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Fibrinolysis Inhibition and Fibronectin in the Blood in Patients with the Delayed Microembolism Syndrome

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ABSTRACT

Various parameters of fibrinolysis inhibition and the plasma concentration of fibronectin (α_2-surface binding glycoprotein, cold insoluble globulin) were measured in patients at risk of developing acute progressive respiratory insufficiency following trauma or sepsis - the delayed microembolism syndrome (DMS). Most parameters measuring fibrinolysis inhibition were significantly higher in the five patients with DMS than in five patients who did not develop the syndrome. Thus, the primary fibrinolysis inhibitor (α_2-antiplasmin) was enhanced and the α-form of this inhibitor, with affinity to plasminogen, showed the greatest increment and might be of major importance for the delayed elimination of fibrin from the lungs occurring in these patients. The fibronectin concentrations were not lower in patients with DMS than in those who did not develop the syndrome.

INTRODUCTION

Intravascular coagulation with microembolism occurring during a phase of fibrinolysis inhibition has been reported to be a major cause of acute progressive respiratory insufficiency following trauma or sepsis, the delayed microembolism syndrome (DMS) (23). A protein in the blood which seems to be primarily responsible for the fibrinolysis inhibition has been isolated from posttraumatic patients (3, 4, 24). This inhibitor has been named "the primary fibrinolysis inhibitor" (PFI) and is immunologically identical with the α_2--plasmin inhibitor (18) and α_2 antiplasmin (28). When PFI in serum is fractionated by affinity chromatography on plasminogen-Sepharose®, a minor part binds to the plasminogen, while the major part remains unbound (5, 7). These two forms of the inhibitor have been called PFIα and PFIβ respectively.

A correlation has been found between the plasma depletion of an opsonic protein, fibronectin (FN;α_2-surface binding glycoprotein, cold insoluble globulin)and the development of acute progressive respiratory insufficiency following trauma and sepsis (19, 22). In the present investigation various parameters constituting measures of fibrinolysis inhibition (especially PFIα) and the plasma concentration of fibronectin were studied in patients with a risk of developing DMS. The possible correlations between an increase in fibrinolysis inhibition and a decrease in FN, respectively, and the development of this syndrome were also examined.

MATERIALS

Patients: Patients with large soft-tissue injuries, multiple fractures or sepsis and postoperative patients after extensive surgery, run a risk of developing DMS, especially when these conditions are combined with shock. The study was made on 10 patients (four women and six men) aged 19-78 years, who were admitted consecutively to the Intensive Care Unit (ICU) at the University Hospital, Uppsala. The patients had already developed or were considered to run a risk of developing DMS. Six patients had sustained trauma; in four of them this was mainly in the form of major fractures, and two had undergone major surgery. The remaining four patients were admitted to the ICU in various stages of shock--three suffering from septicaemia and one from pancreatitis.

Plasma and sera were sampled daily for 6-10 days. Day 1 in the figures is the first day of observation in the ICU. In most patients the traumatic event had occurred the day before and in a few patients even earlier. Five patients fulfilled our criteria of DMS (see below). One patient in the DMS group died, though not from pulmonary insufficiency but from progressive renal failure and uraemia, 15 days after admission to the ICU.

Chemicals: Human fibrinogen (Grade L), Human plasminogen, Human plasmin and trans 4-aminoethyl-cyclohexane carboxylic acid (AMCA) (AB Kabi, Sweden); Chromogenic substrate for plasmin (S-2251, Kabi Diagnostica, Sweden); CBNr-activated Sepharose®4B and Lysine Sepharose®4B (Pharmacia Fine Chemicals, Sweden); Urokinase (Leo, Denmark); Thrombin ("Topostasine®", Hoffman-LaRoche, Switzerland); Rabbit antibodies against human α_1-antitrypsin, α_2-macroglobulin and plasminogen (DAKOPATTS, Denmark); Rabbit antibodies against PFI (own product or "RaHu/Apl", Nordic Immunological Laboratories, The Netherlands); Rabbit antibodies against bovine plasma fibronectin which were shown to cross-react with human FN (21); Immobilized plasminogen: human plasminogen purified by the method of Deutsch & Mertz was coupled to CNBr-activated Sepharose®4B at a concentration of 10 mg/g gel. FN was purified from human plasma by affinity chromatography

on gelatin-Sepharose® (13) followed by an ion exchange chromatography and gel chromatography.

Blood fractions: Human normal serum (HNS) and normal plasma, pooled from 30 apparently healthy persons and kindly supplied from the blood bank, University Hospital, Uppsala, were used as references. All blood fractions (references and samples) were stored at -70°C until analysed.

METHODS

Reference levels of the variables are given as Mean ±2 SD based on individual determinations at our laboratory in more than 30 blood donors or on the reference range used at the Central Laboratory of Clinical Chemistry, University Hospital, Uppsala.

Fibrinolysis inhibition activity (FIA): A clot-lysis method using fibrinogen, thrombin, plasminogen and urokinase (7, 20, 27) was employed. FIA is expressed in dilutions of AMCA (mg/l) giving the corresponding delay in clot-lysis time or in per cent of plasminogen-depleted normal serum (100 ± 18 %).

Fast-reacting antiplasmin (AP): The inhibition of plasmin was measured by an enzymatic assay, using a chromogenic substrate (S-2251) for plasmin and is expressed in per cent of that in HNS (100 ± 13 %). The end-point determination method described in the manual of S-2251 (Kabi Diagnostica, Sweden) was used. This method has been reported to measure α_2-antiplasmin (26).

PFI, α_1-antitrypsin (α_1AT), α_2-macroglobulin (α_2) and plasminogen (plg): These concentrations were assayed by immunodiffusion (17) and are expressed in per cent of those in HNS (PFI:100 ± 12 %, α_1AT: 100 ± 23 %, α_2M:100 ± 7 % and plg:100 ± 9 %).

Platelets (Pl): Platelets were counted by a Coultercounter S+. Reference range 150-400 x 10^9/l.

FN: The concentrations of FN were determined by radioimmunoassay. Antiserum against human FN was raised in a rabbit as described by Rubin *et.al.* The IgG-fraction of the antiserum, isolated by chromatography on Protein A-Sepharose®, yielded one precipitation arch when tested against human plasma or purified human FN by crossed immunoelectrophoresis. Antibodies interacting with FN were purified by adsorption on FN-Sepharose®, coupled to CNBr-activated Sephadex G-25, and used as an immunoadsorbent in the radioimmunoassay (15). The amount of FN in the plasma samples (mg/l) was determined relative to purified human FN. Reference value > 300 mg/l.

Statistical calculation: Conventional significance analysis was performed. As the number of observations (n) was always more than 20, the p values were calculated from the distribution of λ.

EXPERIMENTAL PROCEDURE

Fractioning of sera by a two-step affinity method: 1.5 ml of the serum was mixed with 0.5 ml of lysine Sepharose®(1 h at +4°C) and centrifuged (5.000 x g, 20 min. at +4°C). The supernatant (SI) obtained, consisting of plasminogen--depleted serum, was examined in respect to FIA, AP and PFI. One ml of the supernatant was mixed with 1 ml of immobilized plasminogen (1 h at +4°C) and centrifuged (5.000 g, 20 min at +4°C). The amounts of FIA, AP and PFI in the supernatants (SII) were determined. The amount of PFI adsorbed to the plasminogen matrix (PFIα) was estimated as the difference between applied PFI and non-bound PFI (PFIβ). The recoveries of the biological and immunological parameters were calculated for each step. On calculations of the dilutions, all gel volumes were estimated as the liquid phase. Reference range: PFIα = 100 ± 11 %; PFIβ = 100 ± 18 %.

RESPIRATORY MEASUREMENTS

Blood gases were measured by the conventional electrode technique.

The ability of the lungs to oxygenate the blood. The respiratory ratio (RR) was calculated from the formula:

$$RR = \frac{PaO_2}{P_AO_2} \times 100\ \%$$

where PaO_2 = arterial oxygen tension

P_AO_2 = alveolar oxygen tension

This simple test (8) makes it possible to compare the pulmonary function between different levels of inspired oxygen concentration, and was employed by us in an earlier study (9). A ratio range of 50-60 % was regarded as the lowest tolerable border zone for normal lung function and values below this limit were considered to indicate pulmonary insufficiency. Our criteria for DMS are:

1. RR below 50 % in the absence of other causes such as aspiration, bronchopneumonia or left heart failure with pulmonary oedema; together with
2. areas of infiltration spread over both lungs, and giving a "snowstorm" pattern, at chest radiography.

PREVENTIVE AND THERAPEUTIC MEASURES

Every effort was made to combat shock initially with the aid of dextran 70 and/or balanced electrolyte solutions and blood according to requirements. Particulate matter introduced by transfusion was avoided by the use of micropore filters in the transfusion line when multiple units of stored blood were infused.

After haemodynamic restitution, the patient was kept "on the dry side". Thus, careful gradual dehydration was initiated to draw any surplus fluid from the pulmonary interstitial space with a minimal decrease in intravascular volume. Further preventive measures included chest physiotherapy, high caloric nutrition, effective analgesia and daily administration of dextran 70 and albumin. If, in spite of these measures, there was a progressive decrease in RR, indicating impending pulmonary insufficiency, volume-controlled mechanical ventilation was begun, with a positive endexpiratory pressure (PEEP) of 8 to 15 cm H_2O. Disseminated intravascular coagulation (DIC) was manifested by combinations of decreased platelet count, positive ethanol gelation test, fibrin degradation products in serum (FDP) over 10 mg/l and shortering of activated thromboplastin time (APTT). If so, heparin in doses adequate to prolong APTT to values within its upper normal range (35-39 s) was given. The resulting concentrations of heparin in the blood should not influence the fibrinolysis inhibition assays

CASE REPORTS

Negative DIC variables and APTT during heparin therapy are not commented.

Five patients with DMS:

1. (Fig 2) A 44-year-old man. Traffic accident.

State: Ruptures of diaphragm (left side) and spleen; liver lacerations, pelvic and femoral fractures, parts of the abdominal organs were transpositioned into the left pleural space.

Initial treatment: Adequate surgery and haemodynamic restitution including 500 ml of dextran. The patient was admitted to the ICU and artificially ventilated for the following 16 h postoperatively with a normal RR.

Day 1 in our study represents the first posttraumatic day.

Two days after the trauma, when spontaneously breathing progressive pulmonary insufficiency developed with a low RR and chest radiography revealed small symmetrical densities over both lungs, consistent with DMS

Platelet count: 105 x 10^9/l. APTT: 29 s.

PEEP-ventilation started simultaneously with heparin therapy.

The patient was successfully weaned from the ventilator 13 days after the accident.

2. (Fig 2) A 78-year-old man. Urosepticaemia.

State on admission (day 1): Septic shock. Low platelet count (94 x 10^9/l) but normal APTT (33 s).

Initial treatment: Haemodynamic restitution (no dextran), heparin and antibiotic therapy. After 14 h progressive pulmonary insufficiency occurred and chest radiography revealed fairly large densities spread over both lung fields, consistent with DMS. Platelet count were even lower (64 x 10^9/l) and APTT was shortened (26 s), indicating DIC. Heparin therapy and PEEP-ventilation begun and continued for 10 days.

An improvement in pulmonary function was noted after six days and the platelets were normalized (257 x 10^9/l) after eight days.

However, the patient deteriorated, developed progressive renal insufficiency with uraemia and died 15 days after admission to the ICU.

3. (Fig 2) A 42-year-old man. Pancreatitis due to alcoholism.

State on admission (day 1): Hypotensive and oliguric. Extremely low platelet count (14 x 10^9/l)indicating DIC. APTT: 33 s.

Initial therapy: Haemodynamic restitution (no dextran).

Peritoneal lavage was started on the second day.

Heparin therapy was begun on the third day and continued for six days.

Progressive pulmonary failure had developed on day four and chest radiography revealed densities spread over both lung fields, consistent with DMS.

PEEP-ventilation was initiated and the patient was successfully weaned off after six days.

4. A 30-year-old woman who had undergone right lower lung lobe resection (carcionid) was admitted to the ICU on the fifth postoperative day. Blood gases were fairly normal during the first three postoperative days but deteriorated later.

State on admission: Progressive pulmonary failure with a low RR and chest radiography revealing small densities over both lung fields, consistent with signs of DMS. Platelet count: 334 x 10^9/l, and APTT: 34 s. Ethanol gelation test was positive and FDP varied between 40-80 mg/l, indicating DIC.

Initial treatment: PEEP-ventilation was started. No dextran. No heparin was given.

The patient was successfully weaned from the ventilation after two days.

5. A 19-year-old woman. Malignant lymphoma.

State on admission: Septicaemia. shock and respiratory distress with a low RR. Chest radiography revealed small areas of infiltration spread over both lung fields, consistent with DMS. Platelet count were low (29 x 10^9/l), indicating signs of DIC. APTT: 32 s.

Initial treatment: PEEP-ventilation, Haemodynamic restitution (no dextran) and antibiotic therapy was begun.

After two days the patient was extubated and breathed spontaneously.

Five patients without DMS:

6. A 56-year-old man. Building-site accident.

State: Vertebral luxations L5 - S1, fractured L1 - L5, soft-tissue injury and femoral fracture.

Initial treatment: Adequate surgery and haemodynamic restitution (500 ml of dextran).

Day 1 in our study represents the first posttraumatic day.

Platelet count: 151 x 10^9/l (day 1). Minimum 119 x 10^9/l (day 2). Normalized (247 x 10^9/l) on day 6.

APTT: Minimum 28 s (days 1-3).

7. A 39-year-old man. Traffic accident.

State Femoral fracture and cranial fractures.

Initial treatment: Adequate surgery and haemodynamic restitution (no dextran).

Day 1 in our study represents the first posttraumatic day.

Platelet count: 200 x 10^9/l (day 1). Minimum 115 x 10^9/l (day 3). Normalized on day 5 (183 x 10^9/l).

APTT: Minimum 29 s (day 1). 30-33 s (days 2-6).

8. A 39-year-old man. Traffic accident.

State: Femoral fracture. Cranial and facial fractures.

Initial treatment: Adequate surgery and haemodynamic restitution (1000 ml of dextran).

Day 1 in our study represents the first postoperative day.

Platelet count: Minimum 44-85 x 10^9/l (days 1-5). Normalized on day 8 (257 x 10^9/l).

APTT: 34 s (day 1). 27-29 s (days 2-5). Minimum 25 s (days 6-7).

9. A 58-year-old woman. Septicaemia.

State on admission: Septic shock, hyperthermia (41^{o}C).

Initial treatment: Antibiotic therapy and haemodynamic restitution (500 ml of dextran).

Day 1 in our study represents the day of acute shock.

Platelet count: 296 x 10^9/l.

APTT: 26 s. FDP:80-160 mg/l.

10. A 27-year-old woman who had undergone Caeserean section because of ablatio of the placenta (bleeding 1000 ml).

State on admission: Anuria. Signs of a transient DIC. Platelet count: 37 x 10^9/l.
APTT 38 s. FDP: 160-320 mg/l.

Initial treatment: Haemodynamic restitution (700 ml of dextran). Heparin. Day 1 in our study represents the operative day.

Platelet count: 42-60 x 10^9/l (days 1-3).

RESULTS

The mean values for the different parameters in patients without and with DMS are given in Table 1. The values for the latter group only include those obtained on days when RR was below 50 %. Observations made in these patients in periods when this ratio was above 50 % are included in those for the first group (without DMS) in the table.

Table 1. Values for the measured parameters obtained in the absence (RR > 50 %) and presence of DMS (RR < 50 %). FIA = clot lysis inhibition (mg AMCA/l). FIA (SI) = FIA in per cent of that in normal plasminogen-depleted serum. PFI, PFIα, α_1AT, α_2M and plg = concentration in per cent of that in human normal serum (%HNS); AP = enzymatic determination of plasmin inhibition (%HNS); Pl = number x 10^{-9}/l; FN = concentration (mg/l) determined by radioimmunoassay; RR = respiratory ratio (%); p = level of statistical significance.

	PFI	PFIα	FIA	FIA (SI)	AP	α_1AT	α_2M	plg	Pl	FN	RR
No DMS											
Mean	112	154	820	199	111	173	73	73	112	180	72
± S.E.	8	18	360	33	10	11	4	6	18	20	2
n	22	23	22	23	23	22	22	22	20	21	23
DMS											
Mean	159	261	1,120	380	166	269	83	84	149	260	31
± S.E.	9	18	160	39	10	16	3	6	20	20	2
n	21	23	20	23	21	21	21	21	24	20	24
P	<0,001	<0.001		>0.1	<0.001	<0.001	<0.001	<0.1	>0.1	>0.1	<0.001

While most parameters of fibrinolysis inhibition were increased after trauma and significantly increased in DMS, the FN levels were decreased posttraumatically but did not show the expected lowest value in DMS. Regarding the fibrinolysis inhibition parameters, the largest increase was found in PFIα. The increase in FIA in serum was not significant, whereas FIA in plasminogen de-

pleted serum was significantly raised. The platelet counts were low in DMS but slightly higher than in patients without this syndrome.

In Fig. 1 values from consecutive days are given for the five patients with DMS and the five patients without. Values from days when RR was not measured are excluded. FIA in plasminogen-depleted serum and PFIα showed much greater increases in the patients with DMS than in those without.

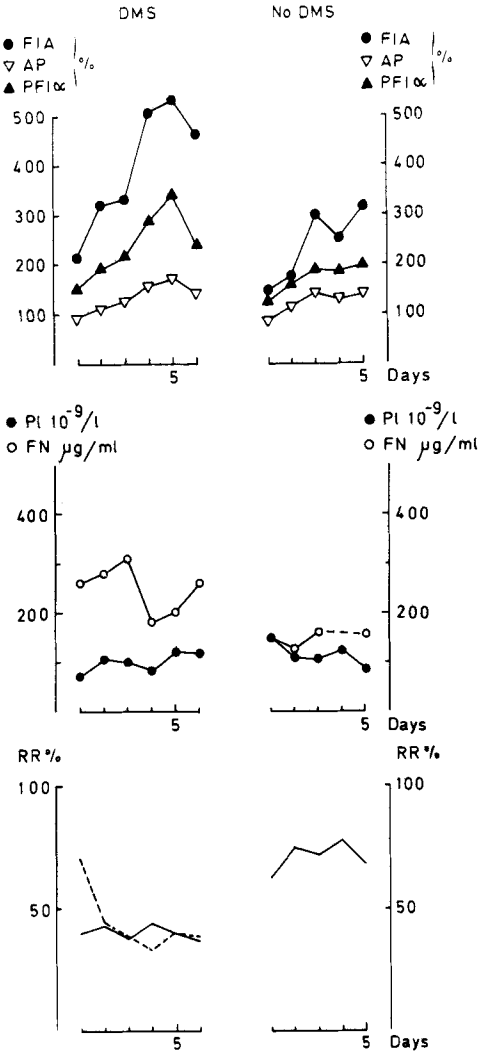

Fig. 1.

Variation of mean values for various parameters in patients 1-5 (left sequence) with DMS and the five patients without. FIA (% HNS) and AP (% HNS) were determined in plasminogen depleted sera. Values of RR below 50 % indicate DMS. The dashed curve of RR represents the mean of patients 1 and 3 who developed DMS during the observation period. For abbreviations see text.

Concerning the initial haemodynamic restitution, there was one important difference between the two groups of patients. Thus, only one patient (patient 1) of the five in the DMS group received dextran, while the other four patients were given balanced electrolyte solutions instead as the initial shock treatment. Four patients in the group without DMS received dextran as the initial

shock treatment, and in two of these patients as much as 1000 ml of dextran was given. In the later posttraumatic course there was no difference between the groups regarding dextran administration.

No difference in platelet count was found between the two groups and the FN concentration was higher in the DMS group than in the group without DMS. As the mean RR values were already low on the first day of observation in the DMS group, no general conclusions can be drawn about the time correlation between the various parameters and the change in RR.

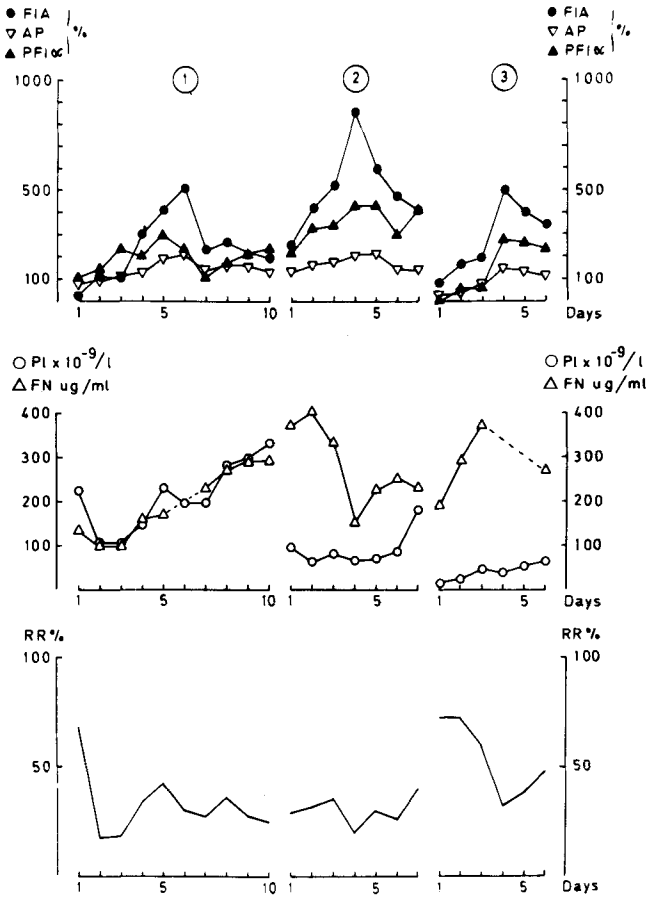

Fig. 2.
Time course of various parameters in patients 1-3 with DMS. FIA (%HNS) and AP (%HNS) were determined in plasminogen depleted sera. For abbreviations see text.

Findings for three of the patients showing decreased RR values during the observation period are presented in Fig. 2. In patient 1 there seemed to be a time correlation between the decrease in RR and the initial decrease in platelets and increase in PFIα. The plasma FN concentration was already low before the reduction of RR began. It then increased, whereas the low RR persisted. In patient 2 the small second drop in RR was preceded by both an increase in the fibrinolysis inhibition parameters and a decrease in the FN concentration. In

this patient the highest level of FIA and the lowest level of FN occurred on the same day. In patient 3 the reduction of RR was accompanied by an increase in PFIα, which reached its maximum on the same day as the lowest value of RR was noted.

DISCUSSION

This investigation has shown that the fibrinolytic system is inhibited in patients developing DMS and that the most pronounced increase among the inhibitory factors occurs in PFIα. This protein seems to be mainly responsible for the FIA in plasminogen-depleted serum (3,4). We have previously shown that in patients with DMS the elimination of fibrin from the lungs is delayed (19) and a good correlation has been found between the increase in FIA and the delay in fibrin clearance from the lungs in rats (2). The present investigation therefore supports the hypothesis that PFIα is of importance in the development of DMS.

A factor which might have contributed to the lower grade of fibrinolysis inhibition in patients not developing DMS is the larger administration of dextran in the initial treatment of shock (10,11).

In experimental studies we have found that intravascular coagulation alone cannot induce DMS and that this syndrome only occurs when the fibrinolytic system is inhibited. These findings are supported by the present observation that the platelet counts were not lower in the DMS patients than in those without DMS. Several of the patients with low platelet counts and other signs of intravascular coagulation, such as reduced APTT, did not have respiratory insuffiency unless the fibrinolytic system was inhibited. Thus, a woman who underwent an emergency Caesarean section because of ablation of the placenta (patient 10) showed signs of severe intravascular coagulation, with very low platelet count and a high concentration of FDP in the immediate postoperative period. The fibrinolytic variables were normal and neither the respiratory ratio nor the chest radiograph gave any indication of DMS.

On the other hand, even a large increase in the fibrinolysis inhibition alone was not inducing DMS in the absence of intravascular coagulation (e.g. patients 6 and 7, PFIα = 230-240 %). Patients who developed DMS had both intravascular coagulation and a large increase in fibrinolysis inhibition, especially PFIα. As a large increase in this inhibitor was seen also in patients without DMS, this increase should not be a consequence of the syndrome.

Not only PFIα but also the other measured variables of fibrinolysis inhibition, namely α_1AT and α_2M, were increased. The two latter antiplasmins do not ordinarily affect the FIA assay (27) and may be of no major significance for

the delay in fibrin elimination. The AP assay seems to measure both PFIα and PFIβ,while the FIA assay, after removal of the endogenous plasminogen, is more specific for PFIα (6). Since PFIβ seems to play a smaller role than PFIα in the increase in FIA after trauma, measurement of AP might be of less importance than measurement of PFIα in these patients.

Recently Kluft, Los & Jie (16) found that serum contains less inhibitor than plasma. In serum particularly the α-form is reduced, suggesting that this form is preferentially bound to fibrin. If so, the large post-traumatic increase of the α-form found (in serum) in the present investigation may indicate an even larger increase *in vivo*. Experiments are in progress to evaluate this possibility.

The impact of phagocytosis on the development of DMS is not clear. It has been reported that the plasma fibronectin concentration is decreased in patients with respiratory insufficiency after septicaemia and trauma and that this is of pathogenetic importance (19, 25). A conceivable mechanism underlying this relationship is a decreased clearance of fibrin and coagulation agents due to impaired function of the reticuloendothelial system. In the present investigation a generally lowered level of fibronectin was found following trauma. However, the concentrations were higher in patients with DMS than in those who did not develop the syndrome. This finding speaks against a specific connection between low levels of fibronectin and a tendency to develop DMS. Generally in severe trauma the collagen in the tissue will be exposed to the plasma, resulting in adsorption of fibronectin to the tissue collagen and a consequent depletion of circulating fibronectin. It is also possible that fibronectin may play a greater part in longstanding low grade intravascular coagulation (23), for instance as in endotoxin shock and septicaemia, than in posttraumatic patients, where the coagulation is probably of a higher grade and induced in a shorter period of time (23).

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