ACUTE PANCREATITIS: DIAGNOSIS AND ASSESSMENT OF SEVERITY WITH MARKERS OF INFLAMMATION

by

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Thesis

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Abbreviations

AP	acute pancreatitis
APACHE II	acute physiology and chronic health evaluation II score
ARDS	adult respiratory distress syndrome
AUC	area under the curve
CD	clusters of differentation
CE	contrast enhanced
CI	confidence interval
CRP	C-reactive protein
CT	computed tomography
CV	coefficient of variation
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ERCP	endoscopic retrograde cholangiopancreatography
FITC	fluorescein isothiocyanate
HLA	human leukocyte antigen
ICAM	intercellular adhesion molecule
IEMA	immunoenzymometric assay
IL	interleukin
IL-1Ra	interleukin-1 receptor antagonist
LPS	lipopolysaccharide
MHC	major histocompatibility complex
MO	monocyte
MODS	multiple organ dysfunction syndrome
NLR	negative likelihood ratio
NPV	negative predictive value
PAF	platelet activating factor
PCT	procalcitonin
PE	phycoerythrin
PLR	positive likelihood ratio
PMN	polymorphonuclear
PPV	positive predictive value
RFU	relative fluorescence units

sE-selectin	soluble E-selectin
sIL-2R	soluble interleukin-2 receptor
SIRS	systemic inflammatory response syndrome
TAP	trypsinogen activation peptide
TNF	tumour necrosis factor
URL	upper reference limit
US	ultrasonography

Introduction

Acute pancreatitis (AP) is a disease with wide clinical variation, which makes its diagnosis complex. Serum / urinary amylase measurement is a standard diagnostic method, although it was shown to be unable to recognize one fifth of AP patients (Clavien et al. 1989b). The severity of AP forms a continuum, and the average mortality rate approaches 2-10% (Mann et al. 1994, Banerjee et al. 1995, Grönroos et al. 1999). Most of the cases are mild and conservative treatment results in a rapid recovery in most of them. However, severe AP constitutes 15–20% of all cases (Steinberg and Tenner 1994, Barie 1996). In recent decades, mortality rate of severe AP has decreased from 30-80% to 15-20% (Banks 1999). Severe AP is now recognized to be a two-phase systemic disease. In the first phase, extensive pancreatic inflammation and/or necrosis are followed by a systemic inflammatory response syndrome (SIRS) that may lead to multiple organ dysfunction syndrome (MODS) within the first week. About 50% of deaths occur within the first week of the attack, mostly from MODS, which is not different from the systemic complications found in other diseases or injuries (e.g., sepsis, major trauma, burns) (Deitch 1992, Heath et al. 1995, Gullo and Berlot 1996, Nathens and Marshall 1996, Balakrishnan and Philip 1998, Neoptolemos et al. 1998, McKay et al. 1999). Unless the first phase is arrested and reversed by natural defences or therapeutic intervention, the second phase ensues usually after the second week of onset, and includes the formation of infected pancreatic necrosis or fluid collection with possible progression to overt sepsis, MODS and death (Am. College of Chest Physicians 1992, Deitch and Goodman 1999, Osman and Jensen 1999, Schmid et al. 1999). Beger and co-workers (1986) showed an overall contamination rate of pancreatic necrosis of 24% within the first week after the onset of AP in patients undergoing surgery for severe AP, increasing to 46% in the second and to 71% in the third week.

Organ failure is present only in half of the patients with pancreatic necrosis, and the extent of pancreatic necrosis does not influence the development of remote organ complications (Tenner et al. 1997, Lankisch et al. 2000). With an increasing number of failing organ systems involved in AP, the associated mortality rises (Knaus et al. 1985). The mortality figures associated with MODS vary between 30 and 100% (McFadden 1991, Marshall et al. 1995, Tenner et al. 1997, Neoptolemos et al. 1998, Erwin et al. 2000). The association between increasing age and death from AP is well documented (De Beaux et al. 1995, McKay et al. 1999). The clinical course of MODS usually begins with acute lung injury (Gullo 1996, Deitch and Goodman 1999). Respiratory failure is the most common type of organ failure in AP (Lankisch et al. 1983, Viedma et al. 1994, Lankisch et al.

1999b, Toh et al. 2000). In 719 AP patients, respiratory failure was present in 148 (21%) followed by renal failure in 44 (6%), cardiovascular failure in 28 (4%) and coagulopathy in seven (1%) patients (Heath et al. 1995).

There is an urgent need to improve the early recognition of patients with severe AP, especially those with subsequent organ failure, so that they can be sent at an early stage to a centre with facilities for maximal intensive care and specialists in endoscopic, radiological and surgical management of AP patients (Larvin 1996). Increasing knowledge of the inflammatory process in AP has led to new therapeutic strategies aiming at modifying SIRS (Osman and Jensen 1999). The importance of the proinflammatory and anti-inflammatory cytokine balance in determining the systemic manifestations and clinical outcome of AP has been emphasized (Simovic et al. 1999). It is necessary to diagnose the systemic inflammatory state of the patient. In an active proinflammatory state, anti-inflammatory therapy may be beneficial. On the other hand, if the patient already has an excessive anti-inflammatory response, immunosuppressive therapy may be harmful. Moreover, since new immunomodulatory therapies may have undesirable side effects, it is of utmost importance to accurately identify patients who will benefit from immunomodulation (Guice et al. 1991, Vuorte et al. 1999).

The purpose of the present investigation was to study the diagnosis and severity assessment of AP. In more detail, the first aim was to evaluate whether diagnosis of AP could be improved by using the rapid actim Pancreatitis test. Secondly, inflammatory variables were assessed in predicting severe AP with special reference to subsequent organ failure. In addition, the mechanisms of the inflammatory cascade and development of immunoparalysis were studied with new cellular markers in AP patients with organ failure.

Review of the literature

BACKGROUND

The first description of the pancreas has been attributed to Herophilus of Chalkaidon about 300 B.C. The naming of this organ, *pancreas* (Greek: *pan*, all; *kreas*, flesh), was not recorded until 400 years later by Rufus of Ephesus (100 A.D.) (Fitzgerald 1980). The earliest case reports of patients dying of suppurative inflammation or tumours of the pancreas were presented by S. Alberti (1578), J. Schenck (1600), and N. Tulp (1641) (Sachs 1993). The first classification system for AP was reported by Fitz in 1889 (Ermak and Grendell 1993). In 1901, Opie described the association of gallstones to AP (Opie 1901). Alcohol was firmly established as an important pathogenetic factor in 1917 (Symmers 1917). More than 100 years ago, Chiari (1896) proposed that intrapancreatic activation of zymogens leads to pancreatic autodigestion and is a key factor in the pathogenesis of AP. The association of hyperamylasaemia with AP has been recognized since 1929 (Elman et al. 1929). The first report of hereditary AP was from the Mayo Clinic by Comfort and Steinberg (1952). In the history of radiography, the pancreas was a hidden structure seen only indirectly through studies exploring the surrounding organs, such as barium examinations of the upper gastrointestinal tract. Sonography was the first method that permitted direct imaging of the pancreas (Thoeni and Blankenberg 1993). Pancreatic imaging essentially developed further with the introduction of computed tomography (CT) (Haaga et al. 1976, Stanley et al. 1977). The rationale for surgery in severe AP has evolved over the last 50 years. Initially, total pancreatectomy was often recommended but it resulted in very high mortality rates (Alexandre and Guerreri 1981). The current thinking is that the patients with infected pancreatic necrosis benefit from surgical debridement and drainage of the infected and devitalised tissue (Neoptolemos et al. 1998, Baron and Morgan 1999, Büchler et al. 2000). Further, surgery is often necessary if aggressive organ support in an intensive care unit seems inadequate for an AP patient with organ dysfunction.

EPIDEMIOLOGY OF ACUTE PANCREATITIS

AP is a common emergency presentation, being responsible for 3% of all hospital admissions with acute abdominal pain (Banerjee et al. 1994). The incidence rate of AP varies considerably in different countries. Low figures have been reported in England (10/100,000) (Corfield et al. 1985, Giggs et al. 1988) and Germany (15/100,000) (Assmus et al. 1996). In USA, AP affects around 40-80 per 100,000 of the general population (Lankisch 1999). In Finland, AP is a common disease, and its incidence has been increasing from 47 to 73 per 100,000 inhabitants/year in 1970-1989, and the

increase correlates with alcohol consumption (Jaakkola and Nordback 1993). However, its increased incidence may be partly due to improved diagnostic methods such as CT.

AETIOLOGY OF ACUTE PANCREATITIS

AP has many distinct aetiologies, though approximately 80% of all cases can be attributed to either gallstones or alcohol (Karne and Korelick 1999). The frequency of different forms of AP varies markedly in different countries. Gallstones are the most common cause in the United Kingdom (Leese et al. 1988) and Asia (Fan et al. 1993), whereas in USA (Steinberg and Tenner 1994) and Finland (Jaakkola and Nordback 1993) alcohol is the most common causative factor. The idiopathic group still comprises 10-30% of all cases. Increasing interest has been focused on biliary sludge, which has been reported to be present in 70% of AP patients with idiopathic AP (Lee et al. 1992). In addition, more than 85 drugs have been reported to cause AP (Steinberg and Tenner 1994). It has also been recognized that AP can rarely be autosomally dominantly hereditary caused by a mutation in the trypsinogen-1 gene that allows prematurely activated trypsinogen to cause acinar cell autodigestion (Whitcomb et al. 1996). About 10% of AP cases are associated with other miscellaneous aetiologies (Steinberg and Tenner 1994). However, although there are various kinds of inducing agents and events, response of the immune system appears to be identical regardless of the cause (Norman 1998).

PATHOGENESIS OF ACUTE PANCREATITIS

Primary events

The major function of pancreatic acinar cells is the synthesis and secretion of inactive digestive enzyme precursors (trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidases A and B and prophospholipase A2) into the duodenum (Dubick 1987, Clavien et al. 1989a). Zymogens are synthesized in the endoplasmic reticulum and then packaged into secretory granules. Following acinar cell stimulation, the contents of these granules are discharged by exocytosis into the acinar lumen and pass via the pancreatic ductal system into the duodenum, where the conversion of trypsinogen to trypsin is catalysed by enterokinase (Steer et al. 1984, Steer 1999). Trypsin is the key enzyme for rapid activation of all the proenzymes, including its own proenzyme, trypsinogen (Marshall 1993). There are two major isoenzymes of trypsinogen: trypsinogen-1 and trypsinogen-2. In healthy subjects, the ratio of trypsinogen-1 to trypsinogen-2 in pancreatic fluid is nearly fourfold (Durie et al. 1982). Trypsinogen is activated by proteolytic cleavage of a peptide called trypsinogen activation peptide (TAP) (Gudgeon et al. 1990, Neoptolemos et al. 2000). Owing to their potent proteolytic and lipolytic functions, the secretory enzymes represent a considerable degradative

(autodigestive) capacity. Compartmental intracellular transport, synthesis of secretory enzymes as inactive zymogens, and the presence of protease inhibitors intracellularly (pancreatic secretory trypsin inhibitor) and in blood (e.g. alpha-1-antitrypsin and alpha-2-macroglobulin) are major protective mechanisms (Ohlsson 1988, Glasbrenner and Adler 1993).

The pathogenesis of AP is only partially known. The initial phase involves triggering events, which are, for the most part, extrapancreatic in origin. Clinically, the most important of these appears to be either passage of a biliary tract stone or ingestion of ethanol. Although the clinical association of AP with biliary disease and with ethanol ingestion has been firmly established, mechanistic explanations for these associations have proven elusive (Steer 1998). In experimental AP, microscopic examination of pancreatic tissue obtained after common bile-pancreatic duct ligation indicates that the earliest signs of cell injury involve acinar cells (Lerch et al. 1992). The severity of experimental AP has been directly related to the duration of duct obstruction (Runzi et al. 1993). Trypsinogen activation, mediated by the lysosomal hydrolase cathepsin B within the acinar cells, appears to be an early as well as critical event that leads to cell injury (Greenbaum and Hirschkowitz 1961). The disruption of the acinar cell follows after premature activation of the proteases as a result of interaction between the digestive and lysosomal enzymes, and activated proteases then escape into the interstitium of the pancreas (Steer 1998). Once released into the pancreatic interstitium, retroperitoneum, peritoneal cavity, and circulation, these enzymes cause necrotizing injury through a variety of events, including local autodigestion by lipase and proteases (Warshaw 1993, Warshaw 1996).

Secondary events

Background

Pancreatic digestive enzymes explain only part of the pathogenesis of complicated AP. The release of various inflammatory mediators is another important mechanism (Giroir 1999, Osman and Jensen 1999). In fact, the pathophysiology of severe AP resembles other conditions with SIRS such as sepsis, multitrauma, ischaemia-reperfusion injury and burns, which do not involve the release of digestive enzymes from the pancreas (Nathens and Marshall 1996). A proinflammatory cytokine cascade follows acinar cell injury (Norman 1998). Localized inflammation is the body’s initial physiological protective response, which is generally tightly controlled at the site of injury. Loss of this local control results in an excessive uncontrolled activation of inflammatory cells and mediators, which is clinically identified as SIRS (Roumen et al. 1993, Davies and Hagen 1997). A frequent complication of SIRS is the development of organ system dysfunction, including acute

lung injury, shock, renal failure and MODS (Am. College of Chest Physicians 1992). The pathogenetic mechanisms leading from localized pancreatic necrosis and inflammation to SIRS with MODS are of particular importance, but these mechanisms are not completely understood.

The role of phagocyte activation

During the last few years it has been recognized that activation of monocytes / macrophages and polymorphonuclear (PMN) granulocytes is an early event during severe AP and plays an important role in the progression of the disease from a local inflammatory necrosis into SIRS (Gross et al. 1993, McKay et al. 1996b, Widdison and Cunningham 1996). Leukocyte activation leads to increased leukocyte aggregation and tissue infiltration within the microcirculation, where these leukocytes (PMNs and macrophages) increase their production of cytokines and other inflammatory mediators, including prostaglandins, leukotrienes, thromboxanes, platelet activating factor (PAF), free radicals, nitric oxide and proteases (cathepsin, elastase) (Davies and Hagen 1997). Factors released by activated leukocytes, therefore, reflect the severity of the disease (Gross et al. 1993, Widdison and Cunningham 1996, Ikei et al. 1998, Beger et al. 2000).

Monocytes have a typical bean-shaped nucleus and are produced in the bone marrow from precursor stem cells. Monocytes migrate into different tissues where they undergo transformation into tissue macrophages with morphological and functional properties that are characteristic of that tissue. In the liver they become Kupffer cells and in the lung, alveolar macrophages (Lasser 1983). Monocytes / macrophages carry out the fundamental protective functions of ingesting and killing invading micro-organisms. Macrophages play a central role in the immune response by presenting antigens to lymphocytes during the development of specific immunity. Bacterial endotoxin is a potent activator of mononuclear phagocytes, and it induces the secretion of cytokines involved in host defence and inflammation such as tumour necrosis factor-α (TNF-α), interleukin (IL) -1, IL-6 and IL-8 (Ziegler-Heitbrock 1989). Monocyte activation and increased proinflammatory cytokine secretion is associated with the development of systemic complications in AP, and occurs early during the course of the disease (Norman 1998). The association suggests that mononuclear phagocyte activation may play an important role in the pathophysiology of organ failure in these patients (McKay et al. 1996b).

Excessive stimulation of PMN-granulocytes plays a key role in aggravating AP and contributing to local destruction and systemic complications (Rinderknecht 1988). Cytokines, such as IL-8, function as chemoattractants and control the movement of PMNs through the extravascular space to

the inflammatory site. Once localized, the PMNs ingest and phagocyte particles such as bacteria and immune complexes (Lloyd and Oppenheim 1992). Upon activation, the ability of neutrophils to damage tissues in vivo rests on the formation of free radicals and degranulation of proteolytic enzymes. The most abundant neutral proteolytic enzyme of human neutrophils is elastase, which is a specific marker for neutrophil activation and its plasma concentration is raised early in AP (Gross et al. 1993, Widdison and Cunningham 1996, Ikei et al. 1998, Beger and Marshall 2000). Neutrophils mediate tissue-destructive events in a wide range of inflammatory diseases and are especially implicated in the pathogenesis of acute lung injury (Fujishima and Aikawa 1995), which is the most frequently occurring complication in severe AP (Formela et al. 1995, Ikei et al. 1998). The study of Acioli and co-workers (1997) has shown evidence for the participation of complement system activation products at an early stage in the priming of neutrophils and their subsequent entrapment in the lung vasculature during experimental AP. This may be the first step in the development of acute lung injury.

PMN has traditionally been thought to participate in the inflammatory response only as an effector cell. However, PMNs can synthesize and release both pro-inflammatory and anti-inflammatory cytokines. Their antagonists hence modulate both the cellular and humoral immunity during the evolution of the immune response (Lloyd and Oppenheim 1992, Fujishima and Aikawa 1995). It seems likely, however, that PMNs have only limited potential as antigen-presenting cells, since they synthesize major histocompatibility complex (MHC) class I but not class II molecules and, therefore, cannot initiate a cellular immune response by presentation of antigen associated with MHC class II to CD4+ T cells (Lloyd and Oppenheim 1992).

Leukocyte adhesion

Leukocytes, including neutrophils and monocytes, the major phagocytes in the bloodstream, circulate in a resting state characterized by low metabolic activity and inability to attach to a normally quiescent vascular endothelium. Leukocytic migration, margination, and adhesion are facilitated by the expression of specific adhesion molecules on both leukocytic and endothelial cell surfaces (Davies and Hagen 1997). Binding to adhesion molecules triggers leukocyte degranulation and generation of free oxygen radicals (Formela et al. 1995).

Leukocytic migration into the pancreas is an early and most probably a critical event in severe AP. An influx of leukocytes into the inflamed pancreas has been demonstrated by leukocyte scintigraphy in patients with severe AP (Schölmerich et al. 1991). Inflammatory cells infiltrating

the interstitial spaces during caerulein-induced AP include neutrophils, monocytes and/or macrophages (Van Laethem et al. 1995). Severe AP complicated by acute lung injury is associated with the sequestration of activated inflammatory cells in the pulmonary microvasculature (Lankisch et al. 1983).

Selectins

The role of leukocytes in the inflammatory process is complex and includes rolling along the endothelium, adherence to the endothelium, and transendothelial migration into the tissue. A large number of cell surface molecules that are involved in these cell/ endothelium adhesive interactions have been identified and characterized. The initial tethering to and subsequent rolling on the activated endothelium, both reversible events, are mediated by the selectin family of adhesion molecules (Bevilacqua and Nelson 1993). The selectin family is composed of three distinct carbohydrate receptors expressed by either endothelial cells (E-selectin), leucocytes (L-selectin) or platelets and endothelium (P-selectin) (Donnelly et al. 1994). The selectins and their receptors can exist in a cleaved, soluble form, indicating that they are cleaved or shed from the cell surface by proteases and are measurable in the circulation (Bevilacqua and Nelson 1993, Gearing and Newman 1993). Soluble E-selectin (sE-selectin) is an activation marker of the vascular endothelium and the plasma concentrations are increased in patients with SIRS and especially in organ dysfunction and failure (Cowley et al. 1994). sE-selectin levels are also increased in patients with severe AP (Inagaki 1997). L-selectin is found on the surface of most circulating human neutrophils, monocytes, and lymphocytes, and initiates the interaction of these leukocytes to activated endothelium (Van Eeden et al. 1995). The measurement of L-selectin expression is difficult, as the expression on cell surface increases and subsequently decreases during leukocyte activation. Therefore, reduced presence of L-selectin on the leukocyte surface may reflect previous leukocyte activation. It is likely that different investigators “catch” the leukocytes at different stages of activation (Bevilacqua and Nelson 1993, Asimakopoulos and Taylor 1998, Maekawa et al. 1998). The profile of leukocyte L-selectin expression is further affected by the release of L-selectin-rich neutrophils from bone marrow (Van Eeden et al. 1995). However, an inverse relationship between soluble L-selectin and subsequent progression to severe lung injury has been reported (Donnelly et al. 1994).

Integrins

After leukocyte activation and rolling on the endothelium by means of selectin expression, the integrin family regulates the subsequent firm adhesion to activated endothelium. Integrins regulate

also leukocyte migration into tissues, their degranulation and phagocytosis. Integrins comprise the largest group of adhesion receptors and are found on most cell types, including leukocytes. The integrins are noncovalent heterodimers composed of a family of three α subunits (CD11a, b, and c). The β2 leukocyte integrins share a common β chain (CD18). CD11b and CD18 combine upon activation, and this complex binds to members of the intercellular adhesion molecules (ICAM) family. Peripheral blood lymphocytes express primarily CD11a/CD18 whereas neutrophils, monocytes and natural killer cells express all three β2 integrins. Intracellular storage pools of CD11b/CD18 and CD11c/CD18 are present in neutrophils and monocytes whereas there is no storage pool of CD11a/CD18 (Arnaout 1990). Upon phagocyte activation, additional CD11b/CD18 molecules are rapidly mobilised from intracellular storage granules and expressed on the cell surface. CD11b expression is a marker of neutrophil activation and likely vascular transmigration, and increases by multiple stimuli, including bacterial products, cytokines, chemotactic peptides, and lipid mediators (Carlos and Harlan 1994). The role of adhesion molecules including measurement of CD11b expression, as determined by flow cytometry (Repo et al. 1993, Repo et al. 1995), has been extensively studied during the SIRS related to cardiopulmonary bypass surgery (Cremer et al. 1996) and is found to be increased (Asimakopoulos and Taylor 1998, Ilton et al. 1999). Increased expression has also been demonstrated in disorders such as sepsis, burn injuries and multitrauma (Lin et al. 1993, Maekawa et al. 1998, Repo and Harlan 1999, Takala et al. 1999). Importantly, increased CD11b/CD18 expression may predict development of organ failure as has been shown in patients with cirrhosis of the liver and in septic patients (Rosenbloom et al. 1995, Takala et al. 1999). On the contrary, neutrophils from patients with obstructive jaundice have shown decreased CD11b expression and an impaired response to stimulation with bacterial products, which may lead to high incidence of septic complications in these patients (Plusa et al. 1996).

Upregulation of the adhesion molecule complex CD11b/CD18 has been demonstrated in the pancreatic and lung tissues of rabbits with necrotizing AP. Upregulation of the adhesion molecule in the lungs was associated with marked neutrophil infiltration, oedema formation and vascular thrombosis, i.e., morphological changes of acute lung injury

(Osman et al. 1999). Additionally, ICAM-1, the endothelial ligand for the integrin CD11b/CD18, has been shown to be elevated in the serum of patients with severe AP (Osman and Jensen 1999) and in the pancreas and lungs in rats with necrotizing AP (Werner et al. 1999).

Cytokines

Considering proinflammatory cytokines, SIRS can be categorized in three stages. Stage 1 is a production of cytokines in response to an injury or infection at the local site of inflammation. Stage 2 is the protective release of a small amount of cytokines into circulation. Stage 3 is the failure of homeostasis with the massive systemic reaction where cytokines turn destructive rather than protective (Bone 1996, Balakrishnan and Philip 1998).

Cytokines are low molecular weight secreted proteins, usually 15-25 kD. They are secreted by many different cell types; the major site of synthesis, however, appears to be cells of the macrophage and monocyte series. All cytokines exert their action by binding to specific cell-surface receptors (Lowry et al. 1993). In an animal model of AP, it has been shown that TNF-α, IL-1, and IL-6 are actually produced also within the pancreatic parenchyma. This is known to occur within an hour of the onset of AP induction and often prior to appreciable changes in pancreatic histology (Norman et al. 1994). Most cytokines are not stored as preformed molecules; hence their production requires new gene transcription and translation (Lowry et al. 1993). In experimental AP it has been demonstrated that TNF-α and IL-1 genes are expressed in the pancreas after induction of AP, resulting in large amounts of intrapancreatic IL-1 and TNF-α proteins (Demols et al. 2000).

IL-1 and TNF-α are primary inducers of IL-6 and IL-8 production and are both produced systemically during AP and not just within the pancreas. Regardless of the animal model used, IL-1 and TNF-α are produced in the spleen, lung, and liver with pancreatic production always preceding that in distant sites by hours or even days, depending on the rapidity of AP development (Norman 1998). In addition to the ability of cytokines to recruit leukocytes to the sites of inflammation, the proinflammatory cytokines also induce expression of cell adhesion molecules, both locally and systemically, increase capillary permeability, promote leukocyte adhesion and extravasation, lead to liver acute-phase protein secretion and, thus, play an important role in the systemic manifestations of AP and associated distant organ dysfunction (Kusske 1996, Norman 1998, Osman and Jensen 1999).

TNF-α may be one of the chief mediators of inflammation in AP, and the serum concentrations of TNF-α have been shown to correlate to disease severity (Exley et al. 1992, Bone 1996) and systemic complications (McKay et al. 1996a). However, TNF-α is often undetectable in the sera of patients with AP, even in those with severe disease (Paajanen et al. 1995, De Beaux and Fearon 1996, Brivet et al. 1999). This may be due to the short serum half-life of TNF-α. TNF-α is also broken down by neutrophil elastase (Van Kessel et al. 1991), which is elevated in patients with severe AP (Dominguez-Munoz 1993). In septic patients soluble TNF-α receptors may be found in high concentrations in the plasma (Opal and DePalo 2000). Moreover, a stepwise increase in soluble TNF-α receptors with increasing severity of AP has been detected suggesting that the degree of TNF-α-induced inflammation correlates with disease severity (De Beaux and Fearon 1996, Kaufman et al. 1997). During experimental AP it has been shown that knockout mice lacking receptors for IL-1 or TNF-α have significantly improved survival compared with wild-type animals (Denham et al. 1997).

In the cytokine cascade, IL-1 stimulates IL-2, which is produced by T-lymphocytes and is principally known as a cytokine responsible for promoting T-cell growth (Lowry et al. 1993). IL-2 acts by binding to its receptor (IL-2R) expressed mainly on CD4-positive T helper lymphocytes and is considered a marker of T-cell activation (Curley et al. 1993, Salomone et al. 1996). High concentrations of sIL-2R have been shown to predict organ failure in patients with septic shock (Takala et al. 1999). Moreover, the level of sIL-2R has been shown to be elevated in patients with severe AP (Pezzilli et al. 1994, Osman and Jensen 1999).

IL-6 is the principal cytokine mediator of the acute-phase response (Castell et al. 1989, Gross et al. 1993, Schölmerich 1996) and is released from monocytes, macrophages and endothelial cells (Biffl et al. 1996, Norman 1998). IL-6 is often used as a measure for systemic activation of proinflammatory cytokines (Opal and DePalo 2000). Prolonged and excessive elevations of circulating IL-6 levels in patients after trauma, burns, and elective surgery have been associated with complications and mortality (Roumen et al. 1993, Biffl et al. 1996, Cremer et al. 1996). Serum levels of IL-6 have also been shown to reflect the severity of an attack of AP, with elevated levels occurring 24-36 h earlier than those of C-reactive protein (CRP) (Viedma et al. 1992, Heath et al. 1993, Inagaki et al. 1997, Ikei et al. 1998, Osman and Jensen 1999).

IL-8 is secreted not only by mononuclear phagocytes, but also by other cells, particularly endothelial cells (Osman et al. 1998). IL-8 is thought to be the principal secondary mediator of TNF-α-induced neutrophil activation (Lowry 1993, McKay et al. 1996b). IL-8 is a chemotactic agent for neutrophils and is believed to play a significant role in the development of organ dysfunction and especially sepsis-associated acute lung injury (Gross et al. 1993). Indeed, it has been shown that IL-8 is increased in the serum and bronchoalveolar lavage fluid in patients with sepsis and severe AP complicated by ARDS (Osman and Jensen 1999). In severe AP, circulating levels of IL-8 appear to closely parallel IL-6 production (Formela et al. 1995, McKay et al. 1996a, Norman 1998).

IL-10, the most important anti-inflammatory cytokine is produced by T cells, B cells, monocytes, and macrophages (Opal and DePalo 2000). It down-regulates the production of a number of proinflammatory cytokines such as IL-1, TNF-α, IL-6 and IL-8, thereby representing a normal endogenous feedback factor of the immune responses and inflammation (Osman and Jensen 1999). In addition, IL-10 is able to decrease human leukocyte antigen (HLA) -DR expression on monocytes. IL-10 gene expression correlates with the fall in monocyte HLA-DR antigen expression in patients undergoing major abdominal surgery and may account for the immunosuppression associated with surgical injury (Klava et al. 1997). The study of Van der Poll and co-workers (1997) documented that plasma IL-10 remained high in non-surviving septic patients, while in survivors it significantly decreased during the follow-up. IL-10 has been identified as a mediator that may ameliorate the physiologic consequences of severe AP (Brivet et al. 1999, Chen et al. 1999).

IL-11 is rarely measurable in circulation but has been found to be physiologically active in localized areas of inflammation, such as inflammatory arthritis associated with inflammatory bowel disease (Opal and DePalo 2000). Additionally, IL-11 concentrations have been shown to reflect the severity of AP (Chen et al. 1999). IL-13 is produced by activated T cells but not by activated monocytes. Like IL-10, IL-13 strongly inhibits the secretion of proinflammatory cytokines by monocytes (Van der Poll et al. 1997, Opal and DePalo 2000).

Procalcitonin

Procalcitonin (PCT) is a polypeptide consisting of 116 amino acids and a molecular size of 13 kDa. It is the precursor protein of the hormone calcitonin (Oczenski et al. 1998). The half-life of PCT in the human body is 25-30 hours (Oberhoffer et al. 1999). No definitive role is known for PCT before its proteolytic conversion to calcitonin (Boucher 2000). The exact source of PCT is not known.

However, thyroidectomized patients unable to produce calcitonin were shown to possess a calcitonin-like immunoreactivity, thus giving indirect evidence of an extrathyroidal production (Assicot et al. 1993). Recently, human liver slices stimulated by TNF-α and IL-6 were detected to produce PCT (Nijsten et al. 2000). Elucidation of the biological role of PCT should contribute to our general understanding of the acute phase reaction (Braithwaite 2000). In a recent experimental model of sublethal sepsis, PCT appeared to exacerbate mortality after a septic insult, while PCT antiserum attenuated this effect (Nylen et al. 1998).

Earlier, serum PCT was reported to increase in patients with severe bacterial infections and to correlate with the severity of the infection (Assicot et al. 1993, Oczenski et al. 1998, Oberhoffer et al. 1999). It has also been proposed that following cardiac surgery, PCT appears to be useful in discriminating systemic infections from acute phase response or local problems, with simultaneous measurement of CRP increasing the specificity (Rothenburger et al. 1999). Recently, there has been increasing evidence that PCT is not only specific for bacterial infection but also an appropriate marker of early development of severe non-infectious SIRS. Raised PCT levels have been reported in other conditions associated with an inflammatory response including trauma, major surgery, cardiac surgery and heat stroke (Mimoz et al. 1998, Vincent 2000). Both bacterial pneumonia and non-bacterial pulmonary inflammation caused by inhalational burn injury are associated with a rapid increase in plasma PCT (Nylén et al. 1996). In trauma patients, the concentration of circulating PCT correlates with the extent of tissue injury and hypovolaemia (Mimoz et al. 1998). Elevated PCT levels have also been reported to predict severe non-infectious SIRS and pulmonary dysfunction secondary to cardiopulmonary bypass (Hensel et al. 1998).

Brunkhorst and co-workers (1998) reported that PCT allowed early discrimination between biliary and non-biliary AP, but in subsequent other studies no correlation between PCT concentration and the aetiology of AP has been found (Rau et al. 1997; Müller et al. 2000). In patients with mild post-ERCP AP, no significant PCT elevation could be observed (Oezcueruemez-Porsch et al. 1998). Rau and co-workers (1997) demonstrated that the degree of PCT elevation reflected the systemic severity of infection in terms of associated organ failure in patients with AP. Müller and co-workers (2000) showed PCT to be a valuable variable for differentiating between oedematous and necrotising AP within the first 24 hours of the onset of symptoms, but unlike in the study of Rau and co-workers (1997), PCT was of no value in the early prediction of infected pancreatic necrosis.

CD14

The Gram-negative bacterial wall consists of inner and outer membranes, the latter of which contains many proteins as well as lipopolysaccharide (LPS) and is chemically unique for each bacterial strain. Endotoxin denotes an impure extract of LPS and various proteins. Endotoxaemia occurs in many patients with AP (Exley et al. 1992). The release is episodic and endotoxin is rapidly cleared from circulation (De Beaux and Fearon 1996). CD14 is a receptor for LPS and is expressed on macrophages, monocytes, and neutrophils (Wright 1999). LPS released from gram-negative bacteria binds to protein circulating in plasma (Schumann et al. 1990), which has been found to reflect the severity of AP (Erwin et al. 2000). The LPS-lipopolysaccharide binding protein complex subsequently binds to the CD14 receptor, triggering cell activation (Wright et al. 1990, Carlos and Harlan 1994, Davies and Hagen 1997). The causative agents in Gram-positive sepsis are cell-wall components of Gram-positive bacteria, such as peptidoglycan and lipoteichoic acid. Like LPS, these bacterial components stimulate excessive release of proinflammatory cytokines (Schwandner et al. 1999). Polymorphism in the CD14 receptor causes a genetically determined variation in the reaction of monocytes / macrophages to infectious stimuli (Hubacek et al. 1999). CD14 modulates the sensitivity of macrophages to LPS, but it does not transmit a signal across the plasma membrane because it lacks an intracellular signalling domain. Instead, a glycosyl phosphatidylinositol linkage tethers it to the cell surface. Therefore, there is another receptor mediating the signal to the macrophage to secrete cytokines. Five such proteins have been identified in humans and are referred to as toll-like receptors (Eubanks et al. 1998, Modlin et al. 1999, Schwandner et al. 1999).

The gastrointestinal tract is a reservoir of pathogens that may enter the circulation by migrating across the gut mucosal barrier initiating a septic cascade and MODS. Intestinal perfusion is decreased early in haemorrhagic AP (Juvonen et al. 1999). Gut hypoperfusion may result in increased intestinal permeability. The transmigration of living bacteria or their endotoxins from the gut lumen into the mesenteric lymph nodes, spleen, peritoneal cavity, and blood, is called bacterial translocation. The translocation of viable bacteria or their endotoxins has been shown to occur in a number of conditions, including haemorrhagic shock, burns, malnutrition, sepsis, jaundice, and AP (McFadden 1991, Runkel et al. 1991, Schmid et al. 1999).

However, approximately 50% of patients with multiple organ failure do not have an identifiable focus of infection. Therefore, bacteraemia and endotoxaemia cannot be universal mediators of the syndrome, and other explanations for the pathogenesis of multiple organ failure have been sought.

One proposed explanation is that distant organ injury and failure may be caused by the unfettered synthesis of cytokines by activated macrophages (Deitch and Goodman 1999). Moreover, studies on endotoxin resistant mice (i.e., lacking the CD14 molecule) show that the progression of AP, including the production of inflammatory cytokines and early death of the disease, are independent of endotoxin action (Eubanks et al. 1998).

Immunosuppression

Background

Surgery and traumatic injury often result in a dysregulated hyperinflammatory response syndrome that may progress to immunosuppression and early MODS (Biffl et al. 1996). The results of Chen and co-workers (1999) and Brivet and co-workers (1999) support the hypothesis that, in the early stage of severe AP, activation of various proinflammatory and anti-inflammatory cytokines plays an important role in the pathogenesis of the disease. It has also been demonstrated that the inability to mount an appropriate cytokine (TNF-α or IL-6) response portends a poor prognosis in patients with intra-abdominal sepsis (Hamilton et al. 1992). Thus, immunologic events are believed to be involved in the pathogenesis of AP and they can fluctuate during the course of the disease from a hypersensitive state to immunoparalysis (Curley 1996). In an animal model of thermal injury, a relationship between immunosuppression and susceptibility to sepsis has been shown (Moss et al. 1988). Similarly, in AP an increased number of infections has been observed in a later stage of the disease presumably as a result, at least partially, of impaired cellular immunity (Ditschkowski et al. 1999, Beger et al. 2000). A decrease in delayed-type skin hypersensitivity reflecting altered cellular immune function is common in patients with burn injury, blunt trauma and after major gastrointestinal surgery, and the decrease correlates with septic morbidity and mortality (Wakefield et al. 1993). Garcia-Sabrido and co-workers (1989) were the first to report a correlation between poor outcome and anergy to delayed-type hypersensitivity testing in AP patients.

Monocytes

The initial event in the generation of the specific immune response is the uptake and degradation of foreign antigens by macrophages. The important role of the monocyte / macrophage system in the course of polytrauma and sepsis has been shown in a variety of studies (Volk et al. 1996, Döcke et al. 1997, Polk et al. 1997). In immunosupression, these monocytes are characterized by a markedly reduced HLA-DR expression, and a profound reduction of their ability to produce LPS-induced TNF-α in vitro (Hershman et al. 1989, Döcke et al. 1997). Monocyte HLA-DR expression is of fundamental importance in antigen processing and presentation leading to effective recruitment of a

specific immune response. Helper T-lymphocytes require macrophage surface expression of HLA-DR contiguously in order to initiate a response and to proliferate (Tonegawa 1988). The ultimate response is T cell activation and subsequent antibody production by B cells and enhanced phagocytosis of opsonized bacteria. HLA-DR bearing monocytes therefore play a central role in the generation of the immune cascade (Hershman et al. 1990).

The decrease in HLA-DR expression represents a marker of suppressed immune competence in patients with septic shock and severe thermal injury (Lin et al. 1993). Further, HLA-DR expression on monocytes is suppressed in traumatized patients with subsequent sepsis (Ditschkowski et al. 1999) and the suppression correlates with the severity of trauma and sepsis as well as with the clinical outcome (Hershman et al. 1990, Lin et al. 1993). The pattern of recovery of HLA-DR on monocytes may also predict development of septic complications after trauma (Hershman et al. 1990, Schinkel et al. 1998) and infectious complications after elective gastrointestinal surgery (Wakefield et al. 1993). The percentage of peripheral blood monocytes that express HLA-DR antigen appears to be an accurate marker of the infection associated with immunosuppression (Cheadle et al. 1991, Sachse et al. 1999). In severe AP, immunoparalysis evidenced by depression of HLA-DR expression of monocytes, and associated with a persistently high CRP level has been reported to reliably predict a fatal outcome (Richter et al. 1996, Richter et al. 1999).

Although monocyte HLA-DR antigen expression correlates clinically with postoperative immunosuppression, the mechanism causing the down-regulation is unclear. There is circumstantial evidence for the involvement of at least IL-10 in the process of monocytic deactivation in sepsis (Randow et al. 1995). Sachse and co-workers (1999) demonstrated that in patients with severe burns individual HLA-DR expression and IL-10 were negatively correlated. In vitro, interferon-gamma, IL-4 and granulocyte/macrophage colony-stimulating factor increase monocyte HLA-DR expression, while IL-10, transforming growth factor-beta and prostaglandin-E2 play inhibiting roles (Richter et al. 1999).

Lymphocytes

Along with changes in the monocyte / macrophage system, a decreased level of total T lymphocytes and especially the proportion of T helper lymphocytes may depress immune function (Hamilton et al. 1992, Curley et al. 1993, Curley 1996). The study of Curley and co-workers (1993) describes immunological abnormalities in AP patients similar to those found in patients with thermal, surgical or traumatic injury. In AP, the concentrations of CD4+ T helper cells correlate with circulating

levels of IL-6 and endotoxin as well as disease severity. However, during the first days of severe AP, a decreased lymphocyte count (Curley et al. 1993, Pezzilli et al. 1995) and a strong inverse correlation between the levels of CRP and the proportion of T-helper cells indicating a defective immune response has been reported (Curley et al. 1993). Furthermore, in animals with AP, a concomitant reduction in CD4+ T cells with a decreased IL-2 production has been reported (Curley et al. 1996). Similar changes have been observed in humans after thermal and blunt traumatic injury (Wood et al. 1984, Moss et al. 1988, Ertel et al. 1990). In contrast, studies show that CD4+ or CD8+ T cells depleted mice have a significant reduction in the severity of AP, which clearly suggests a pivotal role of T lymphocytes in this disease (Demols et al. 2000).

DIAGNOSIS OF ACUTE PANCREATITIS

Clinical presentation

The diagnosis of AP is problematic while there are no specific clinical signs. Patients with AP may suffer from a multitude of symptoms, including upper abdominal pain, meteorism, abdominal resistance, fever, nausea and vomiting, ileus and jaundice (Steinberg and Tenner 1994). None of these frequent symptoms are related to the severity of the disease. Rare clinical findings, such as ecchymosis of the flank (Grey Turner sign) or periumbilical area (Cullen sign), which occur in 1-3% of patients, also fail to effectively predict the severity of AP (Büchler 1991). Within the first days of admission patients with severe AP may develop SIRS characterized by a combination of fever, tachycardia, and tachypnoea (Bone 1996).

Laboratory diagnostics

Amylase and lipase

Traditionally, biochemical diagnosis of AP is based on the determination of serum and/or urinary amylase activity (Elman et al. 1929), the activity of which increases in serum within 2-12 hours of the onset and returns to normal within 3-5 days (Tietz et al. 1993). However, 19% of the AP patients have a normal amylase value (Clavien et al. 1989b). Furthermore, it is well known that hyperamylasaemia occurs in many extrapancreatic diseases such as acute cholecystitis, small bowel obstruction and peptic ulcers resulting in low specificity (Clavien et al. 1989a). Pancreatic lipase is synthesized, similarly with amylase, in the exocrine acinar cells and catalyses the hydrolysis of triglyserides into diglyserides and fatty acids (Clavien et al. 1989a). Wide variation in sensitivity and specificity has been reported for serum lipase determination in the diagnosis of AP, which may partly be due to different assay methods (Patt et al. 1966, Lott et al. 1986, Thomson et al. 1987, Wong 1993, Keim et al. 1998). Because serum lipase remains elevated longer than serum amylase,

it has been suggested that it may be useful when there is a delay between the onset of symptoms and admission (Kolars et al. 1984, Flamion et al. 1987, Clavien et al. 1989a, Tietz et al. 1993).

Trypsinogen

Trypsin is the main protease in human pancreatic fluid. It is secreted by the exocrine cells of the pancreas as a proenzyme, trypsinogen, which is activated in the intestine by enterokinase. When active trypsin reaches circulation, it is inactivated by the major trypsin inhibitors in serum, alpha2-macroglobulin and alpha1-antitrypsin (Ohlsson 1988). The two major isoenzymes of trypsinogen are (cationic) trypsinogen-1 and (anionic) trypsinogen-2. In AP, the serum concentrations of trypsinogen-2 are more strongly increased than those of trypsinogen-1. Both the urine and serum trypsinogen-2 measurements and also measurement of the complex trypsin-2-alpha-1-antitrypsin have been shown to be useful markers for AP (Itkonen et al. 1990, Hedström et al. 1994, Hedström et al. 1996c,d). Recently, a rapid urinary trypsinogen-2 test strip has proven to accurately identify AP patients in a retrospective (Hedström et al. 1996b) and a prospective (Kemppainen et al. 1997) study. It, therefore, appears to be suitable as a screening test for AP.

Other methods

Several assays have been developed to improve the biochemical diagnosis of AP. Serum elastase stays elevated for up to one week after the onset of AP and may be useful in cases with delayed admission (Flamion et al. 1987, Clavien et al. 1989a), but the test is not routinely used. Other serum markers such as ribonuclease, chymotrypsin, phospholipase A2 and pancreatic isoamylase have been evaluated, but their use is infrequent because of practical reasons such as long assay times or limited diagnostic advantages over amylase (Flamion et al. 1987, Clavien et al. 1989b, Kazmierczak et al. 1991, Clavé et al. 1995, Chase et al. 1996, Sternby et al. 1996, Millson et al. 1998).

Radiology

Ultrasonography (US) is often utilized for diagnosis of patients with acute abdominal pain. Overlying abdominal gas often limits the ability to image the entire pancreatic gland completely. In AP patients US is important in the evaluation of the gallbladder and the biliary tract to detect possible gallstones and biliary obstruction. US is also useful for follow-up evaluation of a known fluid collection or pseudocyst. Contrast enhanced (CE)-CT has become the standard imaging method in diagnosing and staging AP and its complications (Kivisaari et al. 1983, Balthazar et al. 1990). The diagnostic accuracy of CE-CT findings has proved high, reaching a specificity approaching 100% (Clavien et al. 1988). The use of CT for primary diagnostics is impossible due to

limited availability and high costs (London et al. 1989, Lucarotti et al. 1993). Furthermore, CT may be normal in 8-28 % of patients with AP, especially in mild forms of the disease (Clavien et al. 1988, Balthazar 1989, Thoeni and Blankenberg 1993, Balthazar et al. 1994).

SEVERITY ASSESSMENT OF ACUTE PANCREATITIS

Background

Early identification of potentially severe AP is of utmost importance. AP patients with delayed transfer to intensive care have higher mortality to those admitted directly, and mortality even increases when transfer is delayed (de Beaux et al. 1995). There is evidence for benefits of early intensive monitoring and support, enteral feeding, prophylactic antiobiotics and emergency endoscopic sphincterotomy in patients with biliary aetiology in severe AP (Neoptolemos 1988, Fan et al. 1993, Sainio et al. 1995, Barie 1996, Nowak et al. 1998, Brivet et al. 1999, Kanwar and Windsor 1999). One of the main problems with AP has been the lack of accurate predictors of disease severity and the development of organ failure in the early stages of the disease. On admission, clinical assessment of severity has been shown to be unreliable (Larvin and McMahon 1989, Büchler 1991, Angood 1999) and the severity of AP is independent of the level of serum amylase and lipase (Clavien et al. 1989b, Kazmierczak et al. 1991, Lankisch 1999b). CE-CT has improved the assessment of the disease severity by accurately identifying areas of necrosis (Kivisaari et al. 1983, Bradley et al. 1989, London et al. 1991, Vesentini et al. 1993). Most investigators define severe AP as a pancreatic necrosis of at least 30% of the gland (Balthazar et al. 1990). It has been reported that necrosis of the head of the pancreas is as dangerous as when the entire pancreas is involved (Kemppainen et al. 1996). However, organ failure occurs in only half of the patients with pancreatic necrosis (Tenner et al. 1997). Magnetic resonance imaging is increasingly used for assessing the severity of AP with quite promising results (Saifuddin et al. 1993, Piironen et al. 1997, Ward 1997).

Atlanta classification

Presently, the classification of AP is based on the internationally recognized Atlanta criteria (Bradley 1993). According to the Atlanta classification, mild AP is associated with minimal organ dysfunction and an uneventful recovery, while AP is classified as severe if systemic and/or local complications are present. Local complications including necrosis, abscess or pseudocyst formation are usually diagnosed by CE-CT. Severe AP can be further characterized by three or more Ranson criteria at the onset of the attack, or eight or more of the Acute Physiology and Chronic Health Evaluation II Score (APACHE II) points any time during the course of the disease (Bradley 1993).

Scoring systems

There are several clinicobiochemical scoring systems for the assessment of the severity of AP (Roumen et al. 1992). The Ranson scoring system (Table 1) comprises 11 biochemical criteria, which require up to 48 hours for complete data collection (Ranson et al. 1974). According to a recent meta-analysis, Ranson`s signs showed poor predictive power (De Bernandinis et al. 1999). The APACHE II illness grading system (Table 2) is more accurate and can be used throughout the patient's hospitalization (Wilson et al. 1990, Brisinda et al. 1999). Due to their complexity, however, the clinicobiochemical systems are seldom used routinely in clinical practice (Toh et al. 2000).

Table 1. Ranson criteria (Ranson et al. 1974)

On admission:

Age > 55 years
Blood glucose > 10 mmol/l
WCC > 16 10^9
LDH > 350 IU/l
AST > 250 IU/l

Within 48 hours:

HCT decrease > 10%
Serum Urea increase > 0.7 mmol/l
Serum Ca^{2+} < 2 mmol/l
Fluid sequestered > 6 litres
PaO2 < 8 kPa
Base deficit > 4 mmol/l

WCC, white cell count; LDH, lactate dehydrogenase;
AST, aspartate transaminase

Table 2. The APACHE II severity of disease classification system (Wilson et al. 1990)

	High abnormal range					Low abnormal range			
Physiological variable	4	3	2	1	0	1	2	3	4
Temperature, rectal (°C)	≥41	39-40.9		38.5-38.9	36-38.4	34-35.9	32-33.9	30-31.9	≤29.9
Mean arterial pressure (mmHg)	≥160	130-159	110-129		70-109		50-69		≤49
Heart rate	≥180	140-179	110-139		70-109		55-69	40-54	≤39
Respiratory rate	≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygenation									
a) FiO2>0.5A-aDO2 (kPa)	≥66.7	46.7-66.6	26.7-46.6		<26.6				
A-aDO2=(FiO2x95)-PaCO2-PaO2									
b) FiO2<0.5 PaO2 (kPa)					>9.3	8.1-9.3		7.3-8.0	<7.3
Arterial pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	<7.15
Serum sodium (mmol/l)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
Serum potassium (mmol/l)	≥7	6.0-6.9		5.5-5.9	3.5-5.4	3.0-3.4	2.5-2.9		<2.5
Serum creatinine (μmol/l)	≥300	169-299	124-168		53-123		≤52		
Haematocrit (%)	≥60		50-59.9	46-49.9	30-45.9		20-29.9	<20	
White blood count (E9/l)	≥40		20-39.9	15-19.9	3-14.9		1.0-2.9	<1	
Glasgow Coma Score (GCS):									
Score = 15 – actual GCS									

APACHE II score is given by the sum of the acute physiology score and the age points. Age points are assigned: age ≤44, zero; 45-54, 2 points; 55-64, 3 points; 65-74, 5 points; and ≥75, 6 points.
FiO2, fraction of inspired oxygen; A-aDO2, alveolar-arterial oxygen difference; PaO2, arterial partial pressure of oxygen

Laboratory tests

Much effort has been directed to developing a single, simple and reliable laboratory test for the severity assessment of an attack of AP. There are laboratory methods based on the determination of pancreatic enzymes in serum and urine, which measure the intrinsic biological severity by estimating the degree of the primary pancreatic proteolytic insult. The concentration of trypsinogen-2 shows a marked correlation with the severity of the disease (Hedström et al. 1996d). Other possibly useful biochemical indicators of severity in AP include the antiproteases: trypsin-2-alpha-1-antitrypsin levels rise during the attack in both mild and complicated attacks, with levels significantly higher in complicated attacks (Hedström et al. 1996c), whereas alpha2-macroglobulin levels are significantly lower in complicated attacks suggesting its consumption (McMahon et al. 1984, Wilson et al. 1989, Dominguez-Munoz et al. 1993, Viedma et al. 1994). Additionally, the complexed alpha2-macroglobulin level has been shown to increase in severe AP (Banks et al. 1991). TAP, which is a peptide released from trypsinogen when it is activated to trypsin, is released from the pancreas during severe attacks of AP (Formela et al. 1995). Urinary TAP has been shown to be useful for the assessment of the severity of AP 24 hours after the onset of symptoms (Neoptolemos et al. 2000).

Another group of prognostic laboratory tests indicates the level of the inflammatory response in AP, which appears to be identical regardless of the cause (Norman 1998). The most commonly used

single marker of severity is CRP, which is a measure of the hepatic acute phase response (Wilson et al. 1989). There is, however, a 48-72-hour delay until peak levels are attained after the insult, limiting its early prognostic role, but in the follow-up of the course of the disease CRP is useful (Puolakkainen et al. 1987, Clavien et al. 1989a, Neoptolemos et al. 2000). A new inflammation parameter, PCT, has been reported to detect bacterial contamination of pancreatic necrosis in the later course of the disease (Rau et al. 1997).

Phospholipase A2 is the rate-limiting enzyme in the formation of prostaglandins, and high serum concentrations have been observed in patients with severe AP (Puolakkainen et al. 1987, Viedma et al. 1994, Formela et al. 1995). Group II phospholipase A2 has been found to reflect the ongoing systemic inflammation in severe AP-associated SIRS (Grönroos and Nevalainen 1992, Hietaranta et al. 1999, Nevalainen et al. 1999). However, at present the assay is not commercially available.

Granulocyte activation is an early pathogenetic factor in AP. It is related to the development of local and systemic complications, particularly acute lung injury (Fujishima and Aikawa 1995), the most common type of organ failure in AP (Toh et al. 2000). The measurement of polymorphonuclear elastase in serum as a marker for neutrophil activation has been used successfully to assess the severity of AP (Dominguez-Munoz et al. 1991, Viedma et al. 1994, Schölmerich 1996, Widdison and Cunningham 1996, Ikei et al. 1998), but it is not used in clinical routine at present.

Proinflammatory cytokines (eg. TNF-α, IL-1 and IL-6) are believed to play an important role in the pathogenesis of severe AP and its systemic complications. In experimental AP it has been shown that there is a rapid release of proinflammatory cytokines from the injured pancreas into serum. The serum levels of proinflammatory cytokines correlate with the severity of the disease also in clinical AP (Norman et al. 1994, Norman 1998). Especially IL-6, which is one of the most important cytokine mediators of the acute-phase response and the elevation of which precedes CRP increase, has been suggested as a marker for predicting the severity of AP (Inagaki et al. 1997, Ikei et al. 1998). IL-8, as a chemotactic agent for neutrophils (Lowry 1993), is believed to contribute to the development of organ dysfunction and especially acute lung injury (Osman and Jensen 1999) and circulating levels of IL-8 appear to closely parallel IL-6 production in AP (Norman 1998). There is also an early anti-inflammatory cytokine response in patients with severe AP (Brivet et al. 1999). IL-10 has emerged as a major anti-inflammatory cytokine (Opal and DePalo 2000). IL-1Ra, IL-10 and IL-11 concentrations have been shown to reflect the severity of AP, and especially IL-10 is

considered a useful marker for early prediction of the prognosis of AP (Chen et al. 1999, Hynninen et al. 1999). In addition, IL-2, which is a product of T lymphocytes, is recognized as essential to normal immunologic function. Serum sIL-2R level has been shown to be elevated in patients with severe AP (Pezzilli et al. 1994, Osman and Jensen 1999).

In summary, present diagnosis of AP is based on a typical clinical presentation and measurements of serum amylase or lipase. These methods are, however, not accurate enough in detecting AP. CE-CT is useful in identifying the presence of pancreatic necrosis but cannot be performed for all cases due to limited availability and increased costs. Clinicobiochemical scoring systems are too complex for routine use. The ideal prognostic laboratory test for AP should be an accurate marker for rapid, reproducible and cheap measurement. CRP is the most commonly used laboratory test in the assessment of the severity of AP, but its sensitivity is unacceptably low in the early course of the disease. Of the more recent tests, especially those for cytokines are expensive and/or laborious to perform. Therefore, it seems obvious that no single test is ideal and accurate enough, and a combination of tests may be needed to predict severe AP and its systemic complications (Windsor 2000).

NEW TREATMENT STRATEGIES

Background

Most patients with mild AP recover without intervention and novel treatment strategies should focus on patients with severe attacks. In the absence of specific therapy for AP, efforts have been directed towards rapid identification of patients who develop a severe form of the disease with early organ failure, in the hope that earlier intervention with appropriate resuscitation in specialized hospital units may improve prognosis (Neoptolemos et al. 1998, Giroir 1999). A striking discovery in the study of Brivet and co-workers (1999) was that delayed admission to intensive care unit resulted in a four-fold excess in mortality. Treatment of AP is still largely supportive and mainly targeted to aggressively prevent systemic complications of the disease by intensive care. There is increasing clinical and experimental evidence indicating that enteral nutrition is physiologically superior to parenteral nutrition (Deitch 1992). In severe cases, early endoscopic retrograde cholangiography (ERC) and sphincterotomy applied for biliary AP, especially with coexisting cholangitis, significantly decrease complication and mortality rates (Nowak et al. 1998). Interest has also been focused on the prophylactic administration of antibiotics in severe AP, and antibiotics have proven to be useful (Pederzoli et al. 1993; Sainio et al. 1995). Surgery is indicated for infected pancreatic necrosis, when percutaneous aspiration is not applicable or has failed, but the

management of sterile pancreatic necrosis is mostly conservative but still controversial (Beger et al. 1986, Neoptolemos et al. 1998). From the clinical point of view, surgery is often undertaken if the patient's status deteriorates despite maximal conservative treatment, although it is generally accepted that surgical treatment of severe AP should be postponed for as long as possible (Schmid et al. 1999).

It has been demonstrated that there is no strict correlation between either necrosis of the pancreas or the extent of necrosis and organ failure in AP. Thus, it could be beneficial to consider AP patients with local and systemic complications separately in new therapy studies (Tenner et al. 1997, Osman and Jensen 1999, Lankisch et al. 2000). Because the mortality in MODS is high, new therapeutic agents have been developed to target various phases of the inflammatory continuum of the SIRS/MODS cascade (Rangel-Frausto et al. 1995). Since the lungs is the main target in these syndromes, extensive research has been undertaken to treat ARDS (Fujishima and Aikawa 1995) and to prevent the progression to multiple organ failure with a significant rise in mortality (Heath et al. 1995).

Immunomodulation

As evidence accumulates in support of the role of excessive leukocyte activation and inflammatory cytokines in the pathogenesis of systemic complications in severe AP, new therapeutic strategies attempting to prevent the activity of these mediators or to block their synthesis are now being evaluated as therapeutic options. Interrupting the disease during the very initial phase by cytokine modulation improves the outcome of animals with experimental AP. Thus, there is a theoretical basis for beneficial effects of cytokine modulation to improve the outcome also in humans (Beger et al. 2000). Generally, immunomodulators of AP can be grouped in three main categories: 1) specific anticytokine antibodies; 2) anti-inflammatory cytokines, and 3) non-specific immunomodulators (Osman and Jensen 1999).

In a rat model of AP, in contrast to the expected findings, anti-TNF antibody was found to be harmful (Guice et al. 1991). Grewal and co-workers (1994), however, later showed that prophylactic treatment with anti-TNF antibodies decreased the severity of AP. Blockage of the cytokine cascade at the level of the IL-1 receptor with a specific antagonist (IL-1Ra) decreases the intrinsic pancreatic damage in experimental AP (Norman et al. 1995, Norman 1998). However, conflicting results have been obtained when inhibiting IL-1 and TNF-α in trials in septic patients, which suggest that the timing of the administration of inhibitory drugs may be crucial (Döcke et al.

1997, Grau and Maennel 1997). The ability of a platelet-activating factor (PAF) antagonist to suppress some aspects of the inflammatory response and to reduce organ dysfunction in human AP has been studied (Kingsnorth et al. 1995, McKay et al. 1997, Johnson et al. 2001), so far no final results have been obtained.

IL-10 decreases the severity and increases animal survival in different experimental models of AP (Van Laethem et al. 1995, Demols et al. 2000). An IL-10-agonist and anti-interleukin-8 antibody were shown to reduce cytokine response, CD11b- and CD18 positive cells in the lung and acute lung injury in experimental AP (Osman et al. 1998a,b). In a rat model of AP the blocking of neutrophil-endothelial cell-cell adhesion with CD18 antibody decreased pulmonary inflammatory changes and improved survival (Inoue et al. 1995). Moreover, the treatment of necrotizing AP with monoclonal antibodies against ICAM-1 decreased both local pancreatic injury and systemic lung injury in rats (Werner et al. 1999).

The balance between proinflammatory and anti-inflammatory cytokines seems to be crucial in AP (Simovic et al. 1999). Early in the clinical course of AP, when SIRS is predominant, agents that inhibit the release and/or action of proinflammatory mediators could be beneficial (Norman 1998). However, if immunosuppression is predominant later in the course of AP, novel immune stimulants or antiantagonist therapies may be needed to overcome the impaired immune function (Kusske et al. 1996, Davies and Hagen 1997). This points to the role of interferon-gamma (Hershman et al. 1989), which has been used as a therapeutic tool for immunomodulation in anergic states of trauma and sepsis (Döcke et al. 1997). In the future, rapid assessment of the state of the immune response and regulation of this response in severe AP may very probably be one of the main goals of pancreatologists.

Deeper understanding of the cell biology and physiology of AP permit the design of effective interventions concerning the inflammatory response process and suppression of its adverse effects (Beger et al. 2000). Improved monitoring of the time course of individual cytokine signalling during AP is urgently needed. Future treatment strategies will most probably focus on a multimodal combination therapy aimed at specifically suppressing excessive activation of the inflammatory response while preserving immune competence and antimicrobial defence (Deitch and Goodman 1999). Further, it is of utmost importance to accurately identify those patients who benefit from immunomodulator therapy, since the therapies may have unsuspected side effects (Guice et al. 1991, Vuorte et al. 1999).

Present investigation

AIMS OF THE STUDY

The specific targets were as follows:

1,2) To assess the value of actim Pancreatitis test for screening AP and to compare the accuracy of the test strip with that of serum lipase determination for the detection of AP in patients with acute abdominal pain.

3) To evaluate the presence of PCT, sIL-2R and sE-selectin in the plasma of patients with AP and to determine whether these plasma values predict severe AP and, in particular, the development of organ failure.

4) To assess the ability of a rapid semiquantitative test strip (PCT®-Q test) to predict the development of severe AP.

5) To investigate which changes in the cellular immune system take place during the course of an attack of AP, with special reference to the monocyte / macrophage system, and whether there is a correlation between the severity of AP and the observed immunological changes. Further, we evaluated the relationship of these variables to the development of organ failure, and studied the kinetics of immunoparalysis in AP.

MATERIALS AND METHODS

Patients

Patients with not more than 72 hours of acute abdominal symptoms and admitted to the emergency unit at Helsinki University Central Hospital (I-V) and Helsinki City Hospital (I) were included in the current study (Table 3).

Table 3. Patients

Study	Studied patients	AP	Mild AP	Severe AP
I	525	45	36	9
II	237	29	23	6
III	57	57	30	27
IV	162	162	124	38
V	89	89	58	31

AP, acute pancreatitis

I) Prospectively collected 525 patients with acute abdominal pain between December 1997 and April 1998, including 45 AP patients (mild AP 36, severe 9).

II) A total of 237 prospective, consecutive patients with acute abdominal pain between December 1997 and April 1998, consisting of 29 patients with AP (mild AP 23, severe 6).

III) The prospective study population consisted of 57 AP patients between August 1997 and October 1998. There were two groups of consecutive patients: 30 patients with mild AP (SEV0) and 27 patients with severe AP. Of the latter, 11 did not develop organ failure (SEV1), while the remaining 16 patients developed acute respiratory failure and nine of them also renal failure (SEV2).

IV) The prospective study included 162 consecutive AP patients (mild AP 124, severe 38) between August 1997 and October 1999.

V) Between August 1998 and August 1999, 89 consecutive AP patients (mild AP 58 = grade I, severe 31) were included in the prospective study. Of the patients with severe AP, 19 patients recovered without organ failure (grade II group), while the remaining 12 patients developed respiratory failure and/or renal failure (grade III group).

The control material consisted of 30 asymptomatic healthy subjects (43 samples) recruited from the laboratory staff (V).

Diagnostic criteria for acute pancreatitis

AP was diagnosed if there were clinical findings consistent with AP and a raised serum amylase activity at least three times above the upper reference limit (URL) (and/or with urinary amylase activity above 6,000 units/l in study I). With lower amylase levels the diagnosis was confirmed with

CE-CT (and/or with US in study I). Exclusion of AP in patients with acute abdominal pain was based on clinical, radiographic, endoscopic and surgical findings.

Severity assessment of acute pancreatitis

Pancreatic necrosis was verified by low (<30 Hounsfield units) enhancement of the pancreatic tissue after the injection of contrast media on CT or at operation or autopsy. The severity of AP was categorized by the classification of the Atlanta Symposium 1992 (Bradley 1993). In Atlanta classification, mild AP is associated with minimal organ dysfunction and an uneventful recovery, while it is classified as severe if systemic and/or local complications are present (Bradley 1993).

Scoring systems

In AP patients, appropriate laboratory and physiological data were recorded on days one and two to permit calculation of the APACHE II (Table 2) (Wilson et al. 1990). Data was collected on admission and within the first 48 hours to calculate the Ranson criteria (Table 1) (Ranson et al. 1974). The MODS score provides a means to grade the intensity of dysfunction of six organ systems: the respiratory system (PO2/FIO2 ratio), the renal system (serum creatinine concentration), the hepatic system (serum bilirubin concentration), the cardiovascular system (pressure-adjusted heart rate), the haematologic system (platelet count) and the central nervous system (Glasgow Coma Scale) (Table 4) (Marshall et al. 1995).

Table 4. The Multiple Organ Dysfunction Score (MODS) (Marshall et al. 1995)

	SCORE				
Organ system	0	1	2	3	4
Respiratory					
PaO2/FiO2, mmHg	>300	226-300	151-225	76-150	≤75
(kPa)	(>40)	(30.1-40)	(20.1-30)	(10.1-20)	(≤10)
Renal					
Serum creatinine, μmol/l	≤100	101-200	201-350	351-500	>500
Hepatic					
Serum bilirubin, μmol/l	≤20	21-60	61-120	121-240	>240
Cardiovascular					
PAR, l/min	≤10	10.1-15	15.1-20	20.1-30	>30
Hematological					
Platelet count, x 10^9/l	>120	81-120	51-80	21-50	≤20
Neurological					
Glasgow Coma Scale score	15	13-14	10-12	7-9	<6

PaO2, partial oxygen pressure in arterial blood; FiO2, fraction of inspired oxygen;
PAR, pressure adjusted heart rate = heart rate multiplied by the ratio of central venous pressure
To the mean arterial blood pressure

Criteria for organ failure

In studies III-V, organ failure was defined as acute respiratory failure necessitating intubation and mechanical ventilation, and/or acute renal failure, defined as need for haemodialysis. The criteria for initiating mechanical ventilation were tachypnoea (respiratory rate of > 35 /minute) and/or the need of inspiratory oxygen fraction (FiO_2) > 0.6 in order to maintain PaO_2 > 60 mmHg. The haemodialysis was started in patients with significant reduction of renal function indicated by increased concentrations of serum creatinine (>300 µmol/l) and serum urea (>40 mmol/l) and progressive metabolic acidosis in serial measurements (pH less than 7.28) with or without anuria or oliguria (urine output less than 500 ml/24 h). For each patient, the MODS score value was determined to quantify the severity of organ failure (III,V).

Samples

I,II) On admission to the hospital, the urinary samples were immediately tested with the actim Pancreatitis test strip. Serum amylase values were determined quantitatively. For quantitative measurement of trypsinogen-2 concentrations, the urinary samples were stored at -20^{o}C until analysis. In the study II, serum samples for the determination of lipase activity were taken on admission and stored at -70^{o}C until analysed.

III) The blood samples were collected on admission to hospital (T0), and at 12 h (T12) and 24 h (T24) after admission. The samples were stored at -70^{o}C until the quantitative measurements of CRP, PCT, sIL-2R and sE-selectin.

IV) The blood samples for the rapid test of PCT were collected on admission to hospital and 24 hours after admission. The EDTA anticoagulated plasma was separated by centrifugation and stored at -70^{o}C until analysis.

V) The blood samples for the estimation of phagocyte surface markers were taken on admission to hospital and 12, 24, 36, and 48 hours after admission. Each tube was immediately placed in an ice-cold water and after that kept at 0^{o}C until staining of leukocytes, which was performed within 24 hours.

Laboratory tests

The actim Pancreatitis test strip (Medix Biochemica, Kauniainen, Finland) is based on the immunochromatography. The test is carried out by dipping the tip of the test strip into urine.

Trypsinogen-2 in the sample is bound to a monoclonal antibody-labelled blue latex particles, which migrate across a nitrocellulose membrane with a catching zone containing an antibody specific for an epitope on trypsinogen-2. A trypsinogen-2 concentration of more than 50 μg/l results in a detectable blue line developing in this zone. The test is considered positive if the blue line is seen within five minutes. A control line indicates proper functioning of the strip. (I,II)

The quantitative measurement of urine trypsinogen-2 was carried out by an immunoenzymometric assay (IEMA) (inhouse assay, Medix Biochemica, Kauniainen, Finland; reference range 0.3-11 μg/l)(I,II). Amylase activity in serum and urine was measured enzymatically using α-Amylase EPS no 1360221 reagents (Boehringer Mannheim, Marburg, Germany; reference value <300IU/l). Serum lipase activity was determined by a turbidimetric assay based on degradation of a triolein emulsion (Boehringer Mannheim, Marburg, Germany; reference value <200IU/l)(II). PCT in plasma was measured with an immunoluminometric assay (LUMItest PCT, BRAHMS Diagnostica, Berlin, Germany; reference value < 0.5 ng/ml) (III). Plasma concentration of sIL-2R in EDTA was determined by the Immulite automated immunoassay analyzer (DPC, Los Angeles, CA; sensitivity 10 U/l)(III). Analysis of plasma concentration of sE-selectin was performed with enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems, Vienna, Austria; sensitivity 0.5 ng/ml)(III). CRP (upper reference range 10 mg/l) was determined by a laser nephelometric technique on a daily clinical routine basis with reagents from Orion Diagnostica (Espoo, Finland). The detection limit was 2 μg/ml. (III,IV)

The PCT®-Q test (B.R.A.H.M.S Diagnostica, Berlin, Germany) is performed by pipetting 200 μl of the patient sample (plasma/serum) onto the test strip at room temperature and reading the result after 30 minutes. The PCT®-Q test uses a monoclonal mouse anti-catacalcin antibody conjugated with colloidal gold (tracer) and a polyclonal sheep anti-calcitonin antibody (solid phase). When the patient sample (serum or plasma) is applied to the test strip, the tracer binds to the PCT in the sample, forming a labelled antigen-antibody complex. On passing the test band region, the labelled antigen-antibody complex binds to the anti-calcitonin antibody immobilized on the solid phase and forms a sandwich complex. At PCT concentrations of > 0.5 ng/ml, this sandwich complex can be seen as a reddish band. The colour intensity of the band is directly proportional to the PCT concentration in the sample. The semi-quantitative measurement range of the PCT®-Q test is correlated to the three reference concentrations (0.5 ng/ml, 2 ng/ml, 10 ng/ml). Unbound tracer

diffuses into the control band zone, where it is bound by the anti-tracer antibody and produces an intensely coloured red control band, which is used to check the correct functioning of the test. (IV)

For measuring phagocyte surface markers, 25µl aliquots of the whole blood were double-labelled by the addition of saturating amounts of the monoclonal antibodies on the basis of the leukocyte count. The cells were washed by the addition of 1:10 diluted ice-cold FACS lysing solution (Becton Dickinson) and collected by centrifugation at 4^{o}C. To lyse all red cells, the procedure was repeated using the FACS lysing solution at room temperature. The leukocytes were resuspended in 300µl of ice-cold saline supplemented with formaldehyde (final concentration 0.5%), and the samples were kept at 0^{o}C until analyses by the flow cytometer. (V)

A FACSort flow cytometer (Becton Dickinson) and CellQuest software were used for the acquisition and analysis of the data. Appropriate data sets of $5x10^3$ neutrophils identified on the basis of their light-scattering properties and/or 10^3 CD14-positive events with light scattering properties compatible to that of monocytes were acquired for each specimen. CD11b (marker of phagocyte activation), CD14, and CD62L (L-selectin) fluorescence histograms were appropriately developed. The results are presented in relative fluorescence units (RFU), i.e., as the geometric mean of the channel number. To determine the proportion of HLA-DR-positive monocytes, firstly, the IgG_{2a}-PE histogram of CD14-FITC positive monocytes was developed, and a marker was set to exclude 95-97% of the events, i.e., to indicate the upper limit of the non-specific fluorescence intensity of monocytes. Then, the respective HLA-DR-PE histogram was created and the proportion of positively fluorescing monocytes was recorded.

Statistics

The results are expressed as median values with range (I-V). Comparison of continuous data was performed by the Kruskal-Wallis analysis of variance and the Mann-Whitney U test, as appropriate (I-V). In study I, agreement between quantitative trypsinogen-2 concentration and the test strip result was evaluated with the kappa statistic. For assessing correlations, Spearman's rank correlation and 95 % confidence interval (CI) were used (III,V). Probability values <0.05 were considered significant. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were counted in studies I-IV and, in addition, likelihood ratios in study IV. In study V, the area under the curve (AUC) values for the serial measurements of cellular markers were determined. In the comparison of AUC values between different severity groups, Guzick`s test for trend was used, and p was adjusted using Hommel`s modification of the Bonferroni method

(Hommel 1988). To evaluate the day-to-day variation of a given marker during the follow-up period, the coefficient of variation (CV%) was determined.

RESULTS

Actim Pancreatitis test in screening for acute pancreatitis (I)

Of the 45 patients with the diagnosis of AP, 43 showed a positive actim Pancreatitis test strip result, giving a sensitivity of 96%. Thirty-seven false positive actim Pancreatitis test strip results were obtained in 480 patients with non-pancreatic abdominal pain, resulting in 92% specificity (Figure 1). The PPV of the test strip was 54% and NPV 99.6%. Nine patients with severe AP were all detected by the dipstick. The median concentration of urinary trypsinogen-2 in patients with AP was 1030 µg/l (range 11.5-48,800 µg/l) and 1.5 µg/l (range 0-4,500 µg/l) in those with abdominal pain from causes other than AP. The results of the quantitative assay were closely correlated with the results of the dipstick test (kappa = 0.86).

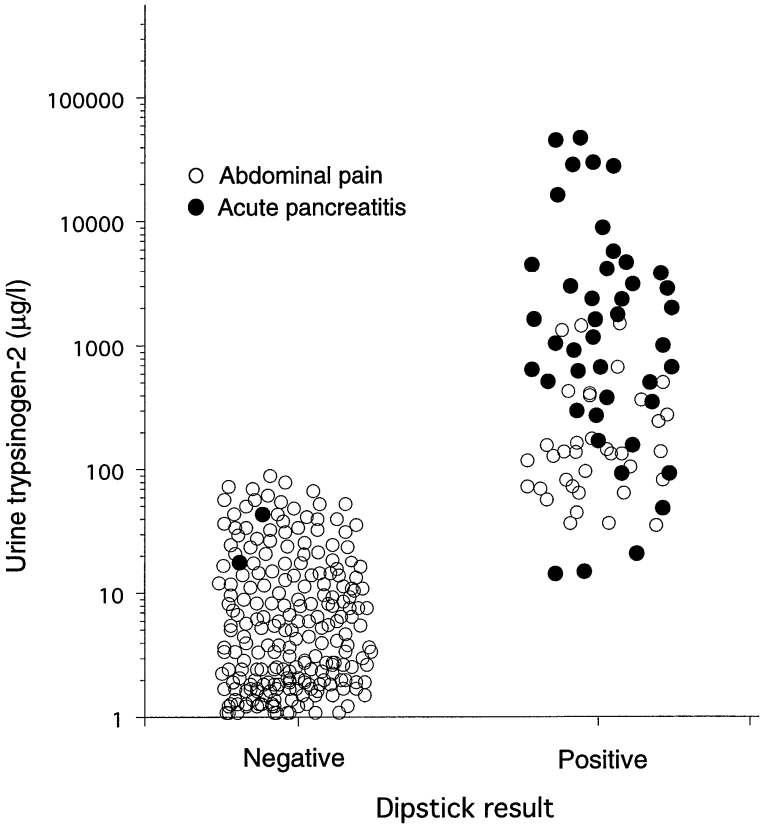

Figure 1. Results of the urinary trypsinogen-2 dipstick test in relation to quantitative urinary trypsinogen-2 concentration in 45 patients with acute pancreatitis and 480 patients with abdominal pain from other causes.

Comparison of actim Pancreatitis test strip with serum lipase in the diagnosis of acute pancreatitis (II)

The actim Pancreatitis test strip result was positive in 27 of 29 patients with AP giving a sensitivity of 93% (Table 5). The specificity was 92% due to 16 false positive results. The PPV and NPV of the actim Pancreatitis test strip were 63% and 99%, respectively. The dipstick detected all six patients with severe AP. The sensitivity, specificity and predictive values of serum lipase with two cut-off values are presented in Table 5. With the cut-off of 600 IU/l, the specificity of serum lipase to detect a patient with AP was very high, 99%, but the sensitivity was low, 55%. The serum lipase values did not correlate with the severity of AP and with the cut-off of 600 IU/l, two patients with severe AP would have remained undetected. A combination of the actim Pancreatitis test strip and serum lipase with a cut-off value of 600 IU/l improved the specificity of the dipstick alone from 92% to 99.5%, and PPV from 63% to 94% (Table 5). However, due to the poor sensitivity of serum lipase, the sensitivity of the combination (55%) was also poor.

Table 5. Diagnostic accuracy of urinary trypsinogen-2 dipstick test and serum lipase activity for detection of acute pancreatitis in 237 patients with acute abdominal pain

		Acute Pancreatitis (N = 29)		Other abdominal disorders (N = 208)			
Test	Cutoff value	Positive test (N)	Sensitivity %	Positive test (N)	Specificity %	PPV %	NPV %
Urinary trypsinogen-2 dipstick test (actim)	~ 50 µg/l	27	93	16	92	63	99
Serum lipase	200 IU/l	23	79	24	88	49	97
Serum lipase	600 IU/l	16	55	3	99	84	94
Urinary trypsinogen-2 dipstick test AND serum lipase	~ 50 µg/l 600 IU/l	16	55	1	99.5	94	94

Reference value of serum lipase activity is < 200 IU/l

PPV = positive predictive value; NPV = negative predictive value

Procalcitonin, soluble interleukin-2 receptor and soluble E-selectin in predicting the severity of acute pancreatitis (III)

Admission values of PCT were higher in patients with severe AP than in those with mild AP (Figure 2). At T12 and T24, PCT values were higher in the SEV2 group than in the SEV1 group (T12: median 2.2 ng/ml, range 0.2-86.6 vs 0.4 ng/ml, 0.3-2.8; $p=0.05$), and both were higher than in the SEV0 group (T12: 0.3 ng/ml, 0.1-1.7; $p<0.001$ and $p<0.01$, respectively). Among SEV2 patients, there was a significant inverse correlation between peak PCT concentration and the time

elapsed between admission and the development of organ failure (Study III, Figure 2). The concentrations of PCT did not vary according to the aetiology of AP (data not shown). sIL-2R levels were higher in patients with severe AP than in patients with mild AP at T12 and T24 (Figure 2). sE-selectin values did not correlate with the development of organ failure in AP (data not shown). At T12, the CRP level of SEV2 group (210 mg/l, 5-378) was significantly higher than that of SEV0 group (53 mg/l, 5-243; $p<0.01$)(Figure 2). At T24, the CRP levels of SEV2 group and SEV1 group were both higher than the respective level of SEV0 ($p<0.001$ and $p<0.05$, respectively).

The sensitivities and specificities of the PCT, sIL-2R, CRP and APACHE II score values in predicting organ failure in patients with AP are shown in Table 3 of Study III. At T24, the PCT test had a sensitivity of 94% and specificity of 73% for the development of organ failure. The NPV and PPV for PCT were, at each time point, higher than the respective values for APACHE II and CRP.

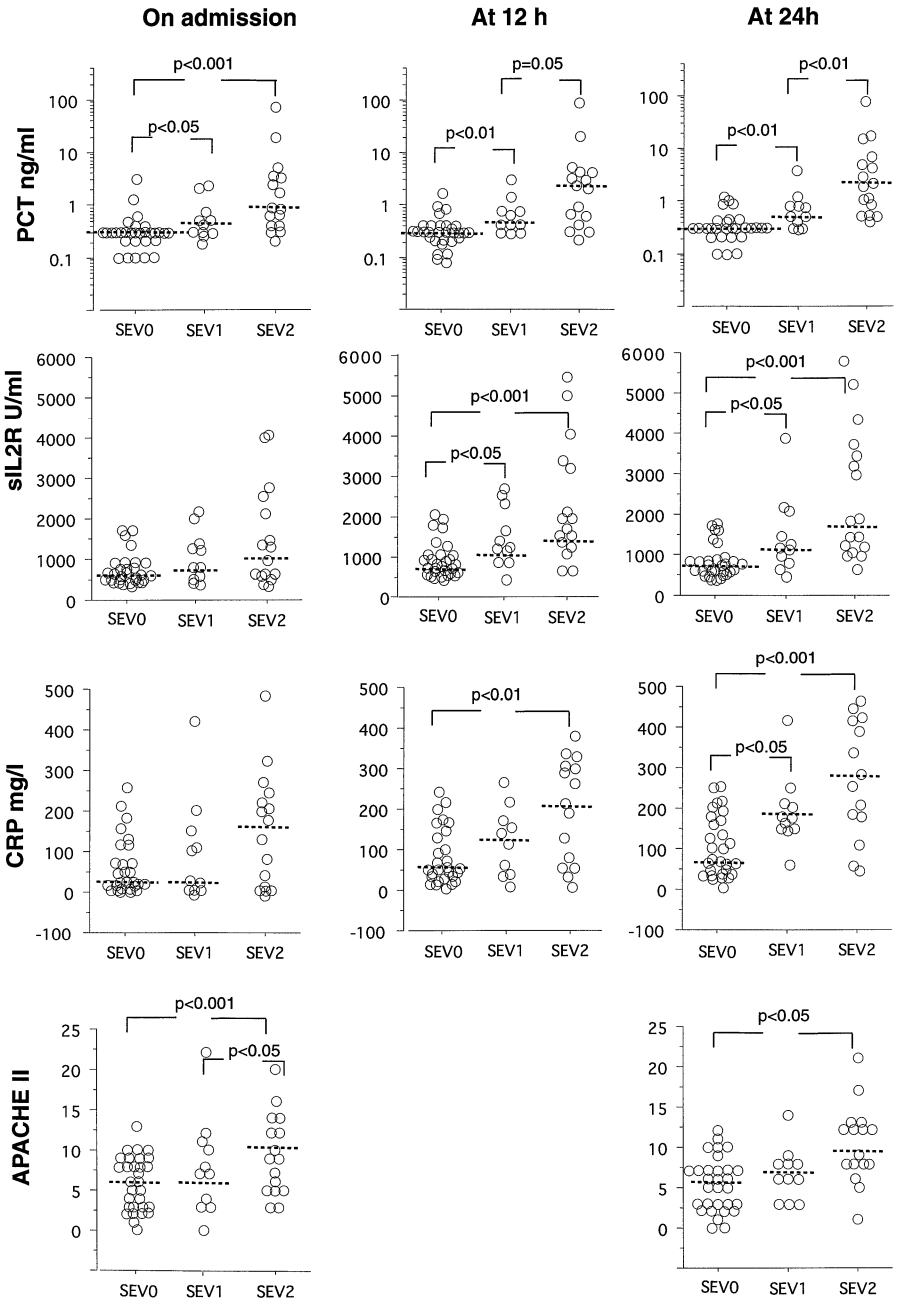

Figure 2. Circulating levels of procalcitonin (PCT), soluble interleukin-2 receptor (sIL-2R), and C-reactive protein (CRP), and APACHE II score values of patients with acute pancreatitis. SEV_0, mild acute pancreatitis (n=30); SEV_1 and SEV_2, severe acute pancreatitis without organ failure (n=11) and with organ failure (n=16), respectively. The dotted line denotes median value. P-values denote significance of difference between groups. Note: PCT concentrations are given on logarithmic scale.

PCT®-Q test in predicting severe acute pancreatitis (IV)

The PCT®-Q test result correlated well with the severity of AP as is shown in Figure 3. When compared to the CRP, APACHE II and Ranson score values, the sensitivity, specificity, PPV and NPV, taken together, in detecting severe AP seem to be higher for the PCT®-Q test (Table 6). In patients with mild AP, the PCT®-Q test was positive in 16% of the cases. For the detection of an AP patient with subsequent organ failure, the PCT®-Q test had a sensitivity of 86% at T0 and 95% at T24. The sensitivity increased to 100% if the higher one of the two PCT®-Q test levels determined is selected.

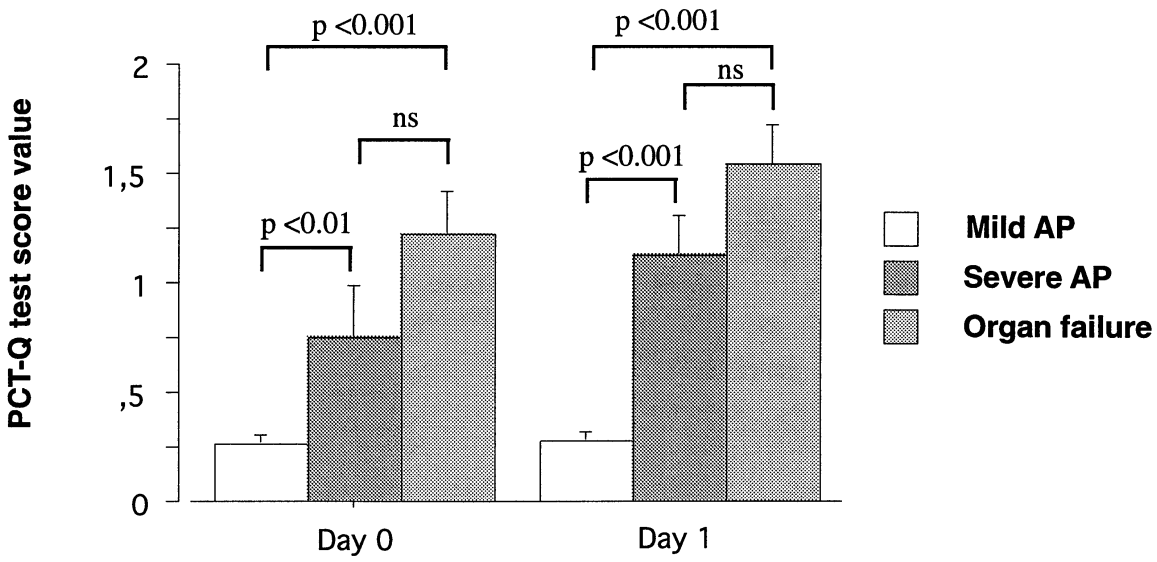

Figure 3. PCT®-Q test score values of patients with acute pancreatitis (AP) on admission to hospital and 24 hours later. The score values were as follows: 0, 1, 2 and 3 if procalcitonin levels (ng/ml) were <0.5, 0.5-2, 2-10, and >10, respectively. Columns denote mean score values and each bar indicates 1 standard error. Organ failure denotes the patients with severe AP who subsequently developed organ failure.

Table 6. Sensitivity, specificity and predictive values of PCT-Q test, C-reactive protein (CRP), Ranson score and APACHE II score for discrimination of severe acute pancreatitis

VARIABLE	SENSITIVITY	SPECIFICITY	PPV	NPV	PLR	NLR
PCT-Q						
Day 0	71 %	84 %	52 %	90 %	4.44	0.35
Day 1	92 %	84 %	53 %	97 %	5.75	0.10
Day 0 or Day 1	95 %	78 %	49 %	98 %	4.32	0.06
CRP > 150 mg/l						
Day 0	37 %	88 %	54 %	82 %	3.08	0.72
Day 1	71 %	68 %	49 %	89 %	2.22	0.43
Day 0 or Day 1	74 %	65 %	47 %	89 %	2.10	0.40
APACHE II ≥ 8						
Day 0	61 %	82 %	51 %	91 %	3.39	0.48
Day 1	47 %	78 %	40 %	83 %	2.14	0.68
Day 0 or Day 1	71 %	73 %	47 %	89 %	2.63	0.40
Ranson ≥ 3						
Day 0	29 %	82 %	33 %	79 %	1.61	0.87
Within 48 hours	45 %	98 %	89 %	85 %	22.50	0.56

PPV, positive predictive value; NPV, negative predictive value

PLR, positive likelihood ratio; NLR, negative likelihood ratio

Cellular markers of systemic inflammation and immune suppression in acute pancreatitis (V)

The MODS score values were related to the severity of AP (Study V, Figure 1). The median level of HLA-DR-positive monocytes of all AP patients decreased during the follow-up. The decrease was seen in all patient groups but, in particular, in the organ failure group (grade III group) (Figure 4A), as expressed by the increased CV% (Study V, Table III). CD11b expression on neutrophils was the highest in grade III patients in all time-points (Figure 4B), as determined by increased AUC values (Table 7). The low CV% for grade III group indicated that the CD11b expression was persistently high in this patient group (Study V, Table III). The same phenomenon as with neutrophils was also seen in CD11b expression on monocytes (Figure 4C). No differences were observed between the severity groups in the monocyte CD14 expression levels (Figure 4D), neutrophil L-selectin levels (Figure 4E), and monocyte L-selectin levels (Figure 4F).

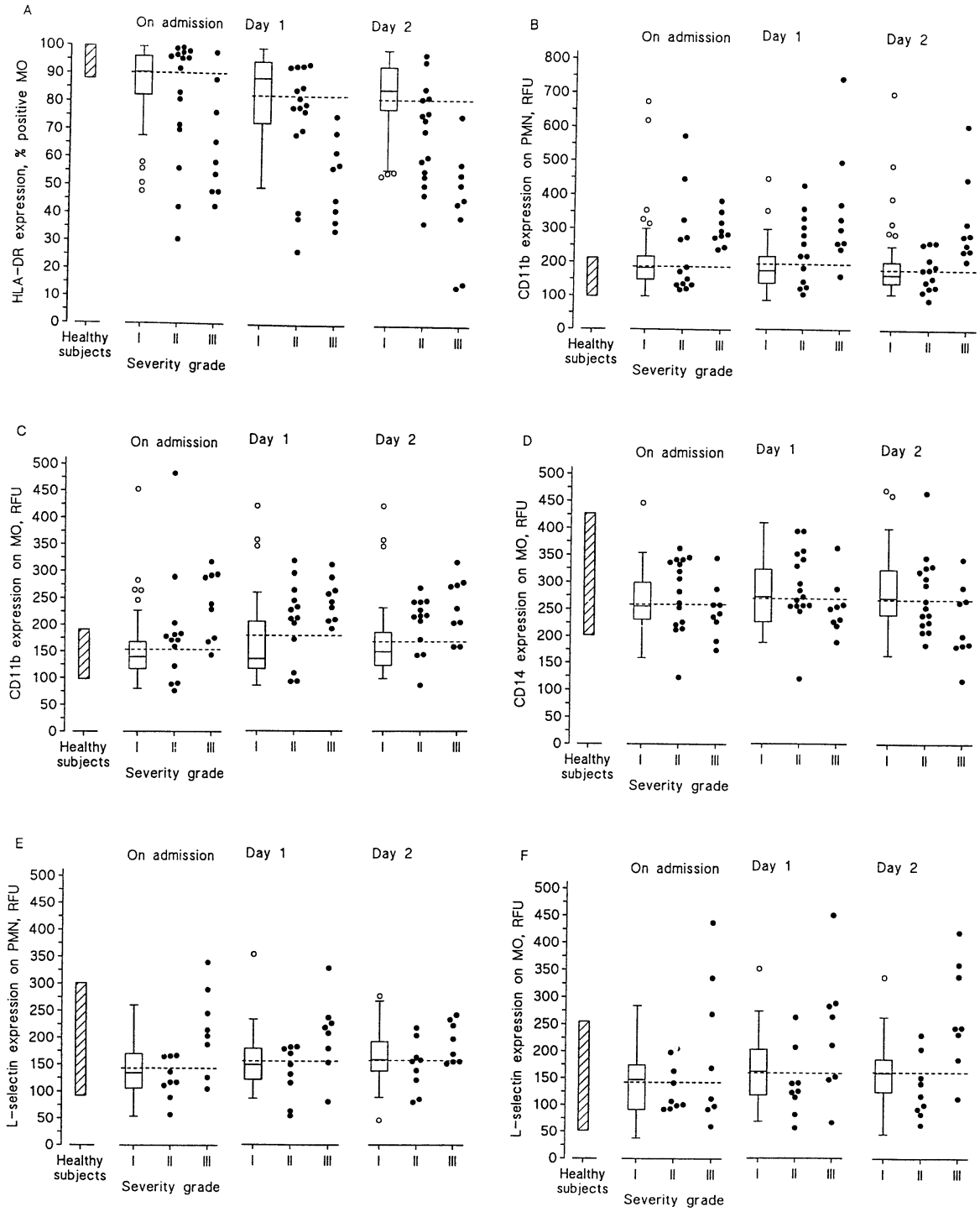

Figure 4. Monocyte and neutrophil cell surface markers. A = proportion of HLA-DR-positive monocytes. B and C = CD11b expression levels on neutrophils and monocytes, respectively. D = CD14 expression level on monocytes. E and F = L-selectin expression levels on neutrophils and monocytes, respectively. Severity Grade I, mild acute pancreatitis shown by box-and-whiskers plot, n=38-52; Grade II, severe acute pancreatitis without organ failure, n =9-16; Grade III, severe acute pancreatitis with organ failure, n=8-9. The dotted line denotes median of all patients. Each shaded box shows 5% and 95% limits of healthy subjects (n=34). Abbreviations: MO, monocyte; PMN, neutrophil; RFU, relative fluorescence units.

Table 7. Area under the curve (AUC) for cell surface markers between time-points on admission to Day 2

Marker	Number of patients	Severity of acute pancreatitis			p for linear trend
		Grade I AUC Mean (SD)	Grade II AUC Mean (SD)	Grade III AUC Mean (SD)	
HLA-DR expression, % positive MO	77	82 (12)	69 (18)	49 (11)	<0.001
CD11b expression, RFU					
PMN	67	184 (68)	213 (52)	359 (142)	<0.001
MO	66	161 (55)	207 (39)	238 (55)	<0.001
CD14 expression, RFU					
MO	76	275 (50)	288 (64)	230 (53)	0.29
L-selectin expression, RFU					
PMN	55	141 (38)	135 (41)	198 (71)	0.062
MO	55	149 (56)	131 (51)	252 (98)	0.16

Abbreviations: MO, monocyte; PMN, neutrophil; RFU, relative fluorescence units

DISCUSSION

Actim Pancreatitis test in screening for acute pancreatitis (I,II)

Determination of urinary trypsinogen-2 is a highly accurate test for the diagnosis of AP (Itkonen et al. 1990, Hedström et al. 1996d). Our research group has earlier introduced a rapid urinary trypsinogen-2 test strip, which is based on the immunogromatography with monoclonal antibodies (Hedström et al. 1996b). A modified rapid dipstick, actim Pancreatitis, has recently been developed with completely new antibodies to detect elevated levels of trypsinogen-2 in urine. With the present study population of 525 patients with acute abdominal pain we evaluated the validity of the dipstick for the detection of AP. Due to the very high sensitivity and NPV of the actim Pancreatitis test strip, AP could be excluded with a very high probability with a negative dipstick result. However, the PPV was relatively low indicating that the dipstick alone cannot establish the diagnosis of AP, but additional examinations (laboratory or radiology) are needed. Earlier, trypsinogen-2 levels have been reported to elevate also in conditions such as hepatobiliary and pancreatic cancer, and in chronic pancreatitis (Hedström et al. 1996a). The quantitative measurements of urinary trypsinogen-2 showed a good correspondence with the test strip result, supporting the use of the simple and rapid dipstick. The minor differences may be due to the fact that the samples were frozen until the quantitative measurement, resulting in some loss of trypsinogen-2 immunoreactivity, while the dipstick test was performed immediately after sampling. The accuracy of the actim Pancreatitis test

proved to be very similar to that of the preliminary dipstick, which has been reported in a retrospective (Hedström et al. 1996b) and a prospective (Kemppainen et al. 1997) study.

In AP, the urinary trypsinogen-2 concentration rises within hours of disease onset and correlates well with the severity of the disease (Hedström et al. 1996d). This was also seen in the present studies (I,II) where all the severe cases were detected by the dipstick, which is highly important in clinical practice. This and the high sensitivity of the actim Pancreatitis test strongly suggest the use of actim Pancreatitis for screening for AP in patients with acute abdominal pain.

Comparison of actim Pancreatitis test strip with serum lipase in the diagnosis of acute pancreatitis (II)

In earlier studies, the sensitivity and specificity of serum lipase activity in diagnosing AP vary markedly mainly due to different assays and diagnostic thresholds (Lott et al. 1986, Wong 1993). In the present study, the sensitivity of serum lipase was too low for screening purposes. The actim Pancreatitis test proved to be a much more accurate method in detecting AP than serum lipase (II). Further, unlike trypsinogen-2, serum amylase and lipase show no correlation with the severity of AP (Clavien et al. 1989a, Steinberg and Tenner 1994, Lankisch 1999a), which was also confirmed in the current studies (I,II). In study II there were two patients with severe AP, which would have been missed with serum lipase determination with a cut-off of 600 IU/L. Similarly, in study I there was one patient with severe AP with normal serum amylase activity.

In the present study, serum lipase activity with a cut-off of 600 IU/l showed a very high specificity and is therefore a suitable confirmatory test for AP in cases with a positive actim Pancreatitis dipstick result. Thus, after a positive actim Pancreatitis test result and serum lipase activity of over 600 IU/l, the diagnosis of AP is very likely. But, because of the poor sensitivity, a low lipase level only indicates the need for additional diagnostic methods such as US or CT after a positive dipstick result.

Procalcitonin, soluble interleukin-2 receptor and soluble E-selectin in predicting the severity of acute pancreatitis (III,IV)

The classification system for AP proposed at the International Symposium on Acute Pancreatitis in Atlanta in 1992 for dividing AP into mild and severe disease is the internationally recognized guideline for clinical assessment of this condition and considers the disease as severe if local and/or systemic complications are present (Bradley 1993). However, systemic complications, which

increase the mortality rate significantly, occur only in half of the patients with a necrotizing form of AP (Tenner et al. 1997). It is now increasingly recognized that it could be beneficial to study the organ failure group separately, as we also confirmed in the present study (Tenner et al. 1997, Osman and Jensen 1999, Lankisch et al. 2000). In the present study (III), the organ failure group was clearly distinct with higher MODS values and level of inflammation as evidenced by increased plasma PCT concentration.

PCT has previously been reported to be an early indicator of severe infection (Assicot et al. 1993, Oberhoffer et al. 1999, Rothenburger et al. 1999) and the monitoring of PCT concentrations has been shown to help in differentiating between sterile and infected pancreatic necrosis (Rau et al. 1997). It was proposed earlier that in an AP patient elevated PCT levels indicate biliary aetiology. This, however, may be explained by the apparent septic complications in the biliary group in the study (Brunkhorst et al. 1995). In our series, PCT values did not correlate with the aetiology of the AP, which is in accordance with other recent studies (Rau et al. 1997, Müller et al. 2000).

The present results show that PCT concentrations were higher in patients with severe AP than in those with mild AP already at the time of admission to the hospital. Further, even more importantly, the PCT levels at 12 h and 24 h of admission were significantly higher in severe AP patients who subsequently developed the most complicated disease accompanied with organ failure(s) than in those severe AP patients who recovered without organ failure. These findings are novel and suggest that PCT may provide a useful means to identify those AP patients with ultimately severe disease and at risk of organ failure at the early stage of AP. The results also confirm the previous findings of PCT increase in non-infectious SIRS (Nylen et al. 1996, Hensel et al. 1998, Mimoz et al. 1998, Vincent 2000). Interestingly, the PCT concentrations correlated inversely to the time between admission and the diagnosis of organ failure.

sIL-2R concentrations correlated to the disease severity, which has also been reported earlier (Pezzilli et al. 1994). However, unlike in septic patients (Takala et al. 1999), sIL-2R concentrations failed to detect the AP patients at risk of organ failure, which in terms of clinical practice is of utmost importance. Elevated levels of sE-selectin have earlier been reported in systemic disorders such as SIRS (Bevilacqua et al. 1987, Cowley et al. 1994, Smith 1997). Obviously, the follow-up period of 24 hours after admission of the present study III was too short for the late-emerging peak of the sE-selectin, because previously sE-selectin has been shown to increase in the later stage of severe AP (Inagaki et al. 1997).

The PCT®-Q test is simple, quick to perform and showed high sensitivity of 92-95% in predicting severe AP within 24 hours of admission. The NPV was very high (97%) indicating that with a negative test strip result severe AP can be excluded with a high probability. The PCT®-Q test was also able to detect all the cases with subsequent organ failure. Therefore, the PCT®-Q test is of clinical value in selecting patients for transferring to university hospitals, for investigations by expensive imaging techniques, such as CE-CT, and for monitoring and treatment in an intensive care unit. Instead, patients with a negative result can be safely treated in low-cost wards. A multicentre study of the urinary TAP in detecting severe AP was published recently (Neoptolemos et al. 2000). When comparing those earlier results, urinary TAP showed no advantage over PCT®-Q test, which, as a rapid test is much easier to perform than the laborious ELISA assay of TAP. However, in the future, when considering the use of immunomodulatory therapies with possible side effects, due to the low PPV of the PCT®-Q test, additional early indicators of subsequent organ failure are needed (Repo and Harlan 1999, Vuorte et al. 1999).

Various scoring systems have been used to define the severity of AP (Roumen et al. 1992). Especially the APACHE II scoring system has proven to be an accurate method in the early assessment of the severity of AP (Wilson et al. 1990, Brisinda et al. 1999). The APACHE II scoring system can be used throughout the patient's hospitalization and is thus considered useful also for monitoring the progress of patients. In the present study, it was demonstrated that the measurement of the PCT®-Q test is at least as accurate as multiple factor scoring systems in detecting severe AP. The multiple factor scoring systems are too complex for routine clinical use (Toh et al. 2000) and the measurement of a single factor, such as the PCT®-Q test, appears more likely to be adopted into daily clinical practice.

CRP is the only widely used single parameter for the differentiation of severe and mild AP, but it is useful only 48-72 hours after the onset of the disease (Clavien et al. 1989a, Neoptolemos et al. 2000). The delay of CRP increase was also seen in the present study, the sensitivity being just 37% on admission to hospital (IV). However, in the follow-up during the course of the disease, CRP has proven to be useful (Puolakkainen et al. 1987).

Cellular markers of systemic inflammation and immune suppression in acute pancreatitis (V)

Severe AP is a two-phase disease. The initial phase is hyperstimulation of the immune system, as expressed by increased monocyte and neutrophil CD11b expression found in the present study. Previously, CD11b expression has been shown to predict organ failure in septic patients and in

patients with liver cirrhosis (Rosenbloom et al. 1995, Takala et al. 1999). The proinflammatory attack, which seems to cause early systemic complications, is followed rapidly by a variably long period of immune suppression, as reflected by low HLA-DR expression on monocytes. These findings agree with the earlier studies indicating decreased immune function in severe AP patients with depressed delayed-type skin hypersensitivity (Garcia-Sabrido et al. 1989), high serum levels of both anti-inflammatory and pro-inflammatory cytokines on presentation (Brivet et al. 1999) and a decreased lymphocyte count (Curley et al. 1993, Pezzilli et al. 1995). The decrease in HLA-DR expression on monocytes following systemic inflammation has been extensively studied in septic patients and has been shown to associate with the severity of the disease (Volk et al. 1996, Döcke et al. 1997, Heumann et al. 1998). In AP patients, persistently low HLA-DR expression has been reported to be related to increased mortality (Richter et al. 1999).

It has been proposed that high CD14 expression, which is genetically regulated, may increase the risk of immune-mediated tissue injury (Fearon 1999, Hubacek et al. 1999). This was not seen in the present study since the monocyte CD14 expression level was equal in different severity groups. Moreover, it has been shown in experimental AP with mice lacking the CD14 molecule that they retained their ability to produce inflammatory cytokines initiating the inflammatory cascade (Eubanks et al. 1998). Also L-selectin expression did not show any correlation to the disease severity. This may be due to the expression alterations in the circulating pool, depending on the activity of tissue infiltration and bone marrow release (Van Eeden et al. 1995).

General discussion

The commercially available urinary trypsinogen-2 test strip, actim Pancreatitis, proved to be an appropriate method for screening for AP. The number of patients in this study with acute abdominal pain was 525, which seems large enough in order to draw this conclusion. The main problem in the present studies with actim Pancreatitis was that there is no “gold standard” for the diagnosis of AP. All diagnostic methods (e.g. US or CT) were not used with every patient. The diagnosis of AP was mainly based on measurements of amylase activity, which is clearly insufficient for that purpose. Thus, some false-positive test strip results may have been obtained from patients with undiagnosed mild AP in the present studies. Assessment of severity of AP was studied with markers of systemic inflammation; sIL2R and PCT concentrations were observed to be higher in patients with severe AP in the early phase of the disease. Furthermore, PCT increase was the highest in AP patients with subsequent organ failure, the subgroup of which would be highly important to detect early in clinical practice. Because of an overlap in the groups with different severity of AP, PCT is not a

perfect marker to be used as a single marker, but is surely useful when used in combination with other markers in predicting the risk of developing organ failure in AP patients. In the future, additional studies with a sufficient number of patients will be needed to find out the most accurate set of markers. The introduction of flow cytometry has made it possible for clinicians to obtain daily routine assays of the cellular immune status. We studied cellular markers of systemic inflammation and immunosupression in patients with AP. Phagocyte activation was observed to be an early phenomenon and the activation was related to development of organ failure. However, immunosupression developed rapidly, as indicated by a rapid decrease of monocyte HLA-DR expression. Our study emphasizes the need for future studies to understand the immunologic changes in AP when evaluating new immunomodulatory therapies.

CONCLUSIONS

1. A negative actim Pancreatitis test result rules out AP with a high probability and is a suitable test for the screening for AP. A positive dipstick result identifies the patients who need further evaluation.

2. A negative actim Pancreatitis test strip result excludes AP with a higher probability than the quantitative measurement of serum lipase. As being highly specific for AP with a cut-off of >3x the URL, serum lipase is suitable as a confirmatory test in patients with a positive trypsinogen-2 dipstick result.

3. On admission to hospital, concentrations of PCT but not those of CRP, sE-selectin or sIL-2R, are higher in patients with severe AP than in those with a mild disease. After twelve hours of admission, also CRP and sIL-2R are helpful in predicting severe AP. Furthermore, a high PCT value provides a useful means for early prediction of subsequent organ failure.

4. The simple and rapid semi-quantitative PCT test (PCT®-Q test) is an accurate method for the screening for severe AP. It seems that cumbersome multiple factor scoring systems (Ranson and APACHE II) could probably be replaced by the PCT®-Q test in the early grading of AP.

5. Phagocyte activation, as confirmed by increased CD11b expression, is an early event in AP and is related to the severity of the disease and to the development of organ failure. In severe AP and especially in cases with subsequent organ failure, monocyte HLA-DR expression decreases rapidly during the first days after disease onset as an indicator of the level of immunosuppression.

SUMMARY

The value of the commercially available actim Pancreatitis test for the measurement of trypsinogen-2 in urine was evaluated as a screening test for AP in 525 patients with acute abdominal symptoms. The trypsinogen-2 test strip was highly sensitive (96%) and specific (92%) for AP. One of the most important clinical features was its ability to detect all the severe cases. Because of the high NPV (99.6%), AP can be excluded accurately after a negative result. Instead, the PPV was only moderate (54%) indicating that after a positive result, further diagnostic methods are needed to ascertain the diagnosis of AP. The trypsinogen-2 strip test can be performed rapidly and appears to be a useful screening method for AP in health care centres with limited laboratory facilities.

The accuracy of the urinary trypsinogen-2 test was compared to the determination of serum lipase in the diagnosis of AP in 237 patients with acute abdominal pain. In the study population there were 29 patients with AP. The sensitivity of the dipstick was 93%, which was superior to that of serum lipase. As a consequence, the NPV of the dipstick was also higher than that of lipase determination. Serum lipase with a cut-off of 600 IU/l was highly specific (99%) but showed an unacceptably low sensitivity of 55% for AP and failed to detect two patients with severe AP. A combination of the urinary trypsinogen-2 test strip and serum lipase with a cut-off of 600 IU/l improved the specificity of the dipstick alone from 92% to 99.5% and PPV from 63% to 94%. The trypsinogen-2 test strip is superior as a screening test for AP. Serum lipase determination is recommended as a confirmatory test after a positive dipstick result.

A prospective study was undertaken to evaluate in 57 AP patients whether the new markers of systemic inflammation, PCT, sIL-2R and sE-selectin, will predict severe AP with a special reference to organ failure during the first day after admission. The results were compared to those with CRP and clinicobiochemical scoring systems. PCT had a sensitivity of 94% and a specificity of 73% for the development of organ failure, which was superior to other tests. The fact that PCT values correlated negatively with the time elapsed between admission and the diagnosis of organ failure strengthens the view that PCT may enhance the body’s inflammatory response. sIL-2R had a potency in detecting severe AP but failed to detect the patients with subsequent organ failure.

Recently a semi-quantitative PCT test (PCT®-Q test) was developed. The ability of the test strip as a screening method for severe AP during the first day after admission was studied in 162 patients with AP, including 38 severe cases. The accuracy was compared to those of CRP and multiple

factor scoring systems. The PCT®-Q test detected severe AP with a sensitivity of 92% and a specificity of 84%. Moreover, all the cases with subsequent organ failure (22 patients) were detected by the PCT®-Q test. The other tests showed no advantage over the PCT®-Q test. As being simple and applicable to an emergency laboratory, the PCT®-Q test is a useful screening method for AP and could replace cumbersome scoring systems.

Phagocyte surface markers were studied in 89 patients with AP. It was shown that monocyte and neutrophil activation as defined by increased expression of CD11b is an early event in AP and is related to the severity of the disease, being highest in those with organ failure. Moreover, the finding that monocyte CD14 expression did not differ between the groups with different severity in the early course of the disease confirms that AP is associated with strong inflammatory response already during the first days. However, the most important finding was the rapid development of immune suppression, as evidenced by decreased HLA-DR expression on monocytes, especially in patients with subsequent organ failure. According to the present results, monitoring the immunological status of the patient is of paramount importance if immunomodulatory treatments are planned.

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