe acute pancreatitis. Disappointingly, the unifying characteristic of the drugs that have been evaluated to date has been their lack of efficacy as effective disease-ameliorating treatments for severe acute pancreatitis. Whilst it is thought that some of this apparent lack of efficacy may relate to the over-broad categorization of severe acute pancreatitis in the 1992 Atlanta consensus criteria (45) resulting in patients with transient or minimal organ failure (and thus a likely mild clinical course) being incorrectly categorized as severe, it remains the case that there is to date, no specific pharmacological treatment for severe acute pancreatitis.

One strategy in the search for specific interventions is to consider treatments that have been effective in disease states that are similar to SAP. Severe sepsis has similarities to SAP; both are characterized by an exaggerated systemic inflammatory response syndrome (SIRS). Inflammation involves a pathophysiological derangement of the endogenous anticoagulant pathways involved in the maintenance of microvascular patency (83) with microvascular thrombosis and disseminated intravascular coagulation being a critical outcome(84). The protein C pathway plays a major role in preventing microvascular thrombosis(85). Endogenous protein C is depleted in a primate model of *E coli*-induced sepsis resulting in microvascular thrombosis, the harmful effects of which are ameliorated by intravenous infusion of recombinant human active protein C molecule (rhAPC) (86). Based on these key experimental findings, human recombinant activated protein C (Xigris™, Eli Lilly, Indianapolis, IN) was evaluated in a major randomized trial in patients with sepsis (the PROWESS study (87)). The PROWESS study demonstrated that treatment was associated with a significant reduction in mortality.

SAP is characterized by pancreatic and peri-pancreatic necrosis in addition to SIRS. Microvascular thrombosis is likely to be one of the mechanisms involved in the mediation of pancreatic necrosis. Thus a hypothetical case can be made that Xigris™ may have a role as an early and specific disease-modulating drug in SAP for its roles in maintaining microvascular patency and down regulating the inflammatory response.

An important caveat is that acute pancreatitis was one of the listed exclusion criteria in the PROWESS study (87). SAP is associated with a risk of peri-

pancreatic hemorrhage and thus the use of a drug with potent anti-coagulant properties may lead to bleeding-related complications.

The potential role of rhAPC as a specific pharmacological treatment for SAP was recognized by Alsfasser and colleagues who undertook the first evaluation of this drug in this setting (88). Since their initial report, experience with RHAPC in experimental acute pancreatitis has accrued and a small clinical randomized trial has been also been undertaken.

Considering the history of unsuccessful pharmacological interventions in SAP the aim of this study is to undertake a detailed systematic review of the evidence for rhAPC as a disease modifier and to address whether there is sufficient evidence for an appropriately constituted randomized trial to evaluate this drug in SAP.

3.3 Methods

3.3.1 Literature search and data retrieval strategy

A computerized search was performed of the MEDLINE and EMBASE databases for the period from January 1985 to January 2011 using the OVID search engine (Version 10.5.1, Source ID 1.13281.2.21; Ovid Technologies, Inc., New York, NY, USA). The search terms 'Pancreatitis', 'Protein C', 'Activated Protein C' and 'Drotrecogin' were used. The map term to subject (MeSH) heading was employed where possible. Results were combined with the keywords, with the aid of Boolean operators. There were 23 hits in MEDLINE and 61 in EMBASE. The Cochrane systematic reviews methodology was utilized to cross-reference combined EMBASE and MEDLINE output with all clinical trials and studies including experimental studies and any non-English studies. Letters and reviews without original data were excluded, leaving a final study population of 8 manuscripts. The reasons for excluding manuscripts are provided in Figure 1.

All retrieved manuscripts were reviewed by two authors (CJM, BIB) and any difference of opinion regarding final inclusion/exclusion was resolved by discussion with the third author (AKS).

Medline

Pancreatitis AND Protein C

23 articles

Exclusion based on:
- Review article: 8
- Intervention with APC not main subject: 8
- Replication of study design and findings : 1

4 articles identified as experimental studies into the effect of recombinant activated protein C as a disease modifier in severe acute pancreatitis (Yamanel, Alsfasser, Chen, Akay, Pettilä)

2 Case reports (Machala, Grochowiecki)

Embase

Pancreatitis AND Protein C : 57 articles

Pancreatitis AND Drotrecogin : 8 articles

Pancreatitis AND Activated Protein C : 15 articles

61 unique articles

Exclusion based on:
- Abstracts : 2
- Intervention with APC not main subject: 52

4 articles identified as experimental studies into the effect of recombinant activated protein C as a disease modifier in severe acute pancreatitis (Yamanel, Alsfasser, Chen, Akay, Pettilä)

3 Case reports (Machala, Grochowiecki, Rybicki)

Figure 3.1: Search strategy. Date range from January 1985 to January 2011. rhAPC = human recombinant activated protein C. AP = acute pancreatitis.

3.4 Results

3.4.1 Human recombinant activated protein C in experimental acute pancreatitis

Four discrete studies have evaluated the role of rhAPC in experimental acute pancreatitis (88-91). Of these, a further study by Chen's group was excluded, as there is a considerable apparent overlap between the 2007 study (90) and the 2010 report (92).

All experimental studies have evaluated experimental acute pancreatitis in the rat model. There are no data on large animal models of acute pancreatitis. All use a well-validated intra-ductal infusion method to induce acute pancreatitis. A wide range of concentrations of human recombinant activated protein C is evaluated. The findings are not consistent. Yamanel and colleagues (89), using 100 mg/kg rhAPC as a single dose at 6 hours after induction, report improvements in histologic features of pancreatic injury, serum markers of inflammation and a reduction in bacterial translocation. There was no evidence of pancreatic or intra-abdominal hemorrhage.

Alsfasser's (88) comprehensive report describes a concentration-dependent thrombocytopenia but no effect of the drug on histologic scores of necrosis and edema with the 100 µg/Kg/hour continuous infusion. The Alsfasser group is the only study to examine mortality and they show a significant reduction in mortality in animals with AP treated with rhAPC compared to animals with AP alone.

Chen's study (90) provides detailed information on the effect of rhAPC on the mitogen-associated protein kinase pathway demonstrating a reduction in expression of stress proteins.

The Akay study (91) findings are in contrast to the others in that they report no beneficial effects from intervention with rhAPC. Of note, the concentration of rhAPC used in their study parallels the dose used in human sepsis (24 µg/Kg/hour).

First Author	Animal model	Study designation/detail	Induction agent/route	Concentration of Xigris™	Groups	Number of animals	Study duration.
Yamanel(89) 2005	Rat	Effect of rhAPC on AP	Intra-ductal 5% sodium taurocholate.	100 mg/Kg single dose at 6 hours after induction.	Group I = control Group II = AP Group III = AP + rhAPC	45	24 hours
Alsfasser(88) 2006	Rat	Safety assessment Of rhAPC.	Intra-ductal saline plus i.v. cerulein 5µg/Kg/hour for mild AP. Intra-ductal 10mM glycodeoxycholic acid-glycylglycine and i.v. cerulein (as above).	0.6 mL/Kg/hour or 100 µg/Kg/hour Continuous infusion.	Dose-ranging study: 12.5, 17, 21, 50 and 100 µg/Kg/hour rhAPC.	15 (3 per group)	6 hours
Alsfasser(88) 2006	Rat	Treatment of severe AP	Intra-ductal 10mM glycodeoxycholic acid-glycylglycine and i.v. cerulein.	100 µg/Kg/hour Continuous infusion.	Group 5: severe AP Group 6: severe AP and rhAPC	24 (12 per group)	6 hours
Alsfasser(88) 2006.	Rat	rhAPC & survival	Intra-ductal 10mM glycodeoxycholic acid-glycylglycine and i.v. cerulein	100 µg/Kg/hour Continuous infusion.	Group 7: severe AP Group 8: severe AP and rhAPC	16 (2 groups)	24 hours
Chen(90) 2007.	Rat	Effect of rhAPC on MAPK	Intra-ductal 5% sodium taurocholate.	10 µg/Kg (low dose) or 50µg/Kg (high dose)	5 groups: Control AP AP + rhAPC low dose (10µg/kg) AP + rhAPC high dose (50µg/kg) AP + CNI1493*	75	16 hours
Akay(91) 2008	Rat	Effect of rhAPC on early phase of AP.	Intra-ductal 5% sodium taurocholate	24µg/Kg/hour as i.v. bolus starting 4 hours after induction of AP.	Laparotomy only AP AP + drotrecogin		9 hours

Table 3.1: Protocol details of studies evaluating human recombinant activated protein C in experimental acute pancreatitis. rhAPC = Human recombinant activated protein C; AP = Acute Pancreatitis; i.v. = Intravenous; MAPK = mitogen-activated protein kinases. CNI1493: a synthetic inhibitor of signal transduction, which inhibits phosphorylation of p38 MAPK.

First Author	Study	Principal Endpoints
Yamanel(89)	Effect of rhAPC on AP.	rhAPC associated with marked reduction in pancreatic edema, necrosis without increase in hemorrhage.
		rhAPC ameliorated serum amylase, TNF-α and IL-6.
		rhAPC associated with a reduction in bacterial translocation to mesenteric nodes.
Alsfasser(88)	Safety assessment.	Concentration-dependent thrombocytopenia.
		No evidence of bleeding.
Alsfasser(88)	Treatment of severe AP.	No difference in histologic scores of necrosis and oedema.
		Significant reduction in pancreatic and pulmonary MPO.
Alsfasser(88)	rhAPC and survival.	6 of 7 (86%) survival in rhAPC group
		3 of 8 (38%) in AP group.
Chen(90)	Effect of rhAPC on MAPK.	rhAPC -treatment resulted in reduction of histological evidence of pancreatic injury.
		rhAPC -treatment was associated with decreased gene, mRNA and protein expression of p38 MAPK and JNK with increased expression of ERK1/2.
		rhAPC -treatment resulted in lower histological scores of pancreatic injury (including necrosis).
Akay(91)	Effect of rhAPC on early phase of acute pancreatitis.	Mean serum amylase lower in treatment group.
		No difference in histopathologic scores of injury.
		No difference in pancreatic MPO activity.
		No difference in serum interleukin-6 concentrations.

Table 3.2: Principal endpoints of studies evaluating Human recombinant activated protein C in experimental acute pancreatitis. rhAPC = Human recombinant Activated Protein C; AP = Acute pancreatitis; TNF-α = Tumor necrosis factor alpha; IL-6 = Interleukin 6; MPO = Myeloperoxidase; MAPK = mitogen-activated protein kinases; mRNA = messenger ribonucleic acid; JNK = c-Jun N-terminal Kinases; ERK 1/2 = Extracellular signal-regulated Kinases

3.4.2 Human recombinant activated protein C in clinical acute pancreatitis

The APCAP study comprised a pilot, double blind, randomized placebo-controlled trial (RCT) of intravenous infusion of rhAPC at a fixed dose of 24 µg/Kg/hour for 96 hours in patients with SAP (93).The inclusion criteria were appropriately focused to deliver a target population with severe disease: <96 hours from onset of pain and at least one organ dysfunction (defined as organ specific Sequential Organ Failure Assessment (SOFA) score of at least 3 or 4, within 48 hours of onset of the first organ dysfunction).

The *a priori* primary endpoint was a three-point difference in change of SOFA score with the authors predicting that this difference could be detected with a sample size of 16. 32 patients were randomized and 16 patients comprised the intervention group. Baseline characteristics were similar and the mean ± standard deviation Acute Physiological and Chronic Health Evaluation II (APACHE II) score in the rhAPC group was 17.6 ± 8.5.

Their results showed no significant bleeding events. The 30-day mortality in the rhAPC group was 3 (19%) compared to 0 in the placebo group. The primary endpoint was not met and there was no significant difference in SOFA score. An interesting observation was that treatment with rhAPC was associated with an increase in serum levels of both total and conjugated bilirubin. There were no differences in ventilator-free days, in renal replacement therapy-free days, in vasopressor-free days or in days alive outside the hospital.

In addition to this principal randomized trial, there are case report-level experiences of the use of rhAPC in severe acute pancreatitis. The first

documented report of rhAPC use in severe acute pancreatitis was by Machała and colleagues who reported their experience with 2 patients with infected pancreatic necrosis (94). In 2006, Grochowiecki and colleagues published a report detailing their experience with human recombinant activated protein C in the treatment of a patient with pancreatitis following the receipt of a transplanted pancreas (95). Rybicki and colleagues report a further case treated with rhAPC because of rapidly progressive multiple organ failure (96). All case reports describe a favourable outcome with no evidence of treatment-related haemorrhage but clearly provide only limited evidence.

3.5 Discussion

This report assesses the evidence for the evaluation of rhAPC as a disease modifying drug in acute pancreatitis. As with almost all other proposed disease modifying drugs, a body of evidence has accrued from studies in experimental acute pancreatitis. Placed together, rather than read in isolation, the relative similarities and disparities of protocol design become readily evident (Table 3.1). The four studies featured here all use the rat model of intra-ductal sodium taurocholate infusion. An advantage of this model is its reproducibility and the resultant severe acute necrotizing pancreatitis(97). A theoretical disadvantage is the physical manipulation of the pancreas in relation to assessment of an intervention, which has pancreatic hemorrhage as a potential side effect. The concentration of rhAPC used in the intervention groups and critically, the timing of intervention, vary considerably between studies. We have previously reported that studies of experimental acute pancreatitis can be broadly dichotomized into those that examine mechanistic components of the pancreatic inflammatory process and those that evaluate a potential specific therapy(98). In this regard, the Alsfasser study(88) is well designed and executed and reports a composite of a series of studies and is the sole experimental study to evaluate the effect of rhAPC on mortality. As such, although there is no formal process of weighting the importance of experimental studies, the Alsfasser study's (88) findings carry considerable importance and their key finding of a reduction in mortality from 86% in the AP group to 38% in the AP group treated with rhAPC (at that time termed Drotrecogin Alfa) without evidence of bleeding is noteworthy. The failure to modify the histological features of pancreatic injury raises the possibility that beneficial effects of rhAPC are from actions out with the pancreas. The studies of Yamanel(89) and Chen(90) are broadly supportive of Alsfasser's findings(88). Yamanel's(89) work contrasts with Alsfasser's(88) in demonstrating histological

evidence of amelioration of pancreatic injury in animals with AP treated with rhAPC.

The findings of the Akay(91) study, which had intervention with rhAPC at 4 hours in a 9 hour protocol, reported no beneficial effect. This apparent disparity may relate to the relatively late timing of intervention in a model with a compressed time course. The studies of rhAPC in experimental acute pancreatitis can be summated as showing no evidence of pancreatic or remote hemorrhage as a consequence of treatment with 3 of 4 studies showing an amelioration of pancreatic injury as a consequence of the intervention. The Alsfasser study (88) provides key evidence of reduction in mortality with treatment and taken together this body of experimental evidence justifies progression to clinical evaluation of rhAPC as a specific disease modifying drug in SAP.

A particular interest with rhAPC is the interaction between its anticoagulant role and its anti-inflammatory properties. In the Alsfasser study, there were significant anti-inflammatory properties manifest by a reduction in myeloperoxidase(88). Chen and colleagues also demonstrated a reduction in pro-inflammatory cytokines (90). At the present time, there are insufficient data to differentiate with certainty whether the beneficial effects of rhAPC in experimental acute pancreatitis are effected predominantly by modulation of microvascular thrombosis, down regulation of inflammation or a combination of both.

This off license role of the drug was evaluated in a well-designed and well-executed study undertaken by the Helsinki group (APCAP(93)). Set against a contemporary backdrop of imminent re-categorization of the terminology around the severity of acute pancreatitis, the Kemppainen(93) study clearly identifies a cohort of patients with clinically severe acute pancreatitis (16 patients in the

intervention arm had an APACHE II score of 17.6 ± 8.5 and a median age of 47 ± 8 years). They clearly learnt the lesson of the lexipafant intervention study (99) and avoided recruiting a population where there was a disproportionate influence of the chronic health evaluation component of the APACHE II score. APCAP is also realistic and reasonable in looking for reduction in organ failure score as a primary endpoint rather than an effect on mortality. The problem with small group intervention studies is that a single adverse outcome (such as death after laparotomy) will have a disproportionate skewing effect on interpretation of endpoints. As such, we would concur completely with the Kemppainen (93) group's own conclusion that their study showed no serious hemorrhagic events associated with intervention but also no evidence of treatment-induced modification in the evolution of organ dysfunction. However, with a treatment arm of just 16 patients, this question clearly remains unanswered.

Little substantial additional information derives from the other anecdotal case reports.

The logical question to answer in continuing the assessment of rhAPC is whether the position of clinical equipoise in relation to intervention has been reached; currently exists or has passed? In this context, the experimental evidence clearly makes a case for evaluation and the carefully executed Kemppainen (93) study takes the body of evidence forward but the nature of its negative result means that currently, equipoise has not been reached. Put in the context of the important moral aspect of contemporary trial design, it remains unethical to randomize large numbers of patients with SAP to receive rhAPC in an intervention arm, in the absence of sufficient clinical evidence to justify altering any given individual's treatment from standard care.

However, a rational argument can be made for a smaller, randomized, placebo-controlled evaluation of rhAPC in SAP. As with APCAP(93), the definition of severity must provide information on APACHE II score, disease duration, organ dysfunction and systemic inflammatory response as these allow later workers to categorize the severity of the disease. Although doses in excess of 24 µg/Kg/hour may be of interest, as anti-inflammatory activity appears to be dose related, the weight of clinical safety data related to the sepsis treatment dose suggests that any higher concentration could not readily be justified.

Critical issues remain around primary endpoint and power calculation. In a placebo-controlled evaluation of a drug with complex, whole-organ, anti-inflammatory effects, a meaningful endpoint could be reduction in critical care occupancy. Given the negative findings of the APCAP (93) study, it is probably unwise to construct a power calculation on the Alsfasser (88) findings and a pragmatic compromise based on recruitment rates is realistic (bearing in mind that the APCAP investigators screened 215 patients to recruit 32). A practical primary endpoint would be reduction in critical care occupancy.

In conclusion, the experimental evidence underlying a potential role for rhAPC as a specific disease modifying drug in acute pancreatitis makes a strong but not conclusive case for evaluation. Set in the context of the lexipafant studies of the 1990s (100-103), the experimental evidence is probably stronger for rhAPC than existed for lexipafant. The single clinical randomized trial (93) strikes an important cautionary note as it is both well designed and well executed. Set against a background of the knowledge of the increasing complexity of the biology of clinical sepsis and the continuing uncertainty over the clinical validity of rhAPC, the point of equipoise to justify a major randomized trial has not been reached. Yet, the Alsfasser(88) evidence remains important and the prospect of a specific disease

modifying drug with anti-inflammatory actions and a role in preservation of microvascular patency cannot be ignored.

As a result of the negative outcome of the current PROWESS SHOCK study, rhAPC has currently been withdrawn from clinical use (104). The withdrawal was made on the grounds of lack of efficacy in overwhelming sepsis rather than on safety grounds thus disease-specific evaluation in a setting such as severe acute pancreatitis remains an option. Further clinical evaluation is justified and supported by the evidence summated in this report.

CHAPTER 4:

Twenty-four hour Infusion of Human Recombinant Activated Protein C (Xigris™) Early in Severe Acute Pancreatitis: The XIG-AP 1 trial

4.1 Abstract

Objective: Patients with severe acute pancreatitis were excluded from major trials of human recombinant activated protein C (Xigris™) because of concern about pancreatic haemorrhage although these individuals have an intense systemic inflammatory response that may benefit from treatment. The object of this study was to provide initial safety data evaluating Xigris™ in severe acute pancreatitis.

Design: Prospective clinical trial recruiting between November 2009 and October 2011. Patients received human recombinant activated protein C (Xigris™) for 24 hours by intravenous infusion (24 µg/kg/hr) in addition to standard clinical care. A matched historical control group treated within the same hospital unit was used to compare outcomes. Of 166 consecutive admitted patients, 43 met the screening criteria for severe acute pancreatitis and 19 were recruited, all contributing to the analyses.

Results: Compared to historical controls there were fewer bleeding events in the Xigris™ group although the finding did not reach significance (Xigris™ 0% vs. Control 21%, p=0.13), similarly further intervention appeared less frequent (11% vs. 47%, p=0.07) in the treatment group. Length of stay was shorter for patients receiving Xigris™ (19 vs. 41 days, p=0.03) as was inotrope use (5% vs. 32%, p=0.02); mortality and incidence of infections in both groups were similar. Biomarker protein C increased while IL-6 decreased following infusion.

Conclusions: A 24-hr infusion of Xigris™ appears safe when used in patients with severe acute pancreatitis. Further research within larger clinical trials might

address optimum duration of treatment and whether this intervention is of benefit

in this group of patients.

Trial Registration: Eudract Number 2007-003635-23

4.2 Introduction

Human recombinant activated protein C (Xigris™™, drotrecogin alfa, Eli Lilly, Indianapolis, Indiana, USA) was developed as a drug treatment for sepsis and was evaluated in the PROWESS study with 1690 patients randomized (850 to Xigris™ and 840 to placebo)(105). Non-stratified analysis of results showed 259 deaths (31%) mortality) in the placebo group compared to 210 (24.7%)) in the treatment arm (105). This difference was significant and persisted at 28 days and in sub-groups analysed by presence or absence of protein C deficiency (105). A potential side effect of modulation of the coagulation cascade is haemorrhage and there were 30 serious bleeding events in the treatment arm of the PROWESS study compared to 17 in the placebo group (105). This difference was not statistically significant ($P = 0.06$).

Amongst the listed exclusion criteria for PROWESS was "acute pancreatitis with no established source of infection" (105). The indications for exclusion were not stated (105). Early acute pancreatitis is characterized by an intense systemic inflammatory response rather than intra-abdominal sepsis (106). There is evidence both from experimental models (88-90) and from clinical studies (107, 108) that microvascular thrombosis in the pancreatic vascular bed is a mediator of pancreatic parenchymal necrosis. Although these findings support the use of activated protein C early in the disease course of acute pancreatitis, one of the major causes of death in the severe form of this disease is haemorrhage (109), raising concerns about the safety of a drug with anticoagulant properties.

In an experimental model of severe acute pancreatitis Alsfasser and colleagues demonstrated decreased inflammation and improved survival in animals with severe disease treated with Xigris™ (88). Of note, there was no evidence of an

increase in haemorrhagic complications (88). These findings were reproduced in

an L-arginine-induced rat model of acute pancreatitis where intervention with

Xigris™ was associated with a modulation of pancreatic and remote organ injury

with no evidence of an increase in pancreatic parenchymal haemorrhage (110).

As there were conflicting reports of the efficacy of Xigris™ in severe sepsis, a

further randomized trial (PROWESS SHOCK) (111) was conducted in 1697

patients with infection, systemic inflammation and shock and this showed no

reduction in mortality at 28 or 90 days (overall and in all sub-group comparisons).

On this basis the drug was withdrawn from commercial use in October 2011.

Despite this, a question remained as to whether Xigris™ could be of benefit as an

early therapeutic intervention in a carefully selected population of patients with

severe acute pancreatitis with a persistent systemic inflammatory response

together with organ failure. Such patients have intense inflammation rather than

infection and constitute the sub-group with acute pancreatitis at highest risk of

death and for whom there is currently no specific therapy (112). To explore this,

the manufacturer (Lilly, Critical care Europe) supported two investigator-initiated

trials (IITs) of Xigris™ as a specific drug treatment for severe acute pancreatitis

(personal communication, Lilly Critical Care, Europe), both initiated prior to

withdrawal of the drug from commercial use. The first, APCAP (activated protein

C in acute pancreatitis), was a small double-blind randomized trial in a Finnish

population of patients with severe acute pancreatitis (93). This study showed no

evidence of an increase in serious bleeding events in the Xigris™ treatment group

compared to placebo but also no difference in the evolution of multiple organ

dysfunctions and in a separate report, no difference in the pattern of distribution of

inflammatory cytokines (113). The present report describes the second IIT, which

was undertaken as a clinical cohort study with a focus on careful definition of early

severe acute pancreatitis and a primary endpoint of assessment of haemorrhagic

complications.

4.3 Methods

4.3.1 Design

A prospective cohort study recruited a consecutive series of patients with severe acute pancreatitis, with evidence of organ dysfunction and a systemic inflammatory response. Patients were administered a 24-hour infusion of Xigris™ in addition to standard clinical care. Outcomes were compared to those in a matched historical control group of patients with severe acute pancreatitis treated in the same unit.

4.3.2 Setting

A tertiary care hepato-pancreato-biliary unit, which was one of two, serving a predominantly urban conurbation of 3.2 million people in Lancashire, UK. Patients were recruited to the interventional group from November 2009 until October 2011 (when Xigris™ was withdrawn from clinical use). No patients received Xigris™ after its commercial withdrawal.

4.3.3 Inclusion criteria

The following were stipulated as inclusion criteria: acute pancreatitis – defined as acute abdominal pain with a threefold elevation of serum amylase or a twofold elevation of serum lipase; early disease – defined as being within 96 hours of onset of severe pain and 72 hours of admission to hospital; severe disease – defined as a patient fulfilling all of the following criteria: an APACHE II (Acute Physiology and Chronic Health Evaluation Score) of ≥ 9 on admission and at least two of four systemic inflammatory response syndrome (SIRS) criteria measured on

two occasions separated by at least 24 hours (the criteria are pulse

>90 beats/min, rectal temperature <36⁰ C or >38⁰ C, white blood count <4000 or

>12,000 per mm^3, and respiration >20/min or PCO_2 <32 mm Hg) plus a Marshall

organ dysfunction score (MODS) of ≥ 2 for at least one of the three organ systems

measured on two occasions separated by at least 24 hours; no clinical evidence of

haemorrhage; patients with no prior history of bleeding duodenal ulcer,

haemorrhagic stroke or other haemorrhagic diathesis; not taking warfarin or other

anticoagulant medication; without evidence of end-stage renal disease; over 18

years of age; no abdominal surgery or endoscopic retrograde

cholangiopancreatography (ERCP) within the previous 30 days; and able to give

informed consent (or complying with current United Kingdom criteria for consent in

critical care unit trials).

4.3.4 Exclusion criteria

The following were exclusion criteria: non-severe acute pancreatitis – defined as

an APACHE II score of < 9 on admission to hospital and/or patient not fulfilling the

SIRS and organ dysfunction threshold criteria; later presentation - in excess of 96

hours after onset of severe pain or more than 72 hours after admission to hospital;

clinical evidence of haemorrhage; prior history of bleeding duodenal ulcer,

haemorrhagic stroke or other haemorrhagic diathesis; taking warfarin or other

anticoagulant medication; thrombocytopenia; coagulopathy; evidence of end-stage

renal disease or liver disease; surgery or ERCP within the previous 30 days;

pregnant or lactating. Additionally exclusion criteria from the PROWESS trial were

applied: platelet count at point of enrolment < 30,000/mm^3; conditions that

increase the risk of bleeding: history of severe head trauma requiring previous

hospitalization, human immunodeficiency virus infection with a last known CD4

count of ≤ 50/mm^3; history of bone-marrow, lung, liver, pancreas or small-bowel transplantation; known or suspected portal hypertension, chronic jaundice, cirrhosis or chronic ascites; participation in another investigational study within 30 days before the current study; use of any medicines at dosages indicating exclusion within PROWESS.

4.3.5 Intervention

In addition to standard clinical care for acute pancreatitis, patients received Xigris™ (human recombinant activated protein C, Eli Lilly, Indianapolis, USA) 24 µg/kg/hr for 24 hours by intravenous infusion. The infusion was delivered after assessment on day 2. Dose-selection was a pragmatic choice, based on the pre-existent general clinical experience with this dose. In the ADDRESS study, the concentration of 24 µg/kg/hr was used for 96 hours but was associated with increased haemorrhagic complications (114). Although patients in the present study were not from the high-risk immediate post-surgery group an empiric decision was taken to limit duration of infusion to 24 hours given the dearth of information on the clinical safety profile of Xigris™ in severe acute pancreatitis. The study was undertaken prior to public reporting of the APCAP protocol or the results of that study.

4.3.6 Clinical care of acute pancreatitis

All patients in this study were managed according to the guidelines for care of patients with acute pancreatitis published by the British Society of Gastroenterology in 2005 (115). Patients treated in critical care were managed in a multidisciplinary fashion with involvement of intensive care physicians,

hepatopancreatobiliary surgeons and interventional radiologists. Computed tomography (CT) was only undertaken when requested by the patient's clinical team.

4.3.7 Concomitant medications

Medications administered to patients were recorded. All patients were administered subcutaneous low molecular weight heparin in a body-weight adjusted dose as prophylaxis against deep vein thrombosis/pulmonary embolism. During the period of this study there was no policy mandating routine use of prophylactic antibiotics in severe acute pancreatitis in this unit. Antimicrobial therapy was prescribed in response to positive cultures according to the clinical situation.

4.3.8 Endpoints

The primary endpoint was the occurrence of a serious bleeding event as defined in the PROWESS trial (105): any intracranial haemorrhage, any life-threatening bleed, any bleeding event requiring the administration of ≥ 3 units of packed red blood cells per day for two consecutive days or any bleeding event assessed as a serious adverse event. Secondary endpoints included mortality, length of stay by level of care, requirement for assisted ventilation and interventions (radiological and/or surgical).

4.3.7 Sample power calculations

To correctly design any future randomized trial it was necessary to establish the product's safety in this patient group and gain variance measures on outcomes to explore alternative power estimates in this patient population. Comparison with a historical control group was used primarily as a safety measure to verify that patient outcomes were not untoward (for example, a higher death rate than expected). A paired analysis could provide more precise (baseline-adjusted) estimates but even so, the original target recruitment of 30 was a modest and pragmatically chosen sample based on anticipated difficulty in identifying patients with severe disease sufficiently early in their disease course. Accepting that internal validity in a non-randomized comparison could be compromised by unforeseen confounders, for power 80% at alpha 5% and control 30% risk of death (9/30), group correlation moderately high (0.4 lower, 0.6 upper), sample size of 30 (pairs) with 2-sided testing the study would reject the null hypothesis for a difference of +/-6 deaths, i.e. ≤3 or ≥15 deaths.

The study would reject the null hypothesis for an average difference of length of stay of 8 days (conservative) to 3 days (optimistic).

4.3.8 Case matching

Controls were identified from patients with severe acute pancreatitis treated in the same intensive care unit during the period May 2007 to April 2009. Control data were matched at the patient level by age, gender and disease severity. Initial matching conditions were: APACHE score, MODS, gender, age band (+/- 5 years), aetiology (alcohol, gallstones, other). Where matching was problematic in

individual patients, requirements were relaxed for APACHE score and age (+/- 1 unit or band).

4.3.9 Measurements and assays

Protein C, Interleukins 4, 6 and 10 (IL 4, IL-6 and IL-10) and Tumor Necrosis Factor alpha (TNF-alpha) were measured from day 2 (pre-infusion). These assays were undertaken according to protocol in the Xigris™ group. On day 3 markers were assessed within 2 hours of completing the infusion.

4.3.9.1 Protein C assay

A 2.7-mL sample of venous blood was collected into a sterile vacutainer (BD Diagnostics, New Jersey, USA) containing 3.2% (0.1 mol/L) trisodium citrate anticoagulant solution at a ratio of 9:1. Following centrifuging at 3500 revolutions per minute for a duration of 10 minutes, the supernatant plasma was separated from cellular matter and platelets. This was stored at -80 °C until sufficient samples had been acquired to permit analysis in a batch. The frozen plasma was thawed to 37 °C for 15 minutes prior to analysis. A fully automated technique using a synthetic chromogenic substrate STA- Stachrom Protein C kit (Diagnostica Stago, Asnieres, France) was employed for the quantitative assessment of functional protein C levels in the subject's plasma. Protein C was activated using the venom of *Agkistrodon contortrix*. The resulting enzyme created was measured by its amidasic activity on a synthetic chromogenic substrate by causing the release of paranitroanaline. The quantity of paranitroanaline produced was directly proportional to the quantity of Protein C in the sample and was measured on a plate reader at 405 nm (laboratory reference range, 69Y154 U/dL). The

United Kingdom National External Quality Assessment Service provided external quality control.

4.3.9.2 Interleukin and tumour necrosis factor –alpha assays

A venous blood sample, 6 ml in volume, was acquired using a sterile vacutainer silicone coated serum separator collection and storage system (BD Diagnostics, New Jersey, USA). This was allowed to stand for 60 minutes at room temperature before being centrifuged for 15 minutes at 1000 x g, following which the supernatant serum was removed; divided into aliquots of 0.5 ml and stored at -80 °C until sufficient samples had been acquired to permit batch processing of the assay. ELISA kits for each cytokine (Quantikine ELISA, R&D Systems, UK) were used according to the manufacturer's instructions. Using a quantitative sandwich enzyme immunoassay technique, the intensity of colour following the addition of a substrate was read after 30 minutes at 450 nm using a Dynex Revelation MRX TC spectrophotometer (Dynex Technologies Ltd., UK). Ranges and sensitivity were as follows: IL-4 (Sensitivity: 10 pg/mL, Range 31.2 – 2,000 pg/mL), IL-6 (Sensitivity: 0.7 pg/mL, Range 3.12 - 300 pg/mL), IL-10 (Sensitivity: 3.9 pg/mL, Range 7.8 - 500 pg/mL) and TNF-alpha (Sensitivity 5.5 pg/mL, Range 15.6 - 1,000 pg/mL).

4.3.9.3 Haematological and biochemical assays

A baseline haematological profile (including full blood count and haematocrit) and clotting profile including Prothrombin Time (PT), Activated Prothrombin Time (APTT) and bleeding time was recorded daily during the first week. Measurements were performed using the clinical haematology and biochemistry services for the hospital.

4.3.9.4 Assessments of organ dysfunction

Other measurements undertaken during the course of the first week included monitoring of organ dysfunction and record of progression of disease. These included: daily Marshall Organ Dysfunction score (MODS), early warning score (EWS) as recorded on nursing charts and APACHE II. Data for the calculation of organ failure scores to determine eligibility into the study was on the basis of physiological observations; haematological and biochemical results at the time of admission and following a 24 hour interval. Subsequent to this, daily observations and results from within a fixed time period were used, following admission to critical care or the acute surgical ward. Absent values that would require an invasive procedure not warranted by the patient's clinical condition (for example, the measurement of central venous pressure that would require a central venous catheter in a patient who was otherwise well) were assumed to be normal.

4.3.10 Statistical analysis

Continuous measures were analysed using the related samples Wilcoxon signed rank test; proportions were analysed using the related samples McNemar test and categorical variables (with more than 2 categories) were analysed using Fisher's exact test. To allow for incomplete data due to discharge from hospital last-observation-carried-forward analysis (LOCF) was reported for disease scores and biomarkers. Subsequently recruitment was limited to 19 contributing patients and matched controls. Reported p-values are hypothesis generating and not intended as a formal test of inference.

4.3.11 Ethics committee approval

This study was approved by the North West Regional Ethics Committee (Reference 07/H1307/201) and the United Kingdom Medicines Health Regulatory Agency (MHRA) (21387/0213/001-0001 Eudract Number 2007-003635-23). Adverse events were also reported to the Lilly Pharmacovigilance unit.

4.4   Results

Abdominal pain and 3-fold rise in serum amylase and/or pancreatic necrosis on CT (N=166)

APACHE II score ≥ 9 (N=44)

24 hr infusion of Xigris 24mcg/kg [Day 2-3] (N=19)

Follow-up 1 day post infusion [Day 4] (N=19)

Follow-up 7 days post infusion [Day 10] (N=13)

Excluded (N=25):
9: Received by tertiary centre >72 hrs from admission
4: refused participation
3: MODS<2 and SIRS<2 over 72 hours
2: Could not give consent
1: Platelets <30,000 mm^3
1: Oesophageal varices
1: On warfarin
1: Upper GI cancer
1: Died before giving consent
1: Recruited to another study
1: End stage Renal Failure

Discharged (N=6)

Figure 4.1: CONSORT flowchart of study recruitment.

4.4.1 Patients

Between 15 November 2009 and 25 October 2011, a total of 166 patients were screened for inclusion into the study. 43 patients met the APACHE inclusion threshold and following exclusions Xigris™ was administered to a consecutive series of 19 patients (Figure 4.1). All 19 patients completed treatment successfully and contribute to analyses. The original protocol aimed to recruit 30 patients but

the study was closed to recruitment when Xigris™ was withdrawn from clinical use.

4.4.2 Baseline comparability
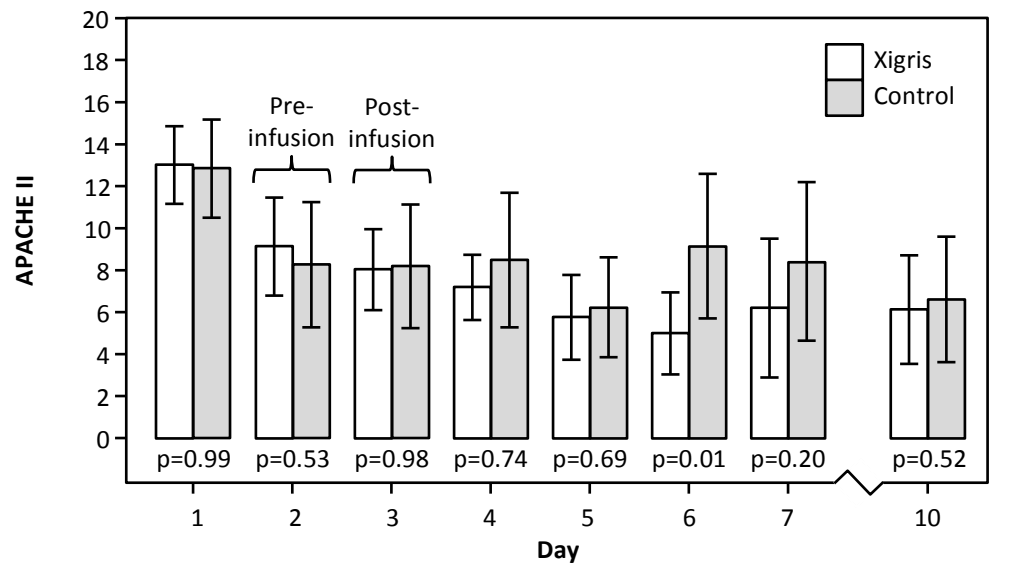
Bars show 95% confidence intervals; p-values from Wilcoxon Signed Ranks (exact) Test

Figure 4.2: APACHE II Scores for trial participants and controls.

Bars show 95% confidence intervals; p-values from Wilcoxon Signed Ranks (exact) Test

Figure 4.3: MODS Scores for trial participants and controls.

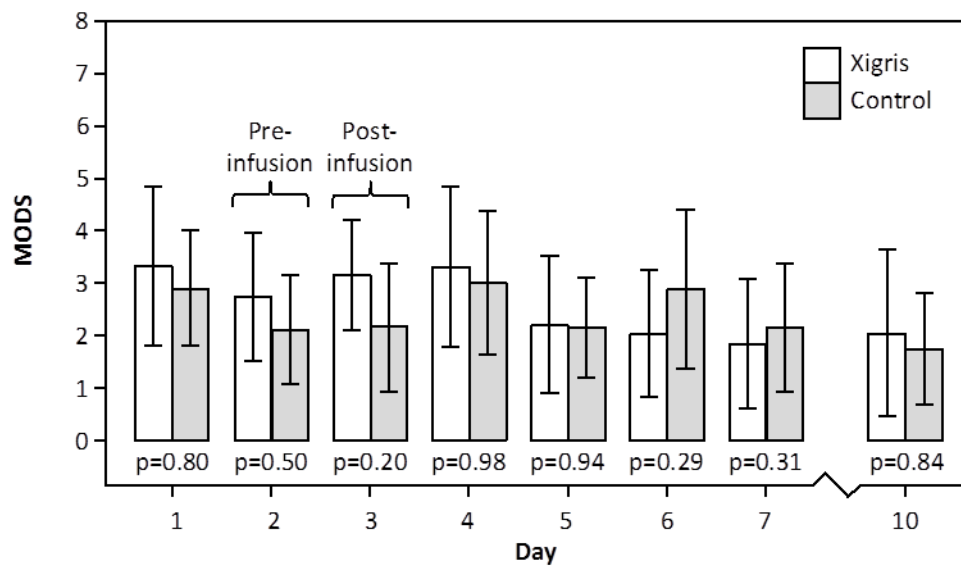|	Control N=19		Xigris™ N=19		
	Mean	SD	Mean	SD	P[2]
Age[1]	58.1	13.1	57.3	16.0	0.68
Gender: Male (%)[1]	13	(68%)	10	(53%)	0.45
Aetiology[1]					0.87
Alcohol related (%)	2	(11%)	3	(16%)	
Drug induced (%)	2	(11%)	2	(11%)	
Gallstones (%)	10	(53%)	12	(63%)	
Post ERCP (%)	1	(5%)	0	(0%)	
Unknown (%)	4	(21%)	2	(11%)	
APACHE II[1]	12.8	5.0	13.0	3.9	0.99
Marshall Organ Dysfunction Score, (MODS)	2.9	2.3	3.3	3.1	0.80
CT Performed (%)CT	12	(63%)	13	(68%)	1.00
Pancreas: normal (%)	1	(8%)	2	(15%)	1.00
Enlarged (%)	8	(67%)	10	(77%)	0.67
Peripancreatic stranding (%)	9	(75%)	8	(62%)	0.67
Single fluid collection (%)	5	(42%)	2	(15%)	0.20
Multiple fluid collection (%)	1	(8%)	3	(23%)	0.59
Necrosis (%)	7	(58%)	6	(46%)	0.70
Biochemistry and Haematology					
Sodium, Na+ (mmol/L)	137.6	3.7	139	3.8	0.20
Urea (mg/dL)	7.1	3.1	16.1	31.3	0.17
Creatinine (mmol/L)	92	25.8	107.9	58.4	0.51
Bilirubin (micromol/L)	23.6	19.8	37.7	39.8	0.29
Haematocrit (%))	0.4	0.1	0.47	0.13	0.21
White blood cell count (10^3/mm^3)	14.9	7.2	20.6	14.8	0.09
Platelet count (10^3/mm^3)	230.5	148.4	213.1	75.4	0.95
Body temperature (°C)	36.8	1.0	36.6	0.9	0.95
Respiratory rate (/min)	19.4	6.5	19.6	8.6	0.99
Mean Arterial Pressure, MAP (mmHg)	98.6	22.7	93.7	19.7	0.68
Central Venous Pressure, CVP (cm $H_2$0)	10.5	2.1	10	0.0	1.00
Heart Rate, HR (/min)	97.8	24.8	84.2	20.5	0.09
(HR.CVP)/MAP	11.4	5.9	9.5	3.6	0.37
Fraction of inspired oxygen, FiO$_2$ (%))	0.5	0.4	0.3	0.2	0.02
Partial pressure of arterial oxygen, PaO$_2$ (kPa)	12.7	6.3	10.8	4.1	0.49
Partial pressure of arterial oxygen, PaO$_2$ (mmHg)	95.5	47.5	83.2	30.7	0.75
Partial pressure of arterial bicarbonate, HCO$_3$ (mEq/L)	20.5	5.2	21.4	5.4	0.13
PaO$_2$/FiO$_2$ (mmHg)	304	182.2	334.1	120.3	0.62
Prothrombin time, PT (seconds)	13	2.1	14	1.8	0.08
Partial Thromboplastin time, APTT (seconds)	33.5	11.5	31	6.5	0.44
Haemoglobin, Hb (g/dl)	12.4	3.3	14.1	2.2	0.05
pH	7.3	0.1	7.9	2.6	0.92

1 Control group selection matching variables
2 Wilcoxon signed ranks test for continuous measures; McNemar exact text for paired proportions; Fisher's exact test for categorical comparisons

Table 4.1: Baseline Characteristics

The Xigris™ and control groups were well-matched in relation to age, gender distribution and aetiology of acute pancreatitis (table 4.1). They were also similar in terms of initial disease severity and organ dysfunction as assessed by APACHE II and Marshall Organ Dysfunction Score (Figures 4.2 and 4.3). Although a statistically significant reduction of APACHE II scores in the treatment group compared to the control group is seen on Day 6, this corresponds to the timing of surgeries within the latter and likely represents peri-operative physiological changes. Initial utilization of CT and radiological findings, baseline physiological and haematological profiles were also similar.

4.4.3 Response to treatment

4.4.3.1 Primary outcome

Compared to historical controls blood transfusion was lower in the Xigris™ group although this was not significant (Xigris™ 0% vs. Control 21%, p=0.13) (Table 4.2). There were no adverse bleeding events in the Xigris™ group. Four patients (21%) in the control group had adverse bleeding events. This difference was not significant (P=0.05; Fisher's exact). All four of these patients had undergone surgery (three open necrosectomy, one colonic resection for ischemia).

4.4.3.2 Secondary Outcomes

Critical care occupancy was shorter in the Xigris™ group compared to controls although this difference was not significant (Xigris™ group 9.6 sd 5.7 vs. controls 16.4 sd 26.8 days; p = 0.07) (Table 4.2). Inpatient stay was significantly shorter in

the Xigris™ group as compared to control (Xigris™ group 19.3 sd 15.3 vs. control 41.4 sd 42.2 days; p = 0.03).

One patient in the Xigris™ group underwent surgical intervention compared to six in the historical control group. There was a significant difference in relation to vasopressor use (5% Xigris™ group vs. 32% control, p = 0.02) but not in use of antibiotics (58% Xigris™ group vs. 53% control p =1.00).

There was no difference between the groups in either 30-day or in-hospital mortality.

	Protein C N=13			IL-6 (pg/ml) N=15			TNFα (pg/ml) N=15			IL-4 (pg/ml) N=15			IL-10 (ng/ml) N=15		
	Mean	SD	P[1]	Mean	SD	p[1]	Mean	SD	p[1]	Mean	SD	p[1]	Mean	SD	p[1]
Baseline	87	27		144	120		44	68		17.3	32.1		0.31	1.01	
Post-infusion	101	29	.006	90	87	.06	42	76	.63	18.6	33.6	.84	0.34	1.03	.75
1 days post-infusion	99	29	.04	66	82	.009	51	112	.63	20.3	36.6	.56	0.37	1.04	.50
2 day post-infusion	101	30	.002	50	76	.005	42	80	.63	17.2	30.3	.44	0.28	1.01	.75
3 days post-infusion	103	32	.001	45	76	.004	46	100	.63	18.7	32.8	.69	0.30	1.01	.75

1 Wilcoxon Signed Ranks (exact) Test for continuous measures, comparing time point with pre-infusion.

Table 4.2 Inflammatory biomarkers for patients who received Xigris™

4.4.3.3 Inflammatory biomarkers

As detailed in Table 4.2, Protein C levels were elevated above baseline after infusion and were significantly elevated compared to baseline at 48 and 72 hours post-infusion (Table 4.3). It should be noted that because of the assay technique used, the values following Xigris™ infusion reflect the sum of the activated protein C and the subject's endogenous protein C both activated and inactive.

4.5   Discussion

This trial provides a safety profile of early intervention with a 24-hour infusion of human recombinant activated protein C in patients with severe acute pancreatitis with a sustained systemic inflammatory response and persistent organ dysfunction.

Previous trials in severe acute pancreatitis have been compromised by including patients with clinically mild disease (99). Thus, the current trial identified those patients with severe acute pancreatitis with physiologically demonstrable severe disease as assessed by a persistent systemic inflammatory response and sustained organ failure. These patients constitute a group at increased risk of death, in whom early surgery is not beneficial (112) and for whom a pharmacological intervention would be ideal. The historical control group was carefully matched for initial disease severity, drawn from the same hospital unit as the treatment group, and were treated only two years prior to the study group. Notably, more control group patients underwent surgery which might explain differences in inotrope requirement, critical care occupancy and in-patient stay. However, given matched disease severity, and since most operations are undertaken late for treatment of necrosis, this might also be a genuine treatment effect.

Comparing the findings of this study to those of the Finnish study, the first observation is the difference in the patient population: 31 of 32 patients in the Helsinki study had alcohol-induced acute pancreatitis and their population was younger than in the present study (93). The inclusion criteria in both studies followed a similar philosophy and although it could be argued that the present study used more stringent definitions of severe disease, the end results in terms of

treated populations are similar with the baseline APACHE II scores in the Finnish study being higher. The key finding from both studies is the absence of haemorrhagic complications after treatment with Xigris™. The rise in bilirubin seen in the Finnish study and not in ours is thought to be related to their 96-hour infusion protocol and this should be explored in any future study design.

Although it is likely that both PROWESS and PROWESS SHOCK included patients with severe acute pancreatitis, their patients would have been enrolled as they met sepsis-led inclusion criteria (e.g. pancreatic abscess, infected necrosis)(87, 111). This type of sepsis picture is a feature of the later stages of severe acute pancreatitis rather than the early stages where a severe systemic inflammatory response predominates. Therefore the severity of the clinical sepsis in both studies was mediated by the presence of an external microbial agent (e.g. bacteria or fungi) that was not targeted by rhAPC administration. Infection in the late stages of SAP is sequelae of initially sterile tissue necrosis. If the results of the animal studies (89, 90) showing protection of pancreatic parenchymal tissue were to be replicated in human subjects, this would preclude their development of sepsis secondary to infection and improve the probability of positive clinical outcomes.

Taking into consideration the withdrawal of Xigris™ from clinical use (and excluding case reports) these two studies, evaluating a combined population of 35 patients with severe acute pancreatitis constitute the only reported experience of the feasibility of this drug in this condition – a setting in which over a century of evaluation has failed to see the emergence of specific therapy. The evidence of these two studies must be regarded as preliminary but the constant finding of a lack of haemorrhagic complications argues for further protocol-specific evaluation. Similarly, the findings of lower inotrope use and shorter critical care occupancy in

the present study, although possibly related to differences in surgical intervention provide scientific justification for further specific evaluation of Xigris™ in severe acute pancreatitis. Given that the drug has been withdrawn from clinical use, such an assessment is not possible. However the evidence presented here suggests that there may be a future role for drugs which modulate microvascular coagulation in the early treatment of severe acute pancreatitis.

CHAPTER 5:

Targeted decompression in Abdominal Compartment Syndrome complicating SAP: a pilot study

5.1 Abstract

Objective: The management of patients with severe acute pancreatitis (SAP) can be complicated by the development of abdominal compartment syndrome (ACS). The gold-standard treatment of a decompressive laparotomy is contentious as surgery in active SAP has been demonstrated to increase mortality and morbidity. A clinical study investigating the outcomes of patients treated non-surgical versus a surgical treatment would help resolve the clinical equipoise that currently exists.

Design*:* A pilot randomized controlled trial of targeted decompression in patients with abdominal compartment syndrome complicating severe acute pancreatitis using a step up approach in the surgical treatment arm. 150 consecutive patients were admitted with acute pancreatitis (AP), between June 2010 to December 2012, and screened for eligibility to participate in the study. 22 patients initially gave permission to have IAP measured on the understanding that they might be randomised to an interventional arm or conservative management arm in the event that they developed SAP and subsequent ACS.

Results: Abdominal compartment pressures (ACP) in the patients admitted with AP were similar to that in a hospital population without ACS. The sub-group of 13 patients that progressed to SAP did not have sufficiently raised ACP to meet the criteria for ACS. Consequently no patient with SAP developed ACS and randomization to a treatment arm was not possible.

Conclusions: The absence of ACS in a population where it was previously observed could be due to Type II observer error. As clinicians responsible for the 13 patients were not blinded to the increasing abdominal pressures, it is possible that preventative steps were instituted before ACPs exceeded the threshold for the

development of ACS, lending credence to a theory that ACS is an epiphenomenon of SAP.

North West Ethics Reference 10/H1010/43.

5.2   Introduction

Intensive support for failed organ systems is the mainstay of treatment for patients with severe acute pancreatitis and there remains no specific therapy for this condition (116, 117).

Early surgical debridement is unhelpful. A randomized trial comparing early surgical debridement to later intervention showed a prohibitively high mortality rate in patients undergoing early surgical debridement of the pancreas (118) (119). The reason for this is that in the early stages of the disease the pancreas is swollen and hyperemic and there is no "target" for debridement. Similarly, formal pancreatic resection is impossible in the setting of gross pancreatic swelling (118) (119).

Disruption of the main pancreatic duct (MPD) is characteristic of severe acute pancreatitis and the presence or absence of MPD rupture is an important determinant of the subsequent clinical course of the disease(120). Patients with severe acute pancreatitis and a proximal rupture of the main pancreatic duct, typically (but not exclusively) develop peri-pancreatic and remote intra-abdominal fluid collections.

Early fluid collections are part of the clinical spectrum of severe acute pancreatitis (SAP). There is no evidence that drainage of these collections in SAP alters the clinical course of the disease (121) and on the contrary, repeated percutaneous radiological drainage risks visceral injury and/or the introduction of infection.

In some patients with severe acute pancreatitis, intra-abdominal pressure can be high and lead to the so-called intra-abdominal compartment syndrome (ACS). The incidence of ACS in SAP has been reported as approximately 60%.(122, 123) It is not clear whether this is related to accumulation of collections of "free" intra-peritoneal fluid or tissue fluid. Given that the capillary leak phenomenon is a

component of critical illness (124), intra-abdominal hypertension may simply be an epiphenomenon of severe acute pancreatitis(125).

However, there is intriguing preliminary evidence from several small reports that decompression may be beneficial in patients with severe acute pancreatitis with evidence of respiratory or renal compromise as a result of intra-abdominal compartment syndrome. In a study by Chen and colleagues (122), 13 patients who underwent a percutaneous (n=8) or surgical decompression (n = 5), showed significant improvement. However this stands in contrast with a larger study by Leppäniemi and colleagues (126), who reported no significant change in Sequential Organ Failure Assessment (SOFA) scores after decompression in 26 patients with SAP.

Targeted intervention in this setting is outside the conventional dogma for management of severe acute pancreatitis where the body of current evidence does not support drainage or laparotomy in the absence of infected necrosis. A decompressive laparotomy may simply introduce infection into the peritoneal cavity and if the abdomen is left open, there are risks of evisceration (127). Furthermore, a small series (n=3) by Gecelter *et al* (128) did not show that decompressive laparotomy reversed organ failure, despite reducing intra-abdominal pressure.

Thus the purpose of this preliminary randomized trial is to assess whether early-targeted decompression in abdominal compartment syndrome complicating severe acute pancreatitis is associated with an improvement in outcome.

5.3   Methods

5.3.1   Study design

A pilot randomized controlled trial of targeted decompression in patients with abdominal compartment syndrome complicating severe acute pancreatitis.

5.3.2   Setting

The randomised controlled trial was carried out in the Intensive Care and High Dependency units of the Manchester Royal Infirmary, a tertiary care hepato-pancreato-biliary unit which was one of two serving a predominantly urban conurbation of 3.2 million people in Lancashire, UK. The setting was previous used for Al-Bahrani *et al*'s (123) study into the treatment of ACS in SAP.

5.3.3   Inclusion and exclusion criteria.

5.3.3.1 Inclusion criteria

Patients with the following criteria were recruited:

1. Acute pancreatitis – defined as acute abdominal pain with a threefold elevation of serum amylase or a twofold elevation of serum lipase.

2. Radiological confirmed acute pancreatitis – diagnosis confirmed by computed tomography (CT).

3. Not pregnant.

4. Over 18 years of age.

5. Patients able to give informed consent (or complying with current United Kingdom criteria for consent in critical care unit trials).

6. Development of the intra-abdominal compartment syndrome, defined for the purposes of this study as:

'***A sustained elevation*** (a minimum of 3 readings separated by at least 8 hours within a 24 hour period) ***in intra-abdominal pressure in excess of 20 mm Hg*** (as measured by intravesicular pressure measurement) ***associated with the <u>new</u> onset of organ failure*** (defined for the purposes of this study as a logistic organ dysfunction score ≥ 2, with a minimum single score of 2 of more in any organ category).'

Thus a patient scoring 1 in both respiratory and cardiac categories would not be eligible, but a patient scoring 2 in a single category would be recruited.

5.3.3.2 Exclusion criteria

1. Patients who are under the age of 18 years.

2. Patients who are unable to give informed consent.

3. Recent laparotomy (within 1 week of onset of acute pancreatitis).

4. Pancreatitis diagnosed at laparotomy.

5. Patients with an underlying diagnosis of malignancy.

6. Patients with pre-episode chronic renal failure or chronic liver failure with ascites.

5.3.4 Sample size, principal and secondary end-points

From hospital audit records and our own earlier published work, we estimated that patients with severe acute pancreatitis, with organ dysfunction and abdominal compartment syndrome would have a logistic organ dysfunction scores (LODS) of 4 or more. Preliminary non-randomized data from our unit indicated that decompression relieved the abdominal compartment syndrome and concomitant organ failure in all patients(123).

As there was no randomized comparative data at the time of commencement, the recruitment of 20 patients (10 in each arm) to the study would generate novel data on intra-abdominal compartment syndrome and the effect of decompression and may then provide a logical basis for progression to larger-scale studies (ACS-SAP 2).

5.3.4.1 Primary endpoints

1. Change in individual organ scoring for the patient's Organ Dysfunction score following either intervention.

2. Mortality in either group.

3. Change in concentrations of circulating inflammatory and anti-inflammatory molecules both before and after any decompressive procedures were performed.

5.3.4.2 Secondary end-points

1. Change in abdominal perfusion pressure (APP) defined as the mean arterial pressure minus intra-abdominal pressure (in mm Hg).

2. Change in markers of the inflammatory response: white cell count, c-reactive protein, serum soluble interleukins, serum tumour necrosis factor alpha (TNF α)

3. Incidence of complications with a particular reference to complications related to intervention arm (intra-abdominal infected collections, episodes of bleeding, wound infection – defined using standard criteria).

4. Cost of intervention and cost of care.

5. Length of stay in critical care and hospital.

6. Mortality.

5.3.5 Stopping rules

Consistent with the need to ensure appropriate treatment and safety of patients, early termination is specified on three grounds:

1. Failure of administration or trial conduct.

2. New evidence about the care of this patient group making it either un-necessary or unethical to continue.

3. A data-dependent stopping condition being met.

5.3.6   Initial approach and recruitment

Information about the study was provided to all patients who were admitted with the diagnosis of acute pancreatitis and required a urinary catheter for their management by the clinical team in charge of the patient. The patient was informed of the randomization to a treatment and non-treatment arm prior to assessment of intra-abdominal compartment pressures. They also had time to decide whether to participate. Although the researcher preferred recruitment within 24 hours of initial approach, it was accepted that patients in this study were ill and needed time to read and understand the study. They also had the opportunity to discuss the study with their relatives. Therefore, no pre-set time limit for enrolment was specified. Once ACS was detected, the patients would enter a treatment arm as indicated in the following flowchart (Figure 1).
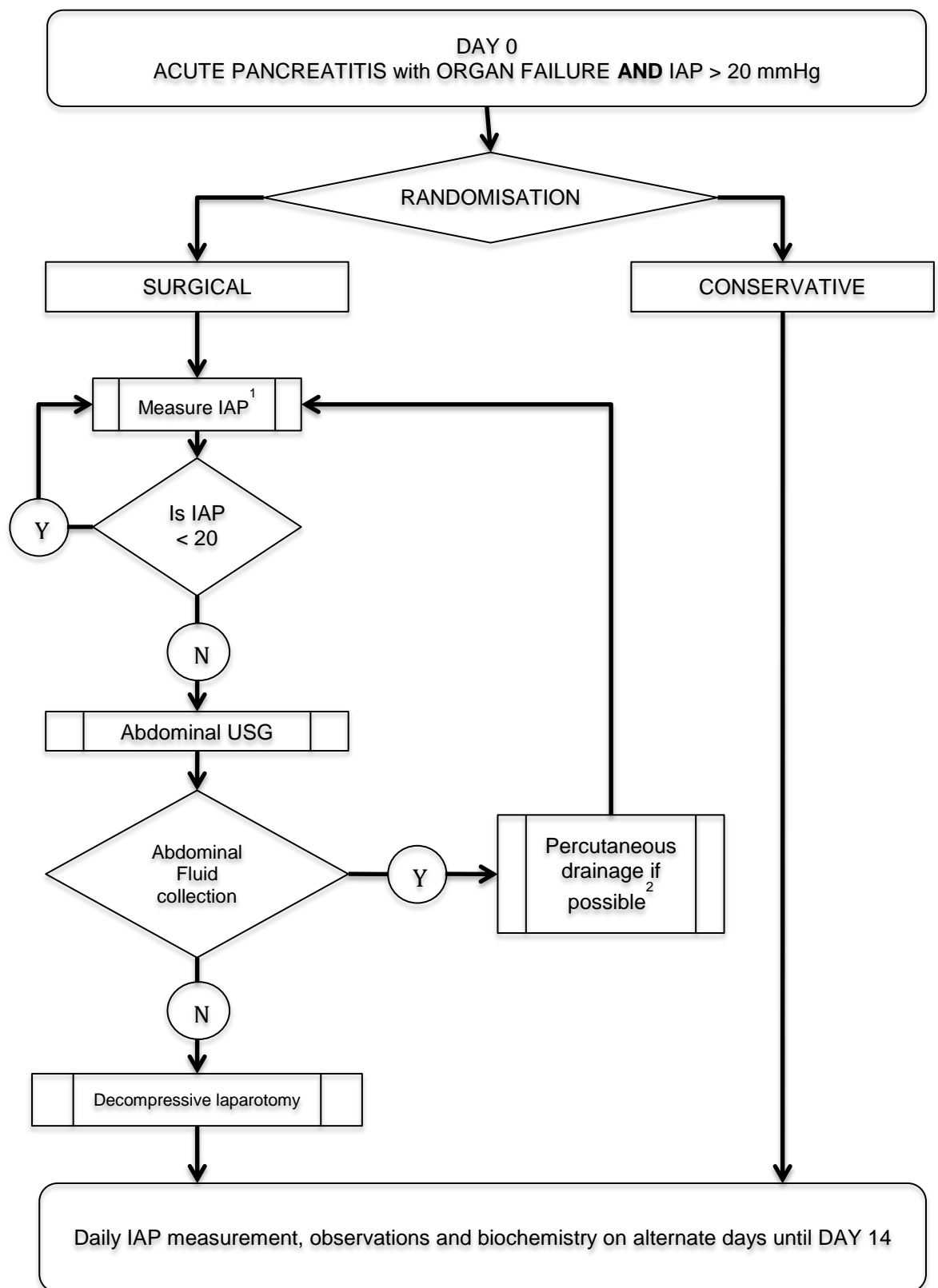
Figure 5.1: ACS-SAP management flowchart

1. As per the technique described, on 3 separate occasions at intervals of 8 hrs.

2. If no fluid can be drained, the response to the previous step (Abdominal fluid present?) will be considered as 'No'. Peripancreatic fluid collections are known sequelae in SAP. Physics dictates that removal of an incompressible fluid from within a closed compartment reduces the volume of contents and hence the pressure within it.

5.3.7 Measurement of intra-abdominal compartment pressure

In order to minimize transient increases in abdominal pressure (IAP) from affecting the analysis of results and permit time for the body to respond to any intervention (fluid drainage/mini-laparotomy), 3 readings of IAP were taken at 8-hour intervals using the method described by Cheatham *et al (129)*, once a patient had been assigned to a treatment arm:

1. The patient was in the supine position.

2. An adult male urinary Foley catheter (Rüsch 16G, Teleflex Medical Inc., Reading, PA, USA) was inserted into the bladder using a clean Aseptic Non Touch Technique (ANTT), irrespective of the patient's gender.

3. A pressure transducer (TruWave Disposable Pressure Transducer, Edwards Lifesciences, Irvine, CA, USA) was connected, via a 3/4 port with Luer lock (Chalice Medical, Nott, UK) and T-valve, to the Foley catheter (Rüsch, Teleflex Medical Company, NC, USA) urine drain port. The pressure transducer was connected in turn to the patient's multi-parameter monitoring device (Vigilance II Monitor VIG2/VIG2E, Edwards Lifesciences, Irvine, CA, USA) to provide a pressure reading in mmHg.

4. The Foley catheter drain was then clamped distal to the sample valve port.

5. The system was flushed with 30ml of sterile saline (using a syringe attached to the 3-way valve) to ensure a continuous fluid column with no air bubbles.

6. The pressure transducer was 'zeroed' to atmospheric pressure in the mid-axillary line at the iliac crest.

7. 25ml of sterile water was then inserted into the urinary bladder and the intra-abdominal pressure (IAP) noted.

8. Three readings were taken and the average reading recorded by a single investigator to prevent inter-observer variability.

9. The catheter drain clamp was released to permit urine drainage.

5.3.8 Intervention

5.3.8.1 *Radiologically guided drainage:*

A drain was to be placed in the largest intra-abdominal fluid collection under ultrasound or CT guidance. This procedure would have been performed under strict aseptic conditions using the Aseptic No Touch Technique (ANTT) guidelines and was to be undertaken either by a consultant radiologist or a senior radiology trainee under direct consultant supervision.

5.3.8.2 *Laparotomy*

Using guidelines described by Leppaniemi et al (130), in those patients that progressed to laparotomy from initial ultrasound guided drainage, a small (<15cm) midline epigastric incision will be made under general anesthesia (as for a standard laparotomy). The length of the incision is a pragmatic one. Full length abdominal incisions for decompression of the intra-abdominal pressure may require skin grafts to close the defect. There is no clinical evidence to support an incision greater than 15 cm and skin grafts would contribute to patient morbidity. Fluid was to be drained and samples sent for microbiology. Unless there were other findings at laparotomy, no other intervention was to be undertaken and the lesser sac and pancreas would not be disturbed. Abdominal closure would not be by primary closure but would utilize either a zipper; silastic wound protector or other similar device at the discretion of the operating surgeon. Primary closure involves the use of suture material with the intention of permanent restoration of the abdominal wall integrity by subsequent wound healing. This would prevent the

internal abdominal contents from equilibrating their pressure with the external atmospheric pressure. Increasing the surface area of the abdominal wound with a temporary aseptic cover, reduces the likelihood of infection and can be altered to accommodate expansion/contraction of the abdominal wall due to internal pressure changes.

5.3.8.3 Standard therapy

Conservative management of SAP was to be managed by the patient's consultants (patients are often under the shared care of critical care physicians and Hepatopancreatobiliary surgeons) in accordance with published guidelines for the medical treatment of raised intra-abdominal compartment pressure(131) and illustrated in Figure 5.2.
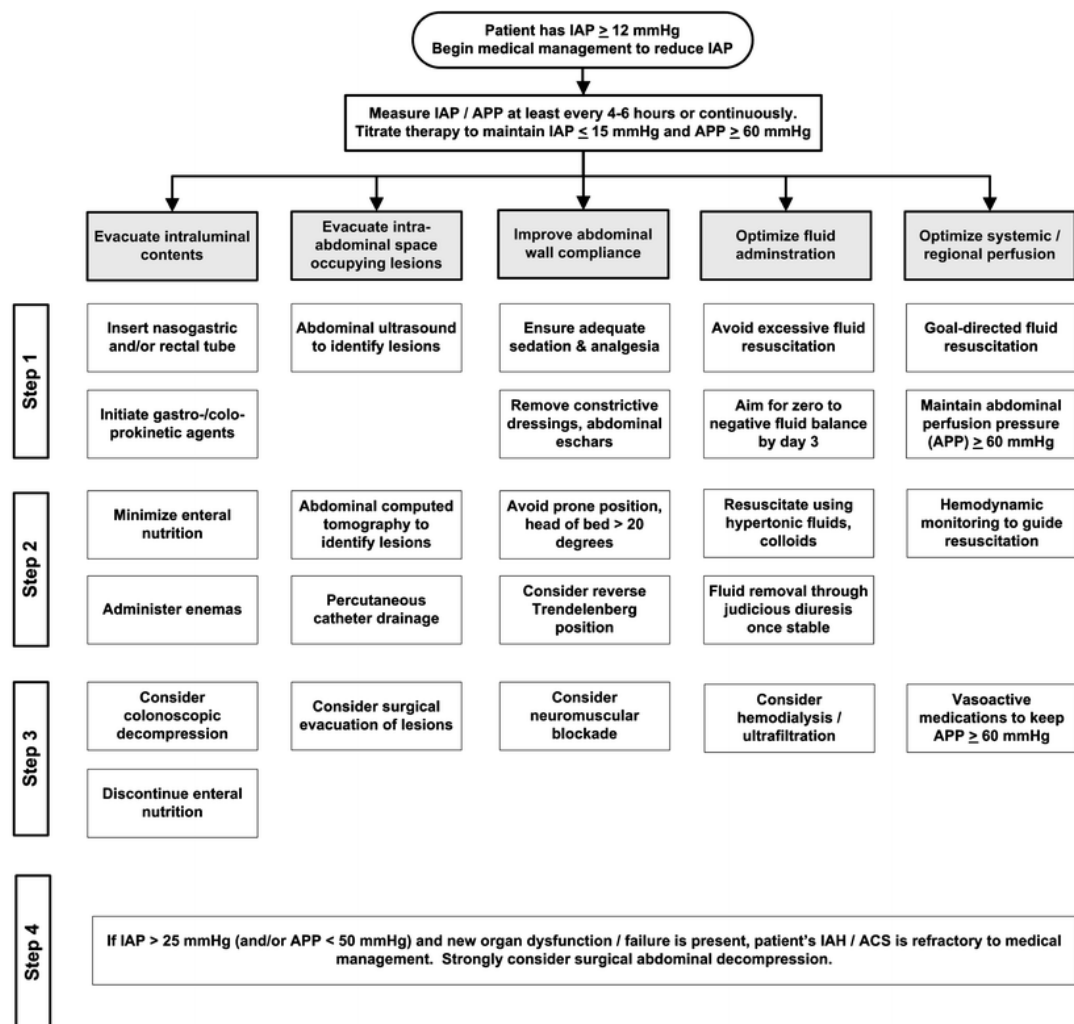

Figure 5.2: Nonoperative intra-abdominal hypertension/abdominal compartment syndrome (IAH/ACS) management algorithm. The choice (and success) of the medical management strategies depicted is strongly related to both the etiology of the IAH/ACS and the patient's clinical situation. The appropriateness of each intervention should always be considered prior to implementing these interventions in any individual patient. The interventions should be applied in a stepwise fashion until the patient's intraabdominal pressure (IAP) decreases. If there is no response to a particular intervention, therapy should be escalated to the next step in the algorithm. APP: abdominal perfusion pressure (Image and caption reproduced from Cheatham *et al*(131))

No specific intervention was kept from patients in either group. The sole difference in care was that patients in the intervention arm would have had targeted radiological drainage if they had met intervention criteria with a step-up approach used to progress to laparotomy if there was no response to radiological drainage.

5.3.8.4   Timing of intervention

Once the criteria for the definition of ACS had been met, it was intended that the patient undergo guided drainage within 24 hours if randomized to the intervention group.

5.3.9   Randomisation

Randomization was to be undertaken using variable length, even number blocks with the treatment arm allocation recorded in sealed envelopes.

5.3.10   Ethics committee approval

This study was approved by the North West Regional Ethics Committee (Reference 10/H1010/43).

5.4   Results

150 consecutive patients admitted with acute pancreatitis to the Manchester Royal Infirmary, between June 2010 to December 2012, were screened for eligibility to participate in the study. 11 patients were excluded as they were unable to give consent or met exclusion criteria. 22 patients initially gave permission to have IAP measured on the understanding that they might be randomised to an interventional arm or conservative management arm in the event that they developed ACS.

Demographic and clinical characteristics (n =22)	
Age (years)	54 (28 to 82)
Gender	11 Male/ 11 Female
Aetiology	
Alcohol	5 (22.7%)
Gallstone	1 (4.5%)
Gallstones	13 (59.1%)
Post ERCP	2 (9.1%)
Unknown	1 (4.5%)
Severe Acute Pancreatitis	13 (59.1%)
APACHE II Score at admission	16 (2 to 18)
Duration of Hospital admission (days)	264 (4 to 268)
Critical Care Admissions	9 (40%)
Duration of Critical Care stay (days)	97 (3 to 100)
Hospital mortality	3 (13.6%)
30 day mortality	1 (4.5%)
Outcomes	
Catheter removed	13 (59.1%)
Abdominal drain inserted	6 (27.3%)
Withdrew consent	3 (13.6%)

APACHE II : Acute Physiology and Chronic Health Evaluation.

Table 5.3: Demographics for patients with acute pancreatitis that underwent measurement of abdominal compartment pressure.

During the study period, no patient developed ACS over the period that IAP was measured.
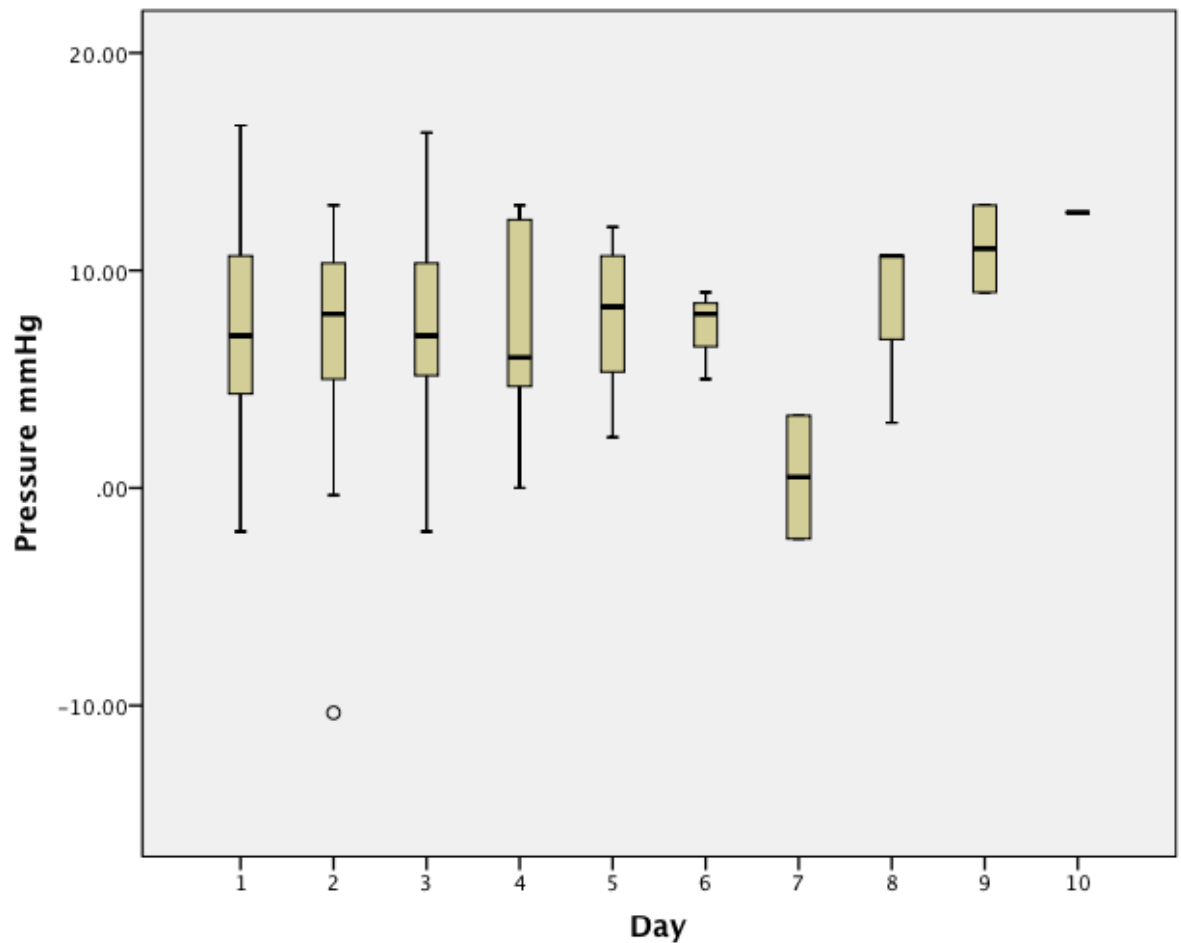
Figure 5.4 : Sequential abdominal compartment pressures for the period in all patients with acute pancreatitis.

Abdominal pressures measured in patients who were screened for inclusion in the study (Figure 5.4) are similar to pressures in a standard hospital population and healthy volunteers, throughout the duration of their admission. This chart includes patients who were admitted to intensive care for the subsequent development of SAP.

118
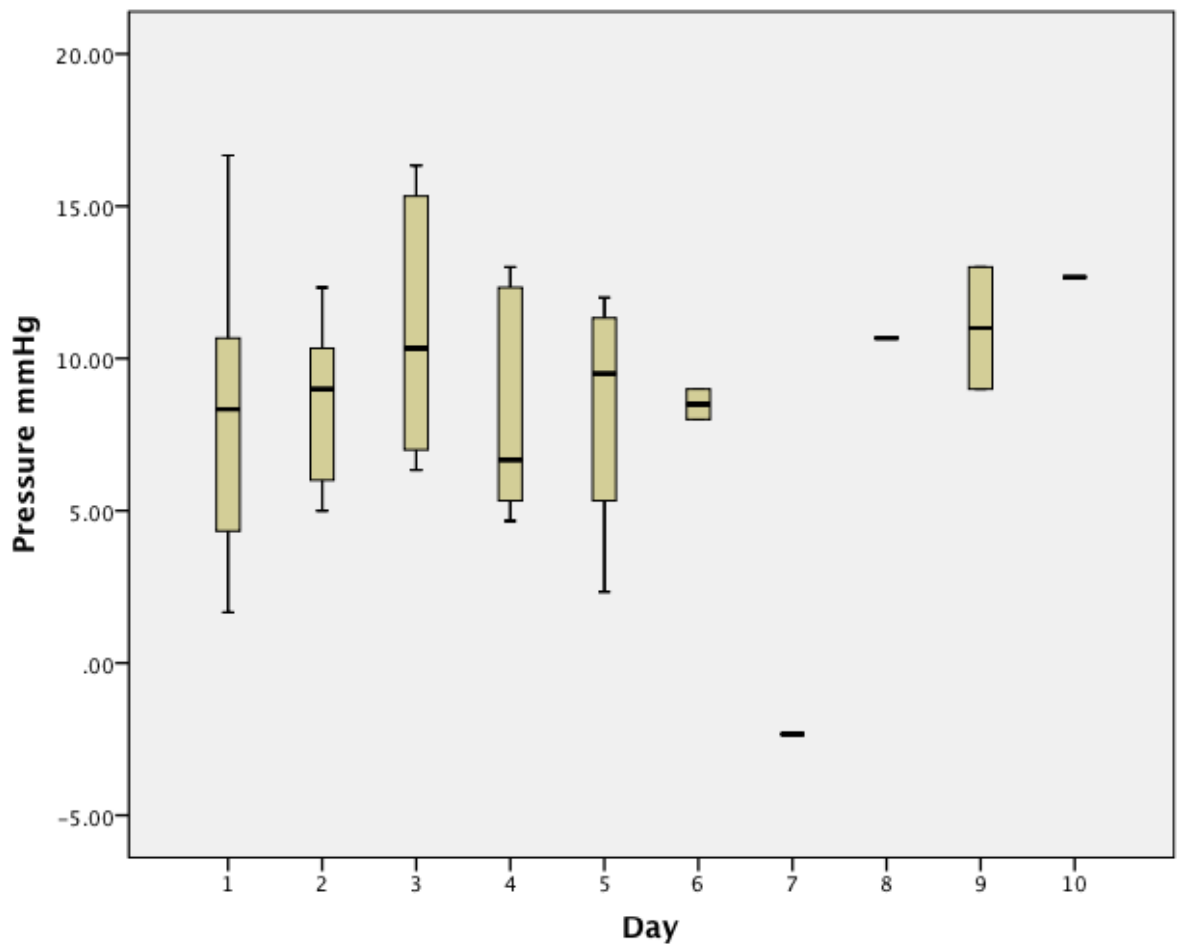
Figure 5.5 : Sequential IAP for patients admitted to critical care.

Abdominal pressures in the subgroup of patients who developed SAP do not show an appreciable variation from the overall group. No individual pressure reading exceeded the threshold of 20 mmHg to be acknowledged as ACS. Intermittent negative values were obtained an individuals on haemofiltration. This is a supportive clinical treatment for renal failure. It involves the removal of blood from the body and filtration of metabolites. The volume of blood returned can be adjusted to remove fluid, resulting in a negative pressure gradient within the abdominal compartment.

5.5   Discussion

At the time this study was commenced, the majority of studies detailing the incidence and progression of ACS in SAP, were based on retrospective analysis of patients who were determined to have ACS following either radiological or clinical indicators(123). The measurement of intra-vesicular abdominal compartment pressures was then performed to confirm the diagnosis. The few studies that did prospectively investigate the incidence and progression of ACS restricted their study to patients with established SAP. In this context, the development of a significant rise in abdominal compartment pressure and the institution of conservative therapy as detailed by Cheatam et al (129), may be missed.

De Waale et al (132) has made a case for the prospective measurement of intra-abdominal pressures in patients admitted to critical care, however the nature of nursing in a critical care environment places this as just one of a myriad of measurements that might take place. Given that the basis of that study's recommendations was in a post-surgical patient group, it is reasonable to consider that there was little evidence for abdominal pressure measurements to constitute standard clinical practice in patients with SAP who have not undergone surgery.

It is possible that the pressure measurements made available to the clinicians responsible for the patients, was responsible for the lack of patients who developed ACS. 2 patients had conservative management instituted after developing IAH associated with a rise in Peak End Expiration Pressure (PEEP) whilst ventilated. Another 3 patients had ultrasound guided drainage for pancreatic collections detected after imaging. Clinicians were also able to stop intravenous

fluid administration upon noting an increase in intra-abdominal pressure when coupled with a decrease in fluid output.

The median abdominal pressures observed in the study patients correlate with Cheetham et al's (129) observation that patients admitted to critical care have pressures ranging between 7-8 mmHg. This is understandable as current therapy for the management of acute pancreatitis remains supportive. This entails intravenous fluid transfusion in an attempt to correct fluid shifts from the intra vascular spaces to the intra- and extra- cellular fluid spaces. However, the quantity of fluid transfused is empirical and usually goal directed (I.e. aimed at increasing mean arterial pressure, a surrogate marker of organ perfusion) as opposed to guidance by a more direct measurement of intra vascular volume such as central venous pressure (CVP). Accurate measurement of CVP requires the insertion of a central venous catheter into a patient's internal jugular vein under aseptic conditions. The procedure is associated with a risk of pneumothorax, air embolism and potential infection, in addition to patient discomfort. Hence it not currently warranted in patients receiving treatment in a non-critical care environment. An alternative guide to fluid resuscitation is the use of urine output to determine the adequacy of transfused fluid volumes. The caveat being that research by Mole et al (133) has suggested that patients with acute pancreatitis may already be fluid deficient prior to admission, leading even CVP to give an inadequate estimation of the extent of fluid deficit. A possibility therefore exists that they present with compensation for intra vascular hypovolemia by maintaining renal perfusion and normal urine output masking the existence of a substantial fluid deficit. Although this can be determined by the presence of altered renal function and glomerular filtration rate, it still offers no guidance to the volume of fluid that should be transfused to maintain perfusion.

Once the fluids have been transfused, the increased capillary permeability associated with the acute phase of acute pancreatitis leads to the water component of the transfused fluids (usually isotonic saline solutions or variants of the same) also ending up in the third intercellular fluid space. This leads to non-specific tissue edema and abdominal collections of fluid. Both contribute to the onset of a raised intra-abdominal compartment pressure and drainage of the latter formed part of this study's step-up protocol of abdominal decompression.

This study's inability to randomize patients to either of the trial's arms, may also be due to type II error. The use of the intra-vesicular measurement of abdominal compartment pressure is a validated technique (129) and recommended by the World Society of Abdominal Compartment Syndrome. A mid-point review of the trial considered type II error was unlikely as the equipment and patient setting was used for a prior study investigating the association between SAP and ACS (123). In that study, a case series of 18 patients were observed to have a reduction in abdominal compartment pressures after decompression surgery. Furthermore, all measurements in this study were performed by a single observer who had been instructed by the medical devices engineer responsible for the calibration and equipment of the department's equipment. Confirmation that the correct pressures were being measured was achieved using a test jig. This consisted of a urinary catheter inserted into a bag of normal saline containing a 100 ml of normal saline. A calibrated paediatric sphygmomanometer cuff was wrapped around the saline bag and inflated to a series of pressures. There was no discrepancy in the pressures applied and those reported by the pressure transducer attached to the urinary catheter.

In conclusion, although no patients were recruited to the designed study, some evidence has emerged to suggest that the presence of abdominal compartment syndrome in patients with severe acute pancreatitis may be an epiphenomenon.

CHAPTER 6:

Further research

On the basis of the findings and as described in the previous chapters, a proposed protocol for the further investigation of Xigris™ as a therapeutic agent for the early treatment of SAP is detailed:

6.1   Introduction

Severe inflammation of the pancreas gland is a progressive illness characterised by associated organ failure and 30% mortality. In contrast to the mild form of the disease, severe acute pancreatitis (SAP) usually recquires admission to intensive care facilities for continuing fluid resuscitation and organ support.

It is currently believed that the primary sequence of events leading to SAP begins with the activation of digestive enzymes within the acinar cells of the pancreas. The cellular injury that ensues triggers the release of inflammatory cytokines, which in turn attract neutrophils; generate free radicals and initiates the complement system. Clinically this is noted by the detection of the Systemic Inflammatory Response Syndrome (SIRS).

Platelet Activating Factor (PAF), nitric oxide and other inflammatory mediators released by macrophages and neutrophils worsens the initial damage sustained by the pancreas, transforming oedematous pancreatic parenchyma into necrotic tissue. Concurrently, the spread of these self-same inflammatory mediators into wider circulation, initiates the beginning of organ dysfunction, clinically characterised by an increase in the severity of organ dysfunction scores for affected organ systems.

Should the patient survive the organ failure brought about by severe acute pancreatitis, two outcomes are generally possible. The first involves the infection

of the necrotic portions of the pancreas and a corresponding increase in morbidity and mortality. The second outcome is dependent on the cessation of further necrosis, permitting the body's enclosure of the sterile necrotic matter by fibrosis.

Numerous experimental studies have investigated the potential of various therapies aimed at modulating the inflammatory response responsible for organ failure. The general opinion being that early intervention restricts the volume of pancreas affected and hence reduces the intensity of the systemic inflammatory response.

Protein C is a 60-kDa protein that is an important regulator of coagulation(134) as a serine protease responsible for the conversion of Factor VIIIa to VIII via the endothelial protein C receptor (ePCR) in conjunction with Ca^{++}. Given that the onset of micro-vascular thrombosis is considered to lead to pancreatic necrosis, early administration of a prophylactic agent may avert this.

Experimental studies (135) (90) (136) suggest that exogenous supplementation of human recombinant activated protein C (rhAPC, Drotrecogin Alfa) commercially available as Xigris™ (Eli Lilly, Indianapolis, USA) reduced pancreatic necrosis in murine and rat models.

In contrast to the PAF antagonist lexipafant, which did not show a demonstrable effect in reducing the mortality in SAP (137), rhAPC was shown to reduce mortality by 19% in severe acute sepsis (87), where the organ failure caused by sepsis is very similar to that seen in SAP. However this efficacy was not replicated in a subsequent trial (111) leading to the withdrawal of its license as a treatment. Subsequent analysis (138) has suggested that the two trials may not be comparable. Given the absence of an underlying microbial agent as a cause for

organ failure in SAP and the results of both the phase 1 clinical trial of rhAPC 'early' and a small randomized controlled trial (93), the relative safety profile of rhAPC early in SAP supports further investigation.

The American College of Critical Care Medicine and Society of Critical Care Medicine advocated a series of sepsis interventions called Early Goal Directed Therapy (EGDT) in 1999 (62). These recommendations were tested in a randomised controlled trial comparing them to standard therapy. A reduction of >16% mortality was seen in the 263 patients recruited. Since then more than 17 studies have demonstrated the benefits of the care bundles, comparing the outcomes in 1677 pre-implementation and 2361 post-implementation adults (139-152). Cumulatively, these studies reported a 20% reduction in mortality irrespective of where EGDT was commenced (i.e. Emergency Department or Critical Care).

While it is advocated that EGDT be started as soon as patients meet criteria, it has been observed to significantly reduce mortality even when initiated up to 18 hours after patients have been detected to meet the inclusion criteria(153).

Early Goal Directed Therapy (EGDT) (152) consists of a care bundle (Appendix D) delivered as soon as the patient meets the criteria for administration, for a minimum of 6 hours duration. The aim of the care bundle is to achieve specified physiological targets.

Current management of both mild acute pancreatitis and SAP consists of the same treatments that compose the care bundle but are not target driven; of variable duration and vary in the time at which the treatments are commenced.

Concerns about the potential of the care bundles to cause harm have centered around the procedures required (i.e. central line insertion) and specific therapy (blood transfusion), however no adverse events associated with these have been demonstrated in the more than 2631 patients who have received EGDT.

6.2   Safety

In the multi-centre study of rhAPC's effect on mortality, severe acute pancreatitis was part of the exclusion criteria due to the risk of peri-pancreatic haemorrhage. Two subsequent studies(2, 93) have not shown any increase in the risk of focal or systemic haemorrhage associated with the administration of rhAPC in the early stages of SAP.

6.3   Hypothesis

Administration of recombinant activated protein C (rhAPC, Xigris™, Drotrecogin Alfa) within 18 hours of the diagnosis of severe acute pancreatitis significantly reduces mortality and morbidity.

6.4.1 Primary endpoints

1. Mortality (30 days and all cause)

2. Hospital stay

3. Critical Care admission

6.4.2 Secondary endpoints

1. Procedures (Ventilation/necrosectomy/drainage/haemofiltration)

2. Intra-abdominal compartment pressure

3. SOFA scores during 14 days from admission.

4. Cytokine levels (IL-1, IL-4, IL-6, TNF-alpha, D-Dimer, Protein C levels)

6.3   Methods

6.3.1 Sample size

Current consensus holds that severe acute pancreatitis carries a 30% risk of mortality with current standard treatment. EGDT alone is purported to reduce mortality by 20%. Therefore for:

 a 2 sided test;

α of 0.05,

power 0.8,

a reduction of 20% mortality would need 62 patients in each arm (248 total).

6.4   Design

Prospective multi-centre randomised double blinded controlled trial with 4 arms.

These are:

ARM 1 – Current non-targeted management of patients in keeping with admitting

clinician's best practice and placebo.

ARM 2 – EGDT instituted upon confirmation of diagnosis and placebo.

ARM 3 – EGDT given concurrently with 96 hours of 24 mcg/Kg/hour of rhAPC.

ARM 4 – Current non-targeted management of patients in keeping with admitting

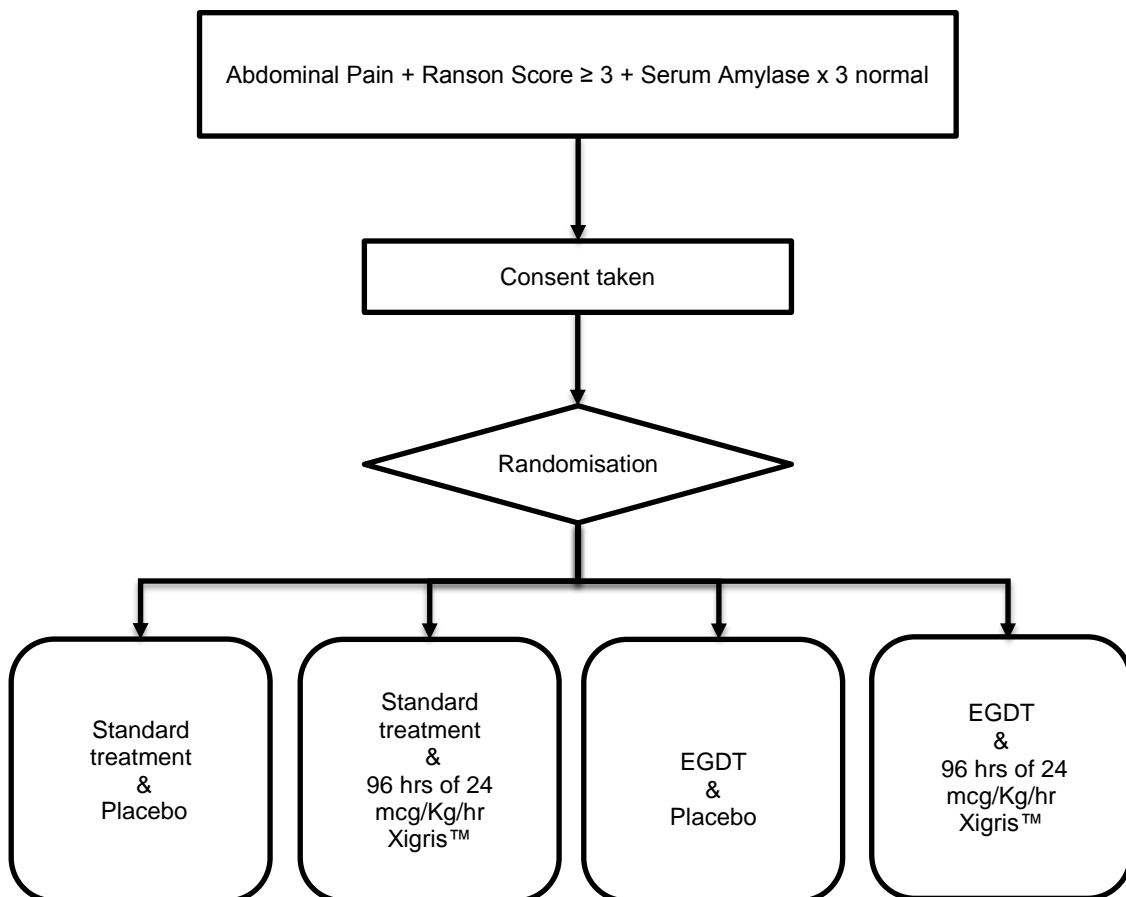clinician's best practice and 96 hours of 24 mcg/Kg/hour of rhAPC.

Figure 7.1 : Proposed flowchart for recruitment and randomization of patients into one of 4 study arms for the

6.5.1   Inclusion criteria

Patients who meet the revised 2013 definition of SAP and give valid consent for participation in the trial (in compliance with the Helsinki declaration [World Medical Association of Helsinki, 2000] are eligible to participate.

6.5.2   Exclusion criteria

To be determined.

6.5.3   Stopping criteria

Infusions of rhAPC will be stopped if any visible or suspected bleeding is detected. Stopping criteria include but are not restricted to:

1. Haemoptysis
2. Haematemesis
3. Maelena
4. Haematuria
5. Decrease in GCS ≥ 2 points.

6.5.4 Treatment arm

Patients who meet the inclusion criteria receive an Early Goal Directed Therapy bundle within the first 6 hours of admission.

6.5.5   Concomitant medications

There are no specific medical interventions for severe acute pancreatitis. Medical treatment will be at the discretion of the patient's clinician. A record will however be made of any medication administered to the patient. It is not routine practice to use anticoagulation in these patients.

6.6   Pharmaco-vigilance

6.6.1   Adverse events (AEs)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after the study has commenced, even if not considered to be related to the investigational medicinal product. Medical conditions/diseases present before starting the study will only be considered as adverse events if they worsen after the start of the study. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of adverse events will be sought by non-directive questioning of the patient during the study. Adverse events also may be detected when they are volunteered by the patient or through physical examination, laboratory test, or other assessment. As far as possible each adverse event will be evaluated to determine:

1. The severity (mild, moderate, severe)
2. Its relationship to the investigational medicinal product
3. Its duration
4. Action taken (no action taken; study drug dose adjusted/temporarily interrupted; study drug permanently discontinued; concomitant medication taken; non-drug therapy given; hospitalisation required)

5. Whether it is **serious**, where a serious adverse event (SAE) is defined as one which:

   1) Is fatal or life-threatening

   2) Results in persistent or significant disability/incapacity

   3) Constitutes a congenital anomaly/birth defect

   4) Requires prolonged hospitalisation (except where it is for routine treatment/monitoring, elective or pre-planned treatment not related to study, for social or respite reasons)

   5) Is medically significant i.e. defined as an event that jeopardises the patient or may require medical or surgical intervention to prevent one of the above

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements (see below).

All adverse events will be recorded in detail, reported to the trial steering committee and treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalisation, or any other medically required intervention. Once an adverse event is detected it will be followed until its resolution, and assessments will be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the investigational medicinal product, the interventions required to treat it, and the outcome.

6.6.2 Evaluation of AEs and SAEs

Seriousness, causality, severity and expectedness will be evaluated for each AE. Cases that are considered serious, possibly, probably or definitely related to drug (i.e. serious adverse reactions, SARs) and unexpected (i.e. SUSARs) should be reported as described below.

6.6.3 Assessment of seriousness

The Investigator should make an assessment of seriousness as defined above (see definitions).

6.6.4 Assessment of causality

The Investigator must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions. All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably, definitely) to the study drug will be considered as ARs/SARs. If concomitant or rescue/escape drugs are given, the Investigator must also make an assessment of whether the AE/SAE is likely to be related to an interaction between the study drug and concomitant or rescue/escape drugs or whether the AE/SAE might be linked to either the study drug or concomitant or rescue/escape drugs but cannot be attributed to only one of these drugs. All AEs/SAEs judged as being related (e.g. possibly, probably, definitely) to an interaction between the study drug and concomitant or rescue/escape drugs, or any AE/SAE that cannot be attributed to

only the study drug or the concomitant or rescue/escape drugs will also be considered to be ARs/SARs .

Unrelated: where an event is not considered to be related to the study drug.

Possibly: although a relationship to the study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.

Probably: the temporal relationship and absence of a more likely explanation suggest the event could be related to the study drug.

Definitely: The known effects of the study drug or its therapeutic class, or based on challenge testing, suggest that study drug is the most likely cause.

Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated.

6.6.5 Assessment of severity

The Investigator will make an assessment of severity for each AE/SAE and record this on the Adverse Event (AE) Form according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

6.6.6 Assessment of expectedness

If an event is judged to be an AR/SAR, the evaluation of expectedness will be made based on knowledge of the reaction and the relevant product information documented in the Summary of Product Characteristics (SmPC).

6.6.7 Serious adverse event (SAE) reporting

Any SAE will be reported by the Principal Investigator (including a completed SAE form) within 24 hours of first knowledge to the Sponsor. The Principal Investigator will ensure that the patient is appropriately treated. They will also determine whether the SAE is a SUSAR (Suspected Unexpected Serious Adverse Reaction). If it is deemed to be a SUSAR it will be reported immediately to the sponsor. The Regulatory Competent Authority (MHRA) and Research Ethics Committee will also be informed in accordance with Trial regulations. All Adverse Events including SAEs will be reported to the Trial Steering Committee. An annual safety report will be sent by the Chief Investigator to the MHRA, the Ethics Committee and sponsor. Completed initial and follow-up Serious Adverse Event forms should be faxed to the sponsor on 0161 276 5766 and addressed 'For the attention of the Quality Manager'. Alternatively, scanned forms can be emailed to adverse.events@cmft.nhs.uk.

6.6.8 Regulatory reporting requirements

The sponsor, or their delegate, has a legal responsibility to notify the Regulatory Competent Authority and the Research Ethics Committee that approved the trial. Fatal or life threatening SUSARs will be reported no later than 7 calendar days,

with a further 8 days for follow up information. All other SUSARs will be reported no later than 15 calendar days after the sponsor is first aware of the reaction.

6.6.9 Follow up procedures

After initially recording an AE or recording and reporting an SAE, the Principal Investigator is required to follow each participant until resolution. Follow up information on an SAE should be reported to the sponsor. AEs still present in participants at the last study visit should be monitored until resolution of the event or until no longer medically indicated.

6.6.10   Criteria for premature termination of study

These criteria include new safety data, or concerns from safety data (number and nature of SUSARs); or evidence from other studies.

6.6.11   Pregnancy

It the event that pregnancy does occur in a patient an any time between commencement of the study and 28 days after completion or termination of the study the pregnancy will be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Details of the pregnancy will be recorded on a Pregnancy Reporting Form. After that the health of the baby will be followed up at 12 and 24 months old. Any SAE experienced during pregnancy will be reported on the SAE Report form.

APPENDIX A

Consent form for patients recruited to 'A 24 hour infusion of human recombinant activated protein C (Xigris™) early in SAP: Phase 1 study'.

Central Manchester University Hospitals NHS

NHS Foundation Trust

Hepato-Pancretico-Biliary Unit
Department of Surgery
Manchester Royal Infirmary
Oxford Road
Manchester M13 9WL

Centre no:
Study no:
Patient identification no:

CONSENT FORM

Title of project

A PRELIMINARY EVALUATION OF THE SAFETY PROFILE OF TWENTY-FOUR HOUR INFUSION OF HUMAN RECOMBINANT ACTIVATED PROTEIN C (XIGRIS) EARLY IN SEVERE ACUTE PANCREATITIS.
THE XIG-AP 1 STUDY

Name of Researcher

Dr. Charles J Miranda
HPB Research Fellow
Dept. of Surgery,
Manchester Royal Infirmary, M13 9WH

Tel: 07917711225

Please initial box

1. I confirm that I have read and understood the information sheet dated .... version (……) for the above study and have had the opportunity to ask questions. ☐

2. Provide blood samples before and after administration of Xigris. ☐

3. Have a CT Scan prior to administration of medication and on the 10th day to review progress of the disease ☐

4. I understand that my participation is voluntary and I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected ☐

5. Store my blood samples for the period of the research. ☐

6. The collected blood samples to be tested for various markers as mentioned. ☐

7. Follow me up in out- patients department 90 days after administration of Xigris ☐

8. I understand that sections of any of my medical notes may be looked at by responsible individuals from Department of Surgery or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

9. I agree to my GP being informed of my participation in the study. ☐

10. I agree to take part in the study. ☐

Version 3: Jan 2008

Page 1 of 2

_____ _____ _____
Name of Patient   Signature   Date

_____ _____ _____
Name of Person taking consent   Signature   Date
(If different from researcher)

_____ _____ _____
Researcher   Signature   Date

1 for patient; 1 for researcher; 1 to be kept with hospital notes

APPENDIX B

Consent form for patients recruited to 'Targeted decompression in Abdominal Compartment Syndrome complicating SAP: a pilot study'.

Central Manchester University Hospitals NHS
NHS Foundation Trust

The Manchester HPB Centre
Dept. of Surgery
Manchester Royal Infirmary
Oxford Road
Manchester M13 9WL

Study No. ……………..
Patient Identification No. ……………………..

CONSENT FORM

Early targeted decompression in Abdominal Compartment Syndrome complicating Severe Acute Pancreatitis: a pilot study.
(ACS-SAP)

Name of Researcher:
Dr. Charles J. Miranda
HPB Research Fellow
Dept. of Surgery
Tel. 07917711225

Please initial each box to indicate your agreement with the statement.

1. I confirm that I have read and understood the information sheet dated …………
    (version ………..) for the above the study. I have had the opportunity to consider the
    information, ask questions and have these answered satisfactorily.

2. I understand that my participation is voluntary and I am free to withdraw at any time without
    giving any reason, without my medical care or legal rights being affected.

3. I understand that relevant sections of any of my medical notes and data collected during the
    study may be looked at by responsible individuals from the Manchester Royal Infirmary or
    from regulatory authorities, or from the NHS Trust, where it is relevant to my taking part in
    research. I give permission for these individuals to have access to my records.

4. I agree to my GP being informed of my participation in the study

5. I agree to take part in the study.

…………………………………….   …………………………………   ……………
Name of Patient   Signature   Date

…………………………………….   …………………………………   ……………
Name of Person obtaining consent   Signature   Date

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes

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