SEVERE ACUTE PANCREATITIS

PREDICTING WITH ECTO-5'-NUCLEOTIDASE (CD73) AND TREATMENT WITH ACTIVATED PROTEIN C

Lea Kyhälä

ACADEMIC DISSERTATION

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SUPERVISORS:

Docent Leena Kylänpää, M.D., Ph.D.
Department of Gastrointestinal Surgery, Abdominal Center
Helsinki University Hospital
University of Helsinki
Helsinki, Finland

Docent Heikki Repo, M.D., Ph.D.
Department of Medicine
Helsinki University Hospital
University of Helsinki
Helsinki, Finland

REVIEWERS:

Docent Juhani Sand, M.D., Ph.D.
University of Tampere
Tampere, Finland

Docent Elina Armstrong, M.D., Ph.D.
Coagulation Disorders Unit, Department of Hematology
Comprehensive Cancer Center
Helsinki University Hospital
University of Helsinki
Helsinki, Finland

OPPONENT:

Professor Juha Grönroos, M.D., Ph.D.
Department of Surgery, Division of Digestive Surgery and Urology
Turku University Hospital
University of Turku
Turku, Finland

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Unigrafia Oy
Helsinki 2016
To my family
Acute pancreatitis (AP) is a common abdominal disease with no specific treatment. The clinical manifestation of AP can vary widely from a mild self-limiting disease to a potentially life-threatening severe form. Severe acute pancreatitis (SAP) develops in about 20-25% of patients with AP, and the severe disease is associated with the development of such complications as pancreatic necrosis and distant organ failure (OF). A complicated inflammatory reaction precedes development of OF and is accompanied by disturbances in the coagulation system.

The patients who will develop SAP need to be identified early in the course of the disease and aggressively treated (i.e. vigorous fluid resuscitation in intensive care unit) to prevent mortality.

The aim of this study was to investigate whether ecto-5’ nucleotidase (CD73) predicts the development of SAP and whether recombinant activated protein C (APC) treatment alleviates the course of SAP. We evaluated also the effects of recombinant APC treatment on occurrence of bleeding complications, course of systemic inflammation, and coagulation cascade disturbances in patients with SAP.

The clinical study consists of four parts. All of the patients investigated had AP when admitted to Helsinki University Central Hospital. The first study comprised 161 patients with AP. Their serum levels of soluble CD73 (sCD73) were analyzed by three different methods (activity, protein concentration, and mRNA levels) and the association of the levels with different severity grades of AP was evaluated. In addition, the ability of sCD73 to predict SAP was examined. The second study was a pilot, double-blind clinical trial in which 32 patients with SAP were randomized to receive recombinant APC treatment or placebo, and the effects of APC on evolution of organ failure and and the safety of APC treatment were analyzed. In the third study, the effects of recombinant APC treatment on markers of systemic inflammation were examined. The changes on humoral and cellular markers of systemic inflammation were determined. In the fourth study, the effects of recombinant APC treatment on coagulopathy in SAP were evaluated. The plasma levels of selected biomarkers of coagulation and physiological anticoagulation were determined.

The results showed that the sCD73 levels on admission to hospital correlated inversely with the severity of AP and the sCD73 activity is able to predict the severe form of AP among patients with no signs of OF on admission.

We found no beneficial effects of recombinant APC treatment on evolution of organ failures or mortality in SAP. No significant bleeding complications occurred. Recombinant APC treatment did not alter the course of systemic inflammation, but we could monitor the changes in parameters of
systemic inflammation reaction produced by SAP. We found that the SAP patients showed coagulopathy and the recombinant APC treatment slowed normalization of the levels of coagulation and anticoagulation markers in SAP.

Based on these studies, sCD73 activity predicts the development of SAP. The recombinant APC treatment does not have a significant effect on evolution of OFs or on mortality in SAP. The effects of APC treatment on parameters of systemic inflammatory reaction are minor. The coagulation in SAP is hindered and APC-treated patients achieve normal homeostasis of coagulation slower than patients receiving placebo.
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:


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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AP</td>
<td>acute pancreatitis</td>
</tr>
<tr>
<td>APA</td>
<td>American Pancreatic Association</td>
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<tr>
<td>APACHE II</td>
<td>acute physiology and chronic health evaluation</td>
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<tr>
<td>APC</td>
<td>activated protein C</td>
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<tr>
<td>ARDS</td>
<td>acute respiratory distress syndrome</td>
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<tr>
<td>AT</td>
<td>antithrombin</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under curve</td>
</tr>
<tr>
<td>BISAP</td>
<td>Bedside Index for Severity in Acute Pancreatitis</td>
</tr>
<tr>
<td>CAT</td>
<td>Calibrated Automated Thrombography</td>
</tr>
<tr>
<td>CARS</td>
<td>compensatory anti-inflammatory reaction</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation</td>
</tr>
<tr>
<td>CD62L</td>
<td>cluster of differentiation 62L, L-selectin</td>
</tr>
<tr>
<td>CD73</td>
<td>cluster of differentiation 73, ecto-5’-nucleotidase</td>
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<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DAMP</td>
<td>damage-associated molecular pattern</td>
</tr>
<tr>
<td>DIC</td>
<td>disseminated intravascular coagulation</td>
</tr>
<tr>
<td>DIM</td>
<td>differences in means</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EPCR</td>
<td>endothelial protein C receptor</td>
</tr>
<tr>
<td>ERCP</td>
<td>endoscopic retrograde cholangiopancreaticography</td>
</tr>
<tr>
<td>ETP</td>
<td>endogenous thrombin potential</td>
</tr>
<tr>
<td>FiO₂</td>
<td>fraction of inspired oxygen</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte-macrophage colony-stimulating factor</td>
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<tr>
<td>Gpbar1</td>
<td>G protein-coupled bile acid receptor 1</td>
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<tr>
<td>HAPS</td>
<td>harmless acute pancreatitis score</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>human leukocyte antigen – antigen D-related</td>
</tr>
<tr>
<td>HMGB-1</td>
<td>high-mobility group box 1 protein</td>
</tr>
<tr>
<td>HUSLAB</td>
<td>Helsinki University Central Hospital Laboratory</td>
</tr>
<tr>
<td>IAP</td>
<td>International Association of Pancreatology</td>
</tr>
<tr>
<td>ICU</td>
<td>intensive care unit</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>interleukin 1 receptor antagonist</td>
</tr>
<tr>
<td>INF</td>
<td>interferon</td>
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<tr>
<td>IQR</td>
<td>interquartile range</td>
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<tr>
<td>MCP-1</td>
<td>monocyte chemoattractant peptide 1</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>MLKL</td>
<td>mixed lineage kinase domain-like protein</td>
</tr>
<tr>
<td>MMS</td>
<td>Modified Marshall Score</td>
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<tr>
<td>MOF</td>
<td>multiple organ failure</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>MWU</td>
<td>Mann-Whitney U-test</td>
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<tr>
<td>NF-kB</td>
<td>nuclear factor kappa-light-chain-enhancer of activated B cells</td>
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<tr>
<td>NT5E</td>
<td>5'-nucleotidase ecto (gene)</td>
</tr>
<tr>
<td>OF</td>
<td>organ failure</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>arterial partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PAF</td>
<td>platelet-activating factor</td>
</tr>
<tr>
<td>PAI</td>
<td>plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PAR</td>
<td>protease-activated receptor</td>
</tr>
<tr>
<td>PC</td>
<td>protein C</td>
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<tr>
<td>PCT</td>
<td>procalcitonin</td>
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<tr>
<td>PROWESS</td>
<td>Protein C Worldwide Evaluation in Severe Sepsis</td>
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<tr>
<td>PRSS1</td>
<td>protease serine 1 gene</td>
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<tr>
<td>PS</td>
<td>protein S</td>
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<tr>
<td>PT</td>
<td>prothrombin time</td>
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<tr>
<td>qPCR</td>
<td>quantitative polymerase chain reaction</td>
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<tr>
<td>RCT</td>
<td>randomized clinical trial</td>
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<tr>
<td>ROC</td>
<td>receiver-operating characteristic</td>
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<tr>
<td>SAP</td>
<td>severe acute pancreatitis</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>SOFA</td>
<td>sequential organ failure assessment</td>
</tr>
<tr>
<td>SPINK</td>
<td>serine peptidase inhibitor, Kazal type 1</td>
</tr>
<tr>
<td>TAFI</td>
<td>thrombin-activated fibrinolysis inhibitor</td>
</tr>
<tr>
<td>TF</td>
<td>tissue factor</td>
</tr>
<tr>
<td>TFPI</td>
<td>tissue factor pathway inhibitor</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>tPA</td>
<td>tissue plasminogen activator</td>
</tr>
<tr>
<td>TT</td>
<td>thrombin time</td>
</tr>
<tr>
<td>uPA</td>
<td>urokinase plasminogen activator</td>
</tr>
<tr>
<td>VCAM</td>
<td>vascular cell adhesion molecule</td>
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Acute pancreatitis (AP) is an inflammatory disease of the pancreas and it is one of the most common gastrointestinal-related reasons for morbidity and hospitalization. The annual incidence of AP in Finland is 73 per 100,000 inhabitants. Usually AP is a mild, self-limited disease; however, about 20-25% of patients develop a potentially life-threatening severe form of AP. The overall mortality of AP is about 5%, but the mortality for severe acute pancreatitis (SAP) can reach up to 20-30%.

Prolonged excessive alcohol intake and gallstone disease are the most common causes of AP. Other causes may be complication of ERCP (endoscopic retrograde cholangiopancreatography), metabolic disorders, drugs, pancreas divisum, and genetic mutations. Of all AP cases, 10-25% remain idiopathic.

Epigastric pain, nausea, vomiting, and abdominal bloating are typical symptoms of AP. Patients with SAP can have hemodynamic instability and dyspnea. There is no specific treatment for AP; options are supportive (i.e. fluid replacement, enteral feeding). According to revised Atlanta criteria (Atlanta 2012), AP is classified into three severity categories: mild AP (only pancreatic involvement, no local complications or organ failure (OF)), moderately severe AP (local complication and/or transient OF), and severe AP (persistent OF).

In pathogenesis of AP, the initial acinar injury is followed by a complicated inflammatory reaction, which can be restricted to a local event in mild AP or be excessive, producing a systemic inflammatory response (SIRS) in SAP. SIRS can lead to the evolution of vital OFs and accounts for early mortality in SAP. A compensatory anti-inflammatory reaction follows SIRS, resulting in secondary infections, i.e. sepsis, and accounts for mortality in the later phase of SAP. Concomitantly with activation of inflammatory reaction, a complex coagulation cascade becomes activated. Disseminated intravascular coagulation, consumption coagulopathy, and increased fibrinolysis are known phenomena in SAP. Inflammatory reaction and coagulation are co-players in inflammatory diseases such as SAP.

Cluster of differentiation 73 (CD73) is an ectoenzyme (surface-associated ecto-5’-nucleotidase), which is expressed on the surface of vascular endothelial cells. CD73 affects vasculature permeability, leukocyte extravasation, and cytokine release by adenosine metabolism.

Activated protein C (APC) is an anti-coagulant with cytoprotective and anti-inflammatory properties. Sepsis and SAP share similarities in systemic inflammatory reactions and coagulopathy, and recombinant APC treatment appeared promising, decreasing mortality in patients with severe sepsis.
Severity of AP can fluctuate rapidly and predicting the course of AP is difficult, but essential to improve outcome of patients. Patients who will develop SAP need to be identified early in the course of disease and aggressively treated (i.e. early intensive fluid resuscitation in ICU) to prevent mortality. Many kinds of severity scoring systems and biomarkers have been developed to assess severity, but a marker that could predict severe AP precisely, easily, cost-effectively, and sufficiently early is still missing.

This clinical study investigates whether CD73 is capable of predicting the development of SAP. We evaluated the effects of APC treatment on SAP. At the same time, we determined effects of APC treatment on systemic inflammation and coagulopathy associated with SAP.
2 REVIEW OF THE LITERATURE OF ACUTE PANCREATITIS

2.1 ANATOMY AND PHYSIOLOGY OF PANCREAS

The pancreas is a retroperitoneal organ that lies in the upper part of the abdominal cavity. The regions of the pancreas are the head, body, tail, and the uncinated process. The spleen is adjacent to the pancreatic tail. The distal end of the common bile duct passes through the head of the pancreas and joins the main pancreatic duct (also called duct of Wirsung) entering the duodenum. The arterial blood supply of the pancreas comes from the celiac and superior mesenteric arteries. Venous drainage of the pancreas is via the splenic vein and the superior mesenteric vein draining into the portal vein. The pancreas is innervated by both the parasympathetic and sympathetic nervous systems (i.e. branches of the vagus nerve and thoracic splanchnic nerves).30

The human pancreas has the largest capacity for protein synthesis of any organ in the human body; mainly the synthesized products are digestive enzymes. The pancreas is divided into an exocrine (acinar and duct tissue, comprising 85% of the mass) and endocrine pancreas (islets of Langerhans). The exocrine pancreas secretes digestive enzymes and NaHCO₃ into the duodenum. The function of the exocrine pancreas is tightly regulated by the neuroendocrine system; when the meal is ingested, the secretion of digestive enzymes is promoted by acetylcholine and cholecystokinin. Secretin promotes the discharge of NaHCO₃ into the pancreatic fluid. The endocrine pancreas secretes hormones (insulin, amylin, glucagon, somatostatin, and pancreatic polypeptide) into the bloodstream, and these hormones are essential for regulating blood glucose levels.30, 31

The functional unit of the exocrine pancreas is composed of an acinus and its draining ductule. The ductule drains into interlobular ducts, which in turn drain into the main pancreatic ductal system. The acinar cell is designed to synthesize, store, and secrete digestive enzymes.32 Mostly these enzymes are proteases (i.e. amylase, lipase, and trypsin), which become active only in the small intestine. Normally, trypsinogen (inactive zymogen) activates to trypsin in the duodenum as the first step in the digestive enzyme cascade. In AP, activation of zymogens occurs within the acinar cell and secretion is inhibited.30
2.2 EPIDEMIOLOGY

AP is a common potentially life-threatening disease with no specific treatment. The incidence of AP varies in different countries. In Finland, the incidence is one of the highest: 73 per 100,000 inhabitants annually. In 2009 in the United States, AP was the prevailing “gastrointestinal-related” reason for hospitalizations (the most common principal gastrointestinal diagnosis at discharge), the second highest cause of total hospital stays, and the fifth leading cause of in-hospital deaths. In Finland, the incidence of hospitalizations for alcohol-induced AP is from 60 to 102/100,000/year in middle-aged men and from 5 to 21/100,000/year in middle-aged women. The incidence of AP has increased in many European countries and in the United States in recent years. The reason for this increase may be partly due to improved diagnostic capabilities of AP. The incidence of biliary AP increases with growing obesity of the population. Admissions for alcoholic AP reflect rising rates of alcohol consumption. Alcohol-induced AP is linked to social deprivation and has seasonal variation.

AP has a wide spectrum of severity. Severity of AP varies from mild, self-limiting disease to severe, even fatal disease. SAP develops in about 20-25% of patients with AP and the severe disease is associated with the development of OF. Respiratory failure is the most common OF in SAP. The incidence of SAP is also increasing. Patients with SAP often need prolonged stay in an intensive care unit (ICU) and impose a burden on ICU resources; SAP patients constituted 17% of all ICU days.

The overall mortality of AP is about 5% (3% in interstitial pancreatitis, 17% in necrotizing AP, 30% in infected necrosis), and the mortality rate for SAP can reach up to 20-30%. The mortality for SAP is especially associated with the development of infected necrosis and multiple organ failure (MOF). Deaths manifest as two peaks; within the first 2 weeks, deaths are generally attributed to OF, and after that they are caused either by infected necrosis or other infection complications.

In Finland, the annual overall number of deaths from AP is between 113 and 167 (mean 142), for men between 75 and 122 (mean 102) and for women between 29 and 47 (mean 40). Mortality is 2.1-3.7 deaths per 100 discharges in men and 2.6-4.1 deaths per 100 discharges in women.

Several studies have reported a slight decrease in mortality for AP, although the incidence seems to have increased. The reason for this decrease in mortality could be due to earlier diagnosis and improved treatment options. On the other hand, an improved sensitivity of diagnostic modalities leads to an increase in the diagnosis of milder forms of AP.
2.3 ETIOLOGY

Determination of the etiology of AP is important for treatment during the initial phase of the disease and for preventing recurrence. Prolonged excessive alcohol intake is the most common cause of pancreatitis in Finland, accounting for around 70% of all cases. Although AP is associated with heavy alcohol consumption, only a minority (2-3%) of the heavy drinkers develop AP. Gallstone etiology is the second most common cause, accounting for around 20% of AP cases in Finland.

The etiology of AP differs considerably between different geographic regions. Biliary AP predominates in many countries, e.g. in Greece (71%), Italy, and widely in Asia. The risk of biliary pancreatitis is about 2% in patients with asymptomatic gallstones, and the risk increases if the gallstones are small (diameter < 5 mm) or numerous.

Alcohol administration and biliary obstruction can induce portal venous endotoxemia, and this is correlated with complications and more severe AP. Idiosyncratic reaction to drugs is a rare cause for AP, overall incidence probably ranging from 0.1% to 2% of pancreatic cases. A total of 525 different drugs listed in the World Health Organization database are suspected to cause AP as a side effect, but the causality for many of these drugs is based only on case reports. For 31 drugs, a definite causality is established. The risk of pancreatitis is highest for the use of mesalazine, azathioprine, or simvastatin. In drug-induced AP, the disease course is usually mild or even subclinical.

Pancreas divisum is the most common developmental anatomic variant of pancreatic duct (incidence 4-14% in an autopsy series). In case of pancreatic divisum, the majority of pancreatic fluids drain through the duct of Santorini into the duodenum at the minor papilla. The presence of pancreas divisum is a risk factor for recurrent AP and for isolated dorsal involvement of AP. It has been shown, however, that pancreas divisum alone does not cause AP, but concomitant mutations, e.g. CFTR (cystic fibrosis transmembrane conductance regulator) genes, suggest a cumulative effect.

Genetic risk factors are associated with mostly recurrent AP. The strongest risk factors, besides CFTR, include genetic mutation in protease serine 1 gene (PRSS1) or serine peptidase inhibitor gene (SPINK).

Rare causes associated with AP include mitochondrial cytopathy (diseases with deletion or depletion of mitochondrial DNA, i.e. Karnes Sayre syndrome). Hypercalcemia is a rare cause of AP. A couple of case reports have introduced rare anatomical causes of AP, e.g. splenic aneurysm progression and intraluminal duodenal diverticulum. Of all AP cases, 10-25% remain idiopathic. One potential etiology of idiopathic AP is gallbladder microlithiasis missed on transcutaneous ultrasound examination and another might be sphincter of Oddi dysfunction.
The exact pathogenic mechanism of AP is unresolved despite decades of intensive research. An inflammation reaction of AP is surprisingly similar, although the etiology can differ. Multiple triggering factors sensitize the pancreatic acinar cell to cellular injury and AP. Whatever the initiating event, the progression of AP can be viewed as a continuum: local inflammation of the pancreas, followed by a generalized inflammatory response, leading to the final stage of multi organ failure.

### 2.4 PATHOGENESIS

The exact pathogenic mechanism of AP is unresolved despite decades of intensive research. An inflammation reaction of AP is surprisingly similar, although the etiology can differ. Multiple triggering factors sensitize the pancreatic acinar cell to cellular injury and AP. Whatever the initiating event, the progression of AP can be viewed as a continuum: local inflammation of the pancreas, followed by a generalized inflammatory response, leading to the final stage of multi organ failure.

### 2.4.1 TRIGGERING FACTORS

**Initial mechanisms of pancreatic injury**

Alcohol has a direct dose-related toxic effect on the pancreas, but additional triggering factors are needed to initiate AP. Alcohol has direct effects on small pancreatic ducts and on acinar cells. Alcohol increases viscosity of pancreatic secretions and causes formation of small protein plugs within pancreatic ducts.59 Alcohol increases digestive and lysosomal enzyme content within acinar cells, thereby increasing the potential of intracellular activation of digestive enzymes.60 Acute ethanol exposure decreases pancreatic blood flow, resulting in decreased pancreatic tissue pH in animal models.61 Low
extracellular pH (metabolic asidosis) sensitizes acinar cells to AP by enhancing calcium signalling.62

The toxic effect of bile acid itself on acinar cells is a possible pathogenic factor of biliary AP. Gallstones may migrate into the distant bile ducts causing bile and pancreatic juice obstruction. Bile acids flow upstream into the pancreatic duct and can bind to an acinar cell surface bile acid receptor (Gpbar-1).63 Bile acids increase intra-acinar calcium concentrations and induce nuclear factor kappa-light-chain-enhancer of activated B cell (NF-kB) activation and synthesis of proinflammatory cytokines.64, 65

Alcohol administration and biliary obstruction can induce portal venous endotoxemia, and this is correlated with complications and more severe AP.49

Trypsin-related theory (pancreatic autodigestion theory)

Over a hundred years ago, Hans Chiari proposed a theory that acute pancreatitis is the result of autodigestion of the pancreas by prematurely activated digestive enzymes.66 Under physiological conditions, the proteases of pancreatic acinar cell become active only when they reach the small intestine. Over the decades, man has tried to clarify mechanisms responsible for this premature activation of pancreatic proteases.

In trypsin-related theory of AP, trypsinogen activation to trypsin within the acinar cells appears to be an early pathogenic event in AP. Conditions that lead to AP alter intracellular acinar cell signalling.67 Intracellular co-localization lysosomal enzymes (i.e. cathepsin B) with zymogen granules lead to activation of pancreatic proteases in the vacuoles of acinar cells, and normal acinar cell secretion (apical exocytosis of zymogen granules) is inhibited.68 Autophagy function is decreased in AP and autophagic vacuoles containing activated proteases accumulate in the acinar cells.69, 70 Inhibited apical exocytosis of activated proteases instead leads to basolateral exocytosis of them.71, 72 The active proteases are released into the interstitial space, resulting in acinar cell injury.67, 73 Trypsin can also prematurely activate precursors of other digestive enzymes.67, 73 Activation of digestive enzymes and the subsequent progressive acinar cell injury initiate autodigestion of the pancreas, causing hemorrhage, necrosis, edema, and destruction of the pancreas parenchyma. Injured acinar cells release cytokines (tumor necrosis factor alpha (TNFα), IL-1β), and this results in activation of local inflammation cells and accelerates the proinflammatory response.14, 74
NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation

NF-κB is a “rapid-acting” transcription factor that responds to harmful cellular stimuli. It regulates the expression of multiple inflammatory and immune genes. Normally, NF-κB is present in the cytoplasm in an inactive form, and many extracellular stimuli, e.g. inflammatory cytokines, viruses, and oxidants, can activate it. Activated NF-κB can induce expression of proinflammatory cytokines, i.e. TNFα, IL-1β, IL-6, chemokines, and adhesion molecules. Early intra-acinar NF-κB activation, which occurs parallel to but independent of trypsinogen activation, might be partly responsible for progression of local and systemic inflammation in AP. A mouse model lacking pathologic intra-acinar typsinogen activation showed that inflammation can progress independently and does not require trypsinogen activation in AP. The level of activation of NF-κB in acinar cells correlates with the severity of the AP in an experimental model of AP. The blocking of NF-κB activation in animal models has shown cytoprotective effects in AP.

2.4.2 LOCAL INFLAMMATION

Inflammation is a host defence mechanism for mechanical or chemical injury or microbial infection. An inflammatory reaction is a complicated progressive process in which numerous humoral and cellular responses participate. In the early phase of AP, inflammation is usually localized to the pancreas tissue, which clinically manifests as mild AP.

Acinar cell injury

Acinar cell injury and necrosis trigger inflammation. In the initial phase of AP, premature activation of pancreatic proteases leads to acinar cell injury and release of proinflammatory mediators (i.e. cytokines like TNFα and IL1β). Intracellular contents released from necrotic cells into the extracellular space serve as damage-associated molecular patterns (DAMPs), which can activate an inflammatory response in AP (Figure 3). High-mobility group box 1 protein (HMGB1) is one of the DAMPs (others include DNA, ATP, and histones). Normally inside the nucleus, HMGB1 is a nuclear DNA binding protein, which participates in determining the structure of chromatin and regulates the transcription. Intracellular HMGB1 protects cells from NF-κB activation and DNA damage and can mediate autophagy. Extracellular HMGB1 is a potent proinflammatory mediator and may act as key mediator for inflammation in AP. HMGB1 mediates inflammation through Toll-like receptor 4. Toll-like receptor 4 is expressed on pancreatic ductal and on endothelial cells and on macrophages and monocytes.
Binding of the HMBG1 to its receptor on monocytes induces expression of proinflammatory cytokines.\textsuperscript{85} Apoptosis, i.e. programmed cell death, of acinar cells occurs also in the early phase of AP. Apoptotic cells release nucleosomes, which are complexes formed by DNA and histone proteins.\textsuperscript{86} Normally, the nucleosomes are packed into vesicles and engulfed by macrophages. The extraordinary apoptosis leads to overloading of phagocytizing mechanisms and higher concentrations of nucleosomes in the circulation.\textsuperscript{86} Higher levels of circulating nucleosomes correlate with the inflammatory response and organ dysfunction in sepsis.\textsuperscript{87} Intracellular HMBG1 prevents inflammatory nucleosome release in AP and blocks inflammation.\textsuperscript{88}

\textbf{Recruitment of inflammatory cells}

Recruitment of inflammatory cells is a critical step in the inflammatory reaction. The release of inflammatory signals (i.e. “first-line” proinflammatory cytokines TNF\textgreek{a} and IL-1\textbeta, DAMPs, and NF-\kappa\text{B}) from acinar cells mediates the activation of circulating inflammatory cells, such as peripheral blood mononuclear cells, comprising lymphocytes and monocytes, and polymorphonuclear neutrophils (PMNs) (Figure 3.).\textsuperscript{89} Monocytes/macrophages are the significant inflammatory cell population involved in pathogenesis of AP.\textsuperscript{89} Promonocytes, precursors of macrophages, are released from the bone marrow into the circulation. They develop into monocytes. Monocytes leave the circulation and migrate to the tissues, differentiating into specific types of tissue macrophages, i.e. Kupffer cells in the liver and alveolar macrophages in the lungs.\textsuperscript{90}

Monocytes/macrophages have a central role as phagocytic cells and as antigen presenting cells in immune reaction. Macrophages are the major resident immune cells within the pancreas and in the peritoneal cavity.\textsuperscript{89} Macrophages on pancreas tissue and in the nearby peritoneal cavity release TNF\textgreek{a} and IL-1\textbeta in the early stage of AP, inducing a cascade of other cytokines and chemokines (i.e. IL-8, monocyte chemoattractant peptide MCP-1), which leads to activation of neutrophils and amplification of the proinflammatory response.\textsuperscript{89} Trypsin has also been shown to stimulate the production of cytokines in peritoneal macrophages.\textsuperscript{91} The recruitment of PMNs has also a central role in the onset and progression of inflammation in AP. PMNs are the most numerous leukocytes in the circulation. They seek signs of foreign organisms and antigens and are recruited to sites of infection or inflammation.\textsuperscript{92} In the early stage of AP, activated neutrophils release high concentrations of oxidants and cytotoxic agents, which worsens the local damage of pancreatic tissue. Activated neutrophils also secrete proinflammatory cytokines (i.e. IL-1\textbeta, TNF\textgreek{a}).\textsuperscript{93}

Activation of inflammatory cells is promoted by membrane-bound cell surface receptors whose expression of them is altered during inflammation. Activation of neutrophils and monocytes is manifested by an increased
expression of $\beta_2$ integrin CD11B/CD18 on the cell surface. Up-regulated expression of these adhesion molecules facilitates neutrophil extravasation at the site of inflammation. Monocytes/macrophages and neutrophils express CD14 glycoprotein on the cell surface. CD14 has been identified as a receptor for a wide range of microbial products (LPS). CD14 acts as a co-receptor with the Toll-like receptors. CD14 appears in soluble (sCD14) and membrane-bound forms (mCD14), both of which have immune stimulatory and inhibitory functions. High CD14 expression may increase susceptibility to immune-mediated tissue injury. Both CD11b and CD14 expression levels on leukocytes can be measured as markers of leukocyte activation.

Leukocyte extravasation

Leukocyte extravasation from the blood into areas of inflammation is a highly regulated phenomenon in immune response. During an early stage of AP cytokines activate microvascular endothelium, and leukocytes can transmigrate into the pancreatic interstitial space. A complex extravasation cascade regulates an adhesion of leukocytes to endothelium and extravasation to tissues. Extravasation depends on the interaction between leukocytes and endothelium, and many kinds of adhesion molecules are expressed on the surface of leukocytes and vascular endothelial cells (Figure 3.). Selectins mediate the initial step of adhesion cascade, promoting tethering and rolling of leukocytes on the endothelium. For example, E-selectin is expressed on endothelial cells after an inflammatory stimulus. The soluble form of E-selectin can be measured in serum, and it serves as a marker of endothelial cell activation. L-selectin (CD62L) is an adhesion receptor expressed on the leukocytes that mediates the leukocyte-endothelium interaction. Decreased monocyte and neutrophil expression of L-selectin is a marker of phagocyte activation. The $\beta_2$ integrin CD11B/CD18 on the surface of neutrophils and monocytes allows them to adhere firmly to the endothelial cells.

Ectoenzymes (i.e. nucleotidases, cyclases, peptidases, proteases and oxidases) are also involved in the extravasation cascade. Ectoenzymes are expressed on the surface of leukocytes and endothelial cells and have their enzymatically active domains outside of the cell membrane. In the extravasation cascade, ectoenzymes can act as adhesion molecules or by regulating cell recruitment through their catalytic activity.

CD73

Cluster of differentiation 73 (CD73) is an ectoentzyme (surface associated ecto-5'-nucleotidase) that is expressed on the surface of vascular endothelial cells. In addition, 5-15% of blood lymphocytes express CD73. CD73 also appears in soluble form (sCD73) in plasma, and the soluble form is suggested
Review of the literature of acute pancreatitis

to be enzymatically active.\textsuperscript{106} sCD73 is produced by shedding of lymphocytes.\textsuperscript{107}

CD73 regulates the purinergic signaling cascade. CD73 regulates extracellular ATP metabolism, dephosphorylating AMP to adenosine.\textsuperscript{108} Adenosine has anti-inflammatory effects: it decreases endothelial cell permeability and inhibits leukocyte extravasation. Adenosine decreases cytokine release and inhibits immune activation.\textsuperscript{22} Other non-enzymatic functions of the CD73 molecule have also been proposed such as induction of intracellular signaling and mediation of cell–cell and cell–matrix adhesion.\textsuperscript{107}

Experimental animal models have shown that CD73 deficiency is associated with aggravated lung injury in acute respiratory distress syndrome (ARDS), worse hepatic, renal, and cardiac ischemia-reperfusion injury, and increased mortality in polymicrobial sepsis.\textsuperscript{109, 110} The activity of CD73 appears to be beneficial in the fight against excessive inflammatory reactions. Exogenously administered CD73 has protective effects in, for example, acute lung injury and myocardial and renal ischemia. Expression of endogenous CD73 can be upregulated by interferon beta (IFN-\(\beta\)).\textsuperscript{109, 110} IFN\(\beta\) has been shown to induce the expression and activity of CD73, thus decreasing vascular permeability in cultured human pulmonary endothelial cells.\textsuperscript{111, 112}

**Microvascular injury**

Microvascular injury is partly responsible for progression of tissue injury at the site of inflammation. Under normal circumstances, the extravasation cascade is highly regulated and during the inflammation extravasation is accelerated.\textsuperscript{104, 113} The transmigrated leukocytes release vasoactive factors (i.e. endothelin-1 and phospholipase A2) and reactive oxygen species, which contribute to the development of microvascular injury and increased capillary permeability.\textsuperscript{114, 115} This results in edema and local ischemia, which leads to ongoing destruction of pancreatic tissue.\textsuperscript{116} Acinar cell damage can amplify this proinflammatory cascade.\textsuperscript{117}

**Restriction of inflammatory reaction**

In mild AP, the inflammation reaction is restricted to a local event. The termination of inflammation is an active biochemical progress that involves the release of anti-inflammatory mediators and downregulation of NF-\(\kappa\)B gene activity. Additionally, cytokine and chemokine receptor antagonist (i.e. interleukin 1 receptor antagonist, IL-1ra) limits neutrophil recruitment to the site of inflammation.\textsuperscript{118}

Simultaneously with the acinar cell injury, the cell death mechanisms (necrosis and apoptosis) become activated.\textsuperscript{13, 80} Apoptosis is a process of programmed cell death that is thought to limit the tissue injury in an inflammatory reaction. Apoptosis is an active process requiring specific
proteins, caspases. If death of acinar cells occurs in the mode of apoptosis instead of necrosis, the following inflammatory reaction may be milder.\textsuperscript{119} The dying cells are phagocytized by macrophages, and this is associated with anti-inflammatory cytokine switching.\textsuperscript{120}

Concomitantly with synthesis of proinflammatory cytokines, anti-inflammatory cytokines and specific cytokine inhibitors are produced. IL-6 is a cytokine known to have both pro- and anti-inflammatory properties, but that acts predominantly as an anti-inflammatory cytokine.\textsuperscript{121} IL-6 induces production of acute-phase proteins in the liver.\textsuperscript{122} IL-6 is an early marker of a complicated course of AP. IL-6 concentrations peak on the first day of AP and decrease to baseline values within 4 days.\textsuperscript{123} IL-10 is a potent anti-inflammatory cytokine and a potent activator of B-lymphocytes.\textsuperscript{124} Proinflammatory cytokine synthesis is downregulated by IL-10 in activated macrophages and neutrophils.\textsuperscript{121} IL-10 has a protective role in SAP; IL-10 might restrict the inflammation reaction. IL-1ra is an important anti-inflammatory cytokine. It binds competitively to the IL-1 receptor and prevents IL-1-mediated responses.\textsuperscript{125}

During inflammation the lifespan of the circulating neutrophils is prolonged, and induction of neutrophil apoptosis facilitates the resolution of inflammation by normalizing the neutrophil lifespan.\textsuperscript{93}
2.4.3 SYSTEMIC INFLAMMATION

AP is a dynamic condition in which the severity of inflammation may change rapidly during the course of disease. A local pancreatic inflammation in the initial phase of AP can rapidly proceed to uncontrolled systemic inflammation reaction in SAP. An ongoing overactive systemic inflammatory reaction can progress to the development of systemic inflammatory response syndrome (SIRS), which may further proceed to distant organ failures and MOF and/or infection of pancreatic necrosis and sepsis with late complications of AP (Figure 1).4, 14

![Diagram showing the time course of inflammation in acute pancreatitis (AP). According to revised Atlanta criteria, severe acute pancreatitis often has a clinical course with two phases, early and late. The early phase, which usually lasts for about one week, is characterized by a systemic inflammatory response syndrome (SIRS). In the late phase of severe acute pancreatitis, a compensatory anti-inflammatory reaction (CARS) can lead to secondary infections and sepsis.](image-url)
In severe AP, loss of control results in an excessive release of proinflammatory cytokines (i.e. TNFα, IL-1β, IL-8, and platelet-activating factor (PAF)) from the inflamed pancreas into the systemic circulation via portal vein and lymph fluid drainage. Once cytokines reach the liver, they strongly activate local macrophages, Kupffer cells. Activated Kupffer cells release inflammatory cytokines, reactive oxygen intermediates, and hydrogen peroxides into the circulation, causing progression of inflammatory reaction. Synthesis of acute phase proteins, i.e. C-reactive protein (CRP) and procalcitonin (PCT), is induced in the liver. PCT levels can be measured as a marker of systemic inflammation and the plasma levels of PCT increase during systemic inflammation. Proinflammatory cytokines are also released in the peritoneal cavity in the vicinity of the pancreas, leading to activation of peritoneal macrophages, and this in turn amplifies the systemic proinflammatory response. Alveolar macrophages are involved in the lungs. During inflammation activated neutrophil lifespans are prolonged, ensures their presence at the site of inflammation. Neutrophils transmigrate from the circulation to the tissues and aggregate around the focus on inflammation, resulting in a release of reactive oxygen species and the production of more proinflammatory cytokines.

**SIRS (systemic inflammatory response syndrome)**

The result of this excessive expanding of local inflammation can lead to SIRS (Figure 2).

**Table 2**  
*SIRS is manifested by two or more of the following conditions.*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature</td>
<td>&gt;38°C or &lt;36°C</td>
</tr>
<tr>
<td>Heart rate</td>
<td>&gt;90 beats/min</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>&gt;20 breaths/min or PaCO₂ &lt;4.3 kPa</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>&gt;12 x 10⁹/l or &lt;4 x 10⁹/l, or &gt;10% immature forms</td>
</tr>
</tbody>
</table>

*PaCO₂:* arterial partial pressure of carbon dioxide

**Distant organ failures and multiple organ failure**

Released proinflammatory cytokines activate the vascular endothelium, leading to enhanced extravasation of leukocytes. Activated neutrophils and monocytes further release proteolytic enzymes and oxygen radicals, contributing to the damage of endothelial cells and the loss of endothelial integrity. An increase in vascular permeability in distant organs can result in a massive infiltration of inflammatory cells and fluid to the tissues. This
may lead to hypotension and oxygen deficiency, resulting in dysfunction of vital organs and finally OF.135

The most vulnerable organs are the lungs and kidneys with a high content of vasculature tissue. Acute lung injury is a consequence of the systemic inflammatory response with increased endothelial and epithelial barrier permeability. The leakage of protein-rich exudate into the alveolar space and interstitial tissues leads to compromised oxygenation and gas exchange.136 Acute lung injury can lead to a more severe disease, the ARDS. Patients with ARDS need frequent mechanical ventilation support, and ARDS contributes to early death in SAP.137 Secondary pulmonary infections contribute to the late phase of deaths in SAP.138

Impairment of renal microcirculation, hypoxemia, decrease in renal perfusion due to intra-abdominal hypertension, and hypovolemia cause acute kidney injury in SAP.139 Pancreatic phospholipase A2 released by neutrophils and macrophages can deposit to renal proximal tubular cells, and the measurement of pancreatic phospholipase A2 in serum may provide a test for identifying a threatening renal complication.140 Acute kidney injury can lead to renal replacement therapy and partly contribute to deaths in the early stage of SAP.139

In case of more than one affected organ system, the situation is termed multiple organ failure, MOF. Organ failures can be transient, such as in the case of moderately severe AP, or persistent such as in SAP.12

**CARS (Compensatory anti-inflammatory response syndrome)**

In systemic inflammation, the proinflammatory phase is followed by an anti-inflammatory reaction that may lead to immune suppression (Figure 2).15-17 This paralysis of the inflammatory response is also termed a compensatory anti-inflammatory response syndrome (CARS). The anti-inflammatory response may control an excessive injurious inflammatory reaction (to restrict the SIRS), resulting in the recovery of organ injuries (termed transient organ injury in the case of moderately severe AP). CARS may be excessive, leading to severe immune suppression.15

Monocytes express major histocompatibility complex (MHC) class II antigens (human leucocyte antigen, HLA-DR) on the cell surface.141 HLA-DR density is related to the capacity of monocytes to present antigen to helper T-cells.141 In immunosuppression, HLA-DR expression of monocytes is reduced and the antigen presentation capacity is impaired. The ability of monocytes to produce proinflammatory cytokines is decreased, and the serum levels of anti-inflammatory cytokines (IL-10, IL-1ra, and IL-6) are increased.17, 142 Low HLA-DR expression of circulating monocytes is a sign of immunosuppression.142 INF-γ treatment can at least partially reverse HLA-DR antigen expression and help septic patients restore their immune defenses and increase their resistance to infection.143
In the second phase of SAP, immune suppression enhances susceptibility to nosocomial infections or evolution of local complications such as infected necrosis, pseudocysts, or abscesses. The increase in permeability of the gut barrier may allow translocation of bacteria and endotoxins into the circulation. This gut barrier failure may lead to infections, including sepsis, and ultimately to death in the late phase of SAP.

![Diagram](image)

**Figure 2** Inflammatory reaction and outcome in acute pancreatitis (AP: acute pancreatitis; CARS: compensatory anti-inflammatory response syndrome; OF: organ failure; MOF: multiple organ failure; SIRS: systemic inflammatory response syndrome).
Figure 3 Immuno-inflammatory pathogenesis of acute pancreatitis (simplified) (DAMP: damage-associated molecular pattern; HMBG1: high-mobility group box 1 protein; IL: interleukin; MCP-1: monocyte chemo-attractant peptide 1; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PMN: polymorphonuclear neutrophil; TNFα: tumor necrosis factor alpha).
2.4.4 COAGULATION AND ANTICOAGULATION

In case of vascular injury, an immediate response is required to limit blood loss. Blood coagulation leads to the formation and propagation of a blood clot at the site of vascular injury. Coagulation involves platelet-vessel interactions related to disruption of the endothelium and the coagulation system (the primary hemostasis, the activation of coagulation cascade, the natural anticoagulants, and the fibrinolytic system). The formation of thrombin is an essential phenomenon in coagulation. Thrombin can activate platelets, convert fibrinogen to fibrin, and via feedback amplify the coagulation. In healthy persons, coagulation is precisely controlled at the site of vascular damage by anticoagulation and fibrinolytic mechanisms. In case of systemic disease (i.e. systemic inflammation), the control system is disturbed, resulting in coagulopathy (i.e. thrombosis and consumption coagulopathy).\textsuperscript{145-147}

Primary hemostasis

Primary hemostasis is the result of complicated interactions between platelets, the vessel wall, and adhesive proteins, leading to formation of the initial “platelet plug”. Intact endothelial lining has antithrombotic properties that prevent plug formation. In case of vascular injury, the highly thrombogenic subendothelial layer containing collagen and Von Willebrand factor is exposed. Platelets adhere to collagen, and Von Willebrand factor acts as a bridge between subendothelial collagen and platelet surface receptors (platelet glycoprotein complex I receptors). Activated platelets produce thromboxane A2, which stimulates further platelet aggregation, resulting in formation of the initial plug.\textsuperscript{148}

Coagulation pathways (formation of fibrin)

Coagulation involves specific interactions between cellular surfaces and plasma-derived zymogens and cofactors. Coagulation cascade is a traditional model of coagulation in which series of proenzymes convert into the respective serine proteases. The main step is the conversion of prothrombin into thrombin, and the final step is the conversion of soluble fibrinogen into insoluble fibrin, resulting in a plug. Two pathways of the traditional coagulation cascade have been described: extrinsic and intrinsic pathways. In the extrinsic pathway, tissue factor (TF) (exposed by vascular injury) binds to and activates factor VII (FVII) to factor VIIa (FVIIa) (the activation of proenzyme is depicted by the suffix \textquotedblleft a\textquotedblright). The TF-FVIIa complex in turn activates FX to FXa. In the intrinsic pathway (also called contact activation pathway), the negative charged phospholipid
surfaces, usually on the activated platelet surface, result in activation of the coagulation cascade. The activation of intrinsic pathways starts with activation of FXII into FXIIa; the resulting activation cascade is shown in Figure 4. These two pathways converge to form a common pathway. In the common pathway, FXa forms a complex with its cofactor FV, leading to the conversion of prothrombin into thrombin. Thrombin in turn cleaves circulating fibrinogen to insoluble fibrin and activates FXIII, which covalently crosslinks fibrin polymers. A fibrin network stabilizes the clot and forms a definitive secondary plug.147, 149, 150

A modern model of coagulation is emphasized as sequential events situated on the surfaces of endothelial cells (Figure 4.). The sequential phases of coagulation are: 1) Initiation phase: TF is exposed to coagulation factors in plasma, permitting formation of a complex between FVIIa. The FVIIa/TF complex activates FIX and FX. FXa forms a complex with FVa, and this complex further converts prothrombin to thrombin. 2) Amplification phase: small-scale thrombin that is generated in the initiation phase is not sufficient. That generated thrombin activates cofactors VIII, V, and platelets and accelerates the activation of FII (prothrombin) by FXa and of FXa by FIXa, 3) Propagation phase: the accumulated enzyme complexes on platelet surface produce a large-scale thrombin burst that promotes the conversion of fibrinogen to fibrin.147, 151
Figure 4  Simplified model of coagulation, the endogenous anticoagulant mechanisms, and fibrinolysis. The cascade model consists of two separate initiations: intrinsic and extrinsic pathways, which merge at the level of FXa (common pathway). V and VIII act as cofactors. Coagulation is triggered (initiation) when circulating FVIIa and locally exposed TF form a complex. Activation of FXa leads to formation of the threshold level of thrombin, resulting in activation of platelets, FV, FVIII, and FXI (amplification). Thrombin augments its own generation (propagation). Activation is shown by black arrows and inhibition by dotted arrows. (APC: activated protein C; AT: antithrombin; Ca$^{2+}$: calcium ion; PAI-1: plasminogen activator inhibitor-1; PS: protein S; TAFI: thrombin-activated fibrinolysis inhibitor; TF: tissue factor; TFPI: tissue factor pathway inhibitor; tPA: tissue plasminogen activator).


**Anticoagulation pathways**

Blood clot formation is tightly regulated to prevent thrombus propagation and vessel occlusion. Regulation of coagulation is focused on different levels of the pathway by enzyme inhibition or by modulation of the activity of cofactors. The endogenous anticoagulant mechanisms are: 1) antithrombin (AT), 2) tissue factor pathway inhibitor (TFPI), and 3) protein C (PC) pathway (Figure 4).

**Antithrombin**

Antithrombin (AT) is a liver-synthesized glycoprotein that primarily binds and irreversibly inactivates thrombin, but also coagulation factors FXa and FIXa. AT limits the coagulation process to the site of vascular injury and protects the circulation from liberated enzymes. Heparin and heparin-like molecules on the surface of endothelial cells are cofactors of AT and stimulate its activity.\(^{150,152,153}\)

**Tissue factor and tissue factor pathway inhibitor**

Tissue factor (TF) (formerly known as FIII) is a transmembrane protein present in cells surrounding vessels (adventitial but not endothelial cells express TF).\(^{154}\) TF comes into contact with blood and the circulating procoagulant factor (FVIIa) when vascular integrity is disrupted.\(^{155}\) TF circulates as a free form in the bloodstream (so-called blood-borne TF), and it was also assumed to activate the coagulation cascade.\(^{156}\) Free TF has been shown to be present in the plasma of healthy individuals in low concentrations, and the plasma levels increase in systemic inflammation, i.e. sepsis.\(^{157}\) The monocytes can synthesize and express TF, and they are supposed to be a source for TF-induced thrombosis when the endothelium is intact.\(^{158}\) Several pathological conditions, e.g. sepsis, are associated with activated monocytes, shedding TF-rich microparticles that are transferred to activated platelets, neutrophils, and endothelial cells.\(^{159}\)

TFPI is a serine protease inhibitor of TF that slows down the initiation of clot formation. TFPI consists of three domains that bind and inhibit FXa and TF-FVIIa complex.\(^{160}\) Endothelial cells, monocytes and macrophages synthesize TFPI. Most of TFPI (50-80%) is bound to the endothelial surface. In circulation, TFPI occurs as a lipoprotein-bound form (80%) and as a free form (20%). Free TFPI has a more potent anticoagulant effect than its bound counterpart.\(^{161}\)
**Protein C pathway and activated protein C (APC)**

Protein C pathway restricts the generation of thrombin. APC is an endogenous anticoagulant (a trypsin-like serine protease) that promotes fibrinolysis and inhibits thrombosis. Protein C, an inactive precursor, is converted to APC by thrombin coupled to thrombomodulin.\textsuperscript{162} This process is accelerated in the presence of endothelial cell protein C receptor (EPCR). The binding of protein C to EPCR potentiates the activation of protein C by 20-fold in vivo.\textsuperscript{163} The presence of protein S, a cofactor of PC, is required for the anticoagulant activity of APC.\textsuperscript{164} APC inactivates the procoagulation factors FVa and FVIIIa by shutting down the coagulation pathway; the inactivation of FVa/FVIIIa by APC with protein S slows down thrombin generation and inhibits thrombus growth.\textsuperscript{165} APC also inactivates plasminogen activator inhibitor (PAI), resulting in increased fibrinolysis.\textsuperscript{166} An $\alpha_1$-antitrypsin and protein C inhibitor can inhibit anticoagulant activity of APC.\textsuperscript{166}

Genetic deficiencies in PC, PS, and AT predispose to thrombofilias.\textsuperscript{153, 167}

**Fibrinolysis**

Fibrinolytic system is a parallel system that is activated along with activation of the coagulation cascade and limits the size of the clot. Fibrinolysis enables the removal of thrombi. Fibrin promotes the generation of fibrinolytic enzyme plasmin to degrade the clot, so fibrin acts as both a cofactor and a substrate for the plasmin. Plasmin is activated from plasminogen by either of two primary serine proteases, tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). tPA is synthesized by endothelial cells and uPA is produced by monocytes, macrophages, and urinary epithelium. tPA requires fibrin as cofactor. uPA acts mainly in extravascular locations. Circulating plasmin and plasminogen activators are inhibited by serine protease inhibitors (serpines). The most important serpines are plasminogen activator inhibitor-1 (PAI-1), plasminogen activator inhibitor-2 (PAI-2), and $\alpha_2$-antiplasmin. PAI-1 is mainly produced by endothelial cells and platelets, and its expression is upregulated by proinflammatory cytokines. In pregnancy, PAI-2 is the main plasminofen activator inhibitor. Thrombin-activated fibrinolysis inhibitor (TAFI) is a non-serpin inhibitor that is activated by thrombomodulin-associated thrombin, and it decreases the affinity of plasminogen to fibrin. Fibrinolysis dissolves the fibrin clot into fibrin degradation products, some of which have immunomodulatory and chemotactic functions.\textsuperscript{168-170}
2.4.5 COAGULATION AND INFLAMMATION

The inflammation and coagulation systems have bidirectional communication with several connections at the cellular and humoral levels. Inflammatory reaction leads to activation of coagulation and suppression of natural anticoagulant mechanisms, and, in turn, the coagulation affects inflammatory activation. In severe inflammatory diseases, such as sepsis and SAP, simultaneously with SIRS, coagulation is activated.

**Inflammation-induced activation of coagulation**

The main mediators of inflammation-induced activation coagulation are proinflammatory cytokines. Inflammation upregulates TF synthesis in endothelial cells, monocytes/macrophages, and dendritic cells. Platelets can be activated by proinflammatory mediators (i.e. PAF) or by formed thrombin. The activation of platelets enhances the expression of TF on monocytes by inducing NF-κB activation during systemic inflammation reactions. The expression of P-selectin on platelets mediates the adherence of platelets to leukocytes and endothelial cells and enhances the expression of TF on monocytes. Binding of activated platelets to monocytes also enhances the production of IL-1b, IL-8, MCP-1, and TNFα(Figure 5)

Anticoagulant pathways, like PC and AT systems, are shut down by proinflammatory cytokines. The components of the protein C pathway are also directly influenced by inflammatory mediators. TNF-α and IL-1β downregulate expression of thrombomodulin on endothelial cells and upregulate its surface expression on macrophages, leading to a deficient protein C system.

Inflammation inhibits fibrinolysis; proinflammatory cytokines stimulate endothelial cells to secrete PAI-1.

**Effects of coagulation on activation of inflammation**

The coagulation system can modulate inflammatory activity. Binding of coagulation proteases or anticoagulant protein to specific receptors on mononuclear cells or endothelial cells may affect cytokine production. FXa, thrombin, and fibrin can stimulate IL-6 and IL-8 synthesis of mononuclear and endothelial cells. Thrombin increases IL-8 and MCP-1 mRNA levels and expression of E-selectins on endothelial cells. Thrombin can bind to protease-activated receptors (PAR-1,3-4) on different cell types, i.e. on platelets and endothelial cells. The resulting intracellular signaling leads to increased production of proinflammatory cytokines. Thrombin represents the most potent agonist of PAR-1 and PAR-4. PAR-1 activation on endothelial cells triggers the release of chemokines and the surface expression of various adhesion molecules. PAR-1 signaling promotes Von Willebrand factor
release, P-selectin exposure, and enhanced production of PAF and prostaglandins.\textsuperscript{173, 181}

\textbf{Figure 5} Effects of coagulation on activation of inflammation. The coagulation system modulates inflammation by several mechanisms. On the other hand, inflammatory mediators (i.e. IL-6, IL-1, TNF\(\alpha\)) induce expression of TF and stimulate activation of coagulation (TF: tissue factor; IL: interleukin; PAF: platelet-activating factor; TNF\(\alpha\): tumor necrosis factor alpha).
Effects of endogenous anticoagulant pathways on inflammation

Natural anticoagulant proteins suppress inflammation by several mechanisms.

TFPI inhibits the LPS-stimulated TNFα production by monocytes and
downregulates CD11b and CD18 expression on neutrophils in vitro. TFPI
inhibits inflammatory TF signaling via PARs. In turn, activated neutrophils
can cleave TFPI to a less active form.

AT has many anti-inflammatory properties, which are mediated by its
actions in the coagulation cascade, the inhibition of thrombin by AT prevents
activation of many inflammatory mediators (such as activation of platelets
and endothelial cells). AT prevents FXa-induced production of IL-6, IL-8,
and E-selectin involved in monocyte recruitment and adhesion to endothelial
cells. AT prevents FVIIa-TF complex formation and subsequent upregulation
of cytokines (i.e. IL-6, IL-8). AT has anti-inflammatory properties
independent of its anticoagulatory characteristics. It induces endothelial cells
to release prostacyclin, which suppresses platelet activity and inhibits
attachment of neutrophils to endothelial cells and decreases release of IL-6,
IL-8, and TNFα by endothelial cells. AT prevents leukocyte adhesion to
endothelial cells, binding receptors like syndecan-4 expressed on
neutrophils, monocytes, and lymphocytes and reduces the expression of IL-6
and TF on monocytes. AT reduces also IL-8-induced chemotaxis of
neutrophils, monocytes, and lymphocytes. During severe inflammatory
response AT levels are decreased; synthesis is impaired, elastases from
activated neutrophils degrade AT, and ongoing thrombin generation
consumes AT.

APC also has cytoprotective effects such as anti-inflammatory, anti-
apoptotic, and endothelial barrier protection effects. APC has an indirect
effect on inflammation; it inhibits formation of thrombin, thereby reducing
thrombin’s potent proinflammatory activities, i.e. platelet activation,
cytokine production, and upregulation of leukocyte adhesion molecules.
Administration of APC has been shown to downregulate the expression of
inflammatory cytokines and chemokines. It reduces production of
endotoxemia-induced proinflammatory cytokines (IL-6, IL-8, IL-1β, and
TNFα). APC inhibits TNFα production by blocking NF-κB transcription
factor in monocytes. APC blocks cytokine production from Th2
lymphocytes. APC has been shown to upregulate anti-inflammatory
mediators, like IL-10, on blood monocytes. The anti-inflammatory effects
of APC on endothelial cells include the inhibition of NF-κB activation,
with decreased expression of endothelial adhesion molecules (i.e. intercellular
adhesion molecule 1, E-selectin, and vascular cell adhesion molecule-1)
resulting in decreased leukocyte trafficking and reduced expression of
proinflammatory cytokines by endothelial cells. APC increases
endothelial production of prostaglandin-2 and MCP-1. APC exerts anti-
apoptotic effects on both leukocytes and endothelial cells by reducing several
pro-apoptotic signals. The direct protective effects of APC on endothelial cells
are mediated in the presence of EPCR and PAR-1. The effects of APC on leukocytes are mediated by EPCR alone or in the presence of PAR-1.\textsuperscript{24, 25} APC levels are deficient in septic patients corresponding to increased mortality.\textsuperscript{190} APC modulates coagulation and inflammation associated with severe sepsis.\textsuperscript{162} Sepsis decreases PC levels and it has been suggested that treatment with human recombinant APC will increase PC levels and prevent MOF.\textsuperscript{191}

**2.4.6 COAGULOPATHY IN SEVERE ACUTE PANCREATITIS**

During the early phase of AP proinflammatory cytokines, chemokines, TNF\textalpha{}, and PAF are released into the circulation.\textsuperscript{192} The proinflammatory cytokines activate monocytes, neutrophils, and endothelial cells, resulting in the production of large quantities of TF to initiate the coagulation cascade.\textsuperscript{178} AP is often accompanied by coagulopathy, which is characterized by increased thrombosis and fibrinolysis.\textsuperscript{18, 171} Hemostatic disorders may range from scattered intravascular thrombosis to disseminated intravascular coagulation (DIC).\textsuperscript{193} AP is associated with microvascular dysfunction including increased vascular permeability, reduced blood flow, ischemia/reperfusion injury, intravascular thrombus formation, and hypercoagulation.\textsuperscript{194} During AP platelets accumulate intravascularly and extravascularly in the pancreas and fibrin deposits in the connective tissue and intercellular spaces of the pancreas.\textsuperscript{195}

Coagulation abnormalities are related to severity of AP. Consumptive coagulopathy and increased fibrinolysis are known to occur in SAP, and they are related to its severity and OF.\textsuperscript{18} The decreased plasma levels of APC and AT and the increased levels of D-dimer and plasminogen activator inhibitor-1 are associated with poor outcome in SAP.\textsuperscript{196} AT inhibits the secretion of cytokines and HMGB1 and ameliorates AP in an experimental animal model of AP.\textsuperscript{197} The high levels of plasminogen activator inhibitor-1 may contribute to fibrin deposition in the microcirculation.\textsuperscript{196} Elevated D-dimer levels have been shown to be a sign of SAP, and non-survivors have been found to have higher D-dimer levels than survivors.\textsuperscript{198} Decreased platelet counts, decreased prothrombin times (PT), and consumption of fibrinogen occur in AP.\textsuperscript{18}

Modulating hemostasis may provide a therapeutic target for the treatment of SAP. PAF is a proinflammatory mediator produced mainly by endothelial cells. PAF can activate platelets and neutrophils and increase vascular permeability. Increased levels of phospholipase A\textsubscript{2} occur in AP. Pancreatic phospholipase A\textsubscript{2} promotes the synthesis of PAF. The PAF antagonist, lexipafant, reduced tissue damage and OFs in AP models.\textsuperscript{199, 200} Lexipafant was a promising treatment in SAP (phase II and phase III showed reduction in incidences of OFs)\textsuperscript{199, 200}, but later studies failed to show any such benefit.\textsuperscript{201}
2.5 DIAGNOSIS

2.5.1 MAIN DIAGNOSTIC CRITERIA

In accordance with the Revised Atlanta classification,12 AP can be diagnosed if at least two of the following three features are fulfilled: 1) abdominal pain (acute onset of persistent and severe epigastric pain, often radiating to the back), 2) serum amylase activity or plasma p-isoamylase (pancreas-specific isoenzyme) level or serum lipase activity at least three times greater than the upper limit of normal, and 3) characteristic findings of AP on contrast-enhanced CT (MRI or transabdominal ultrasonography).12

2.5.2 CLINICAL SIGNS AND PHYSICAL EXAMINATION

Clinical history of known cholecystolithiasis, alcohol intake, new medications, known hyperlipidemia, previous acute pancreatitis, and character of the pain should be evaluated. At the early stage of AP, the pain is usually localized in the epigastric area or left upper quadrant and radiates to the back, later it may involve the whole abdominal area. The intensity of pain is usually severe and does not reflect the severity of AP.4 AP patients usually experience general abdominal palpation pain and will often have rebound tenderness as a sign of peritoneal irritation. Nausea, vomiting, and abdominal bloating are common symptoms caused by gastric and intestinal hypomotility.3 In severe cases, patients can have hemodynamic instability (10%) or hematemeses and melena (5%) as signs of coagulation disturbances.4 The Cullen sign and the Grey Turner sign manifest as brownish-green discoloration of the umbilical region and the flank, respectively. The skin signs are rare (1.2%) and thought to indicate an unfavorable prognosis.202 Fever (76%) and tachycardia (65%) are common signs, and in severe cases the patient can have dyspnea.3, 4

2.5.3 LABORATORY TESTS

Assessment of pancreatic enzymes (i.e. amylase and lipase) is the cornerstone of clinical laboratory diagnosis. The cut-off values for both of these enzymes are normally defined to be three times the upper limit of the normal range. Serum or plasma amylase levels rise rapidly (within 3-6 hours) of the onset of symptoms and can normalize rapidly because of a short half-life (12 hours) so the diagnostic accuracy time is short. Pancreatic amylase concentration on admission is not associated with the severity of AP, and a patient with only a slight increase can also develop SAP.203 Serum lipase
levels increase 3-6 h after the onset of AP, peak within 24 h, and can persist at increased levels for 1-2 weeks. Assessment of lipase levels is today the preferred diagnostic laboratory test over amylase levels due to its higher sensitivity. Lipase levels also remain elevated for longer than amylase levels.\textsuperscript{204}

Other laboratory tests with diagnostic value are urine amylase, serum or urinary trypsinogen-2, phospholipase A\textsubscript{2}, and pancreatic elastase.\textsuperscript{205-208}

Valuable clinical laboratory tests in determining the severity of AP and possible organ systems involved are white blood cell count, concentrations of electrolytes, creatinine levels, liver function tests (i.e. aspartate aminotransferase, alkaline phosphatase, total albumin), C-reactive protein, blood sugar, and arterial blood gas analysis (when the patient’s oxygen saturation is less than 95\% or the patient has tachypnea).\textsuperscript{72}

Clarifying the coagulation status of the patient is important to assess the severity of coagulopathy and the dosing for anticoagulant therapy. PT (and TT) measures the extrinsic pathway activation, and APTT (activated partial thromboplastin time) measures the intrinsic pathway activation. Fibrinogen levels increase within the acute-phase reactions and decrease in DIC and when the liver function is decompensated. AT levels have been shown to decrease in DIC. Fibrin d-dimers is a degradation product of fibrin, and the levels are elevated in DIC and in the presence of a thrombus, i.e. deep vein thrombosis or pulmonary embolisms.\textsuperscript{209, 210}

Monitoring the CRP and leukocyte levels may provide information on the intensity of an inflammatory reaction. CRP is a non-specific acute-phase protein that reacts with a delay (24-48 h) to inflammation, but also to a bacterial infection or to tissue injury.\textsuperscript{211}

In unclear cases, determining the liver enzyme, calcium, and triglyceride levels can help to uncover the etiology.\textsuperscript{212}

\section*{2.5.4 RADIOLOGICAL FINDINGS}

Contrast-enhanced CT is the standard and highly specific imaging technique for evaluation of AP.\textsuperscript{213} CT scan is valuable in establishing the severity of AP; it helps to identify local or extrapancreatic complications, e.g. necrosis, peripancreatic fluid collections, ascites, and pleural effusions. An early CT scan (within 4 days of symptom onset) should be obtained only when the clinical diagnosis is unclear and other serious intra-abdominal disorders must be excluded.\textsuperscript{213, 214} According to the 2012 IAP/APA guidelines, indications for initial CT assessment are 1) diagnostic uncertainty, 2) confirmation of severity based on clinical predictors of SAP, or 3) failure to respond to conservative treatment or in the setting of clinical deterioration. An optimal timing for initial CT is 72-96 hours after onset of symptoms.\textsuperscript{212} In cases of patients with impaired renal function or contrast medium allergy, the use of contrast medium is contraindicated. Magnetic resonance imaging
(MRI) is a viable alternative in these situations. MRI is better than CT in distinguishing the fluid part of pseudocysts from the solid parts.\textsuperscript{215} MRI cholangiography is used to evaluate the biliary tree and the presence of stones in the common bile duct.\textsuperscript{216} Abdominal ultrasonography (US) has limited utility for diagnosis and severity assessment of AP due to its poor accuracy in obese patients and in the presence of intestinal gas. Ultrasonography is valuable for detection of gallstones and dilatation of the common bile duct.\textsuperscript{216} Chest radiographs can show pulmonary infiltrates and pleural effusions in case of SAP. Panoramic abdominal radiographs can show ileus and pancreatic calcifications if the patient has so-called acute-on-chronic involvement of AP.

### 2.6 CLASSIFICATION

AP is a disease with a highly variable clinical course. Classification of AP by severity is an important tool in clinical work, aiding to identify patient with potentially severe disease who require effective early treatment. Classification systems are important in communication between clinicians and researchers, enabling comparison of data from different centers.

#### 2.6.1 ATLANTA CLASSIFICATION 1992

The Atlanta classification (Atlanta 1992) has been the most widely accepted classification system for severity of AP. It was introduced in 1992 by the International Symposium of Acute Pancreatitis.\textsuperscript{217} Atlanta 1992 categorizes AP patients into two groups: mild and severe AP. Mild AP is associated with a shorter uncomplicated course of disease, without signs of significant organ failure. Severe AP is defined by the presence of distant organ failure or local pancreatic complications, e.g. pancreatic necrosis, acute fluid collections, pseudocyst, and pancreatic abscess (Table 3). Diagnosis of local complications is mostly based on CT imaging. Also poor prognostic scores (Ranson’s score \(\geq 3\) and/or APACHE-II \(\geq 8\)) result in a patient being allocated to the severe AP group.\textsuperscript{217}

To achieve better accuracy in clinical studies, patients in the severe AP group are often categorized into two subgroups: those with only local complications and those with OF.
Advancements in the understanding of the pathophysiology and course of AP and improvements in diagnostic and prognostic methods have resulted in the need to update the classification system. In 2012 the Acute Pancreatic Classification Working Group reported a revision of the Atlanta classification (Atlanta 2012).\textsuperscript{12} The revised Atlanta classification is based on consensus of 11 national and international pancreatic associations.

The revised classification defines criteria for the diagnosis of acute pancreatitis (mentioned in 2.5.1.), and it differentiates between interstitial edematous pancreatitis and necrotizing pancreatitis. In the case of interstitial edematous pancreatitis, an inflammatory edema leads to homogeneous enhancement of pancreas tissue and the severity of AP is mild, usually resolving within the first week.\textsuperscript{218} In the case of necrotizing pancreatitis (5-10\% of AP patients), necrosis involves the pancreas and/or peripancreatic tissues. Necrotic lesions evolve over several days and the clinical course of necrotizing pancreatitis can be variable: necrosis may remain solid or liquefy, remain sterile or become infected, or be radiological persistent or transient.\textsuperscript{12}

According to the revised Atlanta criteria, AP is classified into three severity categories: mild AP (only pancreatic involvement, no local complications or OF), moderately severe AP (transient OF and/or local or systemic complications without persistent OF), and severe AP (with persistent OF) (Table 1). Modified Marshall Score (Table 4) is used to assess the presence of organ failure; a score of two or more in any organ system defines the presence of OF.

The revised criteria discriminate two overlapping time phases of AP with two peaks of mortality: early and late phase. During the early phase, an overactive inflammatory reaction of SIRS may lead to OF, and the determinant of severity of AP in this phase is based on the presence and duration of OF (transient or persistent). Transient organ failure resolves within 48 hours. Local complications and level of necrosis are not predominant determinants of severity during the early phase. In the late phase of AP, patients may have signs of persistent systemic inflammation or local complications, and in this phase SIRS may change to CARS and increase the risk of infections. Persistent OF remains the main determinant of severity in the late phase of AP. The late phase of AP occurs only in patients with moderately severe and severe AP.\textsuperscript{12} (Table 5)

The revised classification also differentiates pancreatic and peripancreatic collections on cross-sectional imaging as acute peripancreatic fluid, pancreatic pseudocyst, acute necrotic collections, and walled-off necrosis.\textsuperscript{12}
Table 3  Atlanta classification systems for severity of AP

<table>
<thead>
<tr>
<th></th>
<th>Mild AP</th>
<th>Severe AP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atlanta 1992</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local complications(^a)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Organ failure(^b)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>APACHE II ≥8 or Ranson’s ≥3</td>
<td>No</td>
<td>Yes or No</td>
</tr>
<tr>
<td><strong>Atlanta 2012</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local complications(^c) or comorbidities(^d)</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Organ failure(^e)</td>
<td>No</td>
<td>Transient ≤48 h</td>
</tr>
</tbody>
</table>

\(^a\) acute fluid collection, pancreatic necrosis, pseudocyst
\(^b\) cardiovascular failure: systolic blood pressure <90 mmHg, respiratory failure: PaO\(_2\)≤60 mmHg, renal failure: creatinine ≥177 μmol/l, gastrointestinal bleeding 500 ml/24h
\(^c\) acute peripancreatic fluid collection, pseudocyst, acute necrotic collection, and walled-off necrosis
\(^d\) exacerbation of pre-existing comorbidity
\(^e\) Modified Marshall Score ≥ 2

Table 4  Modified Marshall scoring system for organ dysfunction.

<table>
<thead>
<tr>
<th>Organ system</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory (PaO(_2)/FiO(_2))</td>
<td>&gt;400</td>
<td>301-400</td>
<td>201-300</td>
<td>101-200</td>
<td>≤101</td>
</tr>
<tr>
<td>Renal (serum creatinine, μmol/l)</td>
<td>≤134</td>
<td>134-169</td>
<td>170-310</td>
<td>311-439</td>
<td>&gt;439</td>
</tr>
<tr>
<td>Cardiovascular (systolic blood pressure, mmHg)</td>
<td>&gt;90</td>
<td>&lt;90, fluid responsive</td>
<td>&lt;90, not fluid responsive</td>
<td>&lt;90, pH&lt;7.3</td>
<td>&lt;90, pH&lt;7.2</td>
</tr>
</tbody>
</table>

Table 5  Revised Atlanta classification of AP.

Two phases

<table>
<thead>
<tr>
<th>Early phase</th>
<th>Late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) week</td>
<td>After 1(^{st}) week</td>
</tr>
</tbody>
</table>

Severity

| Mild | Moderate | Severe |

Two types

| Odematous | Necrotizing |
2.7 SEVERITY ASSESSMENT

The appropriate management of AP should be started early to prevent mortality; nearly half of the deaths of SAP patients occur within the first week due to the development of OF; the incidence of OF is maximal (17%) on the first day.\textsuperscript{6, 219, 220}. Therefore, it is critical to predict severity of AP in the initial stages of disease.

2.7.1 RISK FACTORS

Obesity, especially abdominal obesity, predisposes AP patients to the development of local and systemic complications.\textsuperscript{221} Smoking and type 2 diabetes are other known risk factors for AP.\textsuperscript{222, 223} Older patients (over 55 years) are at increased risk for severe disease.\textsuperscript{4}

2.7.2 SCORING SYSTEMS

Several multifactorial scoring systems for the prediction of the severity of AP have been developed, and all of them have advantages and disadvantages. One of the oldest scoring systems is a Ranson score, which was developed in the early 1970’s.\textsuperscript{224} Ranson score has a high sensitivity and specificity (84\% and 78\%, respectively), but the severity can be predicted only after 48 hours from admission.\textsuperscript{225} Other scoring systems are Glasgow, BISAP (Bedside Index for Severity in Acute Pancreatitis), and APACHE II. BISAP and APACHE II were calculated using data within 24 hours of admission. APACHE II has high predictivity, but its complexity in clinical use has stirred criticism.\textsuperscript{226, 227} HAPS (harmless acute pancreatitis score) can rapidly identify patients with a mild disease. According to HAPS, patients without rebound tenderness and/or guarding, a normal hematocrit, and a normal serum creatinine concentration have a high positive predictive value (98-99\%) of having a harmless course of AP. HAPS has a low negative predictive value, so it is unable to predict SAP.\textsuperscript{228} Several CT scoring systems (i.e. CT severity index CTSI, Balthazar grade) have evolved to establish severity of AP, but their predictive accuracy is similar to clinical scoring systems.\textsuperscript{229}

Several organ dysfunction scores also exist, e.g. SOFA score\textsuperscript{230} and Modified Marshall score.\textsuperscript{231} These scores can describe the severity of OF and are not applicable for prediction of SAP. Modified Marshall score is included in the revised Atlanta criteria to identify patients with OF.\textsuperscript{12}
2.7.3 BIOMARKERS IN PREDICTING SEVERE ACUTE PANCREATITIS

Several biomarkers have been studied as potential predictors of severity of AP. Some parameters focus on water balance and microcirculation or presence of an inflammatory process.

C-reactive protein (CRP) is one of the acute-phase proteins most frequently used as a single biomarker for assessment of severity of AP. Inflammatory stimuli (i.e. IL-1 and IL-6) triggers hepatocytes to produce CRP. However, production of CRP is delayed and it is a reliable predictor only after 48 hours from symptom onset. In addition, CRP is not diseasespecific (other infectious diseases are accompanied by increased levels of CRP such as pneumonia, infected necrosis, etc.). Serum creatinine has been identified as a predictor for pancreatic necrosis. Also glomerular filtration rate can predict the severity of AP. Blood urea nitrogen has been shown to predict severity of AP (after 48 hours) and mortality in SAP. Blood urea nitrogen is included in the Ranson and BISAP scores.

Procalcitonin (PCT) is a propeptide of calcitonin and it is usually not detectable in serum in normal controls. PCT is a marker of systemic inflammation and PCT levels increase in bacterial infections and also in non-infectious SIRS. PCT may serve as a predictor of complicated course of AP, and it can be measured within hours of symptom onset. PCT is one of the most promising biomarkers, but the PCT assay is expensive, hindering its use in routine clinical practice.

Numerous cytokines have been studied regarding their relationship with severity of AP, but most of them have proven to be mainly of pathophysiological interest rather than of clinical utility. IL-6 is an anti-inflammatory cytokine, which also has proinflammatory properties. IL-6 concentrations peak in the first day of onset of AP and decrease rapidly within 2-4 days. IL-6 is an early predictor of complicated course of AP, with a high sensitivity and specificity, and it is in clinical use in some hospitals. Drawbacks of IL-6 assay are the rapid decrease in concentrations and the high cost and complexity of the assay. IL-8 is a proinflammatory chemokine (cytokine with chemotactic properties), which has been shown to act as an early predictive marker of disease severity in AP and is useful in monitoring MOF. Recent meta-analysis showed that IL-6 has better sensitivity and specificity than IL-8. IL-10 is a potent anti-inflammatory cytokine and its biologic effects may result in immunosuppression. IL-10 is an early marker of severity of AP. Serum IL-10 levels have been shown to be elevated in severe and lethal AP. IL-1ra is an important anti-inflammatory cytokine. It binds competitively to the IL-1 receptor and prevents IL-1-mediated responses. Increased serum IL-1ra is associated with development of MOF in AP and in sepsis. Serum IL-1ra may serve as an early marker of SAP and may predict poor outcome.

Adhesion molecules on leukocytes and endothelial cells have a central role in pathogenesis of AP, and their potential diagnostic usefulness has been
evaluated. The soluble form of E-selectin is a leukocyte adhesion molecule that is expressed on vascular endothelial cells after an inflammatory stimulus. Soluble E-selectin can be measured in serum and serves as a marker of endothelial cell activation. Soluble E-selectin may serve as a predictor of the severe form of AP. L-selectin (CD62L) is a leukocytic cell adhesion receptor that mediates the initial step of adhesion cascade, the leukocyte-endothelium interaction. Expression of L-selectin did not show any correlation with severity of AP.

In addition, different activation markers of leukocytes and their association with severity of AP have been investigated. In immunosuppression, HLA-DR expression on monocytes is reduced and the ability of monocytes to produce proinflammatory cytokines is impaired. Decreased HLA-DR expression on monocytes acts as a cellular marker of immunosuppression. Decreased monocyte HLA-DR expression is related to disease severity in patients with AP and predicts the development of OF in AP. CD14 is a cell surface glycoprotein on mononuclear phagocytes that has been identified as a receptor for a wide range of microbial products. CD14 exists in soluble and membrane-bound forms, and both have immune stimulatory and inhibitory functions. Increased soluble CD14 expression is associated with SIRS in patients with AP. Monocyte CD14 expression levels were not related to AP severity. CD11b is an adhesion molecule expressed on the surface of neutrophils and monocytes. Activation of these cell types is manifested by increased integrin CD11b/CD18 expression on the cell surface. Enhanced neutrophil and monocyte expression of CD11b molecules is associated with the severity of AP.

In addition to the above-mentioned scoring systems and markers, dozens of other markers have been studied in severity assessment of AP. These days, CRP and APACHE II score and PCT are the most widely used tools for severity assessment in AP. An optimal test, which would predict severity of AP precisely, easily, cost-effectively, and sufficiently early, is still missing.

### 2.8 TREATMENT

A patient with mild AP can be treated in an outpatient department. Patients with risk factors for SAP should be taken to hospital for close monitoring and timely reassessment of disease severity. Patients with apparent OF (MMS>2) should be taken to an advanced medical care ward, and in case of persistent OF the patient should be treated in an intensive care setting.

No effective pharmacological treatment exists for AP despite decades of research. Thus, supportive therapy with management of local and systemic complications is offered. The early phase of SAP (the first 5-7 days) is critical. OF evolves early in SAP, and unless aggressive management is provided, mortality can reach up to 50%.
2.8.1 CAUSATIVE THERAPY

In the case of alcohol- or drug-induced AP, the intake of the triggering agent must cease.

In the case of biliary AP, the indication of ERCP depends on the degree of obstruction of the common bile duct and the presence of cholangitis. The 2012 IAP/APA guidelines state that ERCP is not indicated in predicted mild biliary AP without cholangitis, ERCP is probably not indicated in predicted severe biliary AP without cholangitis, ERCP is probably indicated in biliary AP with common bile duct obstruction, and ERCP is indicated if the patient has biliary AP and cholangitis.\textsuperscript{212} Cholecystectomy is indicated within the same hospital stay for mild biliary AP. If the patient has peripancreatic fluid collections, cholecystectomy should be delayed until the collections are resolved or after 6 weeks.\textsuperscript{212}

2.8.2 FLUID THERAPY

Early intravenous fluid therapy has been the cornerstone of treatment of AP for years. Over the course of AP, increased vascular permeability causes intravascular fluid loss, hypotension, and possible shock. Coagulation cascade activates in the early phase of AP, leading to DIC and microvascular thrombosis. Together these phenomena lead to extravasation of fluid into the third space out of the circulation. In AP, intravenous hydration should replace the hypovolemia that occurs secondary to vomiting, reduced oral intake, third space extravasation, and respiratory losses.\textsuperscript{250} Correction of hypovolemia can limit the development of necrosis and improve outcome in SAP. Early fluid resuscitation, within the first 24 hours of admission for AP, is associated with decreased rates of OF.\textsuperscript{212} Fluid therapy should not only replenish blood volume, but also stabilize capillary permeability, relieve the inflammatory reaction, and sustain intestinal barrier function in SAP.\textsuperscript{251} Patients with moderately severe or severe AP should be started on effective fluid resuscitation. An ideal fluid for resuscitation in AP – crystalloids or colloids – has been under debate. The recent review of Aggrawak et al. concluded that controlled fluid resuscitation (3.0-4.0 l/24 h) should be initiated after a bolus infusion 20 ml/kg (1000 ml over one hour), and lactated Ringers’ is recommended as the fluid of choice. A urine output >0.5 ml/kg and a decrease in blood urea nitrogen (or creatinine level) are simple goals of fluid resuscitation; other parameters are blood pressure, respiratory function, and intra-abdominal pressure. Duration of fluid resuscitation should last 24-48 hours, until signs of volume depletion disappear.\textsuperscript{252}
2.8.3 PAIN MANAGEMENT

Patient with AP usually suffer from severe pain and often require opiate analgesics. Monitoring of oxygenation while administering high-dose opiate medications is necessary. Epidural anesthesia may improve pancreatic microcirculation and tissue oxygenation, so in serious cases epidural anesthesia may be a useful pain management method (coagulation disturbances are contraindications).\textsuperscript{253}

2.8.4 NUTRITION

Previously, total oral abstinence of food was a typical treatment method in AP. Early enteral nutrition has been shown to reduce systemic and pancreatic infectious complications, length of hospital stay, and mortality.\textsuperscript{254} Nutritional support should be started when the patient is unable to eat for 7 days. Enteral nutrition stabilizes gut barrier function and reduces bacterial translocation and infectious complications in SAP. For SAP, enteral nutrition is better than total parenteral nutrition regarding mortality, infectious complications, and OF.\textsuperscript{255} If the SAP patient requires nutritional support, enteral tube feeding via either nasojejunal or nasogastric route is recommended.\textsuperscript{212}

2.8.5 ANTIBIOTIC PROPHYLAXIS

Previously, antibiotic prophylaxis was mentioned to be useful in AP, but there is no evidence that prophylactic antibiotics reduce infectious complications or mortality.\textsuperscript{256} Thus, antibiotic prophylaxis is currently not recommended in AP.\textsuperscript{212}

2.8.6 ANTICOAGULANT THERAPY

Thrombotic complications, i.e. portal vein thrombosis and DIC, are common, especially in alcohol-induced and necrotizing SAP. Also systemic microvascular disturbances, including vasoconstriction, inadequate perfusion, increased blood viscosity, and activation of coagulation, are involved in SAP. On the other hand, the coagulopathy in SAP is associated with elevated risk of hemorrhage because of consumption coagulopathy and thrombocytopenia. Sometimes major bleedings, such as bleedings from pseudoaneurysms and direct arterial erosion, complicate the disease course.\textsuperscript{257}
Antithrombotic treatment in SAP is a balancing act between thrombotic complications and elevated risk of bleedings. Usually critically ill ICU patients, like SAP patients, have several risk factors for venous thromboembolisms, i.e. severe illness, sedating medications, and invasive procedures. SAP patients in the ICU require thrombus prophylaxis, i.e. LMWH, but the treatment (including dosing and monitoring) should be considered patient-specific.

Heparin, a highly sulphated glycosaminoglycan, is mostly known as an anticoagulant, but it also has anti-inflammatory properties. Heparin has two kinds of pharmaceutical forms: traditional unfractionated heparin (UFH) and low molecular weight heparins (LMWHs), which are prepared from UFH by different chemical or enzymatic processes. LMWHs have reduced capability to inactivate thrombin relative to UFH; UFH can inhibit thrombin via both antithrombin-dependent and -independent mechanisms, LMWHs have no direct inhibitory effect on thrombin, but instead LMWHs inhibit FXa. UFH also has direct inhibitory effects on activation of platelets and endothelial cells. LMWHs have advantageous properties in clinical use compared with UFH; LMWHs have a longer half-life, enabling a single daily dosing, and also have more predictable dose-response and lower risk of thrombocytopenia and bleeding complications. LMWHs are more widely used today. Heparin and its derivatives have anti-inflammatory properties; they can reduce the release of cytokines and inflammatory mediators (NF-kb, TNF-a, IL-6). Heparin can diminish the formation of microthrombosis in pancreatic tissue and attenuate inflammatory reaction. LMWH therapy has been suggested to alleviate damage of the pancreas, lungs, kidneys, and brain and to decrease mortality in SAP. LMWH therapy is also used in the treatment of DIC.

Abdominal venous thrombosis (mesenteric, splenic, or portal vein) is a common complication in AP. Management requires therapeutic anticoagulation with heparin infusion or subcutaneous LMWH before transition to oral warfarin.

Administration of AT concentrate is used in the treatment of hereditary AT deficiency. AT has potent anti-inflammatory properties and it has shown to ameliorate experimental AP (as mentioned in Section 2.4.6.).

### 2.8.7 EXPERIMENTAL PHARMACOLOGIC THERAPY

While supportive therapy is the only treatment available for AP, a better understanding of the pathophysiology has led to emerging research of pharmacological treatments. The target of the pharmacological therapy can focus on different steps in the pathogenesis of AP. In the last years, the main research focus has been on immunomodulatory therapies, the idea being that the overactive inflammatory response should be inhibited and excessive immunosuppression avoided in the early stages of AP.
Several experimental pre-clinical and clinical studies (Table 6) have provided evidence of different medical agents with therapeutic potential.

**Anti-secretory agents**

Glucagon increases superior mesenteric blood flow and decreases pancreatic exocrine secretion. Clinical studies have failed to show beneficial effects of glucagon therapy in AP, instead pancreatic hemorrhage increased. Somatostatin inhibits exocrine secretion of the pancreas, reduces splanchnic blood flow, and modulates the inflammatory cascade in AP. Although some preclinical studies have provided promising results, clinical studies have not shown a clinically significant mortality benefit in AP. Octreotidi, a synthetic analog of somatostatin, has also been evaluated. Results of clinical trials have been controversial, but meta-analysis (n=948) revealed no benefit on mortality or complication rates in AP.

**Protease inhibitors**

Protease inhibitors can prevent intra-acinar activation of digestive enzymes. Clinical studies of aprotinin, gabexate mesilate, and nafomostat have been published, and they have shown a potential benefit in AP, but larger studies are needed to confirm the clinical value of protease inhibitors.

**Immunomodulators**

The optimal time-window for immunomodulatory therapy is narrow, and it is difficult to know exactly a patient’s disease phase (i.e. immune status) at a given time-point.

Blocking the inflammatory reactions by anti-inflammatory factors may be an effective treatment for AP. Many factors (i.e. cytokines and cells) are involved in inflammatory reactions so if the treatment is targeted only to one factor it is possible that the therapy is insufficient to terminate the entire overactive inflammatory reaction. Amobarbital can inhibit NF-κB activity and in animal models attenuates AP-associated injuries in the pancreas and lungs. Infliximab (monoclonal anti-TNF-α antibody) has been shown to suppress neutrophil infiltration and ameliorate necrosis of pancreas tissue in animal models, and to ameliorate AP in patients with active Crohn disease. Lexipafant is a PAF antagonist. PAF amplifies the activity of key mediators of SIRS in AP. Clinical trials of lexipafant to prevent OF in SAP were promising, but the largest randomized trial (phase III) showed no significant reduction in OF or local complications. Indomethacin inhibits pancreatic phospholipase A₂ activity and decreases neutrophil-mediated inflammation. Clinical studies showed no decrease in inflammatory reactions in AP, but rectal indomethacin may prevent post-ERCP pancreatitis.
Steroid therapy dampens widely the inflammation reaction; it can reduce the inflammatory cascade and leukocyte recruitment. It has been shown to be beneficial in autoimmune pancreatitis, but the published case reports have mentioned steroids as inductors of AP.278

For patients with immunosuppression, proper immunostimulation may alleviate the disease and prevent infectious complications. In CARS, HLA-DR expression on monocytes is reduced and the administration of IFN-γ or CM-CSF can raise the HLA-DR expression on monocytes in septic patients and shorten the course of sepsis-associated immunosuppression.279, 280 The same phenomenon has been shown in vitro on monocytes in SAP patients, but clinical studies are needed. 281

<table>
<thead>
<tr>
<th>Pharmacologic agent</th>
<th>Sample size</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon Durr263</td>
<td>29</td>
<td>No effects on length of hospital stay or mortality</td>
</tr>
<tr>
<td>Somatostatin Luengo282</td>
<td>50</td>
<td>Decreased length of hospital stay, favorable changes in CT scan, no effect on mortality</td>
</tr>
<tr>
<td>Octreotide Andriulli265</td>
<td>19</td>
<td>Reduced mortality in SAP</td>
</tr>
<tr>
<td></td>
<td>Uhl283</td>
<td>No effects of mortality or evolution of complications</td>
</tr>
<tr>
<td>Aprotinin Baldin264</td>
<td>48</td>
<td>No effects on mortality or clinical results</td>
</tr>
<tr>
<td>Gabexate mesilate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valderrama268</td>
<td>51</td>
<td>No effects on mortality or development of complications</td>
</tr>
<tr>
<td>Buhler285</td>
<td>223</td>
<td>No effects on mortality or development of complications</td>
</tr>
<tr>
<td>Nafomostat Piascik269</td>
<td>78</td>
<td>Reduced mortality and need for surgical intervention</td>
</tr>
<tr>
<td>Lexipafant Kingsnorth286</td>
<td>83</td>
<td>Reduced evolution of OF and levels of IL-6 and IL-8</td>
</tr>
<tr>
<td></td>
<td>McKay287</td>
<td>Reduced evolution of OF</td>
</tr>
<tr>
<td></td>
<td>Johnson288</td>
<td>No effects on evolution of OF</td>
</tr>
</tbody>
</table>
3 PRESENT INVESTIGATION

3.1 AIMS OF THE STUDY

The main goal of the study was to investigate whether ecto-5’nucleotidase (CD73) predicts the development of SAP and whether APC treatment alleviates course of SAP. Specific aims were as follows:

I. To examine CD73 as a predictor of SAP at the levels of mRNA, protein, and enzyme activity.

II. To evaluate in a prospective randomized trial the effects of APC treatment on (i) evolution of OF, (ii) occurrence of bleeding complications, and (iii) course of systemic inflammation and coagulation cascade disturbances.
3.2 MATERIALS AND METHODS

3.2.1 PATIENTS AND HEALTHY CONTROL SUBJECTS

All of the patients studied fulfilled the criteria of AP and were admitted to Helsinki University Central Hospital within 72 (I) or 96 (II, III, IV) hours from onset of symptoms. In all studies, the diagnosis of AP was based on typical clinical findings, including onset of epigastric pain, nausea, and vomiting, and an elevated plasma amylase concentration of at least three-fold the upper reference limit, and/or typical radiological appearance of AP in CT.

Patients in Study I (n=161) were retrospectively categorized into mild AP, moderately severe AP, and severe AP according to revised Atlanta criteria (Atlanta 2012). In mild AP, patients had no OF or local or systemic complications and they were usually discharged during the early phase. In moderately severe AP, patients had local complications and/or transient OF (resolving within 48 hours). SAP patients had single or multiple persistent (>48 hours) OF. The presence of OF is based on a Modified Marshall Score (MMS) ≥ 2. Seventeen patients had MMS ≥ 2 on admission, indicating the presence of OF. Five of them had transient OF, which recovered within 48 hours (moderately severe AP). The other 12 patients had persistent OF (severe AP) (Figure 6).

![Flow chart of patients in Study I. Organ failure (OF) on admission was according to the Modified Marshall Score (MMS) and patients’ outcome was according to the revised Atlanta criteria. (AP: acute pancreatitis)](image)
In Studies II, III, and IV, all patients had a severe form of AP with at least one OF. The additional inclusion criteria were as follows: 1) admitted to the hospital < 96 hours from onset of pain, 2) at least one OF defined as an organ-specific SOFA score of at least three, and 3) < 48 hours from the first OF.

The Ethics Committee of the Helsinki University Central Hospital approved the study protocols, and the informed consent of each patient was obtained.

**Study I**
The prospective study consists of 161 patients with AP: 107 with mild AP, 29 with moderately severe AP, and 25 with severe AP (admitted between June 2003 and February 2007) (Table 7). For three patients, mRNA samples were not available. The reference samples were obtained from 20 healthy controls.

**Study II**
A total of 215 patients admitted with SAP were screened for the study between June 2003 and August 2007. Of these 215 patients, 158 fulfilled the inclusion criteria. After exclusions (see Figure 7), 32 patients with SAP were randomized to receive either APC (n = 16) or 0.9% physiologic saline as a placebo (n = 16) (Figure 6, Table 7).

**Study III**
Study III was a sub-study of the Study II and the study population consists of the same patients as in Study II (Tables 8 and 9). The reference samples were obtained from 65 healthy controls.

**Study IV**
The study population consists of a subgroup of Study II patients who had follow-up coagulation samples taken (APC group n=10, placebo group n=10) (Table 10). The reference samples were obtained from 10 healthy controls.
Table 7  Characteristics of patients (Study I, n=161).

<table>
<thead>
<tr>
<th></th>
<th>Mild n = 107</th>
<th>Moderately severe n = 29</th>
<th>Severe n = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male (n)</td>
<td>33/74</td>
<td>9/20</td>
<td>1/24</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>48 (18-87)</td>
<td>51 (19-82)</td>
<td>43 (29-81)</td>
</tr>
<tr>
<td>Etiology of AP (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>68 (64%)</td>
<td>21 (72%)</td>
<td>22 (88%)</td>
</tr>
<tr>
<td>Biliary</td>
<td>27 (25%)</td>
<td>7 (24%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>12 (11%)</td>
<td>1 (3.4%)</td>
<td>2 (8%)</td>
</tr>
</tbody>
</table>

Figure 7  Exclusion criteria and randomization of patients for Study II (SAP: severe acute pancreatitis).
Table 8  Characteristics of patients (Studies II and III). (SOFA: Sequential Organ Failure Assessment)

<table>
<thead>
<tr>
<th></th>
<th>APC n=16</th>
<th>Placebo n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male (n)</td>
<td>15/1</td>
<td>14/2</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>47 (19-59)</td>
<td>44 (34-36)</td>
</tr>
<tr>
<td>Etiology of AP (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Biliary</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SOFA score</td>
<td>6.5 (± 4.0)</td>
<td>6.3 (± 3.1)</td>
</tr>
</tbody>
</table>

Table 9  Number of patients (Study III).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 5</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>16</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Placebo</td>
<td>16</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Cell markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>16</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Placebo</td>
<td>15</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>
Study I
Study I was a prospective cohort study. We investigated whether serum levels of sCD73 activity, sCD73 protein concentration, and CD73 mRNA levels were associated with the severity of AP and analyzed the ability of SCD73 to predict the development of the severe form of AP.

Study II
Study II was a prospective randomized pilot human clinical trial where SAP patients were randomized to receive APC or placebo. The primary efficacy endpoint was the change in SOFA between the start of the study drug and day 5. The sample size was determined according to the primary endpoint; a three-point difference in change of SOFA score between the groups was the objective. The primary safety endpoint was the number of incidents of bleeding. Secondary endpoints were organ-failure-free days alive, days alive outside hospital in 60 days, and changes in other laboratory values during the first five days.

Study III
Study III was a pre-determined sub-study of Study II. The time course of the patients’ plasma or serum levels of soluble markers (IL-8, IL-6, IL-10, IL-1ra,
sE-selectin, PCT) and monocyte and neutrophil cell surface (CD11b, CD14, CD62L, HLA-DR) markers of systemic inflammatory response were measured during the first 14 days after the randomization.

**Study IV**

Study IV was a pre-determined sub-study of the patients in Study II who had follow-up coagulation samples taken. Coagulation parameters, physiological anticoagulants, thrombograms, and circulating levels of IL-6 and CRP were determined on admission and at days 1, 3-4, and 6-7.

**Table 11** Designs of Studies I-IV.

<table>
<thead>
<tr>
<th>Study</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td>Prospective cohort study</td>
<td>Prospective randomized pilot human clinical study</td>
<td>Prospective study (a sub-study of Study I)</td>
<td>Prospective study (a sub-study of Study I)</td>
</tr>
<tr>
<td>Number of patients (all/different groups)</td>
<td>n=161, of whom n=107 mild AP n=29 moderately severe AP, n=25 SAP</td>
<td>n=32 SAP, of whom n=16 APC, n=16 placebo</td>
<td>n=32 SAP, of whom n=16 APC, n=16 placebo</td>
<td>n=20 SAP, of whom n=10 APC, n=10 placebo</td>
</tr>
<tr>
<td>Main inclusion criteria</td>
<td>Adult patients with AP</td>
<td>Adult patients with SAP and at least one OF admitted to ICU</td>
<td>Same as Study II</td>
<td>Same as Study II</td>
</tr>
<tr>
<td>Main exclusion criteria</td>
<td>Chronic pancreatitis</td>
<td>ASA, active bleeding, chronic pancreatitis</td>
<td>Same as Study II</td>
<td>Same as Study II</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>n=20</td>
<td>-</td>
<td>n=65</td>
<td>n=10</td>
</tr>
</tbody>
</table>
3.3 ANALYTICAL METHODS

3.3.1 BLOOD SAMPLES

Study I
Serum samples for determining CD73 activity and CD73 protein concentration were taken on admission to hospital. The samples were frozen and stored at -70°C. Also venous blood samples for isolation of CD73 mRNA were collected on admission. They were drawn directly into PAXGene tubes. The reference samples were collected from healthy laboratory personnel (n=20, age 28-59 years).

Study II
Blood samples were taken every day during the patient's stay in the ICU. Laboratory measurements for SOFA (including serum creatinine, serum bilirubin, platelet count, and blood gas analysis for PaO2/FiO2 ratio) were performed between the start of the study drug and day 5.

Study III
Blood samples for determination of cell markers of inflammation were collected when a patient fulfilled the inclusion criteria. After the randomization, follow-up samples were collected on the third, fifth, seventh, and 14th day. Blood samples for flow cytometry and for plasma measurements were anticoagulated with pyrogen-free acid-citrate dextrose. Blood samples were cooled in an ice-cold water bath and kept at 0°C until stained for flow cytometry. The plasma was separated by centrifugation at 4°C and stored at −70°C until concentrations of cytokines and sE-selectin were determined. The reference blood samples for the analyses of cell surface markers were obtained from 65 healthy volunteers from the hospital and laboratory staff without medication and with no signs of infection.

Study IV
Plasma samples for the determinations of the biomarkers of coagulation and the markers of physiological anticoagulation and thrombograms were collected before starting the APC infusion (at 0 hours) and at days 1, 3 (or 4), and 7 (or 6) (in case the sample collection was scheduled on a bank holiday, they were taken on the following or the previous day). The samples were frozen and stored at −70°C. Serum CRP concentrations and blood platelet
counts were determined as a part of laboratory at 0 hours and 1, 3–4, and 6–7 days later. Plasma samples at 0 hours were available from each of the 20 patients. Reference blood samples for analysis of coagulation markers and thrombograms were obtained from healthy hospital and laboratory staff without medication (n=10).

### 3.3.2 LABORATORY ANALYSES

CRP (I), creatinine (I, IV), platelet count (II), blood gas analysis (II), B-hb (II), serum conjugated bilirubin (II), serum pre-albumin (II), serum disialotransferrine (II), and serum gamma-glutamyl-transferase (II) were determined in accordance with the hospital’s routine laboratory practice (Helsinki University Hospital Laboratory, HUSLAB).

The coagulation markers in Study IV were determined in accordance with the HUSLAB laboratory practice: the TT and PT levels in plasma samples were measured by Nycotest PT, plasma levels of PC and AT were measured by Berichrom, and the TFPI (free and total) levels in plasma samples were measured by Asserachrom (Diagnostica Stago). We calculated the ratio of free and total TFPI.

### 3.3.3 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

In Study III, the concentrations of IL-6 (also in Study IV), IL-8, IL-10, IL-1Ra, and E-selectin in plasma samples were determined by enzyme immunoassay. The commercial reagents used were as follows: IL-6 and IL-10: PeliPair ELISA, Sanquin, Amsterdam, the Netherlands; IL-8: Opt EIA, BD Biosciences, Erembodegem, Belgium; IL-1Ra: Duo Set ELISA, R&D Systems Europe Ltd., Abindgon, UK; E-Selectin: ELISA, HyCult Biotechnology, Uden, the Netherlands. The detection limits and intra-assay and interassay coefficients of variation (CV%) were as follows: IL-6: 0.3 pg/ml, 3.6% and 5.4%; IL-8: 1.6 pg/ml, 3.5%, 3.4%; IL-10: 0.3 pg/ml, 3.7%, 5.9%; IL-1Ra: 10 pg/ml, 4.2%, 5.2%; E-selectin: 20.5 pg/ml, 3.5%, 6.9%.

Procalcitonin (PCT) (III) was measured by means of a sandwich chemiluminescent immunoassay using monoclonal antibody to fluorescein covalently linked to paramagnetic particles and two antibodies to procalcitonin labeled with fluorescein (ADVIA Centaur XP immunoassay system with ADVIA Centaur BRAHMS PCT assay).

Analysis of sCD73 protein concentration (in Study I) was performed with sandwich ELISA technique using new anti-CD73 specific monoclonal antibodies. New mAbs against immunoaffinity-purified human CD73 molecule were generated by immunizing CD73-deficient mice. The hybridoma generation was performed by fusing the draining lymph node lymphocytes with SP2.0 myeloma cells. The hybridoma supernatants were
screened using immunohistochemical stainings of human tissues, immunofluorescence stainings of transfectants expressing CD73, and immunoblotting; the positive hybridomas were subcloned by limiting dilution.

One of the new mabs (clone 118, mouse IgG1) was used together with an old anti-CD73 mAb (clone 4G) for development of sandwich. In brief, 4G4 mAb was bound to the bottom of white 96-well Cliniplates, the serum samples were added (1:10 dilution in PBS), and the bound sCD73 was detected with biotinylated-118. Clone 118 was biotinylated with NHS-biotin (Pierce) according to the manufacturer’s instructions. The ELISA reaction was developed using streptavidin-horseradish peroxidase and BM Chemiluminescence ELISA Substrate (Roche).

To validate the ELISA, serial dilutions of recombinant human CD73 (RD Systems) and CD73-depleted sera were used. CD73 was removed from serum by three sequential incubations of normal pooled sera with CnBr-activated sepharose beads armed with an anti-CD73 mAb 4G4.

CD73 activity, protein concentration, and mRNA levels were determined in a research laboratory at the University of Turku.

### 3.3.4 Whole Blood Flow Cytometry

In Study III, monocyte expression of CD14, CD11b, CD62L, and HLA-DR and neutrophil expression of CD62L and CD11b were determined using whole blood flow cytometry. Monoclonal antibodies (mAbs) were as follows: phycoerythrin (PE) and fluorescein isothiocyanate (FITC) conjugates of anti-CD14 mAb (IgG2b, clone MFP9), PE conjugates of anti-HLA-DR mAb (IgG2a, clone L243), anti-CD11b mAb (IgG2a, clone D12) and control mouse IgG2a, mAb, and FITC conjugate of anti-CD62L mAb (IgG2a, clone SK11). Staining of aliquots of the whole blood sample at 0°C for flow cytometry was carried out as described previously. Data acquisition and analyses were done with a FACS®Calibur flow cytometer and Cell Quest software (BD Sciences, San Jose, CA, USA). Neutrophils were identified by the light scattering properties and monocytes by the clonal marker CD14. Monocyte HLA-DR expression was determined as the proportion of HLA-DR-positive monocytes. Fluorescence intensity is presented as relative fluorescence units (RFUs).

### 3.3.5 CD73 Activity

CD73 activity in the sera was determined using radioactive enzyme assays in a research laboratory at the University of Turku. The measurement of CD73 activity was based on chromatographic analyses with ³H-labeled nucleotides and bioluminescent measurement of ATP metabolism. Serum
(typically 10 µl) was incubated with 300 µM AMP along with tracer [2-³H] AMP (Quotient Bioresearch, GE Healthcare, UK) at 37°C in a final volume of 60 µl RPMI1640 medium supplemented with 5 mM β-glycerophosphate. Large excess of β-glycerophosphate as an alternative phosphorylated substrate in the enzyme assays allowed us to exclude the potential contribution of non-specific phosphatases (e.g. alkaline phosphatase) in the measured activities. The incubation times (30-60 min) were chosen so that the amount of hydrolyzed AMP was <15% of the initially added substrate to ensure linearity. Sample aliquots were then applied to Alugram SIL G/UV254 sheets (Macherey-Nagel, Germany), and separated by thin-layer chromatography using isobutanol/isoamyl alcohol/2-ethoxyethanol/ammonia/H₂O (9:6:18:9:15). [2-³H]AMP and its dephosphorylated ³H-nucleoside derivatives were visualized in UV-light and quantified by scintillation β-counting. The CD73 activities are reported as nanomoles AMP hydrolyzed by one milliliter of serum in one hour.

3.3.6 PCR FOR CD73 mRNA

CD73 mRNA levels were analyzed in blood leukocytes by using a qPCR (quantitative polymerase chain reaction) technique in a research laboratory at the University of Turku. Leukocyte mRNA was isolated from the blood samples collected into PAXgene tubes using PAXgene Blood RNA kit (PreAnalytix) according to the manufacturer’s instructions. mRNA was reverse transcribed into cDNA using an iScript™ cDNA Synthesis kit (BioRad). The CD73 mRNA levels were determined using a TaqMan Gene Expression Assay (Applied Biosystems) for CD73 (NT5E, assay Hs00159686_m1) as the primer/probe set. The PCR reactions were carried out as suggested by the supplier using the Applied Biosystems 7900HT Fast Real-Time PCR System in the Finnish DNA Microarray Center, Turku Center of Biotechnology, Turku, Finland. All samples were run in triplicate, and the expression values were normalized using a house-keeping gene (GAPDH) as the endogenous control. The results were analyzed with SDS 2.3 software. Changes in cycle threshold levels (ΔCₜ) were calculated by subtracting the average of GAPDH Cₜ values from the average of target gene Cₜ values. Relative expression of the gene analyzed was estimated using the formula: relative expression = 2⁻ΔCₜ. The quantity of CD73 mRNA was expressed as a percentage of GAPDH mRNA after multiplying the relative target gene expression by a factor of 100.

3.3.7 THROMBOGRAMS

The thrombin generation of patients was analyzed by thrombograms in Study IV. The thrombograms were obtained through Calibrated Automated
Thrombography (CAT, Thrombogram™, Synapse B.V., the Netherlands) in the Hematology Laboratory of Helsinki University Central Hospital. The parameters of the thrombogram are the lag time (reflecting the same physiological phenomena that largely determine various clotting time-based assays), the area under the curve (AUC=endogenous thrombin potential, ETP), the peak height (thrombin burst), and the time to reach the peak. Platelet-poor plasma (PPP) was used. Coagulation was triggered by recalcification in the presence of 5 pM tissue factor.

3.4 STATISTICAL METHODS

Study I was a preliminary study, which included prospectively enrolled AP patients to the emergency unit at Helsinki University Central Hospital between June 2003 and February 2007. The sample size for Study II was calculated according to the primary endpoint: the change in SOFA. We assumed that critically ill SAP patients would have an average admission SOFA score of 7 and that the mean (SD) increase would be 1 (± 3) point in the placebo group. Calculations according to established methods revealed that a sample of 16 patients per group would allow us to detect a clinically meaningful three-point difference in change of SOFA score (7 to 8 in placebo group vs. 7 to 5 in APC group with an estimated SD of 3.0 and with P < 0.05 and a power of 80%). The sample size was also assumed to be adequate for testing the differences in patient-related change in laboratory parameters in this pilot trial (no preliminary data were available for calculations). Studies III and IV were pre-determined sub-studies of Study II.

The results (summary statistics) were expressed as means (II, III, IV), differences in means (II), medians (I, III, IV), ranges (I, IV), or inter-quartile ranges (IQR) (I,III,IV).

Normal distribution of the data was tested with skewness (I) and Kolmogorov-Smirnov test (continuous variables) (II, III, IV). In Study I, statistical tests with non-parametric assumptions were used because most of the data showed non-normal distribution (skewness of the data). In the other studies, the data were normally distributed.

Comparison of continuous data was tested with the Mann-Whitney U-test (between two groups) (I, III), the Kruskall-Wallis test (between three groups) (I), or the Jonkheere-Terpstra test for trend (I). In Study II, comparisons between the study groups were performed using patient-related changes (before and after the study drug, days 0 and 5) in laboratory values and the calculation of differences in means (differences in means (DIM) (95% CIs)) according to evidence-based medicine guidelines for randomized studies, and regarding categorical variables with Fisher’s exact test.

The Wilcoxon signed-rank test was used in comparisons of repeated measurements (comparisons of the follow-up samples) (III).
The correlations between two continuous variables were determined using Spearman’s rank correlation (I). In Study IV, the general estimating equations with exchangeable correlation matrix were used to detect differences between the groups in variables with repeated measurements. The Friedman test was used to find statistically significant differences, and the Page test to find statistically significant trends in variables with ordered categories with repeated measurements. In Study I, logistic regression analysis was used to assess the prognostic value of independent predictors (CD73, CRP, and creatinine) in predicting the development of SAP.

In Study I, the area under the receiver-operating characteristic (ROC) curve (AUC), together with the corresponding sensitivity (y-axis) and specificity (x-axis), was used to measure the predictive accuracy with different threshold values. Clinically optimal cut-off values were chosen to analyze the sensitivity and specificity of sCD73 activity and other markers in predicting the development of SAP. Clinically optimal cut-off point of a ROC curve is a point at which the slope R satisfies the equation $R = \frac{C}{Bx(1-P)/P}$, where $C/B$ is the ratio of the net cost of treating non-diseased individuals and the net benefit of treating diseased individuals and $P$ is the prevalence of the disease. The prevalence of OF among patients with AP is approximately 10%, but the $C/B$ ratio in this scenario is not clear. Since ICU beds are limited, we chose a cut-off point where the number of false positives is as low as possible (specificity >90%) by selecting a threshold value at a point where the longest increase in the sensitivity of the slope declines, as described elsewhere.

P-values < 0.05 were considered significant in all pair-wise comparisons. In Study I with multiple comparisons, the Bonferroni correction was used in order to avoid the effects of mass significance.

The statistical analyses were performed using the SPSS (SPSS Inc., Chicago, IL, USA) and the Stat Exact v4.0 software (I) (Cytel Software Corporation, Cambridge, MA, USA).

3.5 RESULTS

3.5.1 CD73 IN PREDICTING SEVERE ACUTE PANCREATITIS

Medians of serum CD73 activity (320 nmol/ml/h, IQR 195-635) and protein concentration (49.7 ng/ml, IQR 33.4-90.9) were significantly higher in patients with AP than in healthy reference subjects (115 nmol/ml/h, IQR 139-181, p<0.0001 and 33.2 ng/ml, IQR 23.1-38.9, p=0.0002). CD73 activity, protein concentration, and mRNA levels were associated inversely with severity of AP (Table 12).
The medians of CD73 activity, protein concentration, and mRNA did not differ significantly between the moderately severe and severe group (p for CD73 activity = 0.053, Mann-Whitney U-test, p for protein concentrations 0.538 and p for mRNA levels 0.086).

We examined whether the patients with transient OF differed from those with persistent OF. Seventeen patients had MMS \( \geq 2 \) on admission, indicating OF (Figure 6). Five of the patients had transient OF, ultimately belonging to the moderately severe AP group, and 12 persistent OF, ultimately belonging to the severe AP group. sCD73 activity, protein concentrations, and mRNA levels did not differ significantly between transient and persistent OF groups (Table 13).

The sCD73 activity on admission predicted the development of SAP among different subgroups of the patients. AUC value for sCD73 activity was 0.79 (95% CI 0.69-0.88) among all patients (Fig. 8). AUC value for CD73 activity was 0.65 (95% CI 0.51-0.80) among a subgroup of patients comprising moderately severe and severe disease (Fig. 9) and 0.75 (95% CI 0.60-0.89) among patients with no signs of OF (MMS< 2) on admission (Fig. 10). The AUC values for CRP and creatinine were lower than that for sCD73 activity in all subgroups; thus, sCD73 activity was better than CRP or creatinine in predicting the severe form of AP.

---

**Table 12**  
*sCD73 activity, protein concentration (ELISA), and CD73 mRNA levels and severity of acute pancreatitis (AP).*

<table>
<thead>
<tr>
<th>Severity of AP</th>
<th>sCD73 Activity (nmol/ml/h)</th>
<th>sCD73 ELISA (ng/ml)</th>
<th>CD73 mRNA (Relative Expression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>410.5 (105-2562)</td>
<td>57.3 (12.4-918)</td>
<td>0.25 (0.01-3.42)</td>
</tr>
<tr>
<td>Moderately severe</td>
<td>235.1 (88.5-1187)</td>
<td>42.3 (9.6-259)</td>
<td>0.15 (0.01-3.60)</td>
</tr>
<tr>
<td>Severe</td>
<td>159.0 (80.2-1147)</td>
<td>45.9 (9.8-293)</td>
<td>0.09 (0.01-1.53)</td>
</tr>
</tbody>
</table>

\( ^{a} \) Jonkheere-Terpstra test for trend. Data are given as median (range).

**Table 13**  
*Comparison of sCD73 activity, sCD73 concentration, and CD73 mRNA levels in patients with transient or persistent organ failure.*

<table>
<thead>
<tr>
<th>Organ failure</th>
<th>sCD73 Activity (nmol/ml/h)</th>
<th>sCD73 ELISA (ng/ml)</th>
<th>CD73 mRNA (Relative Expression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient (n =5)</td>
<td>270.5 (139-425)</td>
<td>53.4 (29-53)</td>
<td>0.15 (0.07-0.28)</td>
</tr>
<tr>
<td>Persistent (n =12)</td>
<td>141.1(114-235)</td>
<td>44.7 (14-77)</td>
<td>0.06 (0.02-0.28)</td>
</tr>
</tbody>
</table>

\( ^{a} \) Mann-Whitney U-test. All patients had on admission a Modified Marshall Score \( \geq 2 \). Data are given as median (range).
**Figure 8**  Receiver-operating characteristic (ROC) curves for predicting severe acute pancreatitis (AP) in the whole AP patient cohort.

**Figure 9**  Receiver-operating characteristic (ROC) curves for predicting severe acute pancreatitis (AP) among patients with moderately severe or severe AP.
The clinically optimal cut-off values on the ROC curves were chosen to analyze the sensitivity and specificity of sCD73 activity (and CRP and creatinine) in predicting the development of SAP. Among patients with moderately severe and severe AP, the sensitivity and specificity of sCD73 activity (cut-off 139 nmol/ml/h, sensitivity 0.40, specificity 0.828) were comparable with those of CRP (cut-off 224 mg/ml, sensitivity 0.40, specificity 0.724) and creatinine (cut-off 164 μmol/l, sensitivity 0.40, specificity 0.862). The combination of sCD73 and CRP (sensitivity 0.60, specificity 0.89) or sCD73 and creatinine (sensitivity 0.60, specificity 0.90) improved the sensitivity. The clinically optimal cut-off values obtained from the whole population or from the patients with moderately severe and severe AP were almost identical. Logistic regression analyses showed that sCD73 activity was an independent predictor of SAP among all patients (cut-off 138, OR 6.578, 95% CI 1.834-23.592). sCD73 activity showed no correlation with creatinine (R=0.015, p=0.913), but a negative correlation with CRP on admission (R=-0.291, p=0.033). Among the patients with MMS<2 on admission, the sCD73 activity (cut-off 139, sensitivity 0.308, specificity 0.939) had a better combination of sensitivity and specificity in predicting SAP than did CRP (cut-off 224, sensitivity 0.077, specificity 0.954) or creatinine (cut-off 164, sensitivity 0.077, specificity 1.00). Logistic regression analyses showed that sCD73 (cut-off 139, OR 6.639, 95% CI 1.245-35.409)
(in contrast to CRP, cut-off 224, OR 0.763, 95% CI 0.066-8.802) was an independent predictor of SAP among patients with MMS<2.

Thus, among all groups (all AP patients, patients with moderately severe and severe AP on admission and patients with no signs of OF on admission) sCD73 activity is better in predicting SAP than CRP or creatinine.

3.5.2 ACTIVATED PROTEIN C (APC) TREATMENT IN SEVERE ACUTE PANCREATITIS

Clinical response of APC in severe acute pancreatitis (Study II)

The efficacy of APC treatment was evaluated by analyzing its effects on OF. We analyzed changes in SOFA scores (primary endpoint), ventilator-free days, renal replacement therapy-free days, and vasopressor-free days. No significant difference in SOFA score changes between the APC (from 6.5, SD ± 4.0, range 2-14, to 8.2, SD ± 5.0, range 0-16) and placebo groups (from 6.3, SD ± 3.1, range 3-11) to 5.7 (SD ± 4.6, range 0-16) was found (DIM 2.3, 95% CI -0.7 to +5.3). The median hospital stay was 23 days (range 2-40 days), and the median duration of ICU stay 11 days (range 0-43 days). Altogether 21/32 patients (66%) needed mechanical ventilation, 12/32 (38%) needed dialysis, and 20/32 (63%) needed vasopressor treatment during their stay at the ICU. No differences in ventilator-free days, in renal replacement therapy-free days, or in vasopressor-free days were detected between the groups.

Safety of APC treatment in clinical use was also evaluated. No serious bleedings were detected clinically or by CT scans in either the APC group or the placebo group (incidence 0%, 95% CI 0 to 18%, DIM 95% CI -17 to +17%). Minor bleeding (from mouth, nose, or urinary tract) occurred in four patients in the APC group and in two patients in the placebo group (p=not significant, NS). The administered red blood cell units during the APC infusion did not differ between the groups (seven in APC vs. eight in placebo group, p=NS). No significant differences in plasma hemoglobin levels or platelet counts were found. We evaluated the effects of APC treatment on liver function tests (serum bilirubin, serum pre-albumin, serum disialotransferrine, serum gamma-glutamyl-transferase) and serum creatinine levels. Administration of APC was associated with an increase in serum bilirubin (DIM 28.4 mmol/L, 95% CI 3.6 to 53.1), and serum conjugated bilirubin (DIM 25 mmol/L, 5.6 to 44.4). No significant differences in other liver function tests or creatinine levels were found. The 30-day mortality in the APC group was 3/16 compared with 0/16 in the placebo group (absolute risk reduction, ARR -19%, 95% CIs -38% to 0). The non-survivors had MOF (SOFA scores 10, 13, and 14), which was also the autopsy-confirmed cause of all deaths (on days 4, 11, and 14). No significant differences in days alive outside the hospital were detected (DIM -17.4, 95% CI -0.1 to -34.9).
Thus, no differences in the evolution of MOF between APC and placebo groups were found.

**Effects of APC treatment on the course of systemic inflammation in severe acute pancreatitis (Study III)**

Characteristics of the patients are given in the Materials and Methods section and in Table 8 and the numbers of the patients at different time-points in Table 9. Study III was a sub-study of Study II. The effects of APC treatment on immunologic parameters in patients with SAP were investigated.

The inflammatory response was detected by analyzing the changes in plasma concentrations of proinflammatory IL-8. IL-8 levels of all patients decreased during the first five days after admission to hospital (at day 0: 264 pg/ml vs. at day 5: 110 pg/ml, p=0.001). The APC had no significant effect on the changes in IL-8 concentrations during the 14-day follow-up. Plasma concentrations of pro-/anti-inflammatory cytokine IL-6 and anti-inflammatory cytokines IL-10 and IL-1Ra decreased during the first five days (IL-6 at day 0: 670 pg/ml vs. at day 5: 215 pg/ml, p=0.001; IL-10 at day 0: 12.7 pg/ml vs. at day 5: 11.3 pg/ml, p=0.001; IL-1Ra at day 0: 2890 pg/ml vs. at day 5: 1250 pg/ml, p=0.007) in both groups. The APC treatment did not have any significant effect on the anti-inflammatory response (changes in IL-6, IL-10, and IL-1Ra concentrations) during the follow-up. Endothelial response in terms of plasma concentrations of soluble E-selectin decreased over the course of the disease (at day 0: 45.5 ng/ml vs. at day 5: 38.2 ng/ml, p=0.031) in both groups, and the APC treatment did not have any effect on changes. Serum concentrations of PCT, a marker of systemic inflammation, showed no altered concentrations during the first five days (at day 0: 0.97 ng/ml vs. at day 5: 0.66 ng/ml, p=0.487), and administration of APC had no effects on the changes in PCT concentrations.

As a marker of immune suppression, monocyte HLA-DR expression was not altered significantly during the first five days of the follow-up period (at day 0: 54% vs. at day 5: 58%, p=0.316) in either group, and the APC had no effect on the HLA-DR expression. The cell surface expression levels of CD11b, CD14, and CD62L were measured as markers of monocyte activation. The monocyte surface expression of CD11b, CD14, and CD62L was downregulated during the first five days (CD11b at day 0: 291 RFU vs. at day 5: 200 RFU, p=0.001; CD14 at day 0: 168 RFU vs at day 5: 150 RFU, p=0.028; CD62L at day 0: 217 RFU vs. at day 5: 135 RFU, p=0.001) in both groups, and the APC treatment had no effect on the expression levels. Neutrophil activation was evaluated by analyzing the expressions of CD11b and CD62L: both were downregulated during the first five days of follow-up (CD11b at day 0: 325 RFU vs. at day 5: 259 RFU, p=0.001; CD62L at day 0: 146 RFU vs. at day 5: 81 RFU, p=0.001), and the APC treatment had no effects on expression levels.
Coagulation effects of APC in severe acute pancreatitis (Study IV)

**Coagulation markers** In the placebo group, the rate of change in PT was negative (p<0.001), indicating decreasing temporal levels, whereas in the APC group such a decrease was not detected (p=0.997). The rate of change was different between the groups (p<0.001). The temporal levels of TT increased in the placebo group (p<0.001 for slope), but not in the APC group (p=0.521). The rate of change between the groups differed (p<0.001). As to D-dimer levels, the rates of change in the two groups showed increasing temporal sequence (p<0.001 and p<0.039, respectively) and differed between the groups (p=0.019). The temporal levels of platelet count in placebo and ACP groups were increasing during the follow-up (both p<0.001); the rates of increase were comparable (p=0.609). The rates of change in fibrinogen levels were positive in the placebo and APC groups (both p<0.001), indicating increasing temporal values. The rates of change were comparable between the groups (p=0.148) (Study IV: Figure 1, Panel A).

In the placebo group, there were significant changes in DIC scores between the measurement days (p=0.008), but the trend was not significant (p=0.064, Page test). In the APC group, the changes in DIC scores between the different measurement days were not significant (p=0.505, Friedman test) (Study IV: Table III).

**Physiological anticoagulation markers** As to PC levels, the rates of change were positive in the placebo and APC groups (p<0.001 and p=0.027, respectively), indicating increasing temporal values. They were comparable between the groups (p=0.067). As to AT levels, the rates of change were positive (p<0.001 for both groups) and differed between the groups (p=0.0111). The alterations of TFPI free and TFPI total levels or TFPI free /TFPI total ratios were not significant (Study IV: Figure 1, Panel B and Table II, Panel B).

**Inflammation markers** The rates of change in IL-6 levels and in CRP levels were negative in the placebo and APC groups (Study IV: Figure 1, Panel C and Table II, Panel C).

**Thrombograms** The shapes of the thrombograms were deranged in the patients with SAP relative to the healthy reference subjects. Of the patients, 10/20 had no detectable thrombin response, and their thrombogram graphs showed straight lines (“flat curve”) at time-point 0. At days 1 and 3-4, nine of the patients (5 in the APC group and 4 in the placebo group) had detectable thrombin generation. The patients with a “flat curve” were excluded from further analyses of the thrombograms. The rates of change in lag time and time to peak values of the placebo group were positive (p=0.013 and p=0.007, respectively). The rate of change in ETP values was positive in the APC group (all p-values 0.05) (Study IV: Figure 1, Panel D, Table II, Panel D).
3.6 DISCUSSION

3.6.1 PREDICTING SEVERE ACUTE PANCREATITIS

The spectrum of severity of AP ranges from a mild, self-limiting disease to a highly fatal SAP with MOF. Of AP cases, 75-80% remain mild\(^3\)\(^4\) and require only supportive care (i.e. intravenous fluid replacement and pain management). Overall mortality of AP is still high (5%), reaching 30-40% in cases with infected necrosis or those with MOF.\(^3\)\(^7\) Mortality in AP manifests in two peaks; nearly half of the deaths occur within the first week due to overwhelming SIRS, and septic complications account for the deaths in the late phase.\(^4\)

Severity of AP can fluctuate rapidly, and predicting the course of AP is difficult. The patients who will develop SAP need to be identified early in the course of disease and aggressively treated to prevent mortality. On the other hand, proper identification of mild cases of AP is necessary to avoid overtreatment and to reduce financial implications, i.e. high ICU costs. As mentioned earlier (Section 2.7.2), many kinds of multifactorial scoring systems have evolved to assess the severity of AP. The scoring systems have several limitations, e.g. Ranson score takes 48 hours to complete and APACHE II is cumbersome in clinical use.\(^294\) It is difficult to find a single biochemical parameter that could predict the severity of AP early enough in the course of the disease; if the inflammatory reaction has already started, it is difficult to terminate. For example, the widely used CRP has a delayed peak (48-72 hours),\(^294\) and it is a non-specific inflammatory marker in AP (i.e. cholangitis and pneumonia increase CRP levels). An ideal predictor should be easy to obtain, widely available, cost-effective, associated with high sensitivity/specificity, have high positive and/or negative predictive value, and be able to predict the severity of AP as early as possible (within the first 24-48 hours of symptom onset).

Our main finding in Study I was that the circulating levels of sCD73 are significantly higher in patients with AP than in healthy control subjects, and the sCD73 levels on admission to hospital correlate inversely with the severity of AP. Even more importantly, the sCD73 activity predicted the development of SAP among all patients and also among patients without signs of OF on admission (MMS<2). Thus, AP patients with increased sCD73 on admission more often had the mild disease than patients with less strongly elevated sCD73 levels on admission. sCD73 activity has better predictive accuracy than CRP and creatinine levels, and the combination of CD73 activity and CRP showed improved predictive performance. sCD73 levels can be measured by using a single blood test, performed within 7 hours, with reagent costs of about 3 euros per sample.

At present, the mediators for sCD73 induction and the cell type from which sCD73 levels derive in AP remain uncertain. Tissue hypoxia, a
common feature in patients with SAP, is known to increase the expression of CD73. Soluble CD73 is mainly derived by shedding of lymphocytes rather than endothelial CD73, and our study showed decreased CD73 mRNA synthesis in leukocytes of SAP patients. An intriguing question is whether the patients with SAP are low responders in terms of CD73 induction/shedding, with their sCD73 levels therefore being lower than those of the patients with mild AP. Genetic deficiency of CD73 has been shown to cause arterial and periarticular calcification phenotypes, but the possibility of involvement of polymorphisms of the CD73 gene in AP is not known and requires further studies.

3.6.2 TREATMENT OF SEVERE ACUTE PANCREATITIS

Clinical response of activated protein C treatment in severe acute pancreatitis

Several pre-clinical studies have shown that APC has potentially beneficial effects on inflammation. The endogenous protein C levels have been shown to correlate with positive outcome in severe sepsis, and the recombinant APC administration has a protective effect in animal models in Escherichia coli-induced sepsis. The precise mechanism of the cytoprotection is unknown, but possible effects of APC administration may include attenuation of proinflammatory cytokine storm, re-balancing dysregulated coagulation, or degradation of cytotoxic extracellular histones.

The PROWESS trial (Protein C Worldwide Evaluation in Severe Sepsis) showed beneficial effects of recombinant APC (drotrecogin alpha, Xigris®) on mortality in sepsis patients (included 1690 patients with severe sepsis, 28-day mortality rate of 25% in patients treated with recombinant APC compared with 31% in those treated with placebo, 6.1% reduction in mortality). Drotrecogin alpha was approved for use as a pharmaceutical therapy for severe sepsis by decision of the Food and Drug Administration in 2001 in the United States.

Sepsis and SAP share similarities in systemic inflammatory reactions and coagulopathy, although both of these diseases also have their own unique characteristics. Therapeutic use of APC in SAP was a potential idea, and the thought was supported by an earlier trial of our study group showing that patients with SAP have low PC levels and high endogenous APC to PC ratios. Also in experimental animal pancreatitis models, APC has been shown to have beneficial effects; i.e. APC improved the severity of pancreatic tissue histology, superinfection rates, and serum markers of inflammation in acute necrotizing AP.
Study II was a prospective randomized double-blind trial of APC in 32 patients with SAP. In this study, we found no difference in the evolution of MOF (the change in SOFA between day 0 and day 5) or in OF-free days (ventilator-free-days, renal replacement-free-days, vasopressor-free days) or days alive outside hospital in 60 days between APC-treated patients and those receiving placebo. We found no serious bleeding in either group.

An interesting question is had a higher dose of APC been used or the duration of infusion extended, would the APC have had a more obvious effect on SAP. The therapeutic use of an anticoagulant like APC in SAP is a balancing act between the beneficial effects on inflammation and the increased risk of bleeding. The anticoagulant nature of APC may enhance the SAP patients’ already high risk of bleeding, and therefore, increasing the dose is challenging. Clinical trials, done after the PROWESS study, used the same standard protocol in administration of recombinant APC as suggested by Bernard et al. (24 μg/kg/h for 96 hours). Effectiveness of APC has varied, but the risk of bleeding complications is documented.

A randomized double-blind placebo-controlled multi-center trial published in 2012 (the PROWESS SHOCK trial, required by the European Medicines Agency) found no evidence suggesting that APC treatment reduces the risk of death in severe sepsis. The pharmaceutical form of APC (drotrecogin alfa, Xigris®) was withdrawn from the pharmaceutical market in 2011.

Sepsis studies of APC treatment are interesting examples of numerous possibilities of biases and errors in randomized clinical trials (RCTs) and their interpretations. In addition, these studies raise ethical questions related to RCTs. The PROWESS study was stopped early for benefit. The Data and Safety Monitoring Board, an independent agency that mainly consists of biostatisticians, clinicians, basic scientists, and bioethicists, is required to minimize conflicts of interest in many large Phase 3 trials. The Data and Safety Monitoring Board of the PROWESS study made the decision to terminate the trial at the second interim analysis because of the significant mortality benefit detected. It has been shown that trials stopped early for benefit (so-called truncated RCTs for benefit) may overestimate treatment effects and may miss important adverse drug reactions (if they became underpowered). It is possible that the PROWESS study had methodological faults and interpretation of the trial was overly optimistic.

On the other hand, the withdrawal of drotrecogin alfa has attracted criticism. Kalil et al. questioned the big differences in resulting mortality of the PROWESS and PROWESS SHOCK trials, which were carried out by the same manufacturer of medicine, the same drotrecogin alfa dose, and the same placebo-controlled design. In the PROWESS study, the decrease in mortality was 6.1%, and in the PROWESS SHOCK study no survival benefits were found. Kalil et al. performed an analysis of the clinical and statistical heterogeneity between the trials. They noted that clinical variables (i.e. baseline characteristics and co-interventions) presented significant
heterogeneity and the PROWESS SHOCK study was underpowered, and they concluded that these trials are not comparable. Kalil et al. also published in 2012 a meta-analysis of drotrecogin alfa treatment in sepsis (over 40 000 patients) and concluded that APC treatment was associated with a significant reduction in hospital mortality (18%) and increased rates of bleeding (5.6%) in patients with severe sepsis.

After several trials and interpretations, open questions remain concerning the benefit of APC treatment in severe sepsis.

**Effects of activated protein C treatment on the course of systemic inflammation in severe acute pancreatitis**

Originally, the APC treatment was proposed mentioned to be a promising target for the treatment of sepsis based largely on its anticoagulant properties. Later, mounting evidence with other anticoagulants (AT, recombinant TFPI) failed to show a benefit in severe sepsis, resulting in the suggestion that the role of APC as an anticoagulant does not explain its benefits in systemic inflammation.

APC has been demonstrated to have direct cytoprotective effects such as anti-inflammatory, anti-apoptotic, and endothelial barrier protection functions. Anti-inflammatory activity of APC is mediated by its interaction with endothelial cells and leukocytes.

Several in vitro and animal studies have revealed that APC has anti-inflammatory effects. APC has been shown to inhibit leukocyte infiltration. Administration of APC inhibits NF-κB activation on cultured endothelial cells, resulting in suppressed expression of adhesion molecules (i.e. ICAM-1, E-selectin, and VCAM-1) and consequent decreased leukocyte trafficking. APC also reduces expression of proinflammatory mediators from endothelial cells (i.e. IL-6, IL-8, and MCP-1). Administration of APC has been shown to downregulate the expression of inflammatory cytokines and chemokines by leukocytes. APC has direct effects on leukocytes, and the effect is mainly mediated by EPCR. EPCR receptors are located on the surface of monocytes, neutrophils, and eosinophils. APC directly inhibits chemotaxis of neutrophils by its association with EPCR. APC has been demonstrated to reduce production of endotoxemia-induced proinflammatory cytokines (IL-6, IL-8, IL-1β, and TNFα) by monocytes. APC inhibits TNFα production by blocking NF-κB transcription factor in monocytes. APC also inhibits cytokine production from Th2 lymphocytes. APC upregulates anti-inflammatory mediators, e.g. IL-10 on blood monocytes.

No previous clinical trials have scrutinized the effects of APC in inflammation markers in SAP. In the PROWESS study, they found decreases in IL-6 levels in sepsis patients’ plasma and that has been taken as evidence of an anti-inflammatory action of APC. HLA-DR expression has been shown to correlate with PC and APC levels in SAP.
In Study III, we examined effects of APC on systemic inflammation response. We analyzed the effect of APC on soluble and cellular markers during a two-week follow-up and found no significant differences between the groups. The result showed that recombinant APC treatment of patients with SAP did not alter the course of systemic inflammation, in accordance with the clinical findings in Study II.

Recent studies on inflammation effects of APC have revealed that the anti-inflammatory effects are mainly mediated by cell signaling. APC triggers an array of signaling pathways via distinct receptor interactions on different cell types. For example, binding the APC to EPCR facilitates activation of PAR1 and PAR3. APC-mediated PAR1 signaling is distinct from the traditional G-protein-coupled signaling (thrombin uses PAR1 receptor to mediate proinflammatory effects). PAR1 mediates APC’s endothelial-barrier protection and anti-apoptotic and anti-inflammatory activities. The enhanced knowledge of APC’s cytoprotective mechanisms is a step forward in fully utilizing APC or its variants in clinical use.

Coagulation effects of activated protein C treatment in severe acute pancreatitis

The hypothesis in Study IV was that APC treatment should alleviate consumptive coagulopathy and increased fibrinolysis in SAP. Our results showed that recombinant APC standard protocol treatment did not have an alleviating effect on coagulopathy; instead APC treatment interfered with normalization of coagulopathy in SAP.

TT (thromboplastin time) monitors the interaction of FII, FVII, and FX, and TT decreases in the DIC. In our study, TT levels in the placebo group showed a trend towards normal in the placebo group, but not in the APC group. PT (prothrombin time) depicts the extrinsic pathway of coagulation. It monitors the function of fibrinogen, thrombin, FV, FVI, and FX. PT levels showed the same phenomenon as TT levels in our study.

Our results indicated that SAP patients’ platelet counts were reduced, and an increasing trend was observed in both groups (APC treatment showed no effect on the trend). Consumptive coagulopathy and increased fibrinolysis are known to occur in SAP, and they are related to its severity and OF. Fibrin D-dimers are degradation products of fibrin, and they are released as a result of fibrinolysis to the circulation. D-dimer levels increase when coagulation and fibrinolysis are accelerated, i.e. in severe inflammatory reactions and DIC. D-dimer levels depend upon both thrombin formation and fibrinolytic activation. In this study, the D-dimer levels showed an increasing trend in both groups, and the rate of change was greater in the placebo group (reflecting that coagulation and fibrinolysis remained accelerated in the placebo group relative to the APC group).

Fibrinogen levels increase within the acute phase reactions and decrease in DIC and when liver function is decompensated. In this study,
fibrinogen levels were elevated in both groups throughout the follow-up, and the fibrinogen levels increased as pancreatitis progressed. This may indicate that acute phase reactions predominate over consumption of fibrinogen in SAP.

Protein C (PC) is the inactive precursor of APC. PC is a K-vitamin-dependent inhibitor of coagulation, and PC levels decrease in DIC. Decreased PC levels are associated with the severity of AP. In our study, PC levels of SAP patients were low and showed an ascending trend; the administration of APC had no effect on the trend.

Antithrombin (AT) is an anticoagulant that forms an inactive complex with thrombin. AT levels have been shown to decrease in DIC. Secondary AT deficiency is associated with OF in SAP patients. In this study, SAP patients showed secondary AT deficiency, and in the APC group normalization of the levels was delayed.

TFPI (tissue factor pathway inhibitor) is a specific inhibitor of TF, and it is released in endothelial cells as a result of thrombin generation. TFPI occurs in plasma as a lipoprotein-bound form (ca. 80%) and as a free form (5-20%). Free TFPI has a more intense anticoagulant effect than the bound form. Elevated TFPI levels have been detected in plasma of AP patients. Our earlier study showed a rising trend in free TFPI and total TFPI levels and in ratios of free/total TFPI associated with severity of AP. This study did not find a significant trend in TFPI levels in either group.

Thrombograms probe exogenous TF-triggered thrombin generation in plasma samples and can be used to diagnose hyper- and hypocoaguable states of patients. Our earlier study showed, for the first time, that the thrombin generation of AP patients was highly variable and the shapes of thrombograms were disturbed. Elevated free TFPI levels of SAP patients appear to inhibit exogenous TF in CAT measurements. The conclusion of the study was that failure of TF-initiated thrombin generation in the thrombogram assay can be explained by high levels of circulating free TFPI that may be associated with OF and mortality in AP. In the recent study, all of the patients had a severe form of AP and their TF-triggered thrombin generation was generally disturbed and shapes of the thrombograms were abnormal. We found no association between patients with “flat curves” (no detectable thrombin response) and mortality, nor did we observe any significant differences in levels of TFPI between patients with “flat curves” and the others. SAP itself had more powerful effect on thrombograms than APC, and in vivo standard dose of APC had only minor effects on thrombograms. A possible explanation for the differences between these findings and those of the previous study is that the samples of this study were collected later, when patients already had severe pancreatitis, not on admission to hospital.

To our knowledge, there are no previous clinical studies on the effects of APC on coagulation parameters in SAP. Dhainaut et al. investigated host coagulopathy responses of APC in sepsis patients (n=1690) in the PROWESS
study. They showed that APTT and PT were prolonged in the APC-treated sepsis patients compared with placebo-treated patients, and the sepsis patients D-dimer levels were decreased during the APC infusion time. Our study revealed a similar phenomenon; TT normalization was delayed and D-dimer stayed lowered in the APC group. Dhainaut’s study showed that the AT levels were significantly higher in the APC group than in the placebo group. In our patients, AT levels remained depressed.

It is difficult to know exactly which state of coagulation (hypo- or hypercoagulation) is favorable for SAP patients at different time-points. SAP patients may suffer from DIC and microthrombosis, and during this phase strengthening of anticoagulant mechanisms may be beneficial. On the other hand, the consumptive coagulopathy and increased fibrinolysis may lead to hemorrhagic complications, and these patients may then benefit from amplification of coagulation mechanisms. Increasing knowledge of coagulopathy in SAP helps clinicians to target anticoagulant treatment to the patients who need it.

### 3.6.3 CLINICAL AND FUTURE ASPECTS

The most important aspect in predicting the course of a potentially severe disease is in clinical work, i.e. the ability of the predictor to predict severity of the disease so early that clinicians have the possibility to influence the course of the disease by the treatment methods available. According to the revised Atlanta criteria, mild AP pertains to patients with MMS<2 on admission. In addition, this is the group (population of AP patients) to which prediction should be directed. Prediction is not important in the group that already has clinical complications of disease, i.e. OF. The patients with OF should be admitted to the intensive care unit, and intensive care is terminated if OF proves to be transient (does not matter if the correction of the clinical state is due to a milder course of AP or due to the treatment in ICU). We were the first to take this approach in predicting SAP, and our results showed that sCD73 is the kind of marker that can predict the severity of AP among patients with no signs of OF on admission (MMS<2). Our group has also investigated with the same principle the predictability of cytokines and nucleosomes in SAP. Future studies on predictors should have this approach so that clinicians can gain useful tools in clinical practice.

CD73 may have therapeutic potential in SAP. CD73 yields anti-inflammatory adenosine, which mediates anti-inflammatory effects such as the prevention of vascular permeability and the inhibition of leukocyte recruitment to the site of inflammation. These protective mechanisms are enhanced in the presence of hypoxia. INF-β has been shown to induce sCD73 and alleviate acute lung injury following ischemia-reperfusion in a mouse model. CD73/adenosine has been established to decrease vascular permeability in cultured human pulmonary endothelial cells. A recently
published preliminary clinical study showed that administration of INF-β upregulates human lung CD73 expression and is associated with reduction in mortality in patients with ARDS. In addition to vascular permeability, CD73 may modify epithelial permeability in the gut and improve the bowel barrier function. In patients with SAP, increased vascular permeability and tissue edema and hypoxia are universal phenomena. Impaired bowel barrier function leads to infection complications in SAP. At this stage, depressing systemic inflammation and improving barrier functions without increasing the risk of secondary infections are attractive goals. Our study showed that low levels of CD73 are associated with the development of vital organ dysfunction in SAP. The possibility that patients with SAP have a shortage of sCD73 and would therefore benefit from administration of exogenous sCD73 or induction of sCD73 by INF-β warrants further research.

Despite the failure of recombinant APC (drotrecogin alfa, Xigris®), the cytoprotective functions of APC give a reason to continue searching for new therapeutic applications. Recombinant APC has been shown to be beneficial in numerous animal models of diseases in addition to sepsis and AP, i.e. inflammatory bowel disease, motor-neuron degeneration, and ischemic stroke. Genetically altered animal models with the separated anticoagulant and cytoprotective activities of endogenous APC have been developed and their use has helped to identify APC’s dual activity. These models had a major role in clarifying APC’s cytoprotective effects. Anticoagulant function of APC is not required for the protective effects on endotoxemia or stroke. Several kinds of APC variants have been generated by targeted mutagenesis. These novel approaches to engineer APC eliminate either its anticoagulant or cell signaling activity. For example, a manipulation of the APC serine protease domain via the introduction of a new disulfide bridge inhibits anticoagulation activity of APC. APC variants that have limited anticoagulant function but normal cytoprotective activity represent a potentially safer alternative. These variants may enable increasing dosage of APC with reduced risk of serious bleeding complications. APC has a short half-life in the circulation (15-20 min) and may require a longer infusion time or repeated boluses for full efficacy, thus, another approach with new APC variants is extending plasma half-life of recombinant APC.

Despite early difficulties with therapeutic use of recombinant APC, it is not time to bury it. Protein engineering can provide second-generation APC mutants, which may have a safer and more effective impact, with a reduced bleeding risk.

Recent research has extended knowledge of pathogenic mechanisms involved in inflammation. An interesting aspect is related to elucidated pathways of cell death, especially the role of necroptosis in the inflammatory reaction. Traditionally, necrosis is considered to be the primary form of cell death caused by inflammation, but recent studies have demonstrated the existence of multiple pathways of regulated necrosis, i.e. necroptosis. Necroptosis is a pathway of regulated necrosis, a programmed form of
necrosis, that is mediated through a pathway dependent on receptor-interacting protein kinase 3 and mixed lineage kinase domain-like protein.\textsuperscript{347} Necroptosis has characteristics of both necrosis and apoptosis, i.e. necroptosis is actively regulated by multiple genes and accomplished through activations of a specific death-signaling pathway. Recent studies have confirmed the existence of acinar cell necroptosis in AP.\textsuperscript{348} The discovery of necroptosis may provide a potential target for regulating the inflammatory reactions in AP. In the future, it may be possible to switch the acinar cell necrosis to necroptosis, which may block the overactive systemic response leading to SIRS. An interesting future objective may be determining whether the early vigorous ICU treatment favors the evolution of necroptosis instead of necrosis, resulting in transient OF instead of persistent OF.

3.6.4 STRENGTHS AND LIMITATIONS OF THE STUDY

Some strengths and limitations of the Studies I-IV are noteworthy.

The approach of the sCD73 study has clear clinical relevance; the prediction of SAP is meaningful among patients who have no signs of OF on admission. The clinical APC study (Study II) was a pilot double-blinded trial and the concomitant treatment did not change during the study period. Patients not included in the study were registered according to the CONSORT guidelines.\textsuperscript{349} The patients in the placebo group received the best available standard treatment, thus avoiding potential bias caused by undertreatment of this group. The mortality in the placebo group was < 20%, which is within the range of the best available practice. The same standard protocol in administration of recombinant APC was used as in the PROWESS study, as assessed by Bernard et al. (24 μg/kg/h for 96 hours).\textsuperscript{29} Sub-studies of the clinical APC study (Studies III and IV) provide new information on the complicated pathophysiological inflammatory reaction and coagulopathy in patients with SAP.

The studies also have some limitations. The severity of AP was classified according to the original Atlanta classification (Studies II-IV), which was later revised. In addition, categorizing patients to the different severity groups involves some difficulties owing to the dynamic nature of AP. In the clinical APC study (Study II), the disease of some of the patients turned out to be milder than originally thought (thrombocytopenia resulted in classification into the SAP group, but no signs of OF manifested). The study groups were not totally comparable after randomization; patients in the APC group had higher plasma amylase levels. The study patients were not divided into subgroups according to the etiology; however, the etiology of AP was alcohol in all patients in the APC group, and in the placebo group the etiology was alcohol in all but one patient. Some of the patients had high SOFA scores on admission, and thus, it is possible that patients with SAP will present with different phases and severity of the disease at ICU admission (at the time of
randomization). In general, ICU patients are a very heterogeneous group (chronic illnesses, medications, etc.), and this should be taken into account when interpreting the results. It is also possible that the dose of APC was insufficient to have an effect on the excessive inflammatory reaction involved in SAP. In a pilot study such as Study II, it is safer to use the same dose as in previous sepsis trials. Sample size was determined according to SOFA change. This enabled us to find a difference in incidences of serious bleeding exceeding 17%; we were unable to exclude possible smaller differences in incidences of bleeding.

Because of the preliminary nature of Study I, the expected levels of sCD73 in different severity groups were unknown, and thus, proper power analyses and sample size calculations could not be performed.

The sample sizes for Studies III and IV were determined according to a primary endpoint (Study I) and not according to systemic inflammatory response or changes in coagulation markers between the groups. In addition, some follow-up samples were missing in Studies III and IV. Therefore, it is not possible to exclude type II error in these studies (Studies III and IV).
3.7 CONCLUSIONS

Based on these studies, the following conclusions may be drawn:

I. CD73 levels are increased in patients with AP and correlate negatively with the severity of AP. sCD73 activity predicts the development of SAP among patients with no signs of OF on admission (MMS<2).

II. APC treatment had no effect on evolution of OF among patients with SAP. We found no differences in ventilator-free days, renal replacement-free days, vasopressor-free days, or days alive outside the hospital over a 60-day period between the study groups. No serious incidents of bleedings occurred in SAP patients receiving APC.

III. APC treatment did not alter the course of systemic inflammation, as determined using soluble and cellular markers of inflammation.

IV. Patients with SAP receiving APC reached normal homeostasis of coagulation slower than patients receiving placebo.
This study was carried out at the Department of Gastrointestinal Surgery of the Abdominal Center at Helsinki University Hospital and University of Helsinki during 2007-2016.

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REFERENCES


47. Lowenfels AB, Lankisch PG, Maisonneuve P. What is the risk of biliary pancreatitis in patients with gallstones? Gastroenterology 2000


70. Gukovsky I, Pandol SJ, Mareninova OA, Shalbueva N, Jia W, Gukovskaya AS. Impaired autophagy and organellar dysfunction in pancreatitis. J


acknowledgments


141. Koppelman B, Neefjes JJ, de Vries JE, de Waal Malefyt R. Interleukin-10 down-regulates MHC class II alphabeta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. Immunity


179. Grey ST, Csizmadia V, Hancock WW. Differential effect of tumor necrosis factor-alpha on thrombomodulin gene expression by human


190. Fisher CJ, Jr, Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. Crit Care Med 2000 Sep;28(9


203. Lankisch PG, Burchard-Reckert S, Lehnick D. Underestimation of acute pancreatitis: Patients with only a small increase in amylase/lipase levels can also have or develop severe acute pancreatitis. Gut 1999 Apr;44(4):542-4.


224. Ranson JH, Rifkind KM, Roses DF, Fink SD, Eng K, Spencer FC. Prognostic signs and the role of operative management in acute


234. Lipinski M, Rydzewski A, Rydzewska G. Early changes in serum creatinine level and estimated glomerular filtration rate predict


acknowledgments


292. Guyatt GH, Sackett DL, Cook DJ. Users’ guides to the medical literature. II. how to use an article about therapy or prevention. B. what were the results and will they help me in caring for my patients? evidence-based medicine working group. Jama 1994 Jan 5;271(1):59-63.


325. Esmon CT. Thrombomodulin as a model of molecular mechanisms that modulate protease specificity and function at the vessel surface. Faseb j


2015 Aug 1;43(4):691-5.


