

Fibrinolytic activity of ascites caused by alcoholic cirrhosis and peritoneal malignancy

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Abstract

Coagulopathy is a well recognised complication of peritoneovenous shunting for ascites. The relative contributions of primary fibrinolysis and disseminated intravascular coagulation remain controversial. Plasminogen activating activity was significantly lower in malignant ascites ($n=10$, median <0.02 (range $<0.02-1.26$) IU/ml) than in alcoholic ascites ($n=10$, 1.07 ($0.30-1.49$) IU/ml) ($p<0.05$). Fibrinolytic activity was determined by a balance between tissue plasminogen activator and plasminogen activator inhibitor-1. There was no significant difference between the two groups in the concentration of tissue plasminogen activator (34 ($12-64$) ng/ml in malignant ascites *v* 29 ($12-43$) ng/ml in alcoholic ascites), but the concentration of plasminogen activator inhibitor-1 was significantly higher in malignant ascites (736 ($213-1651$) ng/ml) than in alcohol ascites (29 ($12-43$) ng/ml) ($p<0.05$). Malignant ascites contained significantly higher concentrations of urokinase (0.7 ($<0.1-1.3$) ng/ml *v* 0.2 ($<0.1-0.6$) ng/ml in alcoholic ascites) and plasminogen activator inhibitor-2 (33 ($<6-140$) ng/ml *v* 9 ($<6-28$) ng/ml alcoholic ascites). The plasminogen activating activity of alcohol ascites may lead to primary fibrinolysis after peritoneovenous shunting. The considerably lower activity found in malignant ascites may explain why coagulopathy after shunting is less pronounced in this group of patients.

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The peritoneovenous shunt is an established method of palliation for intractable benign and malignant ascites. Coagulopathy is a well recognised complication after the insertion of these shunts. The manifestations range from sub-clinical derangements of coagulation tests to life threatening haemorrhage. Controversy exists as to the relative contributions of primary fibrinolysis and disseminated intravascular coagulation to this coagulopathy.

The increased concentrations of fibrin degradation products and concomitant reductions in the concentrations of plasminogen and fibrinogen in ascites compared with plasma, has provided indirect evidence that ascitic fluid possesses fibrinolytic properties.¹⁻⁴ This has been confirmed by direct measurement of ascitic plasminogen activating activity^{5,6} and has led to speculation that an infusion of plasminogen activators could stimulate a coagulopathy mediated by primary fibrinolysis.^{1,2,7,8}

Increased concentrations of cross linked fibrin and fibrinogen degradation products and decreased platelet counts found in the plasma

after peritoneovenous shunting has led others to suggest that the coagulopathy is secondary to disseminated intravascular coagulation induced either by fibrin degradation products⁴ or thromboplastin-like substances,^{3,6} and that ascitic plasminogen activators would be neutralised on entry into the systemic circulation.⁶ At present, the only agreement is that increased plasma concentrations of fibrinogen degradation products after insertion of a peritoneovenous shunt are an indication of shunt patency.

The aim of this study was to measure the overall plasminogen activator activity and concentrations of individual fibrinolytic system mediators in ascites secondary to alcoholic liver disease or malignancy to gain a greater understanding about the aetiology of this condition.

Patients and methods

Ethical approval was obtained from the hospital ethics committee. Ascitic fluid was aspirated from 10 patients (nine men, one woman; age range 37-62 years) with alcohol induced ascites and 10 patients (four men, six women; age range 31-85 years) with cytologically confirmed malignant ascites (six ovarian; one oesophageal; two stomach, and one colonic carcinoma).

A specimen of the fluid was used for bacteriological studies including microscopy and culture on plates for aerobic and anaerobic bacteria. The total white blood cell count in the sample was measured by coulter counter (STKR). Aspirates were immediately treated with anticoagulant (9:1 v/v 10% EDTA) and centrifuged at 3500 rpm at 4°C for 10 minutes. The supernatant was then removed and stored at -20°C until analysis.

Tissue plasminogen activator, urokinase, and plasminogen activator inhibitor-1 and inhibitor-2 were measured by enzyme linked immunosorbent assay (Tintelize, Porton Cambridge Ltd, Maidenhead, UK). The lower limits of sensitivity for the assays were 1.5 ng/ml (tissue plasminogen activator), 0.1 ng/ml (urokinase), 2.0 ng/ml (plasminogen activator inhibitor-1), and 6.0 ng/ml (plasminogen activator inhibitor 2). Plasminogen activating activity was determined by the fibrin plate method after incubation for 24 hours at 37°C⁹ and the results expressed as IU tissue plasminogen activator/ml ascitic fluid (lower limit of detection 0.02 IU/ml).

Statistical analysis was performed with the Mann Whitney U test and values of $p<0.05$ were considered to be significant.

Results

Micro-organisms were not present in any of the samples. The white blood cell count was significant

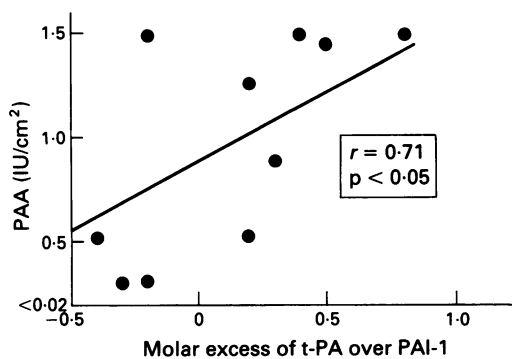
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Correlation between plasminogen activator activity (PAA) and molar excess of tissue plasminogen activator (t-PA) over plasminogen activator inhibitor-1 (PAI-1).

antly lower in alcoholic ascites (median 0.3 (range 0.2–1.0) $\times 10^9$ /ml) than in malignant ascites (0.8 (0.4–2.3) $\times 10^9$ /ml, $p < 0.05$). The plasminogen activating activity was significantly higher in alcoholic cirrhotic ascites (median 1.07 (range 0.30–1.49) IU/ml) than in malignant ascites (<math><0.02</math> (<math><0.02</math>–1.26) IU/ml, $p < 0.05$). There was no significant difference in the concentration of tissue plasminogen activator (29 (12–43) ng/ml in alcoholic ascites *v* 34 (12–64) ng/ml in malignant ascites). A significantly higher concentration of urokinase was found in malignant ascites (0.7 (<math><0.1</math>–1.3) ng/ml) than in alcoholic ascites (0.2 (<math><0.1</math>–0.6) ng/ml, $p < 0.05$).

The concentration of plasminogen activator inhibitor-1 was significantly higher in malignant ascites (736 (213–1651) ng/ml) than in alcoholic ascites (16 (<math><2</math>–43) ng/ml, $p < 0.05$), and a similar but less pronounced pattern was found for plasminogen activator inhibitor-2 (43 (<math><6</math>–140) ng/ml in malignant ascites *v* <math><6</math> (<math><6</math>–28) ng/ml in alcoholic ascites, $p < 0.05$).

The plasminogen activator activity of alcohol induced ascites correlated with the excess (in molar terms) of tissue plasminogen activator over plasminogen activator inhibitor-1 ($r = 0.71$, $p < 0.05$; Figure). There was no positive correlation between the plasminogen activating activity and the severity of alcoholic liver disease in the cirrhotic group, based on serum albumin ($r = 0.04$), bilirubin ($r = 0.45$), or prothrombin time ($r = 0.24$).

Discussion

The incidence and severity of coagulopathy after insertion of peritoneovenous shunts are greater in patients with alcohol ascites than in those with malignant ascites (Table). The incidence of

coagulopathy (prolongation of the prothrombin time or activated partial thromboplastin time) after peritoneovenous shunting in patients with malignant ascites is 0% to 10%.^{7,10–12} By comparison, the incidence of coagulopathy in patients with alcoholic cirrhosis ranges between 40% and 91%^{4,12–14} and a higher proportion of these patients develop clinical problems.^{12,13}

Our results confirm that alcoholic ascites contains plasminogen activating activity. Patrassi *et al* postulated that the enhanced fibrinolysis in ascites induced by alcohol was due to increased release of tissue plasminogen activator from the mesothelium,² and Wilde *et al* were able to quench this fibrinolytic activity with the addition to the ascites of antibody to tissue plasminogen activator.⁶ Neither study, however, directly measured the ascitic concentrations of tissue plasminogen activator. Although it has been shown experimentally that alcohol can enhance the endothelial cell secretion of plasminogen activator,¹⁵ it seems that the difference in plasminogen activator activity between the two groups is secondary to changes in the concentration of plasminogen activator inhibitor-1 rather than tissue plasminogen activator.

The significantly lower plasminogen activator activity of malignant ascites is associated with greatly increased concentrations of plasminogen activator inhibitor-1. This confirms the results of previous studies.^{16,17} Huber *et al* showed more plasminogen activator inhibitor-1 in malignant ascites than in alcoholic ascites¹⁶ and Casslen and Astedt found significantly higher concentrations of plasminogen activator inhibitor-1 in malignant ascites than in benign ascites.¹⁷ Neither study, however, quantified the overall plasminogen activator activity of malignant ascites.

We have shown that the overall ascitic plasminogen activator activity is largely determined by a balance of tissue plasminogen activator and plasminogen activator inhibitor-1. The increased concentration of urokinase in malignant tissues may partly explain the finding of increased concentrations of urokinase in malignant ascites.^{18,19} The concentration of tissue plasminogen activator, however, was 20 to 70 times greater than that of urokinase in both groups of patients and therefore urokinase probably contributes little to the overall fibrinolytic activity. Equally, a significantly higher concentration of plasminogen activator inhibitor-2 in malignant ascites confirms previous work,¹⁶ but as it is a weaker inhibitor of tissue plasminogen activator²⁰ and is present in much lower concentrations than plasminogen activator inhibitor-1, it probably plays only a small part in the overall inhibition of fibrinolytic activity.

These studies show that the plasminogen activating activity of ascites is primarily mediated by tissue plasminogen activator and that it is also probably responsible for a coagulopathy mediated by primary fibrinolysis after insertion of a peritoneovenous shunt. Moreover, the reduced plasminogen activator activity of malignant ascites is caused by increased concentrations of plasminogen activator inhibitor-1, which may account for the lower incidence of coagulopathy after insertion of shunts seen in this group of patients.

Incidence of coagulopathy in ascites induced by alcohol and malignant ascites after insertion of a peritoneovenous shunt

Study	Aetiology of ascites	Incidence of coagulopathy (%)	Incidence of clinical coagulopathy (%)
Kostroff <i>et al</i> ¹²	M	10	0
Gleysteen <i>et al</i> ⁷	M	0	–
Straus <i>et al</i> ¹⁰	M	3	–
Reinhold <i>et al</i> ¹¹	M	0	–
Ragni <i>et al</i> ⁴	A	91	–
Levy <i>et al</i> ¹⁴	A	44	–
Greig <i>et al</i> ¹³	A	40	17
Kostroff <i>et al</i> ¹²	A	62	21

M=malignant; A=alcoholic.

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