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Measurement of thrombus precursor protein in septic patients with disseminated intravascular coagulation and liver disease

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Background and Objectives. Disseminated intravascular coagulation (DIC) is a syndrome characterized by systemic intravascular activation of coagulation leading to the widespread deposition of fibrin in the circulation. Therefore, the determination of soluble fibrin is crucial for the diagnosis of DIC. Thrombus precursor protein (TpP) levels can be determined as a measure of soluble polymers, which are the immediate precursors of insoluble fibrin. In this study, the potential diagnostic usefulness of this TpP test was investigated in septic patients with DIC and liver diseases.

Design and Methods. TpP analysis was performed on 155 plasma samples from 95 septic patients, including 72 patients without liver disease and 23 patients with liver diseases, and on 42 plasma samples from normal healthy subjects. The study population was subdivided according to three phases of DIC described as compensated, decompensated and full-blown DIC. Plasma TpP level was determined using a new assay, the TpP™ (American Biogenetic Sciences, USA), which is based on an ELISA method.

Results. Septic patients with decompensated (16.1±9.1 μg/mL) or full- blown (20.9±12.4 μg/mL) phases of DIC had significantly higher TpP levels than those with the compensated (5.6±6.2 μg/mL) phase of DIC or healthy controls (2.9±1.6 μg/mL). In septic patients with liver disease, a significant difference was found between the TpP levels of patients with full- blown DIC (21.6±10.6 μg/mL) and those of patients with the decompensated phase (13.4±6.5 μg/mL). Plasma TpP levels correlated significantly with other DIC parameters including platelet count, fibrinogen, antithrombin and TAT, and correlated weakly with D-dimer.

Interpretation and Conclusions. Our findings indicate that septic patients who developed decompensated or full-blown DIC or organ dysfunction have significantly higher plasma levels of TpP, and suggest the potential usefulness of the TpP assay as an aid to the diagnosis of DIC in cases of sepsis and liver disease complicated by sepsis.

Key words: sepsis, intravascular coagulation, liver disease, fibrin degradation product.

Disseminated intravascular coagulation (DIC) is a syndrome characterized by systemic intravascular activation of coagulation, which leads to the widespread deposition of fibrin in the circulation.[1] One of the key events in the formation of fibrin is the conversion of circulating soluble plasma fibrinogen to insoluble cross-linked fibrin polymer.[2]

Thrombin cleaves fibrinopeptide A from the fibrinogen molecule, exposing polymerization sites on the newly formed desAA fibrin monomer units. As the polymerization of desAA fibrin proceeds, thrombin removes fibrinopeptide B from the fibrinogen molecule, which results in the formation of a molecule known as desAABB fibrin.[3] These soluble polymers are the immediate precursors of insoluble fibrin and are thus referred to as thrombus precursor protein (TpP). Elevated levels of this protein are indicative of a prothrombotic state and have been reported in clinical conditions in which intravascular coagulation has been indicated.[4-6] The most frequent disease states in which intravascular coagulation occurs are infections (systemic inflammatory response syndrome) and liver diseases.[7] According to the definition of DIC stated above, the determination of soluble fibrin is crucial for the diagnosis of DIC. In this study, the potential diagnostic usefulness of TpP was investigated in septic patients with DIC and in patients with liver diseases.

Design and Methods

TpP analysis was performed on 155 plasma samples from 95 septic patients, including 72 patients without liver disease and 23 patients with liver disease, and on 42 plasma samples from normal healthy subjects. A clinical diagnosis of sepsis was made based on the criteria of systemic inflammatory response syndrome.[8] Bacterial culture studies in septic patients yielded the following information: Gram positive cocci infections in 29 patients, Gram negative bacilli infections in 29 patients, and no growth in 14 patients. Of the 23 septic patients

Table 1. Demographic data of the study population.

Group	Age (years) Mean(SD)	Sex Male/Female	Basal diseases		Failured organ	
Sepsis without liver disease (n=72)	59 (19.6)	39/33	Pneumonia	16	Lung	28
			FUO	15	Kidney	21
			UTI	10	Heart	18
			Trauma	7	Liver	8
			Wound	6		
			Miscellaneous	18		
Sepsis with liver disease (n=23)	52 (14.3)	14/9	LC	15		
			HCC	4		
			AFH	4		

FUO: fever of unknown origin; UTI: urinary tract infection; LC: liver cirrhosis; HCC: hepatocellular carcinoma; AFH: acute fulminant hepatitis; Miscellaneous: 3 acute cholangitis; 3 acute meningitis; 1 AIDS; 6 neoplasms; 3 stroke; 1 diabetes mellitus; 1 neurogenic bladder.

Table 2. Laboratory findings (mean ± SD) of coagulation in 72 septic patients with different phases of DIC.

Parameters	Phase		
	Compensated (n=59)	Decompensated (n=48)	Full-blown(n=14)
Prothrombin time (sec)	15.4±5.3	18.5±9.0	20.8±5.9
APTT (sec)	46.2±21.5	55.7±25.1	60.3±20.8
Platelet count (/μL)	306,000±174,000	138,000±88.000	78,000±56,000
Fibrinogen (mg/dL)	496±166	356±198	166±97
D-dimer (μg/mL)	4.9±6.0	20.2± 53.3	28.4± 22.7
TAT (μg/L)	5.3±10.0	18.6±38.3	68.2±30.9
Antithrombin (%)*	79.4±22.1	69.1±29.6	50.6±20.9

*APTT: activated partial thromboplastin time; TAT: thrombin-antithrombin complex. *Data are missing for 9 patients.*

with liver disease, 2 had Gram positive cocci infections, 9 had Gram negative bacilli infections and no growth was found in 12 patients.

The diagnosis of organ dysfunction was based on criteria described elsewhere,[9] but which can be briefly summarized as follows: lung dysfunction, PaO_2 < 50 mmHg or the requirement of artificial ventilatory support; kidney dysfunction, creatinine level > 3 mg/dL or BUN > 50 mg/dL; heart dysfunction, systolic blood pressure of 90 mmHg or less or heart failure; liver dysfunction, serum total bilirubin > 5 mg/dL.

Patients were subdivided according to three phases of DIC, namely, compensated activation of the hemostatic system, decompensated activation of the hemostatic system, and full-blown DIC, defined by previously described criteria.[10] In addition to prothrombin time (PT), activated partial thromboplastin time (aPTT), platelet count, fibrinogen concentration, D-dimer, and antithrombin, thrombin antithrombin complex (TAT) were determined to differentiate between the three phases of DIC.

The blood samples were anticoagulated with 3.2% sodium citrate and centrifuged at 2,000g for 10 min immediately after venipuncture. Plasma was separated and stored at –70°C until use. The plasma TpP level was determined using an assay kit for TpP™ (American Biogenetic Sciences, USA), which is based on an ELISA method. In this assay, a murine monoclonal antibody, specific for soluble fibrin polymer, is used as the capture antibody and another murine monoclonal antibody, labeled with horseradish peroxidase is used to bind to a different site on the TpP molecule. The monoclonal MH-1 antibody used is an IgG1 (κ light chain) with a relatively high affinity for cross-linked fibrin. The mean within-run coefficient of variation of the TpP assay was 5.8% in samples from 4 healthy subjects with normal TpP levels.

The plasma fibrinogen concentration was determined by Clauss' method and the D-dimer concentration by latex agglutination immunoassay (Diagnostica Stago, France). TAT, an indicator of the amount of thrombin generated in the circulation, was measured using an ELISA kit (Behringwerke, Germany).

The significance of the different values obtained was analyzed using ANOVA and correlation coefficients were calculated by linear regression analysis using SAS software (SAS Institute Inc., North Carolina, USA).

Results

The patients' demographics and the results of the laboratory tests of DIC parameters are summarized in Tables 1 and 2.

Table 3 shows the mean TpP levels in 155 plasma samples from 95 septic patients with disseminated intravascular coagulation and liver disease. The 72 septic patients with decompensated phases of DIC had significantly higher TpP levels (16.1±9μg/mL and 20.9±12.4 μg/mL, respectively) than those in the compensated phase of DIC or in healthy controls (5.6±6.2 μg/mL and 2.9±1.6

Table 3. TpP levels (μg/mL) in 155 plasma samples from 95 septic patients with disseminated intravascular coagulation (DIC) and liver disease.

Group	n	Mean	SD	Minimum	Maximum	p
Sepsis	121					<.0001*
Compensated	59	5.6	6.2	0.9	31.1	
Decompensated	48	16.1	9.1	2.4	42.1	
Full-blown	14	20.9	12.4	4.4	44.9	
Sepsis with liver disease	34					0.0087°
Decompensated	19	13.4	6.5	3.8	29.3	
Full-blown	15	21.6	10.6	8.8	70.0	
Total	155	12.8	10.3	0.9	70.0	<.0001§
Normal controls	42	2.9	1.6	0.5	6.2	

**p value between compensated, decompensated and full-blown DIC in septic patients. °p value between decompensated and full-blown DIC in liver disease. §p value between all septic patients and healthy normal controls. TpP: thrombus precursor protein; SD: standard deviation.*

Table 4. Comparison of TpP levels (μg/mL) between groups with or without organ dysfunction in the septic patients with disseminated intravascular coagulation.

Organ dysfunction	n	Mean	SD	Minimum	Maximum	p value*
No	61	10.3	9.0	0.9	31.1	0.0011
Yes	60	16.9	2.4	2.4	44.9	

**p value between septic patients with and without organ dysfunction. TpP: thrombus precursor protein; SD: standard deviation.*

μg/mL, respectively). Within the septic patients with liver disease, there was a significant difference in the TpP levels between patients with full-blown DIC (21.6±10.6 μg/mL) and those in a decompensated phase of the DIC (13.4±6.5 μg/mL). Among septic patients without liver disease, a significant difference was found in the TpP levels of patients with organ dysfunction (16.9±2.4 μg/mL) and the patients without organ dysfunction (10.3±9.0 μg/mL) (Table 4).

The circulating TpP levels in 95 septic patients including those with liver diseases correlated significantly with other DIC parameters such as platelet count (r=-0.2990, *p*=0.0002), fibrinogen (r=-0.5041, *p*=<0.0001), antithrombin (r=-0.2375, *p*= 0.0327), and TAT (r = 0.3233, *p* = 0.0001) levels, and correlated weakly with D-dimer (r=0.1649, *p* = 0.0559) levels, as shown in Table 5 and Figures 1-5.

Discussion

It is agreed that DIC is an acquired disorder in which the hemostatic system, involving platelets, the coagulation system, fibrinolysis, and endothelial cells, is activated, resulting in the conversion of fibrinogen to fibrin.[10] Quantification of activation products of blood coagulation, such as soluble fibrin or fibrin monomer in plasma samples, are useful for early identification of patients with *in vivo* thrombin generation. However, soluble fibrin is not a homogeneous molecular species as fibrin monomers can form complexes with fibrinogen, other fibrin monomers or with the degradation products of fibrinogen or fibrin.[11] Several methods for measuring soluble fibrin or fibrin monomer in plasma have been developed. These include commercial assays based on the immunologic reactions of antibodies against fibrin neo-epitopes exposed by thrombin on fibrinogen.[12-14] In addition, a monoclonal antibody has been developed that recognizes an epitope region unique to the intact fibrin

Table 5. Correlation of TpP with various hemostatic variables in septic patients with DIC and liver disease.

Correlation coefficients(r)	TpP (n=155)	PT (n=155)	APTT (n=155)	Platelets (n=148)	Fibrinogen (n=130)	AT (n=81)	TAT (n=134)	D-dimer (n=135)
TpP	1.000	0.1221	0.0439	-0.2990*	-0.5041*	-0.2375°	0.3233*	0.1649§
PT		1.000	0.7374*	-0.3304*	-0.5288*	-0.5643*	0.0235	0.1006
APTT			1.000	-0.2420°	-0.4285*	-0.4630*	-0.0057	0.1291
Platelet				1.000	0.4739*	0.4687*	-0.0957	-0.1609
Fibrinogen					1.000	0.6607*	-0.2956°	-0.2850°
Antithrombin						1.000	-0.0273	-0.1286
TAT							1.000	0 .3698*
D-dimer								1.000

**p value < 0.002, °p value < 0.05, §p value = 0.0559. TpP: thrombus precursor protein; DIC: disseminated intravascular coagulation; PT: prothrombin time; APTT: activated partial thromboplastin time; AT: antithrombin; TAT: thrombin-antithrombin complex.*

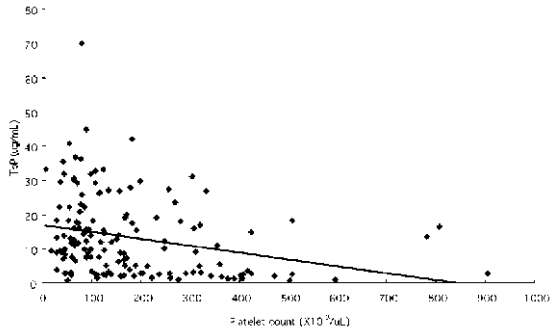

Figure 1. Correlation between TpP levels and platelet count in septic patients.

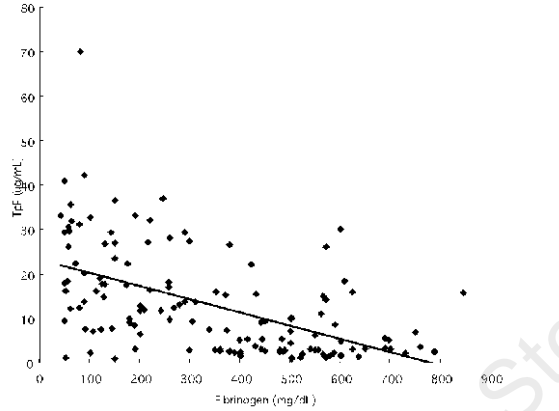

Figure 2. Correlation between TpP levels and fibrinogen concentrations in septic patients.

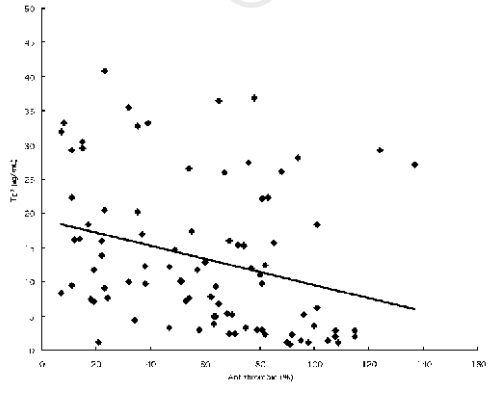

Figure 3. Correlation between TpP levels and antithrombin in septic patients.

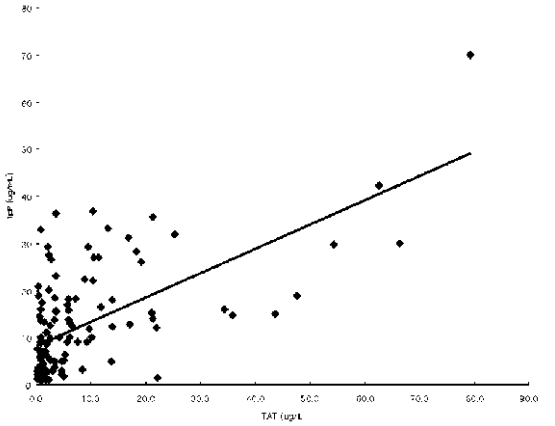

Figure 4. Correlation between TpP levels and TAT in septic patients.

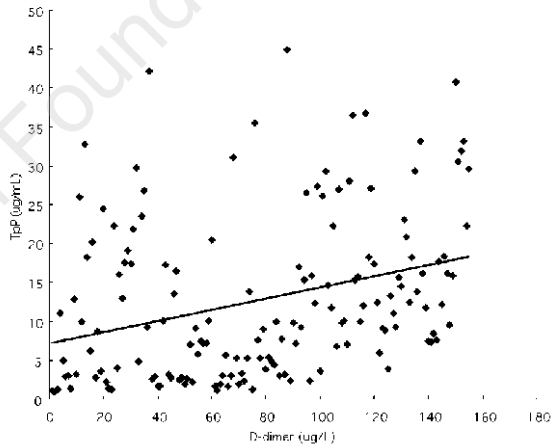

Figure 5. Correlation between TpP levels and D-dimer in septic patients.

polymer structure[15] and it has been suggested that the TpP assay could be useful as an aid to the diagnosis of myocardial infarction[6] or acute chest pain syndrome.[16] However, the magnitude of TpP elevation in septic patients, in whom microthrombosis is believed to play an important role in the development of multiple organ failure, is unknown.

This study demonstrates that septic patients have TpP concentrations from double to 20 times higher than those of normal controls. When patients were categorized according to which of three phases of

DIC they had, TpP elevation was most obvious in the septic patients in the full-blown phase of DIC, compared with those with a stressed, but compensated hemostatic system. In addition, plasma TpP levels were significantly higher in cases of sepsis with organ dysfunction than in cases of sepsis without organ dysfunction.

In 1995, Dempfle *et al.*[14] compared two immunologic tests (Enzymum-Test FM and Fibrinostika soluble fibrin) using monoclonal antibodies against fibrin specific neo-epitopes to evaluate plasma samples from healthy blood donors, patients with cerebral ischemic insult, patients with septicemia and patients with venous thrombosis. In the present study, the upper limit in normal controls was 6.2 μg/mL, which is similar to that using the Enzymum-Test FM (6.0 μg/mL), but different from that using Fibrinostika soluble fibrin tests (2.1 μg/mL). The discrepancy may be caused by different specificities of the monoclonal antibodies used and the standard used to create the reference curves contained in the individual assay kits.

In recent years, it has been shown that D-dimer tests are more sensitive than assays of fibrinogen/fibrin degradation products (FDP) and that a normal D-dimer level has a strong negative predictive value for the presence of intravascular fibrin degradation.[17] In the present study, TpP values correlated well with other DIC hemostatic markers, which suggests that TpP measurement may have a potential diagnostic role as a test for fibrin-related materials in DIC. However, only a weak correlation was found between TpP and D-dimer levels: this may reflect the measurement of different subpopulations of fibrin-related materials.

Because patients with chronic liver disease are prone to develop DIC,[18] and fibrin-related materials, including FDPs, are metabolized by the liver,[10] we also determined the magnitudes of TpP elevation in decompensated DIC and full-blown DIC in liver disease, and found that TpP was significantly higher in the full-blown phase than in decompensated phases. Our data suggest that TpP may be diagnostically useful in fulminant hepatic failure or in chronic liver disease causing acute or chronic DIC.

Hypercoagulation may cause widespread microembolism and is considered to be an important part of the development of multiple organ failure; soluble fibrin levels seem to predict organ system failure and outcome,[19] consistent with our findings in patients with or without organ dysfunction.

In conclusion, our findings indicate that septic patients who develop decompensated or full-blown DIC and organ dysfunction have significantly higher TpP plasma levels, and suggest the potential utility of theTpP assay as an aid to the diagnosis of DIC in sepsis and in liver disease.

Contributions and Acknowledgments

KSS was the principal investigator of the study from conception and design to submitted manuscript. HKK supervised experimental work. SJW collected patients' data. We thank the technicians of the hematology laboratory participating in this study.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Marcel Levi, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Levi and the Editors. Manuscript received April 15, 2002; accepted August 5, 2002.

What is already known on this topic

Markers of thrombin generation may be helpful for the diagnosis of disseminated intravascular coagulation in septic patients.

What this study adds

Thrombin precursor protein is a novel marker that may also be of value for this diagnosis.

Potential implications for clinical practice

Thrombin precursor protein may be useful for the diagnosis of DIC although prospective validation is required.

Marcel Levi, Associate Editor
(Amsterdam, The Netherlands)