Plasma Factor XIII Activity in Patients with Disseminated Intravascular Coagulation

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The objective of this study was to investigate the correlation between factor XIII (FXIII) activity and disseminated intravascular coagulation (DIC) parameters and also to evaluate the clinical usefulness of DIC diagnosis. Citrated plasma from eighty patients with potential DIC was analyzed for FXIII activity. The primary patient conditions (48 male and 32 female, mean age, 51 years) were malignancy (n = 29), infection (n = 25), inflammation (n = 6), heart disease (n = 3), thrombosis (n = 2), injury (n = 2), and other miscellaneous conditions (n = 13). FXIII testing was performed using the CoaLinkTM FXIII Incorporation Assay Kit (PeopleBio Inc.). Among 80 patients who were suspected to have DIC based on clinical analysis, 46 (57.5%) fulfilled the overt DIC criteria (DIC score > = 5) according to the International Society of Thrombosis and Haemostasis. FXIII levels in the plasma were significantly decreased in overt DIC compared to non-overt DIC patients (mean 75.1% and 199.7% respectively, $p < 0.0001$). Interestingly, we found a significant inverse correlation between DIC scores and FXIII activity. In addition, FXIII activity significantly correlated with other hemostatic markers that included platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen, and D-dimer. FXIII levels were significantly lower in patients with liver or renal dysfunction. In conclusion, FXIII cross-linking activity measurements may have differential diagnostic value as well as predictive value in patients who are suspected to have DIC.

Key Words: Disseminated intravascular coagulation, factor XIII, hemostasis

INTRODUCTION

Factor XIII (FXIII) is a plasma protein that plays an important role in the final stages of the clotting cascade and the regulation of fibrinolysis.[1,2] FXIII deficiency, caused by dyspoiesis or increased consumption, results in bleeding tendencies and wound healing complications.[3] Postoperative hemorrhage in patients with congenital and acquired FXIII deficiency has been described in various surgical fields.[4] Low activity levels were found in patients with various conditions such as sepsis, multiple fractures, and disseminated intravascular coagulation (DIC).[5] However, little data has been generated on the clinical relevance of FXIII activity in the differential diagnosis of non-overt and overt DIC. The objective of this study was to investigate the correlation between FXIII activity and DIC coagulation parameters, and to evaluate the clinical usefulness of DIC diagnosis.

MATERIALS AND METHODS

The citrated plasma from 80 patients with potential DIC were analyzed for FXIII activity. The primary patient conditions (48 male and 32 female, mean age, 51 years) were malignancy (n = 29), infection (n = 25), inflammation (n = 6), heart disease (n = 3), thrombosis (n = 2), injury (n = 2), and other miscellaneous conditions (n=13) (Table 1). FXIII testing was performed using the CoaLinkTM FXIII Incorporation Assay Kit (PeopleBio Inc., NY, USA) according to the manufacturer's instructions. The assay kit is designed to quantitate the true functional FXIII and FXIIIa activity in plasma by measuring transglutaminase activity. In this

assay, the thrombin-activated FXIIIa from the patient plasma binds to a substrate coated plate. Next, a horse radish peroxidase (HRP)-conjugated FXIII is cross-linked to the captured FXIIIa. The cross-linked HRP is then detected with a HRP chromogenic substrate at 450 nm. The data was analyzed to evaluate the correlation between FXIII activity and other DIC markers which included platelet count, fibrinogen activities, prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrin degradation product (D-dimer). PT, APTT, fibrinogen, and D-dimer results were generated with an automated clotting analyzer (STA-R) using reagents from Diagnostica Stago (Asnieres, France). Comparisons of mean group values were performed using a Student's t-test. The Pearson correlation coefficients (r) were calculated to evaluate the relationship of FXIII with other hemostatic markers. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Among the 80 patients who, upon clinical examination, potentially had DIC, 46 (57.5%) fulfilled the overt DIC criteria (DIC score $>= 5$) according to the ISTH (International Society of Thrombosis and Haemostasis) scoring system.[6] Patient characteristics at the time of study entry are shown in Table 2. The plasma levels of FXIII was significantly decreased in overt DIC patients (mean, 75.1%) compared to non-overt DIC patients (mean, 199.7%) ($p < 0.0001$, Table 3). DIC scores were inversely correlated to FXIII activity ($p < 0.0001$, Table 4). In addition, FXIII activity was significantly correlated to other hemostatic markers including platelet count (r = 0.2560, $p = 0.0191$), PT (r = - 0.3659, $p = 0.0007$), APTT (r = - 0.3058, $p = 0.0052$), fibrinogen (r = 0.5887, $p < 0.0001$), and D-dimer (r = - 0.3030, $p = 0.0057$). In contrast, FXIII activity did not significantly correlate with antithrombin levels. The FXIII levels were significantly lower in patients with liver or renal dysfunction than those without organ dysfunction ($p = 0.0064$, $p = 0.0473$, respectively). However, no significant difference in FXIII levels was detected when we compared patients with infections to patients without infections (Table 5).

Table 1. Characteristics of the Study Population

Age (yrs, mean ± SD)	51 ± 17
Male: Female	48 : 32
Underlying disease	n
Malignancy	29
Infection*	25
Inflammation	6
Heart disease	3
Thrombosis	2
Injury	2
Miscellaneous	13

*29 cases, including 4 secondary infections.
SD, standard deviation.

Table 2. Hemostatic Markers in Patients with Overt and Non-overt DIC

	Overt DIC	Non-overt DIC	*p* value
DIC score	6.2 ± 0.8	2.8 ± 1.3	< 0.0001
Platelet count (/μL)	69,000 ± 40,000	173,000 ± 98,000	< 0.0001
PT (sec)	29.9 ± 14.2	17.7 ± 3.8	< 0.0001
PT (INR)	3.3 ± 2.4	1.5 ± 0.42	< 0.0001
APTT (sec)	83.8 ± 45.0	55.5 ± 26.4	0.0003
Fibrinogen (mg/dL)	188 ± 167	407 ± 208	< 0.0001
D-dimer (μg/mL)	34.6 ± 40.4	10.7 ± 17.1	0.0003
Antithrombin (%)	46.7 ± 27.3	58.5 ± 22.3	0.0339

DIC, disseminated intravascular coagulation; PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 3. Plasma Factor XIII activity in Patients with Disseminated Intravascular Coagulation (DIC)

Study Group	Factor XIII Activity (%)		
	n	Mean (SD)	Range
Total Patients*	80	127.5 (135.0)	0.0 - 1007.1
Non-overt DIC	34	199.7 (170.0)	10.3 - 1007.1
Overt DIC	46	75.1 (69.5)	0.0 - 285.0

Non-overt DIC vs. Overt DIC*, *p* value < 0.0001.

Table 4. Correlations (r) between Factor XIII Activity and DIC Coagulation Parameters (*p* value)

	FXIII (%)	Platelets (μL)	PT (sec)	APTT (sec)	Fg (mg/dL)	DD (μg/mL)	AT (%)
Platelets (μL)	0.2560 (.0191)	*					
PT (sec)	- 0.3659 (.0007)	- 0.2245 (.0426)	*				
APTT (sec)	- 0.3058 (.0052)	- 0.1364 (.2219)	0.6882 (<.0001)	*			
Fg (mg/dL)	0.5887 (<.0001)	0.3418 (.0018)	- 0.4004 (<.0001)	- 0.3414 (.0017)	*		
DD (μg/mL)	- 0.3030 (.0057)	- 0.2248 (.0424)	0.1520 (.1701)	0.2457 (.0251)	- 0.3011 (.0060)	*	
AT (%)	0.1771 (.1160)	0.1853 (.0999)	- 0.4026 (.0002)	- 0.3259 (.0027)	0.3320 (.0026)	0.1511 (.1783)	*
DIC (Score)	-0.5648 (<.0001)	- 0.5429 (<.0001)	0.5791 (<.0001)	0.5298 (<.0001)	- 0.4888 (<.0001)	0.4449 (<.0001)	- 0.2023 (.0701)

Table 5. Percentages of Plasma Factor XIII Activity in Patients with Infection, Liver Dysfunction, or Renal Dysfunction

	Factor XIII Activity (%)			
	n	Mean (SD)	Range	*p* value
Infection				
Yes	29	160.3 (190.6)	7.6 - 1007.1	0.2373
No	51	109.6 (90.5)	0.0 - 327.3	
Liver dysfunction				
Yes	50	107.7 (148.8)	0.0 - 1007.1	0.0064
No	30	162.0 (107.3)	0.0 - 376.5	
Renal dysfunction				
Yes	20	80.7 (74.4)	0.0 - 1007.1	0.0473
No	60	143.8 (149.0)	0.0 - 309.6	

PT, prothrombin time; APTT, activated partial thromboplastin time; Fg, fibrinogen.
DD, D-dimer; AT, antithrombin; DIC, disseminated intravascular coagulation.

DISCUSSION

In this study, FXIII plasma activity is compared with other hemostatic markers in a group of patients with a clinical potential for DIC. Of the 80 patients in this study, 46 (57.5%) patients were diagnosed with overt DIC as determined by a DIC score >= 5, according to the ISTH scoring system. FXIII activity was highly variable, but decreased significantly in overt DIC patients compared with non-overt DIC patients. We detected a significant correlation between FXIII activity and global coagulation tests, such as platelet counts, PT, APTT, and fibrinogen. Interestingly, these tests are also important for the diagnosis of DIC. These results suggest that measuring FXIII activity may be helpful in diagnosing overt DIC.

FXIII activity was significantly correlated to DIC score, suggesting that the measurement of FXIII activity could be a clinically useful prognostic marker for DIC. FXIII circulates as an intact zymogen composed of a tetramer of paired A- and B- subunits and becomes enzymatically active after thrombin or factor Xa activation. The peptide responsible for enzymatic activation is located in the A subunit.[7] In this study, we used the true functional assay of transglutaminase activity to determine the levels of FXIII incorporation.

Lower FXIII activity in overt-DIC patients compared to non-overt DIC patients in this study may reflect a decreased number of A subunits as the result of a state of consumptive coagulopathy. In DIC cases which improved after treatment, the ratio of A to B subunits has been reported to be between 0.5 and 1.0. These ratios were less than 0.5 in cases of DIC that deteriorated in spite of treatment.[8] A functional ELISA-based FXIII incorporation assay may be a good alternative to using two separate ELISA systems to measure FXIII A and B subunit ratios. In addition, this functional assay may have prognostic value as a predictive marker for DIC.

DIC occurs frequently as a complication of severe sepsis and other conditions,[9] contributes to the pathogenesis of multiple organ dysfunctions, and is an independent predictor of mortality.[10] Recent advances in our understanding of DIC pathogenic mechanisms have resulted in novel therapeutic approaches, such as the administration of activated human protein C in order to restore physiologic pathways.[11] In this study, FXIII is associated with renal dysfunction and may have clinical implications in the treatment of patients with both DIC and organ dysfunction. Hypothetically, a combination of overt DIC and low FXIII activity may provide one of the new therapeutic strategies aimed at reducing mortality or the sepsis-related organ failure assessment (SOFA) score. There are several reports documenting the success of using FXIII in conjunction with antithrombin concentrate or antiplasmin drugs to treat patients with life-threatening DIC and decreased FXIII activity.[12,13]

In this study, FXIII activity was found to be associated with liver dysfunction. Because FXIII is synthesized in the liver, impaired synthesis could lead to acquired FXIII deficiency in acute or chronic liver failure, as previously described.[14] The mean FXIII activity (107.7%) in patients with liver dysfunction was only slightly decreased compared to healthy subjects (121.3%, ranging from 40.5% to 196%, SD, 28.9, n = 100). This finding supports that FXIII is synthesized not only in the liver but also in other cells, including platelets and monocytes.[15,16] These cell-associated FXIII molecules could also be one of the regulating factors of plasma FXIII activity and the cross-linking of fibrin that is associated with DIC pathogenesis.

In conclusion, we found a significant difference in plasma FXIII activity between patients with overt DIC and non-overt DIC. The measurement of FXIII cross-linking activity may have differential diagnostic value as well as predictive value in patients suspected to have DIC. FXIII concentrate could be used beneficially as an adjunct in therapeutic intervention.

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