New Serum Markers for the Detection of Severe Acute Pancreatitis in Humans

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Acute pancreatitis is an inflammatory process which occurs in a normal organ and which is diagnosed mainly by acute abdominal pain associated with a concomitant rise of serum amylase and lipase concentrations (1, 2). Gallstone migration into the common bile duct and alcohol abuse account for most of the etiologies of the disease. Usually the injury is mild, but 20% of the patients have a severe injury and, among them, 15 to 25% will die.

Because it is important to predict the severity of the illness as early as possible in order to optimize the therapy and to prevent organ dysfunction and local complications, several scores of severity have been proposed. Criteria of severity, such as Ranson (3–5), Glasgow (6), and Acute Physiology and Chronic Health Evaluation (APACHE) (7) scores have been used for a long time. These scores assess the multiple organ dysfunction induced by the disease and consequently, the greater the number of organs injured, the greater the score.

New serum markers have recently emerged and their potential for providing additional information on the severity of the disease is currently being evaluated. However, to become useful such markers must be assessed in a large consecutive series of patients, including a significant proportion of severe cases, and the timing of the assessment must be related to the onset of the disease. Moreover, the usefulness of the new marker must be compared with established ones; the results must be reproducible; and the detection of the new marker must be easy to detect in clinical chemistry laboratories.

Interestingly, when seeking medical attention (usually 12 to 24 h after the onset of pain) most patients do not exhibit multiple organ dysfunction, which is likely to emerge by the second or third day and, at admission, numerous mediators can be detected in serum. If the concentration of these biologic factors is correlated to the severity of the disease, and if they are detected before the occurrence of multiple organ dysfunction, it is then conceivable that the therapeutic antagonism of these mediators might prevent or attenuate the severity of the multiple organ dysfunction, and consequently the outcome of the disease. These new factors might be important for the rapid scoring of the disease severity in the acute phase and some of them might be used as potential therapeutic targets.

EMERGING CONCEPTS IN THE PATHOPHYSIOLOGY OF ACUTE PANCREATITIS

The pathophysiology of acute pancreatitis includes the activation and release of pancreatic enzymes in the interstitium, the autodigestion of the pancreas, and the multiple organ dysfunction after their release in the systemic circulation (Figure 1) (8). The initial phase of the disease originates from the activation of trypsinogen to trypsin within the acinar cells, which in turn activates various enzymes such as elastase and phospholipase A2 (PLA2), and the complement and kinin systems (9, 10). Trypsinogen activation peptide (TAP), which is cleaved when trypsinogen is activated into trypsin, is found in pancreatic tissue during both experimental and human pancreatitis. The higher the peptide concentration is in plasma, urine, and ascites, the higher the severity of the disease (11–14). To prevent a premature activation, the harmful digestive enzymes are synthesized in acinar cells and released as inactive precursors. Moreover, when passing through the Golgi complex, these digestive enzymes are separated from other lysosomal enzymes which may activate trypsin from trypsinogen. Intra-acinar colocalization of digestive and lysosomal enzymes is one important feature of experimental pancreatic injury but the relevance of this colocalization in the pathology of human acute pancreatitis remains unclear (9, 10). Another feature observed in experimental pancreatitis is the disruption of the paracellular barrier of acinar cells and intralobular pancreatic duct cells with extravasation of pancreatic enzymes into the interstitium (15).

The activation of pancreatic enzymes is not the only finding involved in the pathophysiology of the disease. After trypsinogen activation into trypsin, a local inflammation is initiated which results in the local production of inflammatory mediators. Experimental studies show that pancreatic injury is mediated by the release of proinflammatory mediators such as interleukin-1 (IL-1), IL-6, IL-8, as well as by the activation of inflammatory cells such as neutrophils, macrophages, and lymphocytes. Tumor necrosis factor-α (TNF-α), released by macrophages within pancreatic tissue, correlates with the severity of the experimental disease (16, 17). In experimental protocols, treatment with IL-1 receptor antagonist (18), mediators blocking the generation of O2· derived free radicals (19), and treatment with platelet-activating factor (PAF) receptor antagonist (20) improve the outcome of the disease. Interestingly, anti-inflammatory cytokines, such as IL-10, decrease the severity of experimental pancreatitis (21–23).

Activation of endothelial cells permits the transendothelial migration of neutrophils, monocytes, and lymphocytes in the pancreas and mediators released by these cells, such as neutrophil elastase, might be much more damaging than pancreatic enzymes (24). Decreased O2 delivery to the organ (25) and generation of O2· derived free radicals (26) also contribute to the injury. Moreover, proinflammatory mediators released by neutrophils and macrophages injure the vascular wall and increase the microvas-
cular permeability, leading to intraparenchymal edema and O$_2$ supply deficiency.

Thus, whatever the initial cause (alcohol, gallstone, etc.) (27, 28), the severity of acute pancreatitis is related to the injury of acinar cells and to the activation of various cells such as neutrophils, monocytes, lymphocytes, and endothelial cells (Figure 1). Local and systemic complications follow the release of numerous mediators by these activated cells. However, a full extrapolation of these experimental findings to humans should be cautious.

**TRADITIONAL SEVERITY SCORES IN HUMAN ACUTE PANCREATITIS**

The early prediction of the severity of the disease is an important goal for physicians in charge of patients with acute pancreatitis in order to optimize the therapy and to prevent organ dysfunction and local complications. For that purpose, multiple scale scores including Ranson (3–5), Glasgow (6), and APACHE II (7) which were first described for critically ill patients, have been applied to patients with acute pancreatitis. These scores record numerous physiologic and biologic parameters to assess multiple organ dysfunction. The Ranson score has been used in most studies dealing with acute pancreatitis since the 1980s. Among the 11 variables collected, five are obtained at admission and six during the first 48 h of hospitalization. The Glasgow score includes nine variables. Both scores predict the evolution of the disease correctly in 71 to 88% of the patients (29, 30). The APACHE score, which first assigned five grades to 34 physiologic and biologic parameters, was simplified in 1985 (12 parameters) (31). In 160 patients, this score was significantly higher in severe acute pancreatitis than in mild injury (7). Another clinical factor was also recently outlined by Funnel and coworkers (32). One-third of the patients who develop local complications and 7 of 19 who die have a body mass index greater than 30 kg/m$^2$. Because these multifactorial scale scores are complex and include numerous parameters easily available only in intensive care units, new criteria for the detection of severe acute pancreatitis have been studied.

**NEW MARKERS FOR THE DETECTION OF SEVERE ACUTE PANCREATITIS**

The 48-h delay necessary to collect the standard scores has prompted physicians to investigate new markers (Tables 1–3). Because these factors might be detected before the development of multiple organ dysfunction, the early start of an aggressive therapy might prevent the development of multiple organ dysfunction. Additionally, the early administration of antagonists targeting these factors might also improve the outcome of the disease.

**Cytokines**

IL-1 is a proinflammatory cytokine produced during acute and chronic inflammation which is responsible for many symptoms

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**TABLE 1. CYTOKINES AS SINGLE PREDICTIVE FACTORS IN CLINICAL STUDIES**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Latency (h)</th>
<th>Clinical Observations</th>
<th>Clinical Usefulness</th>
<th>Main References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 and IL-1 receptor</td>
<td>24–48</td>
<td>Not superior to IL-6 and CRP. IL-1/IL-1 receptor is useful to predict systemic or abdominal sepsis</td>
<td>+</td>
<td>Norman$^{18}$, McKay$^{33}$, Heresbach$^{44}$</td>
</tr>
<tr>
<td>IL-6</td>
<td>18–48</td>
<td>One of the most useful factors for early detection of severity</td>
<td>+++</td>
<td>Leser$^{15}$, Viedma$^{16}$, de Beaux$^{37}$, Heath$^{38}$, Pezzilli$^{39}$</td>
</tr>
<tr>
<td>IL-8</td>
<td>12–24</td>
<td>Correlated to neutrophil activation. One of the most useful factors for early detection of severity</td>
<td>++</td>
<td>Chen$^{41}$, Gross$^{45}$</td>
</tr>
<tr>
<td>IL-10</td>
<td>24</td>
<td>The lower the concentration at the time of admission, the more severe the pancreatitis Correlated to the severity from Day 2 to Day 4. Not an early marker</td>
<td>+</td>
<td>Pezzilli$^{46}$</td>
</tr>
<tr>
<td>IL-11</td>
<td>24</td>
<td>Correlated to the severity from Day 2 to Day 4. Not an early marker</td>
<td>+</td>
<td>Chen$^{47}$</td>
</tr>
<tr>
<td>IL-12</td>
<td>24</td>
<td>Increased at admission; unknown significance</td>
<td>?</td>
<td>Pezzilli$^{48}$</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6–18</td>
<td>Not correlated to the severity of the disease</td>
<td>?</td>
<td>de Beaux$^{49}$, Paajanen$^{42}$</td>
</tr>
<tr>
<td>TNF-α receptor</td>
<td>6–18</td>
<td>Predicts the severity of the disease</td>
<td>+</td>
<td>de Beaux$^{41}$, Kaufmann$^{44}$</td>
</tr>
</tbody>
</table>

0, no clinical usefulness; ?, unknown significance; + (weak), ++ (good), and +++ (very good) clinical usefulness.
during sepsis (Table 1). IL-1 has also been investigated in the pathophysiology of human acute pancreatitis. In the study published by McKay and coworkers (33), in which monocytes were isolated from peripheral blood, the release of cytokines was measured in patients with moderate and severe diseases from Day 1 to Day 7. Although TNF-α, IL-6, and IL-8 release were significantly higher in patients with systemic complications than in those with an uncomplicated disease, IL-1 release was similar in the two groups (33). The results obtained from 37 patients (25 severe and 12 mild disease) confirm that C-reactive protein (CRP) and IL-6 determinations within 48 h after admission predict the severity of the attack more accurately than in those with an uncomplicated disease, IL-1 release does not differ in patients with acute pancreatitis and in volunteers (41, 42). When proinflammatory cytokine release was measured from isolated peripheral blood mononuclear cells, concentrations of IL-6 and IL-8 were higher in severe disease (41). However, the kinetics of IL-6 which peaks early at Day 1 cannot be compared with the Ranson and APACHE II scores which are established at Day 2. Thus, more investigations are necessary to accept IL-6 as a new useful marker.

Early studies found that, in contrast to IL-6, plasma TNF-α does not differ in patients with acute pancreatitis and in volunteers (41, 42). When proinflammatory cytokine release was measured from isolated peripheral blood mononuclear cells, concentrations of IL-6 and IL-8 were higher in severe disease whereas TNF-α concentrations did not increase (41). However, a recent study showed that plasma concentrations of TNF-α were higher, concomitantly with other cytokines such as IL-1β.

### TABLE 2. PANCREATIC PRODUCTS AS SINGLE PREDICTIVE FACTORS IN CLINICAL STUDIES

<table>
<thead>
<tr>
<th>Products</th>
<th>Latency (h)</th>
<th>Clinical Observations</th>
<th>Clinical Usefulness</th>
<th>Main References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>12–36</td>
<td>Not correlated to the severity of the disease</td>
<td>0</td>
<td>Ranson⁹⁹, Clavien⁹⁰, Winslet⁹¹, Pezzilli⁹²</td>
</tr>
<tr>
<td>Lipase</td>
<td>18–48</td>
<td>Not correlated to the severity of the disease. More specific than amylase.</td>
<td>0</td>
<td>Ranson⁹⁹, Clavien⁹⁰, Winslet⁹¹, Pezzilli⁹²</td>
</tr>
<tr>
<td>PAP</td>
<td>24–48</td>
<td>Clinical significance debated</td>
<td>?</td>
<td>Pauwels²³, Clavien⁹⁰, Winslet⁹¹, Pezzilli⁹²</td>
</tr>
<tr>
<td>PLA₂</td>
<td>24</td>
<td>Differentiates edematous and necrotic pancreatitis. Correlated with pulmonary injury.</td>
<td>++</td>
<td>Büchler⁶⁵, Bird₂⁶, Puolakkainen⁶⁵, Makela⁶⁴</td>
</tr>
<tr>
<td>Procarboxypeptidase</td>
<td>24–36</td>
<td>Differentiates between mild and severe disease.</td>
<td>+</td>
<td>Appelros⁷⁰, Rau⁶⁹</td>
</tr>
<tr>
<td>TAP</td>
<td>Few h</td>
<td>The earliest pancreatic enzyme to be detected</td>
<td>+++</td>
<td>Gudgeon⁶⁵, Tenner⁶⁶, Neoptolemos³⁷</td>
</tr>
<tr>
<td>SPINK1/HPSTI</td>
<td>24–48</td>
<td>SPINK1/HPSTI predicts better the severity of the attack than CRP</td>
<td>+</td>
<td>Pezzilli²²</td>
</tr>
<tr>
<td>Trypsinogen-2</td>
<td>Few h</td>
<td>Useful to diagnose ERCP-induced acute pancreatitis</td>
<td>+</td>
<td>Kemppainen⁷³, Saijio⁷⁴, Hedström⁷⁵</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** ERCP = endoscopic retrograde cholangiopancreatography; HPSTI = human pancreatic secretory trypsin inhibitor; PAP = pancreatitis-associated protein; TAP = Trypsinogen activation peptide.

For explanation of symbols, see Table 1.

### TABLE 3. OTHER MARKERS AS SINGLE PREDICTIVE FACTORS IN CLINICAL STUDIES

<table>
<thead>
<tr>
<th>Products</th>
<th>Latency (h)</th>
<th>Clinical Observations</th>
<th>Clinical Usefulness</th>
<th>Main References</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1-antitrypsin</td>
<td>48–96</td>
<td>High concentrations of trypsin/α1-antitrypsin complex correlated to the severity of the disease. Late marker of severity</td>
<td>?</td>
<td>Wilson²⁹, McMahon²⁹, Banks⁸⁰, Kimura³¹, Büchler³²</td>
</tr>
<tr>
<td>α2-macroglobulin</td>
<td>48–96</td>
<td>Lower in severe disease than in mild forms. Better marker than α1-antitrypsin. Late marker of severity</td>
<td>?</td>
<td>McMahon²⁹, Wilson²⁹, Banks⁸⁰</td>
</tr>
<tr>
<td>Complement factors</td>
<td>24–36</td>
<td>Unhelpful in predicting the severity of acute pancreatitis</td>
<td>0</td>
<td>Büchler²⁵, Foulis³¹, Puolakkainen⁶¹, Wilson²⁹, Gross³⁴</td>
</tr>
<tr>
<td>CRP</td>
<td>24–72</td>
<td>Detects severe disease with a sensitivity ranging from 67 to 100%</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Glucose/urea</td>
<td>24–36</td>
<td>Clinical significance debated</td>
<td>?</td>
<td>Far³⁵, Heath³⁶</td>
</tr>
<tr>
<td>HGF</td>
<td>24–36</td>
<td>Predicts the severity with sensitivity of 71%. Correlated to mortality</td>
<td>+</td>
<td>Ueda³⁷</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>24–48</td>
<td>Promising factor; Further studies are needed</td>
<td>+</td>
<td>Frossard²⁴, Kaufmann³⁸</td>
</tr>
<tr>
<td>LDH</td>
<td>24–48</td>
<td>Not a specific marker</td>
<td>+</td>
<td>Chen⁹⁰, Uhl³⁰</td>
</tr>
<tr>
<td>Neopterin</td>
<td>24</td>
<td>Correlated to macrophage activation.</td>
<td>+</td>
<td>Uomo³⁹, Mora³⁹, Kaufmann³⁴</td>
</tr>
<tr>
<td>Neutrophil elastase</td>
<td>24</td>
<td>Early marker of severity</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>24–36</td>
<td>Differentiates sterile and infected pancreatic necrosis</td>
<td>+</td>
<td>Rau³⁴</td>
</tr>
</tbody>
</table>

**Definition of abbreviations:** CRP = C-reactive protein; HGF = hepatocyte growth factor; ICAM-1 = intercellular adhesion molecule-1.

For explanation of symbols, see Table 1.
IL-6, and IL-8 in severe acute pancreatitis than in mild disease (43). Increased concentrations of soluble TNF-α receptors (p55 and p75) were also easily detected on Day 1, while TNF-α was not present (41) and the increased concentrations of the soluble TNF-α receptors were positively correlated to the development of multiple organ dysfunction and mortality rate (41, 44). Additionally, the presence of high concentrations of soluble TNF-α receptors has been associated with pancreatic necrosis (44).

IL-8 also increases in patients with a severe form of the disease (43, 45). Plasma concentrations of IL-8 and neutrophil elastase, which both reflect the activation of neutrophils, are positively correlated. When assayed in the first 24 h, IL-8 and IL-6 are more useful than CRP in predicting the severity of the disease because both markers peak before CRP (45). However, IL-8 does not yet fulfill the five criteria necessary to be accepted as an important marker.

Interestingly, the plasma concentrations of IL-10, a cytokine that inhibits the release of proinflammatory cytokines by macrophages, are lower in patients with severe pancreatitis than in patients with mild disease (46). In contrast, in another study, IL-10 concentrations were much higher when acute pancreatitis was severe than when the injury was mild (47). The discrepancy observed between this study and the previous one seems to be related to the different types of criteria used to score the severity. The increased IL-10 concentrations during severe disease also contrast with the results obtained during acute experimental pancreatitis in knock-out mice for IL-10 (IL-10−/− mice). Indeed, the severity of acute pancreatitis is higher in IL-10−/− mice than in wild-type animals (23). Thus, high concentrations of IL-10 are likely to predict a mild form of the disease.

Similarly to IL-10, IL-11 is also a potent anti-inflammatory cytokine which can block the increased serum concentrations of TNF-α after endotoxin. During acute pancreatitis, the serum concentrations of IL-11 are significantly higher from Day 2 to Day 4 in patients with a severe disease than in those with mild attacks. However, the IL-11 peak is lower than the one observed for IL-10 (47). The correlation is weak between IL-11 and CRP during Days 1 and 2 and IL-11 does not constitute an early marker of the severity of acute pancreatitis (47).

In acute pancreatitis, macrophages can stimulate cell-mediated immunity through the activation of lymphocytes and the release of T-cell-derived cytokines such as IL-12 and interferon-γ. IL-12 exists in two forms: the homodimer, IL-12p70, which directs the T helper type 1 (Th1) response and the monodimer, IL-12p40, which downregulates the Th1 response. The Th1 response is defined as the activation of T lymphocytes which in turn activates B lymphocytes, leading to the release of protective antibodies. In patients with acute pancreatitis, IL-12p40 was significantly higher from Day 1 to 6 than in healthy subjects. The increased concentrations of IL-12p40 during severe pancreatitis might increase the susceptibility to infections by downregulating the Th1 response (48).

In conclusion, the best cytokines to predict the severity of acute pancreatitis in humans are IL-8 and IL-6. However, their validity and superiority over standard scores (APACHE II, Ranson, and Glasgow) have not been fully investigated. Although semiautomatic methods can measure IL-8 and IL-6 within a few hours of admission, these kits are not yet available in clinical chemistry laboratories.

**Products Released by the Pancreas**

Several studies have also investigated products released by the pancreas to assess the severity of the disease (Table 2).

**Amylase and lipase.** Surprisingly, although amylase and lipase are important for the diagnosis of acute pancreatitis, neither one was included in the scores of severity. Indeed, these factors are imprecise in predicting the outcome of the disease (49). In a consecutive series of 352 attacks of acute pancreatitis, 19% of the patients had normal amylase concentrations on admission. Interestingly, acute pancreatitis with normal serum amylase concentrations is characterized by a high prevalence of alcoholic origins (50, 51). The outcome of acute pancreatitis was found to be either similar (50) or less severe (51) in patients with normal amylasemia than in patients with hyperamylasemia. However, these results have not been confirmed and might result from a delayed diagnosis of the disease. Indeed, in the study published by Pezzilli and coworkers (52), serum amylase and lipase concentrations were not able to establish either the cause or the severity of acute pancreatitis and these criteria must be considered as useless in evaluating the severity.

**Pancreatitis-associated protein (PAP).** Recent studies focusing on the pathophysiology of acute pancreatitis have shown that the synthesis of pancreatic proteins decreases in the acute phase of the disease, whereas other nonenzymatic secretory proteins are overexpressed by the pancreas during the acute phase. Among them, PAP was purified from pancreatic juice in 1988 (53). When PAP values were normal at Day 1, patients did not develop complications and had a shorter hospitalization time than patients with elevated PAP levels (54). PAP value was 8-fold higher in patients with pancreatic necrosis than in patients without evidence of necrosis, suggesting that PAP measurement might also provide useful information concerning the development of complications. In another study published by Chen and coworkers (55), the sensitivity, specificity and accuracy of diagnosis for PAP determination were 100%, 94%, and 97% respectively. However, in 1996, Kemptainen and coworkers (56) reported that serum PAP concentrations did not distinguish severe from mild acute pancreatitis better than CRP. These data were confirmed by Pezzilli and coworkers (57) one year later, minimizing the importance of PAP.

**PLA₂.** Two forms of the enzyme have been described: type I originates from the pancreas, whereas type II is a mediator of the acute-phase response. Pancreatic PLA₂ induces cell necrosis by converting the lecithin of cellular membranes into more toxic lysolceithin compounds (58). It might also play a role in the pulmonary dysfunction associated with acute pancreatitis, by destroying pulmonary surfactant (59) and by inducing the release of nitric oxide from alveolar macrophages (60). Other studies have shown a correlation between the plasma concentrations of PLA₂ level and the severity of the attack (61–63) and the selection of patients with mild and severe pancreatitis can be achieved as early as Day 1 with this marker. Büchler and coworkers (61) clearly delineated the sensitivity (75%) and the specificity (78%) of PLA₂. These results were confirmed recently by Makela and coworkers (64), emphasizing the role of PLA₂ as an interesting marker.

**TAP.** Trypsinogens are pancreatic proteases that can initiate the autodigestive cascade characterizing acute pancreatitis. TAP is the amino-terminal peptide released by the activation of trypsinogen into trypsin. Normally, this peptide is released into the lumen of the small intestine through the action of enterokinase. In acute pancreatitis, the inappropriate activation of trypsinogen within the pancreas results in the release of TAP into the plasma, urine, and peritoneum. Thus, plasma TAP concentration seems to be the best and the earliest marker of acute pancreatitis (65, 66). TAP is closely correlated with the severity of the disease. Because TAP is rapidly
excreted in urine (small size), its detection in urine is easier than in serum. A diagnostic test is now available which might soon have routine clinical application (67). Twenty-four hours after the onset of the symptoms, urinary TAP concentrations were 37 nmol/L in severe diverse and 15 nmol/L in mild disease. The sensitivity, specificity, positive predictive, and negative predictive values of the test in differentiating severe and mild acute pancreatitis were at 24 h: 58%, 73%, 39%, and 86%. The values for the APACHE II score at 48 h were 56%, 64%, 30%, and 85%.

Procarboxypeptidase B and carboxypeptidase B activation peptide (CAPAP). In 1988, a new cytostatic protein, the procarboxypeptidase, was isolated from pancreatic acinar cells. This protein differs from most of the pancreatic proenzymes by its large size and constitutes 2% of total cytostatic proteins of acinar cells. Procarboxypeptidase B had the same sensitivity as serum amylase and lipase for the diagnosis during the first 24 h of the disease (68). Because its elevation persisted longer than the two other enzymes, its determination may be useful for diagnosis in the late phase (68). Moreover, procarboxypeptidase B values are higher in necrotizing pancreatitis than in the edematous form of the disease by Day 3 (69).

The activation peptide released by carboxypeptidase B (CAPAP) is a small peptide which has been purified and characterized recently (70). Urine and serum concentrations of CAPAP correlate with the severity of the attack and this marker might be a valuable tool in the early determination of the severity of the disease (70). Recently Pezzilli and coworkers confirmed that CAPAP might be useful for both the diagnosis of the disease and the assessment of the severity (71).

Serine protease inhibitor Kazal type 1 (SPINK)/Human pancreatic secretory trypsin inhibitor (SPINK/HPSTI). SPINK/HPSTI is the first molecule able to inhibit trypsin and to prevent further autoactivation of trypsin and other proenzymes within the pancreas. It has a better sensitivity than CRP at Day 1 (71% versus 29%) and at Day 2 (88% versus 75%) in predicting the severity of acute pancreatitis, whereas specificity was 77% (SPINK/HPSTI) versus 92% (CRP) at Day 1 and 86% (SPINK/HPSTI) versus 85% (CRP) at Day 2 respectively (72). However, these results need to be confirmed and the test is not available in most institutions.

Trypsinogen-2. The usefulness of serum trypsinogen-2 and trypsin-2-α1 antitrypsin complex in assessing the severity of acute pancreatitis after endoscopic retrograde cholangiopancreatography (ERCP) was assessed by Kempainen and coworkers (73) in the 10% fraction of patients who suffered from acute clinical pancreatitis after the procedure. Serum trypsinogen-2 increased 26-fold, 6 h after ERCP whereas trypsin-2-α1 antitrypsin ratio increased 11-fold after 24 h. Interestingly, the increase of both parameters was stronger in severe than in mild pancreatitis. The accuracy of serum trypsinogen-2 in predicting the severity of acute pancreatitis was also demonstrated by Sainio and coworkers (74) in 52 patients. With a cutoff concentration of 1,000 μg/L, patients with uncomplicated disease differed from patients with complicated acute pancreatitis (91% sensitivity and 71% specificity). These results were confirmed by Hedström and coworkers (75) the same year.

In conclusion, TAP is the best and earliest marker of acute pancreatitis and is closely correlated to the severity of the disease. The usefulness of trypsinogen-2, PLAS, procarboxypeptidases, and CAPAP should also be kept in mind.

Other Markers Used to Assess the Severity of Human Acute Pancreatitis

Antiproteases. The relationship between proteases and antiproteases has also been studied extensively with the hypothesi that an imbalance between them was the main problem in the pathogenesis of acute pancreatitis. However, several prospective randomized trials treating human acute pancreatitis with antiproteolytic drugs such as aprotinin (76, 77) and gabexate mesilate (78) have failed to show any benefit. Because high concentrations of pancreatic enzymes are rapidly complexed with protease inhibitors, the role of circulating protease inhibitors in evaluating the severity of the disease has also been investigated in several studies. α2-Macroglobulin is an intravascular antiprotease which binds irreversibly with proteases such as trypsin or elastase. The complex is then rapidly degraded and eliminated by the macrophages, and plasma α2-macroglobulin concentrations are frequently decreased during severe disease (29, 79). Similarly, Banks and coworkers (80) found that patients with severe illness had lower concentrations of α2-macroglobulin and higher concentrations of complexed α2-macroglobulin than those with mild disease. These results were confirmed by Kimura and coworkers (81). Opposite results were found in mild disease, but the changes were delayed from Day 3 to Day 8 (29). Finally, Büchler (82) found that a decreased serum α2-macroglobulin concentration was associated with a necrotic evolution of the organ. Thus, these markers seem useless for the early prediction of the severity, but appear helpful after the third day.

Complement factors. Several studies also pointed out that the serum concentrations of complement factors are lower in patients with severe disease than in those with mild disease (82, 83).

C-reactive protein. CRP is an acute-phase protein that was first described in 1930. In the mid-1980s, several studies showed that the hepatic production of CRP was increased after any type of inflammation, and subsequently the protein was proposed as a prognostic factor of severe pancreatitis. Values greater than 120 mg/L can detect between 67 and 100% of pancreatic necrosis (29). However, in most studies the cutoff concentration for CRP is 150 mg/L. Identical results have been published by Puolakkainen and coworkers (63). Moreover, the increased detection of both CRP and neutrophil elastase in plasma accurately predicts the outcome of the disease (84). However, because a 24- to 48-h latency is necessary before detecting a CRP increase in plasma, other predictive mediators, such as cytokines, have been proposed (42, 43).

Glucose and urea. Glucose and urea are two criteria included in Ranson’s scoring system. In 1993, Fan and coworkers (85) evaluated whether isolated serum urea (> 7.4 mmol/L) and plasma glucose levels (> 11 mmol/L) on Day 1 might predict the outcome of the disease. They concluded that serum urea and glucose concentrations (also known as the Hong Kong criteria) were two independent variables useful to determine the outcome. The urea/glucose criteria were comparable in overall accuracy with the APACHE II (sensitivity and specificity of 79% and 67% for urea/glucose criteria and 45% and 80% for APACHE II score, respectively). In contrast, in 1997, Heath and coworkers (86) reported that the Hong Kong criteria were not effective in predicting the severity of the disease whereas the best prediction was achieved by the APACHE II score determined 24 h after admission.

Hepatocyte growth factor (HGF). HGF is a potent mitogen of cultured rat hepatocytes and a trophic factor for hepatic regeneration. In 38 patients suffering from acute pancreatitis, Ueda and coworkers (87) showed that serum HGF concentrations were nine times higher in patients with acute pancreatitis than in normal subjects and significantly higher in severe than in mild attacks. The sensitivity and specificity for the detection of severe pancreatitis were 71% and 86%, respectively. Serum HGF concentrations on admission were almost 3 times higher in the nonsurvivors than in the survivors. Although the mech-
anism of increased serum HGF concentrations is unclear, this study showed that serum HGF might be an important factor in predicting the severity of acute pancreatitis.

**Intercellular adhesion molecule-1 (ICAM-1).** After the expression of the glycoprotein ICAM-1 on cell membranes, neutrophils adhere to the endothelium and migrate into various tissues during inflammation (24). The soluble form of the adhesion molecule or sICAM increases during inflammation, including pancreatitis. An enhanced plasma release of sICAM was observed in the early stage of acute necrotizing pancreatitis (88). In this study, the sensitivity and specificity to detect acute pancreatitis were 75% and 85% respectively. These promising results remain to be confirmed.

**Lactate dehydrogenase (LDH).** LDH, one of the 11 criteria of the Ranson score, has also been studied independently. Chen and coworkers (89) found that in 42 patients with acute pancreatitis, serum LDH activity was significantly higher in severe than in mild attacks. Moreover, by evaluating the distribution of the five known LDH isoenzymes, they found that LDH-4 and LDH-5 were the only isoenzymes increased during the disease and that LDH-4 was the only isozyme that could differentiate between severe and mild attacks. However, because the predominant pancreatic isoenzymes are LDH-2 and LDH-3, these results showed that the pancreas was not the major source of LDH. In the study by Uhl and coworkers (90), LDH measurements detected 82% of necrotizing pancreatitis in a cohort of 52 patients (23 severe and 29 mild disease). Finally, in a retrospective review of 50 patients with AIDS suffering from acute pancreatitis (5 severe, 45 mild), the Ranson score yielded a reasonable sensitivity (80%) but a poor specificity (54%) with regard to severity (91). The major pathologic markers were decreased serum Ca2+ concentrations and an elevated serum LDH.

**Neopterin.** Because macrophages are another type of cell activated during pancreatitis, the severity of the disease has also been investigated by measuring neopterin, a marker of macrophage activation. In the study by Uomo and coworkers (92), the serum neopterin concentrations were higher in severe than in mild pancreatitis at Day 1. Neopterin serum values did not correlate with IL-6 and TNF-α concentrations at any day. In the study by Mora and coworkers (93), there was a significant correlation between neutrophil elastase and neopterin values on Days 1 and 2 in a cohort of 26 patients with severe pancreatitis and 26 patients with mild pancreatitis. However, neopterin was not superior to neutrophil elastase in predicting the severity of the disease: neutrophil elastase predicted the severity at Day 1 with a sensitivity of 77% and a specificity of 92% whereas for neopterin these values were 21% and 93% respectively (93). In a prospective study of 25 patients with acute pancreatitis (94), serum neopterin concentrations were correlated with the severity of the disease determined by the APACHE II score. In this study, the discrimination between mild and severe pancreatitis was higher for neopterin (sensitivity 80% and specificity 100%) than for CRP (70% and 87%, respectively). Although neopterin is a good marker, its measurement is not available in most institutions.

**Neutrophil elastase.** Neutrophil elastase has also been chosen as an early predictive factor in acute pancreatitis. For example, neutrophil elastase concentrations are higher in severe acute pancreatitis than in the mild form of the disease (90). With a high positive predictive value the day after admission, neutrophil elastase seems to be a reliable marker of severe pancreatitis. Moreover, the plasma detection of neutrophil elastase precedes the detection of CRP. However, neutrophil elastase does not fulfill the five criteria required to be accepted as a new marker and the reproducibility of the assay needs to be improved.

**Procalcitonin.** Apart from the factors mentioned previously, procalcitonin has been proposed as a valuable tool for the noninvasive diagnosis of infection in pancreatic necrosis. Rau and coworkers (95) found that the concentrations of procalcitonin (the precursor of calcitonin synthesized in the thyroid) were higher in patients with infected pancreatic necrosis than in those with sterile necrosis, whereas the CRP concentrations were similar. In contrast, a recent study published by Müller and coworkers (96) showed that procalcitonin and granulocyte colony-stimulating factor were not suitable for the early prediction of infected pancreatic necrosis. Another marker of inflammation, serum amyloid A has also been investigated with procalcitonin in early assessment of severe acute pancreatitis (97). In this study, the sensitivity of serum amyloid A was higher than that of procalcitonin in assessing the severity of the disease whereas procalcitonin and CRP had a higher specificity than serum amyloid A. Serum amyloid A was significantly higher in patients who had complications, such as necrosis, infection, or multiple organ dysfunction syndrome and better discriminated necrotizing from interstitial edematous pancreatitis (98). However, CRP provided an earlier differentiation between both entities.

Among the new criteria of severity described in this review, TAP is the only one which has been fully validated. IL-6 and IL-8 seem potentially useful but do not fulfill all the criteria to be considered as an important marker. Moreover, none of them can be measured with a simple and automated kit readily available in chemistry clinical laboratories.

**THERAPEUTIC INTERVENTIONS IN HUMAN ACUTE PANCREATITIS**

Numerous pharmacologic treatments for severe pancreatitis have been proposed, but clinical results have been disappointing. Administration of proteolytic enzymes inhibitors, steroids, and inhibitors of pancreatic exocrine secretion did not alter the course of severe pancreatitis (76–78). In recent years, attention has been directed toward cytokines and other mediators of inflammation that play an important role in the pathophysiology of the disease. When patients with severe acute pancreatitis were treated with a potent inhibitor of PAF at admission for as long as 3 (99) or 7 (100) d, the severity score for organ dysfunction was lower in this group than in the group of patients treated with saline. However, a recent study failed to confirm the efficacy of inhibition of PAF to improve the outcome of severe disease (101).

**CONCLUSION**

Traditional severity scores have been used successfully by most physicians to detect severe acute pancreatitis. These scores assess the multiple organ dysfunction induced by the disease and consequently, the greater the number of organs injured, the greater the score. To predict the severity of the pancreatic disease itself, before the occurrence of the multiple organ dysfunction, other factors have been measured. These factors are either enzymes released from the pancreas (TAP and CAPAP) or mediators induced by the inflammation in the pancreas and distant organs (cytokines, adhesion molecules, and CRP). For most of them, the higher the blood concentration of these enzymes or mediators, the more severe the disease will be. However, standard scores and CRP remain the most effective methods in routine use between Day 2 and 3. Among the other markers listed in this review, only TAP has been adequately validated. Nevertheless, its assay must become fully
automated to improve its clinical availability. The clinical usefulness of IL-6 and IL-8 needs to be confirmed.

Moreover, when a new predictive factor is added to a traditional severity score, the ability to detect the severe forms of the disease, before the occurrence of multiple organ dysfunction, increases. It is then conceivable that the therapeutic antagonism of these mediators might prevent or attenuate the severity of the multiple organ dysfunction and consequently, improve the outcome of the disease. However, the most important clinical study concerning PAF has been disappointing. Whether other markers pointed out in this review might be more important targets for therapeutic interventions in the near future remains to be investigated.

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