Relationship between Hemostatic Markers and Circulating Biochemical Markers of Collagen Metabolism in Patients with Aortic Aneurysm

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Key Words

D-dimer · Thrombin-antithrombin III complex ·
Propeptide of type III procollagen · Propeptide of type I
procollagen · Aortic aneurysm

Abstract

Our objective was to determine the relationship between plasma levels of hemostatic molecular markers – D-dimer and thrombin-antithrombin III complex (TAT) – and circulating biochemical markers of collagen metabolism – aminoterminal propeptide of type III procollagen (PIIIP) and carboxyterminal propeptide of type I procollagen (PICP) – in patients with aortic aneurysm. The subjects were 43 patients with aortic aneurysm (AA; mean age 71 years) and 26 age-matched controls (mean age 75 years). The mean D-dimer, TAT and PIIIP levels were higher in the patients than in the controls ($p < 0.0001$, 0.0001 and 0.012 , respectively), while the mean PICP level was similar to that in the controls. Increased D-dimer had a significant correlation with PIIIP ($r = 0.412$, $p = 0.006$) and PICP ($r = 0.342$, $p = 0.0246$), while TAT correlated with PIIIP ($r = 0.3194$, $p = 0.0374$), but not with PICP. There was also a significant correlation ($r = 0.306$, $p = 0.0463$) between PIIIP and PICP. As shown by the signifi-

cant positive correlations among D-dimer, TAT and PIIIP, accelerated fibrinolysis and thrombogenesis induce an increase of collagen degradation and procollagen synthesis in atherosclerotic lesions. These findings show that D-dimer and TAT, especially the former, may be useful markers to monitor the progression and predict the prognosis of AA.

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Introduction

Aneurysm formation is a complex process that involves both synthesis and degradation of proteins in the extracellular matrix (ECM) [1]. Collagen is an important protein of the ECM that determines the physiological properties of the aortic wall.

Expression of type I and III of collagen are increased in aneurysm tissue compared with normal aortic walls or aortas affected by occlusive atherosclerotic disease [2]. Procollagen carboxyproteinase splits the carboxyterminal propeptide of type I procollagen (PICP), and PICP is then released into the blood. Accordingly, PICP is considered to be purely a marker of type I collagen synthesis [3]. Procollagen aminoproteinase splits the aminoterminal pro-

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Table 1. Clinical characteristics of AA patients

Type of aneurysms	Patients	Age years	Sex M:F	PIIIP U/ml	PICP ng/ml	D-dimer ng/ml	TAT ng/ml
AAA	22	75.0 ± 5.6	19:3	0.88 ± 0.44 (p < 0.05)	113.0 ± 36.1 (NS)	732.6 ± 858.6 (p < 0.0001)	16.4 ± 16.9 (p < 0.0001)
TAA	6	74.7 ± 7.2	6:1	0.82 ± 0.36 (NS)	114.0 ± 41.8 (NS)	1,261.2 ± 2,301.8 (p < 0.002)	13.6 ± 17.7 (p < 0.0034)
DAA	15	64.4 ± 12.6	11:4	0.77 ± 0.30 (p = 0.03)	115.7 ± 37.8 (NS)	880.5 ± 1,271.2 (p < 0.0001)	14.9 ± 9.6 (p < 0.0001)
Total AA	43	71.2 ± 10.0	35:8	0.79 ± 0.38 (p = 0.012)	114.1 ± 36.6 (NS)	858.0 ± 1,253.0 (p < 0.0001)	15.5 ± 14.6 (p < 0.0001)
Controls	26	74.5 ± 5.1	17:9	0.56 ± 0.11	123.0 ± 21.9	125.7 ± 46.1	2.6 ± 1.3

peptide of type III procollagen (PIIIP), and PIIIP is then released into the blood during both the synthesis and degradation of this type of collagen [4]. There have been some reports regarding these propeptides of procollagen in the blood and tissues of patients with abdominal aortic aneurysm (AAA) [5–9], and a correlation with disease severity in the blood and aortic tissue levels of PIIIP has been reported [6, 7].

Thrombosis is not only responsible for the acute manifestations of atherosclerosis, but also for the continued progression of atheroma [8], and thrombin plays a major role in these events. Thrombin-antithrombin III complex (TAT) is used as a marker of thrombin generation. The D-dimer level is dependent on the amount of fibrin associated with arteriosclerotic thrombin and the activity of the plasminogen activator [9].

Thus, collagen metabolism, coagulation and fibrinolysis are all closely related with each other. However, there have been no reports on the direct relationship between hemostatic molecular markers (D-dimer, TAT) and the markers of procollagen synthesis and turnover (PICP and PIIIP). We assayed the levels of D-dimer, TAT, PICP and PIIIP in patients with AA and age-matched controls to study the influence of coagulation and fibrinolysis on procollagen production and turnover.

Subjects and Methods

Subjects

From July 1993 to March 2002, 43 patients with AA (aged 46–89 years, with a mean age of 71 years; 35 males and 8 females), including 22 with AAA, 6 with thoracic aortic aneurysm (TAA) and 15 with dissecting aortic aneurysm (DAA), as well as 26 age-matched controls (aged 63–84 years, with a mean age of 75 years; 17 males and 9 females) were studied. Blood was collected early in the morning. Patients were excluded if they had acute AA or were within 1 year of surgery and if they were on fibrinolytic therapy. Patients with disseminated intravascular coagulation, liver dysfunction and renal failure were also excluded. As for the controls, persons who suffered from hypertension, liver dysfunction, anemia, thrombocytopenia

and hyperlipidemia, and those with a history of cerebral infarction or myocardial infarction were excluded.

Methods

Blood samples were drawn from a forearm vein, and parameters were measured by radioimmunoassay or enzyme-linked immunosorbent assay. Plasma D-dimer and TAT were measured by ELISA using two kits (Dimer test EIA, AGEN, Australia; Enzygnost TAT, Behringwerke, Germany). PICP was measured by radioimmunoassay using a kit from Orion Diagnostica, Finland, while PIIIP was measured by an immunoradiometric assay kit (Riagnost PIIIP, CIS Biointernational, France).

Statistical Analysis

The measured values of coagulation and fibrinolysis factors are expressed as means ± standard deviation. Intra-assay and inter-assay variance were within 5%. Differences between the two groups were assessed using the Mann-Whitney U test, and correlations between variables were investigated by linear regression analysis. Differences were considered significant at $p < 0.05$.

Results

Clinical Characteristics

The mean age of the AAA patients was 75.0 ± 5.6 years ($n = 22$), while TAA patients were aged 74.7 ± 7.2 years ($n = 6$), DAA patients 64.4 ± 12.6 years ($n = 15$) and the controls 74.5 ± 5.1 years ($n = 26$). Patients with AAA and TAA had almost the same age distribution, but the DAA patients were significantly younger compared with the AAA or TAA patients ($p = 0.0319$) and the controls ($p = 0.0233$). A similar sex distribution was observed in the three types of AA and the controls.

TAT and D-Dimer Levels

The mean plasma D-dimer and TAT levels in the patients were $858.0 \pm 1,253.0$ and 15.5 ± 14.6 ng/ml, respectively, being significantly higher ($p < 0.0001$) than in the controls (125.7 ± 46.1 and 2.60 ± 1.37 ng/ml, respectively). There were no differences of D-dimer and TAT among the three types of AA, as shown in table 1.

Fig. 1. Correlation of D-dimer with PIIP and PICP in patients with AA.

Serum PIIP and PICP Levels

The mean serum PIIP level of the patients was 0.79 ± 0.38 U/ml, being significantly higher ($p < 0.012$) than in the controls (0.56 ± 0.11). The mean PICP level of patients was 114.1 ± 36.6 ng/ml, which was similar to that in the controls (123 ± 46.1 ng/ml). There were no differences of PIIP and PICP among the three types of AA, as shown in table 1.

Relationships among Variables in AA Patients

D-dimer (fig. 1) showed a significant positive correlation with PIIP ($r = 0.412$, $p = 0.006$) and with PICP ($r = 0.342$, $p = 0.0246$), while TAT correlated with PIIP ($r = 0.3194$, $p = 0.0374$), but not with PICP ($r = 0.155$, $p = 0.32$). There were also significant correlations between PIIP and PICP ($r = 0.306$, $p = 0.0463$), and between D-dimer and TAT ($r = 0.678$, $p < 0.0001$).

Discussion

In this study, we evaluated those markers in the whole group of patients with various types of aneurysm. However, the etiopathogenesis of AA is mainly due to atherosclerosis.

Despite higher plasma PIIP levels, there was no correlation of PIIP with the diameter and symptomatology of AAAs [6]. However, good correlations were reported between serum PIIP and AAA symptomatology and aneurysmal diameter [7, 10, 11]. The mean PICP level of our

AA patients was similar to that of the controls, and a normal plasma PICP value was previously reported in patients with AAA. Serum PICP values also tended to be high in the rupture group, and the correlation between serum PIIP and PICP was very strong compared with that in other clinical situations [7]. In asymptomatic AAA patients who were diagnosed at an early stage of the disease, blood PICP levels did not correlate with PIIP levels. In contrast, a good correlation between serum PICP and PIIP was present in our patients who are at various stages of the disease. Thus, serum PIIP and PICP may predict an approaching rupture in AA patients.

In this study, TAT showed a low correlation coefficient with PIIP and no correlation with PICP. Thrombin influences the physical characteristics of the endothelium so that increased permeability can lead to its sequestration within the subendothelial extracellular matrix. This protects thrombin from inactivation by various physiological inhibitors, such as antithrombin III, and may lead to persistently high levels that interact with cell populations responsible for the repair processes [12]. These are the suspected reasons for a low correlation coefficient of TAT with PIIP and for a lacking correlation of TAT with PICP.

The thickness of a thrombus is the net effect of thrombogenesis and physiologic fibrinolysis, and associated fibrinolysis could theoretically be capable of increasing the collagen degradation in the aortic wall through the effect of plasmin [5]. These are the reasons for a strong positive correlation of D-dimer with PIIP. Thus, circu-

lating thrombosis- and fibrinolysis-related molecular markers (D-dimer and TAT) show significant correlations with a marker of collagen turnover (PIIIP). We have reported that aneurysmal size and volume were well correlated with TAT and D-dimer [11].

In conclusion, D-dimer and TAT show a positive correlation with PIIIP, while only D-dimer is correlated with PICP, and that there is a good correlation between serum PICP and PIIIP, findings which have never been reported before. Increased levels of D-dimer and TAT, as well as

PIIIP and PICP, indicate a poor prognosis of AA patients. In particular, D-dimer may be a more useful marker than TAT for assessing the prognosis of AA.

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