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# Coagulation and fibrinolytic responses of human peritoneal fluid and plasma to bacterial peritonitis

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Significantly higher ( $P < 0.05$ ) thrombin–antithrombin III complex levels were found in the abdominal exudate of patients with peritonitis (median 5500 ng/ml) than in that of controls (median 89 ng/ml). In patients, peritoneal fluid concentrations of tissue and urokinase-type plasminogen activator were increased by factors of 65 and 10 respectively ( $P < 0.05$ ). The concentration of plasminogen activator inhibitor (PAI) 1 was increased by a factor of about 800 (median 395 versus 0.5 ng/ml,  $P < 0.05$ ). Despite

markedly raised concentrations of PAI, peritoneal fluid displayed fibrinolytic activity as demonstrated by significantly increased ( $P < 0.05$ ) concentrations of plasmin– $\alpha_2$ -antiplasmin complex (median 10 952 versus 57 ng/ml) and fibrin degradation products (median 40 360 versus 126 ng/ml). There was no correlation between plasma and peritoneal fluid concentrations. Intra-abdominal coagulation and fibrinolysis are stimulated in the abdominal cavity of patients with bacterial peritonitis.

In bacterial peritonitis, fibrin is formed in the peritoneal cavity as a result of activation of the intra-abdominal coagulation cascade. Intraperitoneal fibrin plays a key role in the local host response to bacterial invasion, and prevents early bacteraemia and sepsis by entrapment of bacteria<sup>1</sup>. The role of fibrin is only temporary and fibrin must be degraded for normal tissue function to resume. When intra-abdominal fibrin persists, it acts as a nidus for infection, resulting in an intra-abdominal abscess<sup>2</sup>. In patients with bacterial peritonitis, intra-abdominal abscesses are associated with marked morbidity and mortality<sup>3,4</sup>.

For the degradation of intra-abdominal fibrin, stimulation of the intraperitoneal fibrinolytic system is required. However, fibrinolytic activity is reduced during bacterial peritonitis, as has been demonstrated in several experimental studies<sup>5–7</sup>. Diminished release of tissue plasminogen activator (tPA) and increased release of plasminogen activator inhibitor (PAI) from peritoneal tissue have been considered to be important reasons for the reduced fibrinolysis<sup>7</sup>. Recently, the authors have demonstrated an increase in both tPA and PAI activity in the abdominal exudate of rats with faecal peritonitis, but this did not correlate with the extent of peritoneal damage<sup>6</sup>. In patients with peritonitis, research has thus far been confined to the fibrinolytic properties of the peritoneum<sup>8</sup>. The aim of the present study was to assess coagulation and fibrinolytic activity in the abdominal exudate and plasma of patients with bacterial peritonitis.

## Patients and methods

### Study design

Ethical approval was obtained from the hospital ethics committee. Twenty-five patients who underwent emergency laparotomy for peritonitis were studied. Peritonitis was due to perforated appendicitis in ten patients, duodenal perforation in five, perforated diverticulitis in five, small bowel perforation in two, toxic megacolon with perforation in two, and infected ascites due to pancreatic necrosis in one. Abdominal cultures revealed bacterial growth in all patients. There were 16 male and nine female patients of mean age 48 (range 11–90) years.

Seven patients undergoing elective cholecystectomy for gallstone disease served as controls (three men and four women, mean age 50 (range 12–75) years). Bacterial cultures of the peritoneal fluid were negative in all control patients. After incision and meticulous haemostasis, 10 ml of peritoneal fluid were aspirated from the abdominal cavity of all patients with peritonitis. In patients undergoing cholecystectomy, the amount of fluid aspirated varied between 0.5 and 2 ml, and was diluted with normal saline to a total amount of 10 ml.

Venous blood (10 ml) was drawn simultaneously with peritoneal fluid aspiration. Peritoneal fluid and blood were collected in a citrate tube (1:10) and a Stabilyte (Biopool, Umea, Sweden) tube (1:10) respectively, and placed in melting ice<sup>9</sup>. Within 30 min the samples were centrifuged at 4°C and the supernatants stored at –80°C until assays were performed.

Activation of coagulation was assessed by measuring the thrombin–antithrombin III (TAT) complex, as thrombin generation is the final step in the coagulation cascade before fibrin formation.

To analyse fibrinolysis, tPA, urokinase-type plasminogen activator (uPA), PAI-1, plasmin– $\alpha_2$ -antiplasmin (PAP) complex and fibrin degradation products were measured.

### Assays

The concentration of TAT complex was measured by enzyme-linked immunosorbent assay (ELISA) (Enzygnost TAT; Behringwerke, Marburg, Germany).

The level of tPA antigen was measured by ELISA (Asserachrom tPA; Boehringer, Mannheim, Germany). The assay measures both free and complexed (with PAI-1) human tPA and is calibrated against the international standard for tPA. tPA activity was measured by Coaset-tPA assay (Kabi Diagnostica, Molndal, Sweden), as described by Nilsson *et al.*<sup>10</sup>.

PAI-1 antigen levels were measured by ELISA (Biopool, Umea, Sweden). The assay measures free, latent as well as complexed (with tPA or uPA) human PAI-1. PAI activity was measured by Berichrom-PAI assay (Behringwerke), as described by Stief *et al.*<sup>11</sup>.

The level of uPA antigen in plasma and peritoneal fluid was measured by ELISA, according to the method of Binnema *et al.*<sup>12</sup>. Concentrations were expressed as a percentage of the uPA concentration in normal pooled plasma of healthy volunteers.

PAP complex was measured by ELISA (EIA APP micro; Behringwerke). Fibrin degradation products were measured by ELISA (Fibrinostika FbDP; Organon Technika, Turnhout, Belgium).

The validity of the assays was not affected by the dilution of peritoneal fluid in control patients.

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Table 1 Parameters of coagulation and fibrinolysis in the peritoneal fluid of patients with peritonitis and controls

Peritoneal fluid	Peritonitis (n = 25)	Controls* (n = 7)
tPA antigen (ng/ml)	66.1 (37.5–180.0)	1.1 (0.5–1.8)†
tPA activity (units/ml)	6.5 (3.7–11.9)	<0.25 (<0.25–0.3)†
PAI antigen (ng/ml)	395 (147–588)	0.5 (<0.5–1.3)†
PAI activity (units/ml)	14.3 (7.4–22.2)	<0.3 (<0.3 to <0.3)†
uPA antigen (%)	740 (168–2000)	78 (14–168)†
TAT complex (ng/ml)	5500 (2976–9094)	89 (30–198)†
PAP complex (ng/ml)	10952 (4834–15469)	57 (17–123)†
Fibrin degradation products (ng/ml)	40360 (6475–200000)	126 (31–463)†

Values are median (95 per cent confidence interval). \*Results corrected for dilution at sampling. tPA, Tissue-type plasminogen activator; PAI, plasminogen activator inhibitor; uPA, urokinase-type plasminogen activator; TAT, thrombin–antithrombin III; PAP, plasmin– $\alpha_2$ -antiplasmin. † $P < 0.05$  (patients with peritonitis *versus* controls, Mann–Whitney *U* test)

Table 2 Parameters of coagulation and fibrinolysis in the plasma of patients with peritonitis and controls

Plasma	Peritonitis (n = 25)	Controls (n = 7)
tPA antigen (ng/ml)	7.9 (4.9–12.6)	6.4 (3.4–10.3)
tPA activity (units/ml)	2.3 (1.7–3.3)	1.3 (0.9–2.4)
PAI antigen (ng/ml)	15.0 (10.2–22.3)	13.8 (5.9–39.9)
PAI activity (units/ml)	1.7 (0.9–2.9)	1.4 (0.8–2.3)
uPA antigen (%)	204 (138–272)	120 (96–172)*
TAT complex (ng/ml)	5.2 (3.3–16.5)	2.6 (1.4–5.5)
PAP complex (ng/ml)	709 (450–917)	381 (290–567)
Fibrin degradation products (ng/ml)	1201 (559–2867)	350 (193–594)*

Values are median (95 per cent confidence interval). tPA, Tissue-type plasminogen activator; PAI, plasminogen activator inhibitor; uPA, urokinase-type plasminogen activator; TAT, thrombin–antithrombin III; PAP, plasmin– $\alpha_2$ -antiplasmin. \* $P < 0.05$  (patients with peritonitis *versus* controls, Mann–Whitney *U* test)

#### Statistical analysis

Data are expressed as median values and 95 per cent confidence intervals. The Mann–Whitney *U* test was used for analysis of differences between groups. Correlations between plasma and peritoneal fluid values were analysed by Spearman's rank test.  $P < 0.05$  was considered significant.

#### Results

Activation of coagulation in the abdominal exudate of patients with peritonitis was demonstrated by significantly higher TAT complex concentrations, compared with controls (Table 1). Stimulation of the fibrinolytic system in the peritoneal fluid of patients with peritonitis was demonstrated by significantly raised levels of tPA, uPA, PAP complex and fibrin degradation products, compared with those found in the peritoneal fluid of controls (Table 1). Median peritoneal fluid concentrations of tPA and uPA in patients with peritonitis were about 65- and tenfold respectively higher than values in controls. PAI-1 antigen showed an 800-fold increase. Hence, concentrations of PAP complex and fibrin degradation products in the peritoneal fluid of patients with peritonitis were significantly raised in comparison with those in controls.

By contrast, plasma concentrations of TAT and PAP complex, and tPA and PAI-1 antigen and activity, were comparable in patients with and without peritonitis. Only plasma concentrations of uPA antigen and fibrin

degradation products in patients with peritonitis were higher than those in control patients (Table 2).

Neither in those with peritonitis nor in control patients did the level of any coagulation or fibrinolytic factor measured in the plasma correlate with that observed in the peritoneal fluid.

#### Discussion

This study demonstrates that, in the abdominal cavity of patients with bacterial peritonitis, both the coagulation cascade and the fibrinolytic system are stimulated. The resultant deposition of fibrin probably reflects a relative deficit of fibrinolytic activity, resulting from strongly enhanced inhibition of plasminogen activators.

The markedly raised concentration of TAT complex in the peritoneal fluid of all patients with peritonitis demonstrates intraperitoneal stimulation of coagulation. The coagulation cascade may be triggered by expression of tissue factor (procoagulant activity) on injured mesothelial and endothelial cells in the peritoneum<sup>13–15</sup> and on peritoneal macrophages, which express tissue factor following stimulation with, for example, endotoxin and tumour necrosis factor (TNF)<sup>15,16</sup>. The relative contribution of separate cell types to intra-abdominal coagulation has not yet been clarified. It seems that activation of coagulation was confined to the abdominal cavity, as the plasma TAT complex concentration in most patients with peritonitis was not raised. In a few patients with sepsis, high plasma levels of TAT complex were found, which is in concordance with earlier reports on the relationship between sepsis and systemic hypercoagulability<sup>17,18</sup>.

These data show increased levels of fibrinolytic activators following peritonitis. This finding is in agreement with that of Dörr *et al.*<sup>19</sup>, who reported increased fibrinolytic activity and fibrin degradation in the peritoneal fluid of women with pelvic inflammatory disease. Increased tPA antigen levels in the peritoneal fluid, associated with normal plasma levels of tPA antigen in patients with bacterial peritonitis, suggest local production of tPA. In particular, mesothelial cells and submesothelial endothelium might be responsible for the production of tPA<sup>20,21</sup>. The increased tPA concentrations found in the present study might be explained by prompt release from a preformed storage pool within these cells. Several 'stress factors' and inflammatory mediators, stimulated by bacterial infection, may induce such a release of tPA *in vivo*<sup>22</sup>. However, these findings are not supported by *in vitro* experiments in which inflammatory

stimuli, such as TNF and interleukin 1, inhibited tPA release by cultured mesothelial and endothelial cells<sup>21,23</sup>. Alternatively, increased levels of tPA might be caused by leakage from mesothelial cells, which are damaged during peritoneal infection.

PAI-1 forms a 'one to one' inactive complex with both tPA and uPA. Because the increase in PAI-1 concentration in the peritoneal fluid of patients with peritonitis was much higher than that of tPA and uPA, complete inhibition of fibrinolytic activity in the abdominal cavity might be expected. However, plasmin activity has occurred, as shown by the raised levels of PAP complex and fibrin degradation products. As plasminogen activation, mediated by tPA, is significantly enhanced in the presence of fibrin, tPA may display fibrinolytic effects even in the presence of a high PAI-1 level<sup>24,25</sup>.

The rise of uPA in the peritoneal fluid of patients with peritonitis is probably not linked to mesothelium, but to endothelium, as uPA has not been demonstrated in mesothelial, but in endothelial, cells of biopsies of inflamed appendices and in *in vitro* cultures, following stimulation with inflammatory cytokines<sup>26,27</sup>. The more pronounced increase in uPA levels in peritoneal fluid, compared with that in plasma, might be explained by the direction of secreting uPA by endothelial cells, which is mainly abluminal<sup>27</sup>. Except for a relatively small increase in uPA and fibrin degradation products concentrations, plasma levels were not raised in patients with peritonitis. Based on the findings of predominantly normal plasma levels, as well as the absence of correlation between plasma and peritoneal fluid levels, the authors hypothesize that the abdominal cavity with its surrounding peritoneum acts as a separate compartment. The coagulation and fibrinolytic responses to bacterial peritonitis in this compartment, and the systemic effects, depend on many factors, of which individual contributions are difficult to assess. It seems that the increase in fibrinolytic activity during peritonitis is limited by high PAI-1 levels, and does not meet the overwhelming demand to overcome fibrin production.

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