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Changes in Biomarkers of Coagulation, Fibrinolytic, and Endothelial Functions for Evaluating the Predisposition to Venous Thromboembolism in Patients With Hereditary Thrombophilia

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Abstract

The changes in the coagulation, fibrinolytic, and endothelial functions are correlated with the pathophysiology of the thromboembolic diseases during acute illness. However, these changes in patients with hereditary thrombophilia who were not in the acute stage of venous thromboembolism (VTE) are unclear. A panel of 4 biomarkers, including thrombin–antithrombin complex (TAT), plasmin-α2-plasmin inhibitor complex (PIC), tissue-type plasminogen activator/plasminogen activator inhibitor-1 complex (t-PAIC), and soluble thrombomodulin (sTM), were assayed in 100 healthy controls and 100 patients with thrombophilia. Although significantly higher concentrations of TAT, PIC, t-PAIC, and sTM were observed in patients with thrombophilia than in healthy controls, 70 patients showed absolutely normal levels of the above 4 biomarkers. Among the other 30 patients who had at least 1 biomarker out of the corresponding reference interval, 26 of them presented elevated PIC with or without increased TAT. Except for sTM, other 3 biomarkers did not show significant differences in patients with previous VTE compared to those without. Patients with single episode of VTE had obviously lower t-PAIC than those with multiple episodes of VTE, whereas the levels of TAT, PIC, and sTM were unassociated with the number of thrombosis episodes. Most thrombophilia patients who were not in the acute stage of VTE showed normal coagulation, fibrinolytic, and endothelial functions. Thus, we were unable to show that the one-time response of this panel was clinically helpful in determining thrombosis risk in thrombophilia individuals. Future studies should focus on the dynamic monitoring during the chronic phase of VTE to offer special advantages for patients with thrombophilia.

Keywords

thrombophilia, venous thromboembolism, coagulation, anticoagulants, fibrinolysis, endothelium

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Introduction

Thrombophilia, a complex condition resulting from congenital risk factors or acquired risk factors or both, is mainly characterized as the predisposition to experience multisite or recurrent thrombotic episodes in varying degrees, which would severely damage the quality of patients' lives.[1,2] The most common clinical manifestation associated with thrombophilia is venous thromboembolism (VTE), especially deep vein thrombosis (DVT) and pulmonary embolism (PE).[1] In Asian population, deficiencies in anticoagulant proteins including antithrombin (AT), protein C (PC), and protein S (PS) are the main congenital risk factors for thrombophilia, while FV Leiden and prothrombin G20210A are more prevalent in Caucasians.[3-6]

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As an ongoing challenge for physicians, the timely and accurate diagnosis of VTE are requisite for the patients with thrombophilia. The imaging assays, such as the duplex ultrasound imaging, computed tomography, magnetic resonance venography, and so on, remain the gold standard for the diagnosis of VTE.[7,8] However, imaging screens are commonly time-consuming and associated with radiation exposure, so the painless diagnosis of VTE based on the detection of plasma biomarkers has great clinical usefulness.[9-12] Currently, a panel of 4 laboratory assays comprising of thrombin–antithrombin complex (TAT), plasmin-α2-plasmin inhibitor complex (PIC), tissue-type plasminogen activator (tPA)/plasminogen activator inhibitor-1 (PAI-1) complex (t-PAIC), and soluble thrombomodulin (sTM), which were related to human coagulation, fibrinolytic, and endothelial function, was established to evaluate the risk of VTE in combination. Thrombin, an activated form of prothrombin, plays a vital role in the activation of its natural substrates, including fibrinogen, factor V, factor VIII, and PC, and so on. The activity of thrombin in plasma is regulated through its rapid interaction with AT to subsequently form TAT. As a marker of assessing the level of coagulation activation, increased levels of TAT were observed in patients with coronary artery disease, peripheral arterial occlusive disease, or perioperative patients associated with the development of DVT.[13-16] Plasmin-α2-plasmin inhibitor complex, an irreversible complex of plasmin and a2-plasmin inhibitor, is considered as a satisfactory indicator of an enhanced fibrinolytic state in vivo because it is almost undetectable in normal persons and expected to be more useful for the clinical assessment of VTE in patients with a high-risk perioperative VTE.[17,18] Besides, t-PAIC, the 1:1 covalent inactive complex of tPA and PAI-1, has been proposed as a marker of the fibrinolytic system in the diagnosis of acute myocardial infarction (MI), stroke, and VTE.[19-21] Moreover, regarded as a marker of endothelial lesion, sTM, the glycoprotein appears in human urine and plasma in a truncated form of TM without transmembrane and cytoplasmic domains, is involved in this panel as well.[22] Some studies have indicated that elevated sTM was observed in a certain number of pathologies associated with endothelial damage: atheromatous arterial disease, disseminated intravascular coagulation, and systemic lupus erythematosus.[23-25]

In this study, we firstly explored the performance of 4 biomarkers (TAT, PIC, t-PAIC, and sTM) on evaluating the activations of coagulation and fibrinolytic system as well as endothelial function in patients with deficiencies of anticoagulant proteins.

Materials and Methods

Patient Involvement

A total of 200 participants were planned to be included in this case–control study to detect a medium effect size and taking into account a significance level (alpha) of .05 and statistical power (1-β) of .90. The sample size was determined by a priori power analysis using G*Power version 3.1.9.2.[26] Among the 520 unrelated VTE pedigrees who were registered at the Thrombosis and Haemostasis Centre in Ruijin Hospital from 2016 to 2019, 100 anticoagulant-deficient patients were selected to constitute the thrombophilia patients group (TPs). The diagnosis of anticoagulant deficiencies depended on the decreased activities of anticoagulant proteins measured on an ACL-TOP automatic analyzer (Instrumentation Laboratory) and/or causative gene mutations[27] All patients have been out of the acute phase of VTE more than 6 weeks and have ceased taking anticoagulant treatment for at least 2 weeks. Quantitative test and genotypic analysis were also carried out in 150 healthy volunteers to exclude the anticoagulant deficiency. Finally, 100 healthy participants were selected and matched for age, sex, and geographic region to form the healthy control group (HCs).

Biomarker Measurements

After informed consent was obtained, venous blood samples were collected in 0.109 mol/L sodium citrate tube from all patients and centrifuged at 3000*g* for 15 minutes to separate plasma from blood cells, then samples were rapidly frozen at −80 °C until use. The kits of TAT, PIC, t-PAIC, and sTM were provided by Sysmex Corporation. The measurements were all based on the chemiluminescence enzyme immunoassay method with CDP-Star chemiluminescent substrate and carried out on an HISCL automated analyzer (HISCL-2000i; Sysmex).

Statistical Analyses

Quantitative data were presented as mean with SD or median with interquartile ranges (IQR) and were compared using Student *t* test or nonparametric Mann-Whitney *U* test as appropriate according to the normality of continuous variables by GraphPad PRISM version 5.0. Categorical variables were expressed by means of absolute numbers or percentages and were compared using the χ^2 test. To analyze correlations, the Spearman correlation test was used. Kruskal-Wallis analysis of variance (ANOVA) test was applied to compare more than 2 groups of unpaired data. A *P* value of .05 or less was considered statistically significant. The reference intervals of TAT and PIC were less than 4.0 ng/mL and 0.8 μg/mL, respectively. From 3.8 to 13.3 TU/mL was used as the reference interval of sTM, while t-PAIC had a gender-specific reference interval that female should below 10.5 ng/mL and male should below 17.0 ng/mL.

Results

Descriptive Characteristics

The baseline characteristics of the study population are listed in Table 1. The median ages of TPs (56% males, 44% females) and HCs (56% males, 44% females) were 45 years (IQR, 30-55 years) and 47 years (IQR, 32.0-55.8 years), respectively. The TPs group was comprised of 17 AT-deficient, 39 PC-deficient, 38 PS-deficient, and 6 combined anticoagulant-deficient patients (Supplemental Table 1). Among the 66 TPs who had a positive history of thrombosis, 54.5% (36/66) experienced

Table 1. Descriptive Characteristics of the TPs and HCs.[a]

	TPs	HCs	*P* value
Age, median (interquartile range)	45.0 (30.0-55.0) y	47.0 (32.0-55.8) y	ns
Gender, n (%)			
Male	56 (56.0%)	56 (56.0%)	ns
Female	44 (44.0%)	44 (44.0%)	ns
History of VTE, n (%)			
No thrombosis	34 (34%)	0 (0.0%)	[b]
Single thrombosis	30 (30%)	0 (0.0%)	[b]
Recurrent thrombosis	36 (36%)	0 (0.0%)	[b]
Risk factors for thrombophilia, n (%)			
AT deficiency (single heterozygote)	17 (17.0%)	0 (0.0%)	[b]
PC deficiency (single heterozygote)	29 (29.0%)	0 (0.0%)	[b]
PC deficiency (compound heterozygote)	10 (10.0%)	0 (0.0%)	[c]
PS deficiency (single heterozygote)	37 (37.0%)	0 (0.0%)	[b]
PS deficiency (compound heterozygote)	1 (1.0%)	0 (0.0%)	ns
AT and PC deficiency	1 (1.0%)	0 (0.0%)	ns
AT and PS deficiency	1 (1.0%)	0 (0.0%)	ns
PC and PS deficiency	4 (4.0%)	0 (0.0%)	ns

Abbreviations: AT, antithrombin; HCs, healthy controls; PC, protein C; PS, protein S; ns, no significance; TPs, thrombophilia patients; VTE, venous thromboembolism.

[a] Ages were presented as medians with interquartile range and were compared using Student *t* test. Other categorical variables were expressed by absolute numbers and percentages and were compared using the χ^2 test.

[b] $P < .001$.

[c] $P < .01$;

multisites and/or recurrent thrombotic episodes. None of HCs had deficiency of anticoagulant proteins.

The HCs Versus TPs Group

The TAT, PIC, t-PAIC, and sTM were significantly higher in TPs compared to HCs (Table 2). The TAT was 2.2-fold (1.88 ± 0.34 ng/mL vs 0.85 ± 0.10 ng/mL; $P < .05$), PIC was 1.9-fold (0.88 ± 0.11 ng/mL vs 0.46 ± 0.02 μg/mL; $P < .001$), t-PAIC was 1.3-fold (6.90 ± 0.34 ng/mL vs 5.33 ± 0.26 ng/mL; $P < .001$), and sTM was 1.14-fold (8.82 ± 0.24 TU/mL vs 7.72 ± 0.17 TU/mL; $P < .01$) higher in TPs than in HCs. However, most TPs (70%, 70/100) and HCs (91%, 91/100) showed absolutely normal levels of the above 4 biomarkers. Elevated TAT, PIC, t-PAIC, and sTM were observed in 9, 26, 4, and 5 TPs, respectively, but no potential correlations between the values of biomarkers and the types of anticoagulant deficiency had been found (data not shown).

Table 2. Plasma levels of TAT, PIC, t-PAIC, and sTM from TPs and HCs.

Parameters	TPs	HCs	*P* value
TAT (ng/mL)	1.88 ± 0.34, N=100	0.85 ± 0.10, N=100	**
PIC (μg/mL)	0.88 ± 0.11, N=100	0.46 ± 0.02, N=100	***
t-PAIC (ng/mL)	6.90 ± 0.34, N=100	5.33 ± 0.26, N=100	***
sTM (TU/mL)	8.82 ± 0.24, N=100	7.72 ± 0.17, N=100	***

Abbreviations: HCs, healthy controls; PIC, plasmin-α2-plasmin inhibitor complex; sTM, soluble thrombomodulin; TAT, thrombin–antithrombin complex; t-PAIC, tissue-type plasminogen activator/plasminogen activator inhibitor-1 complex; TPs, thrombophilia patients.

[b] $P < .01$.

[c] $P < .001$.

Males Versus Females

The characteristics of TAT, PIC, t-PAIC, and sTM in males and females are shown in Figure 1. In HCs, remarkably higher sTM and t-PAIC were observed in males than in females (8.41 ± 0.20 TU/mL, N = 56 vs 6.83 ± 0.23 TU/mL, N = 44; $P < .0001$ and 5.90 ± 0.35 ng/mL, N = 56 vs 4.59 ± 0.34 ng/mL, N = 44; $P < .01$, respectively), while TAT and PIC were not ($P > .05$). In TPs, sTM showed obvious difference between men and women (9.26 ± 0.33 TU/mL, N = 56 vs 8.26 ± 0.32 TU/mL, N = 44; $P < .05$), while no significant differences in the other 3 biomarkers were detected ($P > .05$).

Additionally, males showed significantly higher TAT, PIC, tPAI-C, and sTM in the TPs group than those in the HCs group (1.89 ± 0.45 ng/mL vs 0.71 ± 0.07 ng/mL; $P < .05$, 0.91 ± 0.13 μg/mL vs 0.46 ± 0.02 μg/mL; $P < .001$, 7.38 ± 0.48 ng/mL vs 5.90 ± 0.35 ng/mL; $P < 0.05$, and 9.26 ± 0.33 TU/mL vs 8.40 ± 0.20 TU/mL; $P < 0.05$, respectively). In females, t-PAIC (6.29 ± 0.47 ng/mL vs 4.59 ± 0.34 ng/mL; $P < .01$) and sTM (8.26 ± 0.32 TU/mL vs 6.83 ± 0.23 TU/mL; $P = .001$) were obviously higher in the TPs group compared with the HCs group, while TAT and PIC were not ($P > .05$). It might suggest that male patients were more likely to exhibit excessively activation of coagulation and fibrinolysis simultaneously than female patients when they did not have the current VTE.

With Versus Without a History of VTE

Among the patients with congenital deficiencies in anticoagulant proteins, 66 patients experienced single or multiple episodes of VTE during their lives, while other 34 patients were free of thrombosis until admitted to our study. Nonparametric Mann-Whitney *U* test indicated that only sTM showed significant difference in patients with previous VTE compared to those without (8.46 ± 2.41, N = 66 vs 9.52 ± 2.16, N = 34; $P < .01$; Figure 2), and other 3 biomarkers only presented slight increase in patients with a history of VTE ($P > .05$). Kruskal-Wallis ANOVA test revealed that patients with DVT, PE, DVT + PE, or other unusual site thrombosis (portal venous thrombosis, spontaneous abortion, or cerebral venous sinus thrombosis) did not show any differences in TAT, PIC, t-PAIC, and sTM concentrations (data not shown).

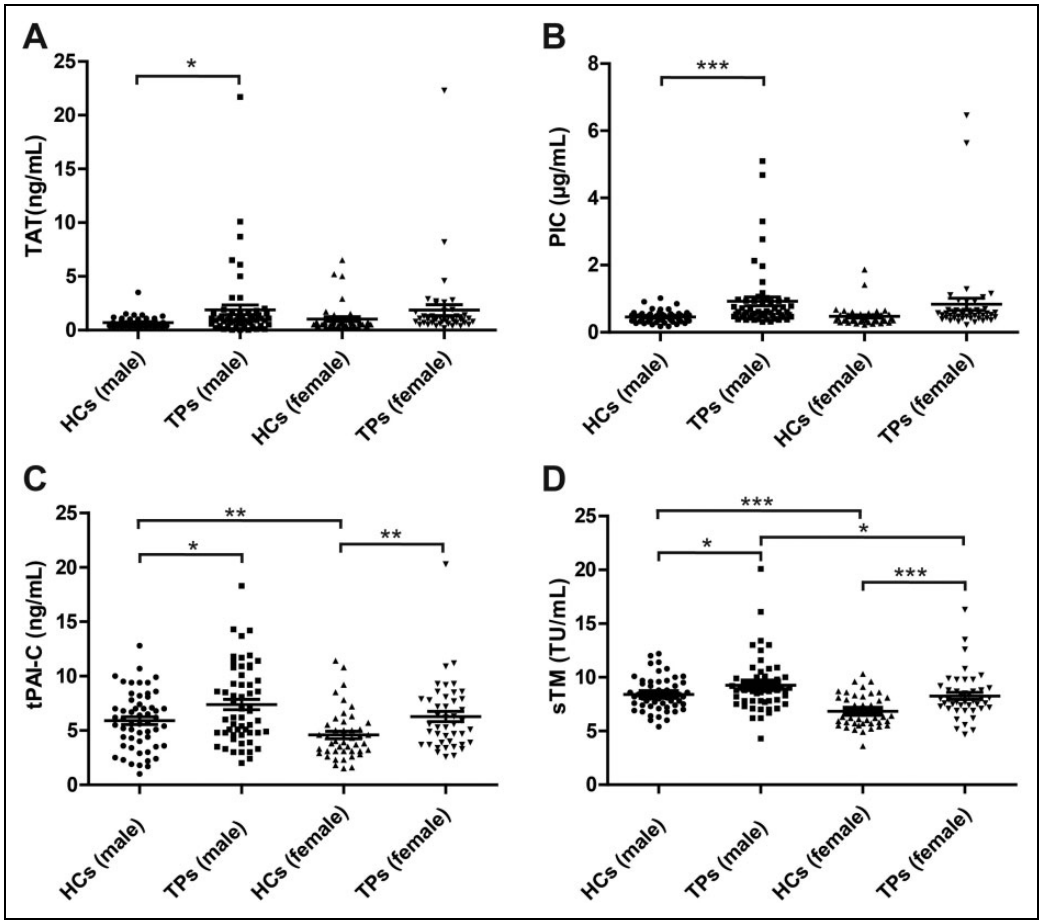

Figure 1. Plasma level of TAT, PIC, t-PAIC, and sTM in different genders from TPs and HCs. • Male HCs; ■ male TPs; ▲ female HCs; ▼female TPs. Statistical analysis was performed using Student *t* test. *$P < .05$; **$P < .01$; ***$P < .001$. HCs indicates healthy controls; PIC, plasmin-α2-plasmin inhibitor complex; TAT, thrombin–antithrombin complex; TPs, thrombophilia patients; t-PAIC, tissue-type plasminogen activator/plasminogen activator inhibitor-1 complex; sTM, soluble thrombomodulin.

Single Versus Multiple Episodes of VTE

Among the thrombophilia patients with a history of VTE, 45.5% of patients suffered single episode of VTE and the other 54.5% of patients experienced multisites and/or recurrent thrombotic episodes, including DVT, PE, portal venous thrombosis, spontaneous abortion, or cerebral venous sinus thrombosis (Table 1). As shown in Figure 3, Student *t* test revealed that patients with single episode of VTE ($N = 30$) presented lower t-PAIC (6.27 ± 2.28 ng/mL vs 7.42 ± 3.77 ng/mL; $P < .05$) concentrations compared to those with multiple episodes of VTE ($N = 36$). The levels of TAT, PIC, and sTM were unassociated with the number of episodes of VTE.

Discussion

It is well known that deficiencies in anticoagulant proteins are the predominantly thrombotic risk factors in the Asian populations.[3,4] Based on our previous investigation, the frequencies of inherited PS, PC, and AT defects among Chinese population with VTE were 17.5%, 16.8%, and 8.3%, respectively. But the incidence bias may be existed because the majority of patients were referred to our center by local clinicians for screening the thrombotic risk factors after the diagnosis of first or recurrent VTE.[27] Ultimately, we chose 17 AT-deficient, 39 PC-deficient, 38 PS-deficient, and 6 combined anticoagulant-deficient patients from our center to constitute the thrombophilia group. It is worth noting that these values only represented the composition of the 3 defects in this group rather than the incidences in the population.

Although previous study showed a significant surge in the incidence of VTE in Chinese population, the VTE risk assessment has not been routine in China so far.[28] D-dimer is the most widely used marker in clinic to exclude VTE and monitor the recurrence of VTE with a high sensitivity but a low specificity.[29] As aforementioned, the panel of 4 biomarkers (TAT, PIC, t-PAIC, and sTM), which was able to evaluate the dynamic balance of procoagulant, anticoagulant, and fibrinolytic system as well as the endothelial lesion, was established and applied to estimate the relative risk of developing thrombosis in Chinese polulation.[30-32] Previous studies mainly concerned about its early and sensitive assessment in patients with thromboembolic diseases during acute illness, such as

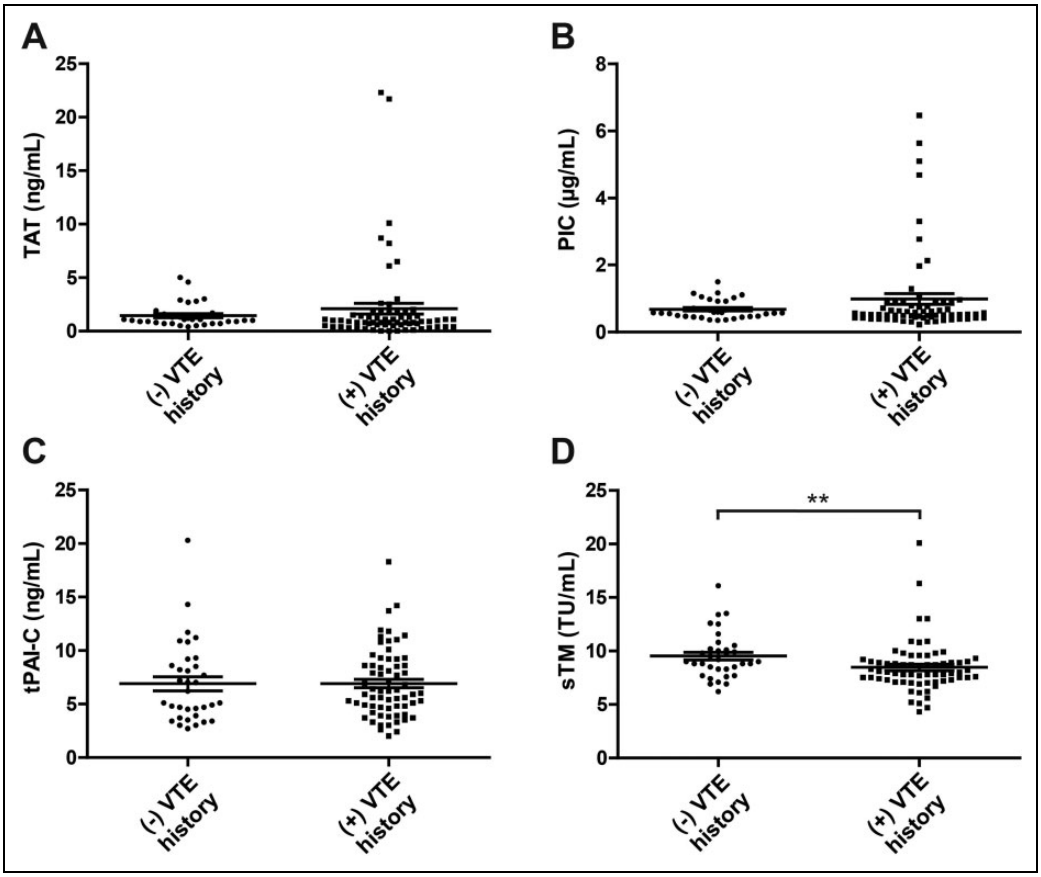

Figure 2. Plasma level of TAT, PIC, t-PAIC, and sTM of TPs with (n = 66) or without (n = 34) a history of VTE. • The TPs without a history of VTE; ■ TPs with a history of VTE. Statistical analysis was performed using nonparametric Mann-Whitney *U* test. $^{**}P < .01$. PIC indicates plasmin-α2-plasmin inhibitor complex; sTM, soluble thrombomodulin; TAT, thrombin–antithrombin complex; t-PAIC, tissue-type plasminogen activator/plasminogen activator inhibitor-1 complex; VTE, venous thromboembolism.

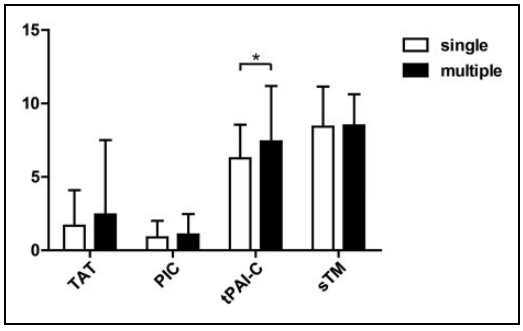

Figure 3. Plasma level of TAT, PIC, t-PAIC, and sTM of TPs with single (n = 30) or multiple (n = 36) episodes of VTE. The unit was ng/mL, μg/mL, ng/mL, and TU/mL for TAT, PIC, t-PAIC, and sTM, respectively. Statistical analysis was performed using Student *t* test. $^{*}P < .05$. PIC indicates plasmin-α2-plasmin inhibitor complex; sTM, soluble thrombomodulin; TAT, thrombin–antithrombin complex; t-PAIC, tissue-type plasminogen activator/plasminogen activator inhibitor-1 complex.

traumatic patients after orthopedic surgery, patients with acute MI, or patients with critical thromboembolism from intensive care unit, in which above biomarkers showed remarkable abnormality compared with healthy controls.[30-32]

Why do we focus on the performance of this panel in patients with inherited thrombophilia but not in the acute stage? On the one hand, patients with thrombophilia are associated with a higher risk of developing multisite or recurrent thrombotic episodes, particularly when additional factors (trauma, surgery, etc) existed.[1] It is necessary to ascertain whether patients with thrombophilia who are not in the acute stage of VTE might benefit from such testing, which would have an impact on whether clinicians recommend this test for such patients. On the other hand, it is common knowledge that VTE was induced by imbalance among coagulation, anticoagulation, and fibrinolysis. Thus, it is important to understand how do anticoagulant defects perturb the hemostatic balance and endothelial function in patients with thrombophilia out of acute illness.

Prompted by our data, though significantly higher TAT, PIC, t-PAIC, and sTM were observed in TPs than in HCs, the majority of TPs and HCs showed absolutely normal levels of the above 4 biomarkers, and there were considerable overlaps with the normal control group. It suggested that the most patients with deficiencies in anticoagulant proteins had relatively normal coagulation, fibrinolytic activity, and endothelial function when they did not have the current VTE episodes,

which was consistent with the previous study.[33] Additionally, male patients with thrombophilia had significantly higher TAT, PIC, t-PAIC, and sTM than male healthy controls, while females only presented significantly higher t-PAIC and sTM in the TPs group compared with the HCs group. It might suggest that male patients were more likely to exhibit excessively activation of coagulation and fibrinolysis simultaneously than female patients when they did not have the current VTE.

As an indicator to evaluate the extent of fibrinolytic activity, elevated PIC indicated the presence of thromboembolic disease.[18,34] In our study, 8 TPs with a history of VTE showed obviously increased PIC (2-8 folds). Interestingly, almost all patients with markedly increased PIC, especially the patients with previous thrombosis, were accompanied by the overtly elevated TAT, suggesting that these patients were more likely to exhibit excessively activation of coagulation and fibrinolysis simultaneously when they did not have the current VTE. Continuous follow-up of these patients is important to prevent the recurrence of thrombosis. Since D-dimer is a traditional biomarker reflecting both ongoing coagulant and fibrinolytic activity with high sensitivity, we assessed the relationship between PIC and D-dimer, and a significant correlation was found between them (Supplemental Figure 1).[11] It was reported that D-dimer showed decreased sensitivity in patients with recurrent thrombosis than first episode because that patients with a history of VTE were more likely to have positive D-dimer than patients without previous VTE.[11,35] In the current study, 13 patients without previous VTE showed positive D-dimer, in which 9 combined with positive PIC; however, among the patients with a history of VTE, 26 showed positive D-dimer including only half patients combined with positive PIC, suggesting that PIC may be more specific to assess the fibrinolytic activity in patients with recurrent thrombosis than D-dimer.

Moreover, endothelial lesion is associated with thrombotic risk in a variety of diseases including atherosclerosis, viral infections, and neoplastic disease.[36] The level of sTM, a marker of endothelial cell injury, was related to the severity of the pathology in atheromatous arterial disease and disseminated intravascular coagulation.[23,24] Prompted our data, only few TPs had slightly increased sTM, while most patients had normal sTM. It suggested that in TPs who were not in the acute stage of VTE, release of sTM from the endothelial cell membrane following the course of injury by activated leukocytes or hydrogen peroxide was not obvious. However, sTM showed significant difference in symptomatic patients compared to asymptomatic patients, suggesting that decreased plasma sTM concentration may be associated with the history of VTE.

Our study has some limitations. Firstly, the sample size of our study was limited so that all the above results should be explained in the context of the small study population. In the future, larger studies are needed to be carried out to support these findings. Secondly, we collected the blood sample at a certain moment instead of a continuous stage. For the patients with thrombophilia who had suffered thrombotic episode, the dynamic monitoring to keep them from the recurrence of VTE is very important. The momentary examinations of the above biomarkers are hard to reflect the tendency of thrombus formation.

Taken these results together, though TAT, sTM, PIC, and t-PAIC were sensitive biomarkers of active VTE, most patients with thrombophilia who were not in the acute stage of VTE performed normal coagulation, fibrinolytic, and endothelial function. Thus, it was unable to show that the one-time response of this panel was clinically helpful in determining thrombosis risk in thrombophilia individuals. Future studies should focus on the dynamic monitoring during the chronic phase of VTE to offer special advantages for patients with thrombophilia.

Authors' Note

Lei Li and Lixia Gao contributed equally to this work. Ethical approval to report this case series was obtained from Ruijin Hospital Ethics Committee (APPROVAL NUMBER/2019-54). Verbal informed consent was obtained from the patients for their anonymized information to be published in this article.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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