Laboratory evaluation of patients with undiagnosed bleeding disorders

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The evaluation of patients with a bleeding tendency represents a challenge as the routinely available tests for evaluating bleeding disorders are limited, complicating the laboratory determination of the clinically observed bleeding tendency. As a result, some bleeding disorders remain undiagnosed. The aim of the study was to evaluate whether global coagulation tests would contribute to the laboratory analysis of patients with undiagnosed bleeding disorders. Patients were evaluated for coagulation and fibrinolysis activities by thrombin generation test and euglobulin lysis time. In addition, plasma activity of factor XIII, plasminogen, α-2 antiplasmin, plasminogen activator inhibitor-1, and thrombin-activatable fibrinolysis inhibitor was also obtained. Forty-five patients were included. Eight per cent presented a mild bleeding disorder and 20% a moderate bleeding disorder. The thrombin generation test results were similar between patients and controls. Euglobulin lysis time results, however, were lower in patients than in controls, both before (median 175 vs. 250 min, respectively; $P = 0.003$) and after (median 145 vs. 115 min, respectively; $P \leq 0.001$) arm constriction, suggesting that they were experiencing hyperfibrinolysis. Interestingly, patients' median thrombin-activatable fibrinolysis inhibitor activity was higher than in controls (21.2 vs. 19.46 μg/ml; $P = 0.016$). However, plasminogen, α-2 antiplasmin, plasminogen activator inhibitor-1, and factor XIII activities did not differ between the groups. Global coagulation and fibrinolysis tests proved to be limited in detecting the hemostatic disorders in some patients with a relevant bleeding tendency and may not be adequate to address their bleeding risk. Bleeding scores are currently the available medical approach for the evaluation of these patients. *Blood Coagul Fibrinolysis* **27:500–505**

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Introduction

The evaluation of patients with a bleeding tendency represents a challenge for clinicians and hematologists. At least half of the patients remain undiagnosed because they may present normal laboratory results, despite of the wide range of tests performed [1–3]. The causes of undiagnosed bleeding disorders are speculative and may be multifactorial. The associations of slightly decreased coagulation factor activities [2] or impaired fibrinolysis [4] have been described to possibly contribute to the bleeding manifestations in these patients. However, routinely available tests to evaluate these disorders are limited, complicating the laboratory determination of the bleeding tendency observed clinically.

Global hemostasis tests, however, are capable of evaluating the interaction of varied mechanisms responsible for blood coagulation and fibrinolysis [5–7], and could therefore be more sensitive for detecting subtle impairments of clot formation or degradation than the traditional tests. In this study, we evaluated the integrity of blood coagulation and fibrinolysis in patients with an undiagnosed bleeding tendency using two global tests: thrombin generation test (TGT) and euglobulin lysis time (ELT). Our aim was to evaluate whether these tests would contribute to the laboratory analysis of the patients. We also correlated the findings of the global tests with the results of coagulation factor and fibrinolysis tests obtained from patients in order to achieve a specific coagulopathy diagnosis.

Patients and methods

Patients' selection

Patients with bleeding symptoms who attended the Hematology Center of the University of Campinas, from September 2004 until September 2007, were selected for the study. Patients diagnosed with hemophilia or some other coagulopathy, such as von Willebrand disease (VWD), platelet disorders, and rare bleeding disorders, were excluded. Intake of any medications that could

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interfere with hemostasis imbalance, chronic renal or liver insufficiency, infections, autoimmunity, and neoplasia were also considered as exclusion criteria. Healthy individuals, belonging to the same geographic area as the patients, were selected from the blood donors and employees from our institution, and were used as controls. The inclusion criteria were the same as for the patients.

The present study was approved by the Committee on Human Research of the Faculty of Medical Sciences of the University of Campinas, and a written informed consent was obtained from patients, controls, or their attending relatives.

Clinical evaluation

All patients and healthy controls were interviewed using a structured questionnaire for bleeding scoring (Score System Questionnaire for bleeding disorders proposed by the MCMDM-1 Steering Committee, Milan, 13–14 November 2003) to confirm or discard the bleeding tendency. By this questionnaire, the scores were attributed to each symptom as follows: −1, no bleeding even during risk situations; 0, no bleeding even after small procedures; 1, bleeding referred by the patient without medical intervention; 2–4, bleeding requiring medical attention or intervention. Final score determined the severity of the symptoms: −3 to −1, no bleeding disorder; 0, no symptoms; 1–10 (1–12 for women), mild bleeding disorder; 11–20 (13–24) for women, moderate; 21–30 (25–36 for women), severe; above 30 (above 36 for women), very severe.

The patients' medical history and the results of coagulation factors and platelet aggregation assays were obtained retrospectively from the medical charts and annotated in the patients study registry.

Blood sampling

Patients' plasma samples were obtained from whole blood by peripheral venous puncture, in citrate tubes, and were stored at −80°C for prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), factor (F)XIII, α-2 antiplasmin (α2AP), plasminogen, plasminogen activator inhibitor-1 (PAI-1), and TGT. ELT was performed with whole blood collected from the left forearm before and after being constricted by a cuff inflated to 40 mmHg for 10 min, to stimulate tissue plasminogen activator release. Control samples were processed by the same investigator following the same protocols.

Evaluation of fibrinolysis

Euglobulin lysis time, before and after stimulation by arm constriction, was performed for global fibrinolysis evaluation. ELT was performed according to the method described by Haverkate *et al.* [8].

Alpha-2 antiplasmin and plasminogen activities were determined by the chromogenic kits 'Berichromα-2 antiplasmin' and 'Berichromplasminogen', respectively (Dade-Behring Inc., Newark, Delaware, USA) using the automated photo-optic analyzer Coag-A-Mate-MTX (bioMérieux Inc., Durham, North Carolina, USA). PAI-1 activity was achieved using the chromogenic kit 'Berichrom PAI-1' (Dade-Behring Inc.) and the analyzer AMAX Destiny (Trinity Biotech Inc., Bray, County Wicklow, Ireland). Thrombin-activatable fibrinolysis inhibitor (TAFI) activity was measured by the commercial kit Actichrome TAFI activity (American Diagnostica Inc., Stamford, Connecticut, USA).

Evaluation of coagulation

Blood coagulation was evaluated by TGT in platelet-poor plasma using a commercial fluorescent assay (Technothrombin TGA, Technoclone GmbH, Vienna, Austria), and fluorescence was read using FLx800 Fluorescence Microplate Reader (Bio-Tek Instruments, Winooski, Vermont, USA). Four parameters of TGT were evaluated: the lag phase, which is the time from the beginning of the experiment to the first burst in thrombin formation; the thrombin peak, which is the maximum concentration of thrombin formed; the velocity index, which is the amount of thrombin formed per minute; and the total thrombin formed, represented by area under the curve (AUC).

Factor XIII activity was determined by the chromogenic kit 'Berichrom FXIII' using the BCS analyzer (Dade-Behring Inc.). All commercial kits were used according to the manufactures' instructions.

Statistical methods

Results are presented as mean ± SD or as median and 95% confidence interval (CI) (for normal and non-normal distributions, respectively). The means were compared by a two-tailed unpaired Student's *t* test and the medians by Mann–Whitney *U* test. Categorical data were compared using Fisher's exact test or chi-square analysis. Pearson's or Spearman's rank correlations were used for data with normal or non-normal distribution, respectively.

Results obtained from the controls were used to determine the normal range of each parameter studied, calculating the mean and two SDs for each variable. We also performed individual analysis in order to reach the diagnosis of a specific coagulation disorder.

Results

From the 1543 individuals with a bleeding tendency attended at the Hemostasis and Thrombosis Clinic at the Hematology Center of the University of Campinas, Brazil, during the period of the study, 376 (24.3%) individuals had hemophilia, 388 (25.1%) individuals had VWD, and 628 (40.7%) patients presented platelet

Table 1 Demographic characteristics and laboratory results of patients and controls

	Controls	Patients	*P* value
Male/female (*n*)	23/27	7/38	0.001
Age (years)	31 (18–53) ($n=50$)	32 (18–64) ($n=45$)	0.440
aPTT (s)	31.2 (25.6–50) ($n=45$)	31.9 (29.6–34.2) ($n=23$)	0.246
PT (s)	14.9 (13.3–16.6) ($n=47$)	14.2 (12.6–18.2) ($n=34$)	0.0323
TT (s)	10.6 (9.7–13.1) ($n=47$)	11.1 (11.9–13.2) ($n=34$)	0.0104

Age, aPTT, PT, and TT are expressed as median and range. Mann–Whitney *U* test was performed to compare continuous variables and Fisher's exact test was used for comparison of non continuous variables between patients and controls. aPTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time.

disorders, including immune thrombocytopenia. The individuals with coagulation factor deficiencies were: six (0.4%) with factor V deficiency, 44 (2.9%) with FVII deficiency, two (0.1%) with FX deficiency, 21 (1.4%) with FXI deficiency, and 33 (2.1%) with FXII deficiency. In this study, the remaining 45 (2.9%) patients with an undiagnosed bleeding tendency and the 50 controls were included. The demographic characteristics and laboratory results are listed in Table 1.

According to the bleeding questionnaire, 80% patients presented a mild bleeding disorder (score 4–12) and 20% moderate bleeding disorder (score 13–19). Twenty patients reported a previous blood transfusion after surgeries (44%), six presented chronic anemia (13%), and five reported spontaneous bleeding episodes (11%) [9].

All patients had normal screening coagulation tests, median PT was 14.25 s (range 12.6–18.2 s), median aPTT was 31.95 s (range 29.6–34.2 s), median bleeding time was 6.05 s (range 3.35–5.22 s), and median TT was 11.1 s (range 11.9 –13.2 s).

There was no difference between lag phase, velocity, AUC, peak thrombi, and time to peak of TGT in patients and control groups (Fig. 1).

Euglobulin lysis time was lower in patients than in controls, both before (median 175 vs. 250 min, respectively; $P=0.003$) and after arm constriction (median 145 vs. 115 min, respectively; $P\leq 0.001$) (Fig. 2), suggesting that patients might have hyperfibrinolysis.

The activities of α2AP, PAI-1, plasminogen, and FXIII were, however, similar between patients and controls (Table 2). Interestingly, patients' median TAFI activity was higher than in controls (21.2 vs. 19.46 μg/ml; $P=0.016$). In these analyses, two outliers were observed, one patient was diagnosed with severe PAI-1 deficiency (undetected activity; normal range 6.90–2.25 U/ml) and one patient diagnosed with FXIII deficiency (normal range 70–140%).

We also investigated the coefficient of correlation between TGT and ELT with traditional coagulation tests and specific fibrinolysis components. However, no significant correlation was found between these parameters (data not shown). Furthermore, we failed to find any correlation between bleeding scores and TGT or ELT results. Noteworthy, we found a positive correlation between age and bleeding score in patients ($r=0.4530$ and $P=0.0018$).

Discussion

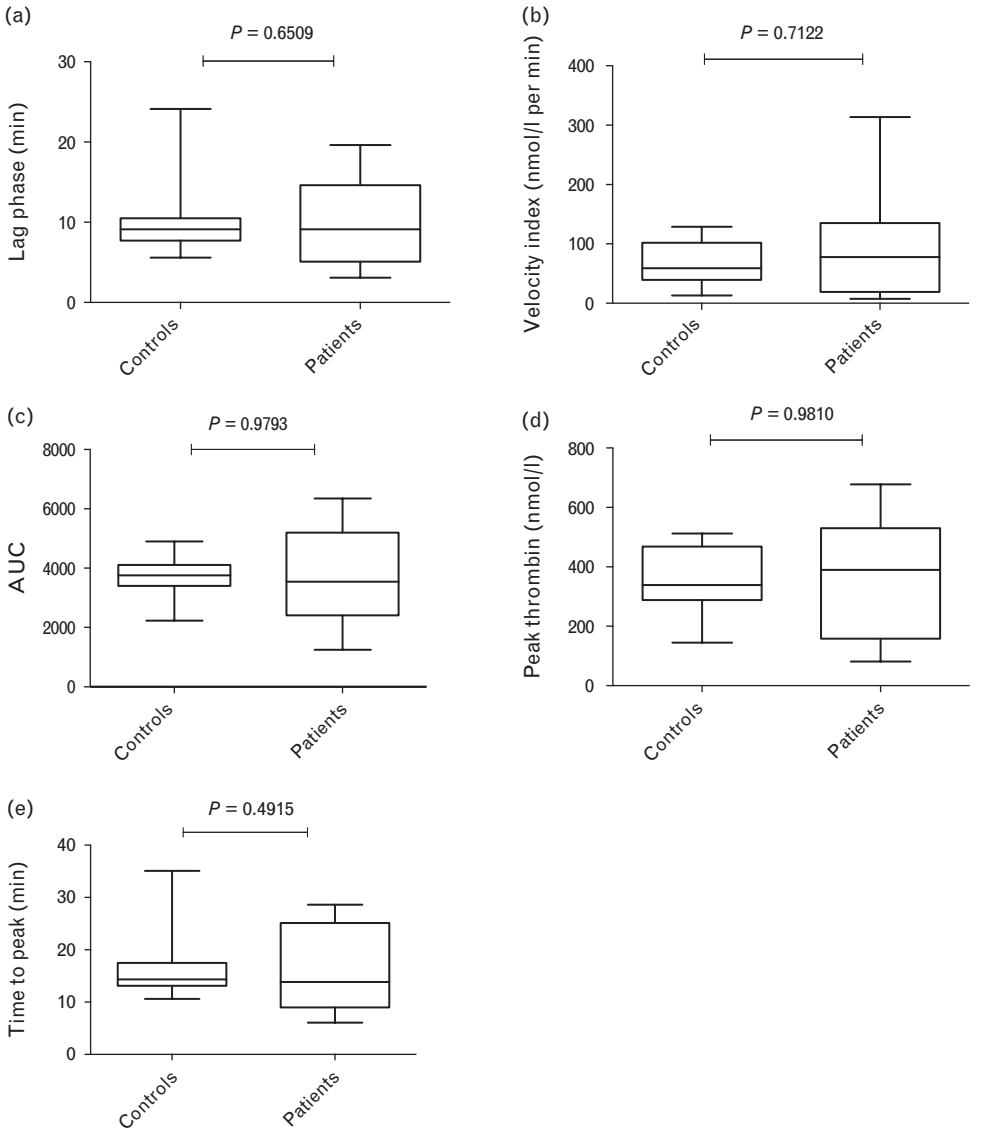
At least half of the patients with bleeding symptoms have been recognized to present normal coagulation tests. Conversely, recent clinical studies have described that up to 25% of healthy individuals may present mild bleeding manifestations [10] and that this bleeding tendency may not be accompanied by a detected hemostasis disorder [11]. This fact complicates the evaluation of the bleeding risk in patients and their medical management.

In this study, we evaluated global and specific coagulation and fibrinolysis parameters in a selected group of patients with undiagnosed bleeding disorders. The patients' bleeding tendency was confirmed by the MCMDM-1 Bleeding Score System; however, none of them had an identified coagulation disorder. Our hypothesis was that the coagulopathy in these cases could be caused by multiple subtle hemostasis disorders and that global assays of hemostasis would be capable of detecting this possible coagulopathy, and becoming possible tests for the clinical management of patients.

Unexpectedly, the thrombin generation results were similar between patients and controls, and did not confirm our initial hypothesis. However, these findings are consistent with previous data that have also failed to detect coagulation abnormalities by TGT in patients with undiagnosed bleeding disorders [12]. TGT results were not related to the bleeding phenotype of these patients, even patients with moderate bleeding manifestations showed thrombin generation values within the normal range. Indeed, the clinical significance of TGT results is unclear, since the correlation of the thrombin generation with the individual risk for bleeding or thrombotic complications has not yet been established [13,14]. Thus, taking previous studies into account, our results may possibly confirm that the TGT might not be an important test to evaluate the bleeding risk in patients with an undiagnosed bleeding tendency. Furthermore, the activities of coagulation factors were within the normal range, leading to the conclusion that possible subtle disorders of coagulation factors leading to decreased thrombin generation may not be the cause of the bleeding tendency in these patients.

On the contrary, the global test of fibrinolysis – ELT – was impaired in patients, suggesting that they were possibly experiencing a hyperfibrinolysis state. The increased activity of TAFI also suggested that fibrinolysis could be involved in their bleeding manifestation. However, the ELT should be interpreted with caution. ELT values are detected by visual analyses and, although sensitive for fibrinolysis defects, the specificity of the

Fig. 1

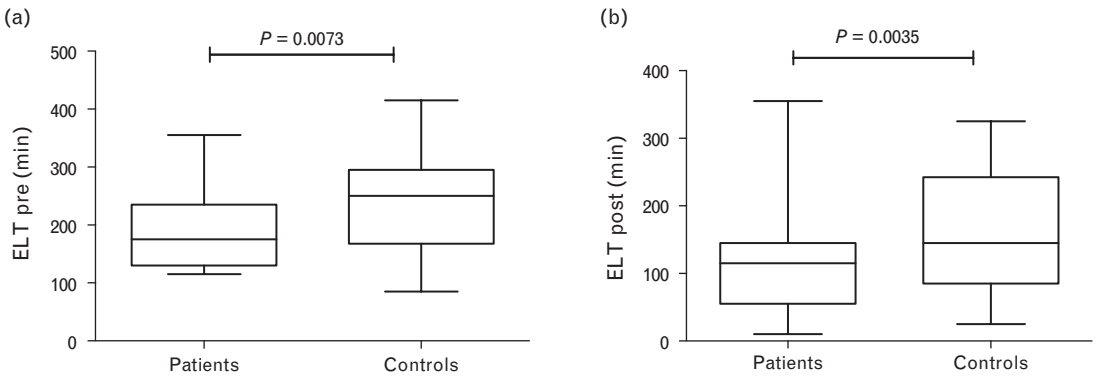


Comparative analysis of the distribution of thrombin generation parameters in patients with an undiagnosed bleeding tendency ($n = 18$) and controls ($n = 20$). (a) Lag phase; (b) velocity index; (c) area under the curve (AUC); (d) peak thrombin; and (e) time to peak. The *P* value was calculated by Mann–Whitney *U* test.

test depends on the methodology used and the variability of the test makes the results difficult to reproduce [15]. In this study, although decreased ELT results suggested that patients presented hyperfibrinolysis, we failed to detect a fibrinolysis disorder that could justify the bleeding manifestations in the majority of the patients. The plasma activities of α2AP and PAI-1 were similar between patients and controls. Only one patient presented severe PAI-1 deficiency and one patient was diagnosed with FXIII deficiency.

Thrombin-activatable fibrinolysis inhibitor activity result was also a controversial finding, since we expected TAFI activity to be decreased in patients. In contrast, TAFI activity was higher in patients and was inversely correlated to ELT. Notably, to interpret TAFI assays, it is necessary to take into account the kind of assay used [16]. The TAFI activity assay performed by us is a functional assay that measures the potential activity of proenzyme TAFI (pro-TAFI) to generate the active form TAFIa. In our study, higher TAFI activity was observed in bleeding patients. Other studies have demonstrated similar discrepancies between TAFI activity values and thrombotic or bleeding manifestations in patients. Antovic and Blomback [17], de Bruijne *et al.* [18], and Meltzer *et al.* [19] showed lower pro-TAFI or TAFI activity in patients

Fig. 2



Comparative analysis of euglobulin lysis time (ELT) (a) before and (b) after arm constriction in patients and controls. Data are expressed as median (range). Mann–Whitney *U* test was performed to compare continuous variables between the controls ($n = 50$) and patients ($n = 45$).

Table 2 Comparative analysis of fibrinolysis results of patients and controls

	Controls	Patients	*P* value
FXIII (%)	96.6 (52.5–156) ($n = 50$)	88.6 (15–156) ($n = 45$)	0.3678
α2AP (%)	127.3 (96.3–152.2) ($n = 45$)	130 (93.5–157) ($n = 45$)	0.5145
Plasminogen (%)	121.0 (86.1–169.9) ($n = 50$)	121.6 (74–406) ($n = 45$)	0.4817
PAI-I (U/ml)	4.5 (1.9–6.8) ($n = 33$)	5.1 (0–7.3) ($n = 43$)	0.2953
TAFI (μg/ml)	19.5 (11.5–28.9) ($n = 44$)	21.2 (12.1–33) ($n = 45$)	0.0161

Data are expressed as median and range. Mann–Whitney *U* test was performed to compare continuous variables between patients and controls. α2AP, α-2 antiplasmin; FXIII, factor XIII; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin-activatable fibrinolysis inhibitor.

with factor V Leiden mutation, coronary disease, and recurrent venous thrombosis, respectively. Conversely, Matus *et al.* [20] and Antovic *et al.* [21] showed, respectively, higher pro-TAFI levels and higher TAFI activity in patients with bleeding of unknown cause, hemophilia, and VWD. Taking these evidences into account, lower TAFI activity has been associated with prothrombotic clinical features, whereas higher TAFI activity has been associated with bleeding manifestations. In our study, as TAFI activity was inversely correlated to ELT, this finding may suggest that higher TAFI activity may be associated with hyperfibrinolysis. It is possible that the higher TAFI activity might be due to higher levels of pro-TAFI and impaired physiological conversion of TAFI into TAFIa. However, the role of TAFI tests in the diagnosis of bleeding disorders has not been established yet.

Therefore, despite the comprehensive evaluation of hemostasis parameters, by global and specific assays, the causes for the bleeding manifestations in patients remained undetected. Initially, our results raised a possible association of undiagnosed bleeding tendency with an impaired fibrinolysis; however, no specific fibrinolysis disorder could be detected. As fibrinolysis activity depends upon the activity of endothelial cells [22], cellular elements of hemostasis may possibly play a role in triggering bleeding disorders. However, up to now, the available coagulations assays are not adequate to evaluate the interaction of endothelial cells, coagulation factors, and platelets in blood hemostasis.

Moreover, as laboratory methods are limited for the diagnosis of a great number of patients with a bleeding tendency, a detailed and objective bleeding history may be the key point in the clinical evaluation of these patients, instead of a comprehensive laboratory evaluation [23,24].

In conclusion, our results showed that available global tests of coagulation and fibrinolysis are limited for detecting the coagulopathy of some patients with a relevant bleeding tendency and may not be adequate to address their bleeding risk. Bleeding scores are, until this date, the only medical approach for the diagnosis of these patients.

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Conflicts of interest

There are no conflicts of interest.

References

1 Parkin JD, Smith IL, O'Neill AI, Ibrahim KM, Butcher LA. Mild bleeding disorders. A clinical and laboratory study. *Med J Aust* 1992; **156**:614–617.

2 Quiroga T, Goycoolea M, Panes O, *et al.* High revalence of bleeders of unknown cause among patients with inherited mucocutaneous bleeding. Prospective study of 280 patients and 299 controls. *Haematologica* 2007; **92**:356–364.

3 Gupta PK, Charan VD, Saxena R. Spectrum of Von Willebrand disease and inherited platelet function disorders amongst Indian bleeders. *Ann Hematol* 2007; **86**:403–407.

4 Agren A, Wiman B, Stiller V, Lindmarker P, Ste-Linder M, Carlsson A, *et al.* Evaluation of low PAI-1 activity as a risk factor for hemorrhagic diathesis. *Haemost* 2006; **4**:201–208.

5 Chitlur M. Challenges in the laboratory analyses of bleeding disorders. *Thromb Res* 2012; **130**:1–6.

6 Gando S, Otomo Y. Local hemostasis, immunothrombosis, and systemic disseminated intravascular coagulation in trauma and traumatic shock. *Crit Care* 2015; **19**:72.

7 Tynngard N, Lindahl T, Ramstrom S. Assays of different aspects of haemostasis: what do they measure? *Thromb J* 2015; **13**:8.

8 Haverkate F, Thompson SG, Duckert F. Haemostasis factors in angina pectoris; relation to gender, age and acute-phase reaction. Results of the ECAT Angina Pectoris Study Group. *Thromb Haemost* 1995; **73**:561–567.

9 Rodeghiero F, Castaman G, Tosetto A, Batlle J, Baudo F, Cappelletti A, *et al.* The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. *J Thromb Haemost* 2005; **3**:2619–2626.

10 Mauer AC, Khazanov NA, Levenkova N, Tian S, Barbour EM, Khalida C, *et al.* Impact of sex, age, race, ethnicity and aspirin use on bleeding symptoms in healthy adults. *J Thromb Haemost* 2011; **9**:100–108.

11 Tosetto A, Castaman G, Plug I, Rodeghiero F, Eikenboom J. Prospective evaluation of the clinical utility of quantitative bleeding severity assessment in patients referred for hemostatic evaluation. *J Thromb Haemost* 2011; **9**:1143–1148.

12 Ay C, Haselböck J, Laczkovics C, Koder S, Pabinger I. Thrombin generation in patients with a bleeding tendency of unknown origin. *Ann Hematol* 2011; **90**:1099–1104.

13 Chantarangkul V, Clerici M, Bressi C, Giesen PL, Tripodi A. Thrombin generation assessed as endogenous thrombin potential in patients with hyper- or hypo-coagulability. *Haematologica* 2003; **88**:547–554.

14 Simpson ML, Goldenberg NA, Jacobson LJ, Bombardier CG, Hathaway WE, Manco-Johnson MJ. Simultaneous thrombin and plasmin generation capacities in normal and abnormal states of coagulation and fibrinolysis in children and adults. *Thromb Res* 2011; **127**:317–323.

15 Tregouet DA, Schnabel R, Alessi MC, Godefroy T, Declerck PJ, Nicaud V, *et al.* Activated thrombin activatable fibrinolysis inhibitor levels are associated with the risk of cardiovascular death in patients with coronary artery disease: the AtheroGene study. *J Thromb Haemost* 2009; **7**:49–57.

16 Smith AA, Jacobson LJ, Miller BI, Hathaway WE, Manco-Johnson MJ. A new euglobulin clot lysis assay for global fibrinolysis. *Thromb Res* 2003; **112 (5–6)**:329–337.

17 Antovic JP, Blombäck M. Thrombin-activatable fibrinolysis inhibitor antigen and TAFI activity in patients with APC resistance caused by factor V Leiden mutation. *Thromb Res* 2002; **106**:59–62.

18 de Bruijne EL, Gils A, Guimaraes AH, Dippel DW, Deckers JW, van denMeiracker AH, *et al.* The role of thrombin activatable fibrinolysis inhibitor in arterial thrombosis at a young age: the ATTAC study. *J Thromb Haemost* 2009; **7**:919–927.

19 Meltzer ME, Bol. Rosendaal FR, Lisman T, Cannegieter SC. Hypofibrinolysis as a risk factor for recurrent venous thrombosis; results of the LETS follow-up study. *J Thromb Haemost* 2010; **8**:605–607.

20 Matus V, Willemse J, Quiroga T, Govcoolea M, Aranda E, Panes O, *et al.* Procarboxypeptidase U (TAFI) and the Thr325Ile proCPU polymorphism in patients with hereditary mucocutaneous hemorrhages. *Clin Chim Acta* 2009; **401 (1–2)**:158–161.

21 Antovic JP, Schulman S, An SS, Greenfield RS, Blombäck M. Does an enzyme other than thrombin contribute to unexpected changes in the levels of the different forms of thrombin activatable fibrinolysis inhibitor in patients with hemophilia A, hemophilia B and von Willebrand disease? *Scand J Clin Lab Invest* 2004; **64**:745–751.

22 Cesarman-Maus G, Hajjar KA. Molecular mechanisms of fibrinolysis. *Br J Haematol* 2005; **129**:307–321.

23 Tosetto A, Rodeghiero F, Castaman G, Goodeve A, Federici AB, Batlle J, *et al.* A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD). *J Thromb Haemost* 2006; **4**:766–773.

24 Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, *et al.* ISTH/SSC joint VWF and Perinatal/Pediatric Hemostasis Subcommittees Working Group. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. *J Thromb Haemost* 2010; **8**:2063–2065.