Three decades of gastroenterology in Soweto South Africa: from descriptive to scientific observations
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Chapter 14

Acute Pancreatitis in Soweto, South Africa: Relationship Between Trypsinogen Load, Trypsinogen Activation, and Fibrinolysis


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Objective: It is not known why acute pancreatitis in Soweto, South Africa, pursues an aggressive course. We sought clues from circulating trypsinogen load at admission as marker of initial acinar injury, trypsinogen activation using the carboxypeptidase B activation peptide as surrogate, proteinase inhibitors, the coagulation-fibrinolysis axis, indicators of inflammation, oxidative stress markers, and antioxidant status. This article reports on the first four aspects. Methods: The study involved 24 consecutive patients with a first attack. All of them were admitted within 24 h, and 22 were alcoholic. Urine was analyzed for anionic trypsinogen and the carboxypeptidase B activation peptide. Serum was tested for anionic and cationic trypsinogen, α, proteinase inhibitor, and α₂ macroglobulin. Plasma from a subset was assayed for soluble fibrin, cross-linked fibrin degradation products (surrogates for thrombin and plasmin activity, respectively), and tissue-type plasminogen activator and inhibitor. Results: Soweto controls had higher serum, anionic trypsinogen (p = 0.004) and plasminogen activator inhibitor ratio (p = 0.047) than U.K. controls. The outcome of acute pancreatitis was mild in 17 but severe in seven with three deaths, two on day 2. In mild pancreatitis, intense plasmin activity (p < 0.001) accompanied the surge in trypsinogen, especially anionic (p < 0.001), but without increased thrombin activity and in five patients without trypsinogen activation. In severe pancreatitis, further significant increments in plasmin activity and trypsinogens were accompanied by increased thrombin activity (p = 0.013) and trypsinogen activation (p = 0.046). There was no correlation between surrogates of plasmin and thrombin activity, or between either and the carboxypeptidase B activation peptide, which showed a curvilinear relationship to total serum trypsinogen. Conclusions: The aggressive nature of alcoholic acute pancreatitis in Soweto seems to reflect early profound fibrinolysis, which precedes coagulation and is initially independent of trypsin. Subclinical acinar-cell injury and a profibrinolytic diathesis in outwardly healthy Sowetans may predispose to this problem.

Introduction

In Soweto, South Africa, alcoholic pancreatitis is common (1), and the first attack pursues an aggressive course with significant long-term morbidity (2). These idiosyncrasies occur against a background of micronutrient antioxidant insufficiency and oxidative stress in outwardly healthy Sowetans (1).

Studies from developed countries associate the following disturbances with severe acute pancreatitis: increase in plasma fibrinogen-fibrin degradation products (3-8), trypsinogen activation (9-15), huge load of trypsinogen in blood and urine (16-18), depletion of α₂
macroglobulin (19), exaggerated inflammatory reaction (20), profound oxidative stress (20), and antioxidant depletion (20). It is assumed that heightened plasmin activity is a response to activation of prothrombin by prematurely activated trypsin (19). However, in none of the cited articles were circulating trypsinogen load, markers of trypsinogen activation and of the thrombin-plasmin balance (Fig. 1) assessed concurrently. Trypsinogen load is relevant because it seems to reflect the redirection of secretions from the pancreatic acinar cell (21), after the disease-initiating blockade in the regulated secretory pathway (21, 22) and also because it could have a bearing on trypsinogen autoactivation (23). Further, in relatively few patients has any of these aberrations been evaluated during the critical first 24 h, and in no report has the potential impact of antioxidant deficiency/oxidative stress on trypsinogen behavior or hemostasis disturbances been considered. Most studies describe changes at or after 48 h from the start of symptoms, or admission, by which stage the worst affected patients may have died (24).

Prospective studies in groups at risk of acute pancreatitis reveal that, whereas blood trypsinogen shows an early presymptomatic surge, 24-48 h elapse before a significant increase in markers of active trypsin in blood and/or urine (25-27). The delay seems to indicate that as in experimental pancreatitis (28), the early intra-acinar phase of trypsinogen activation (22) is not propagated extracellularly. It also raises the possibility that the coagulation disturbance is independent of trypsin and, instead, may be part and parcel of the oxidant-charged hyperinflammatory state, which tends to immobilize proteinase inhibitors (20). In fact, plasmin and thrombin have been considered as potential activators of trypsinogen during acute pancreatitis (23) because these serine proteinases and trypsin have overlapping substrate specificities (29). In vitro studies show that plasmin activates trypsinogen, albeit less well than trypsin (30). Although there is disagreement as to whether thrombin does (29) or does not (23, 30) possess this property, recent studies suggest it may have a physiological role in inhibiting fibrinolysis by activating procarboxypeptidase B (31) (Fig. 1).

We now report data on trypsinogen load, trypsinogen activation, proteinase inhibitors, and the coagulation-fibrinolysis axis within 24 h of symptoms in Sowetan Africans with acute pancreatitis, bearing in mind parallel information on the inflammatory response, oxidative stress, and antioxidant status. Our overall aim was to seek clues to the aggressive nature of the disease in Soweto. A subsidiary aim was to clarify the role of trypsinogen activation in plasmin and thrombin activation and visa versa.
Figure 1. Schematic representation of operational links between the contact system of plasma proteolysis, coagulation, and fibrinolysis pathways. Items in Roman numerals are serine proteases of coagulation pathways; the suffix "C" indicates the activated form. Encircled positive symbols indicate activation of a reaction or system; negative symbols indicate inhibition. Only the main pathways are indicated, whereas in reality there are numerous feed-back and feed-forward loops wherein the endothelium plays dynamic roles. Asterisks indicate substances that were measured in the present investigations. Abbreviations: PF = platelet-derived factor; Ca²⁺ = ionized calcium; TF = tissue factor; PL = phospholipid; FPA = fibrinopeptide A, which splits off fibrinogen to allow formation of monomers; SF = soluble fibrin; XL = cross-linked; FDP = fibrin degradation products of which all but fragment E are reflected in the XL-FDP assay; HMWK = high molecular weight kininogen; tPA, uPA = tissue-type and urokinase plasminogen activators; C3, C3a = complement factors 3 in native and activated forms, shown to indicate recruitment of this system by plasmin; C1-INH = C1 esterase inhibitor, which also inhibits factor XE₉, kallikrein, and bradykinin; a₂M = alpha 2 macroglobulin, which inhibits all classes of proteases; AT-III = antithrombin III, which inhibits thrombin, factors XIIa, Ma, IXa, Va, and kallikrein; proteins Ca, Sa = activated forms of proteins, which inhibit factors Va and VHa; PAI-1 = plasminogen activator inhibitor; a₆P = alpha 2-antiplasmin; a, PI = alpha 1 proteinase inhibitor whose main function is to inhibit trypsin and neutrophil elastase; pro-CPB = procarboxypeptidase B, which is nowadays regarded as synonymous with TAFT (thrombin activatable fibrinolysis inhibitor), and provides an explicit molecular link between activation of coagulation and fibrinolytic systems (31).
Materials and methods

Patients and Controls
The study protocol was approved by the Ethical Committee of the University of the Witwatersrand, South Africa, and every patient gave prior informed consent. From August, 1994, in a 6-month period, 25 consecutive patients with a first attack of acute pancreatitis were entered, but one actually had a perforated duodenal ulcer. Within the group of 24 (21 men and three women, age median and range, 37, 24-88 yr), excessive alcohol consumption was documented in 17 (1400, 700-3675 g/l/k for 18, 7-35 yr) and strongly suspected in four. An alcoholic binge preceded admission in one of the first group and three of the second. Of the remaining three patients, one was an exdrinker, another had gallstones, and the last had idiopathic disease.

Two control groups of Sowetans were studied. The first involved 12 outwardly healthy hospital workers (10 men, two women, 41, 28-49 yr). The second comprised nine patients (seven men, two women, 36, 27-73 yr) with other acute abdominal crises: two with appendicitis; one each with perforated duodenal ulcer, colonic obstruction, incarcerated umbilical hernia, sigmoid volvulus, severe pelvic inflammatory disease, or perforated carcinoma of the colon; one unexplained. Eleven of the 12 healthy controls drank 20-30 units of ethanol per week, usually on the weekend, as did seven of the nine disease controls. Studies in 14 healthy hospital workers at Manchester, U.K (eight men, six women, 44, 22-58 yr) provided a reference frame: 10 of them drank some 30 units of ethanol per week, five did so mainly on the weekend, and five during the whole week.

Sample Collection and Chemical Methods
Approximately 30 ml of peripheral venous blood and a urine specimen were obtained at admission from patients, who had eaten very little for ≥12 h, or after an overnight fast from healthy controls. The blood sample was divided between plain tubes for serum studies and acidified citrate tubes for plasma studies. The prepared material was snap-frozen in suitable fractions, stored at -70°C and batch transferred to Manchester for analysis. The present article focuses on the following aspects: serum analysis for anionic and cationic trypsinogen isoforms (18), α₁ proteinase inhibitor, and α₂ macroglobulin; plasma studies to assess thrombin and plasmin behavior (32-34); and further analysis of anionic trypsinogen in urine (18) with assay of the urinary carboxypeptidase B activation peptide (CAPAP) as surrogate
marker of trypsinogen activation (13). The complete investigation also involved measurement in appropriate blood fractions of oxidative stress markers, glutathione, micronutrient antioxidants, LDH isoenzymes, and staging markers of the inflammatory response. In consideration of the known stability periods for the various assays, the oxidative stress markers were analyzed within 3 wk of sample receipt, antioxidants within 3 months, inflammatory markers, measures of hemostasis, and antiproteases within 6 months. However, the assays for trypsinogen and CAPAP in Malmo, Sweden, were done early in 1998 after the methods had been streamlined (13, 18). Results on inflammatory markers (35), oxidative stress, and antioxidants (36) have been presented in abstract form, and details will be reported separately. However, data on C-reactive protein have been summarized herein (Table 1), as an objective gauge to the duration of the disease (37), and also relationships between antioxidant levels and enzyme markers of the present report.

Total trypsinogen in serum was derived from the sum of its anionic and cationic isoforms, which were measured by solid-phase double-antibody ELISA (18), and CAPAP was measured by radioimmunoassay (Urodiagnostica AB Malmo, Sweden) (13). In acute pancreatitis, urine is a convenient medium for anionic trypsinogen analysis because it contains little of the cationic isoform (13, 38). It is also the preferred medium for assay of CAPAP because high-circulating levels of procarboxypeptidase B show immunoreactivity in the serum assay, but the zymogen does not enter urine (13).

Soluble fibrin, as a surrogate marker for thrombin activity (Fig. 1), was measured by ELISA, incorporating a monoclonal capture antibody specific for the neceptope on the γ chain of fibrin, fanned when fibrinopeptide A is cleaved from fibrinogen, and does not cross-react with fibrinogen (Soluble Fibrin EIA, Ortho Diagnostic Systems, Ranatan, NJ), together with a tag monoclonal antibody specific for the D region of fibrin in fibrinogen (4D2, AGEN Biomedical, Brisbane, Australia) (32). Cross-linked fibrin degradation products (XL-FDP), as a surrogate marker for plasmin activity (Fig. 1), were measured by ELISA, using a monoclonal capture D-dimer antibody specific for the cross-linked D region of fibrin (DD-3B6, AGEN Biomedical) and a fibrinspecific tag D-dimer antibody (DD-ID2, D-dimer Gold, AGEN Biomedical) (33): this assay is said to preferentially report on higher molecular weight fragments (34). Commercially available COALIZA chromogenic methods (Chromagenix, Molndal, Sweden) were used for assay of tissue-type plasminogen activator (tPA) and its inhibitor (PAI-1) (Fig. 1).
C-reactive protein, $\alpha_1$ proteinase inhibitor, and $\alpha_2$ macroglobulin were measured on a Beckman array analyzer with commercially available material (Boehringer Mannheim, Chalfont St. Giles, U.K.), using rate nephelometry for the inflammation marker and immune complex nephelometry for the antiproteases.

Statistical Analysis
This was done by parametric and nonparametric methods with a significance level set at $p < 0.05$ (two tailed), but nonparametric tests were preferred (Mann-Whitney U, paired signed rank, Kruskal-Wallis, Dunn's correction for multiple comparison, Pearson correlation coefficient) because in the acute pancreatitis samples several variables were highly skewed (39). Hence, results in the text are given as medians and ranges, and a "box-and-whisker", format is used for the figures. Data processing was facilitated by GraphPad Prism, Version 3.00 for Windows (GraphPad Software, San Diego, CA).

Results

Healthy Controls
The concentration of anionic trypsinogen in serum was higher in healthy Sowetans than in Manchester controls (20.9 -53 $\mu$g/L vs 10.7-17 $\mu$g/L, $p = 0.004$), whereas concentrations of cationic trypsinogen in serum and of the anionic isoform and CAPAP in urine were of the same order (data not shown). Controls in Soweto also showed similar levels to Manchester controls of tPA, PAW, soluble fibrin, and XL-FDP (data not shown); however, their tPA:PAI.1 ratio was higher (0.14,0.07-0.38 vs 0.07,0.02-0.30, $p = 0.047$).

Disease Controls.- Acute Abdomen
Trypsinogen, CAPAP, and hemostasis profiles in eight of nine patients in this group conformed with those in the African controls. The profile in a patient with a perforated duodenal ulcer and serum amylase of 800 u/L (normal 10-220 u/L with Boehringer Mannheim kit) was the exception. It showed a modest increase in serum and urinary concentration of anionic trypsinogen (118 and 175 $\mu$g/L, respectively), normal levels of serum cationic trypsinogen and urinary CAPAP, and a pattern of hemostasis disturbance that was indistinguishable from that in mild pancreatitis (data not shown).
### Table 1: Summary of results

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Acute Pancreatitis</th>
<th>p (Two-Tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (S) mg/L</td>
<td>6 (2-11)</td>
<td>78 (5-340)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total trypsinogen (S) µg/L</td>
<td>37 (20-80)</td>
<td>2768 (144-8,615)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anionic trypsinogen (S) µg/L</td>
<td>20 (9-53)</td>
<td>2275 (117-2,804)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cationic trypsinogen (S) µg/L</td>
<td>18 (11-27)</td>
<td>546 (27-3,088)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anionic trypsinogen (U) µg/L</td>
<td>0 (0-11.5)</td>
<td>5530 (26 -&gt; 25,000)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAPAP (U) nmol/L</td>
<td>1.30 (0.09-2.22)</td>
<td>16.0 (0.40-404)</td>
<td>0.002</td>
</tr>
<tr>
<td>α₁ proteinase inhibitor (S) g/L</td>
<td>1.41 (1.11-1.80)</td>
<td>2.19 (1.34-4.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>α₂ macroglobulin (S) g/L</td>
<td>2.05 (1.63-3.01)</td>
<td>2.19 (1.23-3.98)</td>
<td>0.594</td>
</tr>
<tr>
<td>tPA (P) µg/L</td>
<td>7.8 (5.1-20)</td>
<td>7.4 (4.7-35)</td>
<td>0.859</td>
</tr>
<tr>
<td>PAI-1 (P) µg/L</td>
<td>54 (22-113)</td>
<td>55 (19-104)</td>
<td>0.580</td>
</tr>
<tr>
<td>tPA:PAI-1 (P)</td>
<td>0.14 (0.07-0.38)</td>
<td>0.24 (0.10-0.64)</td>
<td>0.054</td>
</tr>
<tr>
<td>Soluble fibrin (P) mg/L</td>
<td>0.98 (0.30-4.5)</td>
<td>2.4 (0.40-10)</td>
<td>0.019</td>
</tr>
<tr>
<td>XL-FDP (P) µg/L</td>
<td>26 (10-100)</td>
<td>596 (56-2,000)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data as medians and ranges from analysis of serum (S), plasma (P), or urine (U) at admission in the patients (24 for trypsinogen and antiprotease studies, 17 for hemostasis studies), and after an overnight fast in local controls. Comparisons by two-tailed Mann-Whitney U tests. Urinary α-Tg concentrations >25,000 µg/L were not resolved further. For conversion from mass to SI units, the molecular weight of the human trypsinogen is around 23 kd; for conversion of SI to mass units for CAPAP, molecular weight = 9.96 kd. For the other analytes, precise molecular weight derivation is difficult because a substance such as C-reactive protein exists in more than one form.

* Indicates data significantly different between mild and severe pancreatitis subgroups
* Indicates significant difference from controls at Manchester, United Kingdom

**Acute Pancreatitis**

Patients reported a delay of 12-18 h from the start of symptoms, but the concentration of C-reactive protein in admission serum samples was generally elevated (Table 1). With reference to the pattern when pancreatitis follows ERP (37), the increase indicated a time lapse of some 24 h in the majority. Seventeen patients recovered rapidly: x-rays at admission showed pleural effusion in two, basal atelectasis in one, a "sentinel loop" in three, and "colon cutoff" in two patients. Seven patients became gravely ill despite prompt supportive care. Three of them died, aged 25, 34, and 38 yr, all of whom presented after an alcoholic binge, against a background of some 700 g of alcohol consumption per week for 17 yr in one patient and suspected excess in the others. Two deaths occurred on the day after admission and the third on day 17 from adult respiratory distress syndrome, which was accompanied in the first two by renal tubular necrosis and in the third by ketoacidosis; the other four needed intensive
care for 9-26 days for acute lung injury, coupled with renal injury in two and ketoacidosis in two patients.

Admission serum samples showed a huge excess of trypsinogen, with contributions by both isoforms but with the anionic form predominant (Table 1): levels of anionic, cationic, and total trypsinogen were higher in severe than mild pancreatitis, and this was also true for the anionic isoform in urine (Fig. 2). There was no increase in urinary CAPAP in five patients with mild disease, in contrast to a clear signal in all with severe pancreatitis (Fig. 2). For the group as a whole, serum α₁, proteinase inhibitor concentration at admission, but not that of α₂ macroglobulin, represented a significant increase over the level in controls (Table 1); however, there was no difference between its concentration in subgroups with mild or severe disease (data not shown).

After the analysis of plasma for antioxidants, free radical markers and complexes of neutrophil elastase with α₁ proteinase inhibitor, sufficient material was available for the hemostasis marker studies in 17 patients. This group consisted of 12 with a mild outcome including the five with no increase in urinary CAPAP, and five with a severe outcome including the three who died. Platelet counts at admission were within normal limits in every patient (data not shown). In the subset with mild pancreatitis, a surge in plasma XL-FDP level (333, 56-1126 μg/L, p < 0.001 vs controls) was not accompanied by alteration in the level of soluble fibrin (1.6, 0.4-7.8 mg/L), tPA, or PAM (data not shown). In the subset with severe pancreatitis, a markedly higher concentration of XL-FDP (1200, 476-2000 μg/L) was accompanied by an increase in soluble fibrin (5.0, 3.8-10 mg/L, p < 0.001 vs controls).

The tPA:PAI-I ratio doubled over the control value because a 1.5-fold increase in the inhibitor fell short of the Molar increase in the concentration of the activator (p values for significance vs control or mild subgroup, respectively: ratio 0.031, 0.082, inhibitor 0.114, 0.007, activator 0.004, 0.007) (Fig. 3). In retrospect, it was noted that an arbitrary value for admission XL-FDP ≥200 μg/L would have identified each of the patients with a severe outcome. It would also have identified six of the seven with a mild outcome in whom plasma was available for analysis among eight with admission x-ray evidence of pulmonary or intestinal reaction. Of interest, higher concentrations of XL-FDP were associated with lower concentrations of ascorbate, selenium and β-carotene (Table 2) (40).
A correlation matrix denied a link between trypsinogen activation and the activation of plasminogen or prothrombin and visa versa (Appendix). Instead, it disclosed significant positive influences on trypsinogen activation, as judged by urinary CAPAP measurement, of trypsinogen load, PAW, and C-reactive protein. Further exploration of these interactions by sum-of-squares analysis (Fig. 4) showed that clear increases in C-reactive protein or PAI-I not infrequently coexisted with an absent or insubstantial CAPAP signal. In contrast, there was a
striking exponential increase in CAPAP above a serum total trypsinogen threshold of around 3000 μg/L. This curvilinear relationship was also evident when the serum concentration of anionic or cationic trypsinogen was substituted in the equation, whereas the precise relationship between urinary anionic trypsinogen and CAPAP could not be calculated because zymogen values >25,000 μg/L were not resolved further.

**Discussion**

In theory, the aggressive nature of acute pancreatitis in Soweto could reflect any of the factors enumerated in the introduction, which are known from studies elsewhere to prejudice recovery. Our investigation sought to probe as many of those factors as possible, remembering that antioxidant deficiency impacts upon several of them (20), is prevalent in outwardly healthy Sowetans, and compounded by alcoholism (1). We chose urinary CAPAP as a surrogate marker of trypsinogen activation, rather than measuring the trypsinogen activation peptide directly, because serum/urine of healthy controls has little or no CAPAP but readily measurable amounts of trypsinogen activation peptide (41). However, after the analysis was completed, an article appeared on the activation by thrombin of procarboxypeptidase B in vitro (Fig. 1). This evidence potentially invalidated our work and persuaded us to delay the present report until we were reassured from a study of myocardial infarction that CAPAP is not released into the systemic circulation under conditions of increased thrombin activity in vivo (42).

| Table 2: Relationships between concentrations of micronutrient antioxidants and markers of zymogen activation in admission samples |
|-----------------------------|-------------|----------------|----------------|
|                             | tPA         | XL-FDP         | Soluble Fibrin | CAPAP     |
| Ascorbate                   | -0.742 *    | -0.638 *       | 0.624          | -0.210    |
| Slenium                     | -0.232      | -0.485 *       | 0.084          | -0.086    |
| β-carotene                  | -0.433      | -0.515 *       | -0.410         | -0.285    |
| α-tocopherol                | 0.243       | -0.078         | 0.187          | 0.226     |

Plasma ascorbate, serum β-carotene, and α-tocopherol were measured by high performance liquid chromatography, and serum selenium by acid digestion fluorimetry, as described in our earlier studies showing antioxidant deficiency in Soweto controls (1). Correlations were assessed by Spearman’s test, two-tailed. XL-FDP is marker of plasminogen activation. Soluble fibrin is a marker of prothrombin activation. CAPAP is a surrogate marker of trypsinogen activation.

*p* < 0.05 (40)
The investigation of outwardly healthy Africans showed a higher concentration of anionic trypsinogen in serum than in U.K. controls, in line with our earlier work showing their higher serum level of pancreatic isoamylase (43). In experimental studies (21, 44), such increases represent the diversion into the pancreatic interstitium and portal bloodstream of acinar products when the cell's exocytosis machinery is disrupted, apparently as a result of increased free radical activity (45) from a variety of sources, including ethanol (46). The concurrence of oxidative stress and increased serum levels of pancreatic enzymes in Sowetan controls (1) may thus indicate subclinical oxidant-mediated injury to acinar cells. Data from the present work also identified a profibrinolytic state in these nondiabetic nonalcoholic controls under resting conditions, their higher tPA:PAI-I antigen ratio than in U.K. controls a reminder of a study showing lower PAI-I activity in rural sub-Saharan Africans than in urban whites (47).
Figure 4: Computer-assisted sum-of-squares analysis of relationships that were positive in the correlation matrix (Table 2). Open triangles and closed triangles represent subsets of mild and severe pancreatitis, respectively. There was no urine sample for CAPAP in a patient with severe disease in whom serum CAPAP was elevated at 12nmol/L, and also in two with mild disease and serum values increased at 3 and 10nmol/L; two of these missing samples were from the subset of 17 patients in whom hemostasis markers were measured, and hence there are only 15 data points in the middle frame, which relate CAPAP and PAI-1.

Whether the explanation is genetic or diet related, profound oxidative stress and antioxidant depletion associated with acute pancreatitis, irrespective of geography (20, 46) and extending to Soweto (36), would amplify this tendency because, whereas the inhibitor is vulnerable to oxidant attack (48), the catalytic activity of plasminogen activator and its binding affinity for plasminogen are resistant (49).

In the group with acute pancreatitis as a whole, data on trypsinogen load, trypsinogen activation, fibrin degradation products, and proteinase inhibitors were broadly comparable with data from other countries, as cited earlier. However, a subgroup analysis revealed unexpected findings concerning the relationship of fibrinolysis to coagulation and trypsinogen
activation. Thus, in mild pancreatitis, within 24 h from the start of symptoms, which equates to around 27-31 h from disease onset (37), there was a median 12-fold increase in plasma XL-FDP concentration, such that the level was as high as after streptokinase treatment of myocardial infarction (34). Further, this surge occurred without a concomitant increase in soluble fibrin, about 12 h before a rise in fibrinogen is expected (8) and in five patients without an increase in urinary CAPAP. The high specificity of the XL-FDP assay (Fig. 1) negates measurement artifact (50). Conversely, underestimation of soluble fibrin is very unlikely because this assay (Fig. 1) is thought to be the best among the available measures of thrombin generation and activity in vivo (51).

Perusal of the literature disclosed two sets of clinical observations similar to ours. First, hypocoagulability was a striking feature in a report on 55 patients, some of whom were studied within 3 h of symptoms' onset (52). Second, analysis of venous effluents from pancreas transplants showed a marked increase in fibrinolytic activity during the first 10 min after reperfusion; this increase was transient and occurred in advance of prothrombin activation (53). Hence, the deduction seems to be that early in the course of acute pancreatitis, preformed soluble fibrin quickly polymerizes, via a mechanism that does not involve increased thrombin activity (Fig. 1), to serve as a substrate for plasmin and later also for trypsin. This notion of enhanced physiological fibrinolysis has been proposed previously in the setting of myocardial infarction (34). The dissociation between enhanced plasma fibrinolytic activity and unchanged coagulation in our patients with mild pancreatitis and of hypocoagulability at admission in a previous investigation (52) brings to mind the pattern that has been described in experimental endotoxaemia and also when the systemic inflammatory response syndrome is provoked by sepsis (54). The pattern suggests early and preferential (54) activation of the 'contact system' (54, 55) (Fig. 1), with its profibrinolytic, anticoagulant (via thrombin inhibition), antiadhesive and proinflammatory properties (55).

The evidence from our study suggests that significant activation of this system might be identified by an admission concentration of XLF-DP ≥200 μg/L, as occurred in the majority of patients in whom lung and/or intestinal reaction was indicated by x-rays at admission. It was evident, too, that whatever the explanation for contact system activation might be, it could also apply to the identity of hemostasis profile in patients with perforated duodenal ulcer, as was noted in our patient and in the earlier study that identified hypocoagulability (52).

The coagulation marker profiles associated with severe pancreatitis in Sowetans indicated further activation of fibrinolysis but now with enhanced coagulation. That, too, is consistent
with the natural progression of contact system activation (55), the continuing influence of which was suggested by comparing data on XL-FDP and soluble fibrin with corresponding data in patients with complicated myocardial infarction (34). Levels of XL-FDP were four times higher in acute pancreatitis, whereas levels of soluble fibrin were four times lower. This interpretation is in line with the prolonged depletion of serum α₂ macroglobulin in both acute pancreatitis (8, 19, 56) and after fibrinolysis therapy (8, 57), but with the difference that plasminogen levels remain low for some time in the former but recover rapidly in the latter setting (57). It is also consistent with the far higher concentration in peritoneal fluid than in plasma of many proteinases, including those that indicate activation of the contact system (57, 58), but a lower proportion of functional antiproteases (8, 56).

The correlation matrix in our study seemed to deny a direct role for trypsin in the activation of plasminogen during the first 24 h of alcoholic acute pancreatitis (Appendix). However, increasing trypsin release is expected after this time point (10-15) and would fuel both plasminogen and prothrombin activation (8). A possible explanation for trypsinogen activation is suggested by the striking relationship that we found between an exponential increase in serum trypsinogen load and a linear increase in urinary CAPAP (Fig. 4). This pattern is reminiscent of that described in a study of post-ERCP pancreatitis between serum anionic trypsinogen and anionic trypsin-antitrypsin complexes using a double-log plot (25). The evidence hints at trypsinogen autoactivation (23) or a process with similar kinetics that operates within the pancreatic interstitium, where zymogens; accumulate in experimental pancreatitis, and which has been identified as the site of pathologically significant activation of trypsinogen (44). In contrast, we regard the positive correlation between C-reactive protein or PAI-1 and CA-PAP (Table 2) as being spurious, in that near-zero values for urinary CAPAP often coexisted with clear increases in the inflammatory markers (Fig. 4). We suggest that this reflects the shared property of C-reactive protein and PAI-1 as acute phase reactants (59).

In summary, our study of admission samples pointed to heightened fibrinolysis as a major factor in the aggressive nature of alcoholic acute pancreatitis in Sowetan Africans. It also suggests that subclinical acinar cell injury and a profibrinolytic diathesis in outwardly healthy Sowetans may be relevant to this susceptibility. Whereas today's "autodigestion-cytokine model" for the pathogenesis of life-threatening pancreatitis does not accommodate these observations, they are rationalized in terms of mast cell pathology (Fig. 1) and its relationship to antioxidant insufficiency (60). From a practical perspective, there are three main implications of our work: to limit the patient's exposure to drugs and chemicals that will
exacerbate fibrinolysis and related systemic injury (4, 6) by inducing further bouts of mast cells degranulation; to consider parenteral antioxidant supplements so as to curb plasmin activation (Table 2) and activity (Fig. 1); and to recognize that rebound hypercoagulability will require treatment in patients with severe disease. These therapeutic aspects have recently been reviewed (61).

REFERENCES


Appendix: Correlation matrix seeking explanations for Trypsinogen, Plasminogen, and Prothrombin Activation in Acute Pancreatitis.

<table>
<thead>
<tr>
<th></th>
<th>CAPAP (U)</th>
<th>t-Tg (S)</th>
<th>a-Tg (S)</th>
<th>c-Tg (S)</th>
<th>a-Tg (U)</th>
<th>TPA</th>
<th>PAI-I</th>
<th>SF</th>
<th>XL-FDP</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPAP (U)</td>
<td>1.000</td>
<td>0.713a</td>
<td>0.682a</td>
<td>0.735b</td>
<td>0.790a</td>
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<td>0.457*</td>
<td>0.463*</td>
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<td>t-Tg (S)</td>
<td>1.000</td>
<td>0.977c</td>
<td>0.953c</td>
<td>0.695a</td>
<td>0.635a</td>
<td>0.753c</td>
<td>0.698a</td>
<td>0.412</td>
<td>0.565b</td>
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<td>a-Tg (S)</td>
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<td>0.894c</td>
<td>0.567b</td>
<td>0.615a</td>
<td>0.679b</td>
<td>0.707a</td>
<td>0.412</td>
<td>0.564b</td>
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<tr>
<td>c-Tg (S)</td>
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<td>0.841c</td>
<td>0.538b</td>
<td>0.727b</td>
<td>0.589b</td>
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<td>a-Tg (U)</td>
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<td>0.444</td>
<td>0.614b</td>
<td>0.294</td>
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<td>0.707a</td>
<td>0.642b</td>
<td>0.738c</td>
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<td>PAI-I</td>
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<td>SF</td>
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<td>XL-FDP</td>
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Analysis in samples from 17 patients in whom plasma was available. Correlations with urinary anionic trypsinogen [a-Tg (U)] are imprecise because values >25,000 µg/L were not resolved further. CRP = C-reactive protein; c-Tg = cationic trypsinogen; S = serum; SF = soluble fibrin; t-Tg = total trypsinogen.

• 'p < 0.01, Spearman’s test, two tailed, the outcome was broadly similar using Kendall’s test
• 'p < 0.05, Spearman’s test, two tailed, the outcome was broadly similar using Kendall’s test
• 'p < 0.001, Spearman’s test, two tailed, the outcome was broadly similar using Kendall’s test