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ADAMTS13 activity, high VWF and FVIII levels in the pathogenesis of deep vein thrombosis

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ABSTRACT

Background: Deep vein thrombosis (DVT) is a common multi-factorial disease with a partially understood aetiology. Although the roles of high factor (F)VIII and von Willebrand factor (VWF) levels are recognized, that of ADAMTS13 is still unclear.

Aim: To assess the association between ADAMTS13 activity levels, VWF antigen (VWF:Ag) and FVIII coagulant activity (FVIII:C) levels and DVT.

Materials and methods: 365 Italian DVT patients and 292 age- and sex-matched controls were considered. Plasma ADAMTS13 activity was measured using FRETS-VWF73 assay. VWF:Ag and FVIII:C were measured using immunoassay and one-stage clotting assay (ACL TOP analyzer), respectively. Quartile analyses were performed to evaluate the individual association between ADAMTS13 activity, VWF:Ag, FVIII:C and DVT. The combined effect of high VWF levels (> 4th quartile) and low ADAMTS13 levels (< 1st quartile) was evaluated using binary variables. All models were age- and sex-adjusted. Estimated risks were reported as Odds ratio (OR) with 95% confidence intervals (CI).

Results: ADAMTS13 activity was lower in DVT patients (94% vs. 98% of controls). Patients with an ADAMTS13 activity <1st quartile (86%) showed a 1.6-fold increased risk of DVT (95%CI, 1.05–2.55). The combination of low ADAMTS13 activity and high VWF:Ag levels was associated with a 15–fold increased risk (95%CI, 7.80–33.80). VWF:Ag and FVIII:C were associated to DVT with a dose-response relationship.

Conclusions: ADAMTS13 activity < 86% was associated with a moderate risk of DVT. The co-presence of low ADAMTS13 activity and high VWF levels resulted in a strong synergistic effect on DVT risk. The association of VWF:Ag and FVIII:C with DVT was confirmed.

1. Introduction

Deep vein thrombosis (DVT) is a common multi-factorial thrombotic disorder caused by environmental, behavioral and genetic risk factors, high coagulant factors levels or a combination of them. Environmental factors include surgery, trauma or fracture, hospitalization, immobilization, pregnancy/puerperium, oral contraceptive use, cancer, age, sex and ethnicity [1,2]. The behavioral habits include smoke, sedentariness, obesity and long travels, whereas among the genetic risk factors there are the deficiencies of the natural anticoagulant proteins (antithrombin, protein C and protein S), Factor V Leiden, prothrombin G20210A mutation and blood group non-O [2–6]. Despite all, known risk factors can explain only a part of DVT events and a missing hereditary is still present.

ADAMTS13 is a metalloprotease which plays an important role in hemostasis due to its cleavage activity of von Willebrand factor (VWF), a large sticky multimeric glycoprotein [7,8]. Under flow conditions, VWF passes from a globular form to an elongated form, thus exposing the A2 domain. ADAMTS13 binds to VWF within A2 domain, reducing the highly thrombogenic ultra large multimers into smaller and less active

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molecules. This mechanism is fundamental to prevent an excessive platelets adhesion to ultra-large VWF and to dissolve VWF-platelet aggregates [9].

A severe deficiency of ADAMTS13 results in the development of thrombotic thrombocytopenic purpura (TTP) [10], a thrombotic microangiopathy characterized by VWF-mediated platelet thrombi disseminated in the microcirculation of vital organs such as heart, kidney and brain [11]. Recent finding also described the role of ADAMTS13 in the pathogenesis of other thrombotic disorders. Indeed, low and moderately low ADAMTS13 levels showed to be associated to myocardial infarction and coronary artery disease [12–14].

To date, there is few and discordant information regarding the role of ADAMTS13 in venous thromboembolism, which includes both DVT and pulmonary embolism (PE). Indeed, different authors reported the association of both high and low ADAMTS13 levels with VTE [15–17]. Furthermore, VTE was associated to low or normal ADAMTS13 activity in patients with cancer [18–20].

We hypothesized that the reduction of ADAMTS13 activity or the alteration of the equilibrium between ADAMTS13 and VWF may also play a role in DVT pathogenesis. Therefore, we decided to measure ADAMTS13 activity in a group of 365 Italian DVT patients and 292 age- and sex-matched controls with the aim to evaluate: i) the association between ADAMTS13 activity levels and risk for DVT, and ii) the possible synergistic effect of low ADAMTS13 activity and high VWF antigen levels as novel potential mechanism of DVT pathogenesis. In addition, we further evaluated the independent association of VWF and FVIII levels with DVT.

2. Materials and methods

2.1. Study population

We selected Italian patients with DVT among those referred to the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center in Milan (Italy) for a thrombophilia workup after a first event between 2006 and 2016. Briefly, the selection criteria included: (i) objective diagnosis of DVT of the lower limbs (i.e. by compression ultrasonography or venography); (ii) idiopathic DVT defined by the absence of cancer, surgery or immobilization; (iii) normal levels of the natural anticoagulant antithrombin, protein C and protein S; (iv) absence of FV Leiden (FVL) or prothrombin G20210A mutations. Controls, matched with cases for age (±5 years) and sex were recruited among friends and partners who accompanied patients to the Center, agreed to be tested for thrombophilia and had wild-type FVL and prothrombin G20210A genotypes. The study was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and all patients and controls signed their informed consent to participate into the study.

2.2. Biochemical assays

Plasma ADAMTS13 activity was measured using fluorescence resonance energy transfer on plasma samples, as previously described [21]. VWF antigen (VWF:Ag) and FVIII coagulant activity (FVIII:C) were measured using an automated immunoassay and one-stage clotting assay on ACL TOP analyzer (Instrumentation Laboratory Italy), respectively.

2.3. Statistical analysis

Continuous variables were described as median and interquartile range, whereas categorical variables were reported as counts and percentages.

A logistic regression was performed to assess the independent association of ADAMTS13 activity with risk for DVT. A first analysis was based on dichotomous exposure with an a priori cut-off set at the 5th and 1st percentiles of ADAMTS13 activity levels of the pooled control group distribution. Then, the risk of DVT was calculated across quartiles of ADAMTS13 activity, based on its distribution among controls, and using the highest quartile as reference. The measure of the relative risk was expressed as odds ratio (ORs) with the corresponding 95% confidence intervals (CI), and adjusted for age and sex.

The possible presence of a non-linear association between ADAMTS13 activity levels (kept continuous) and risk of DVT (expressed as log odds) was evaluated by using a restricted cubic spline function with three knots.

The association of VWF:Ag and FVIII:C plasma levels (divided into quartiles of their distribution among controls) with DVT, was performed using a logistic regression model with the lowest quartile of the controls' distribution set as reference. The estimated risks were expressed as OR and 95% CI. Each model was adjusted for age and sex. Binary variables with predefined cut-off points were set up to deeply evaluate the combined effect of high VWF levels (above the 4th quartile) and low ADAMTS13 levels (below the 1st quartile) on DVT risk.

Both quartile and binary variable analyses were also performed after excluding patients in anticoagulant therapy or who had DVT event less than 3 months before blood collection, in order to avoid the possible interference of these factors on risk estimates. All the statistical analyses were performed using the statistical software R, release 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Sample size was determined using PS Power and Sample Size Calculations (Version 3. version 3.1.6; http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize), considering a 0.05 two-tail alpha error and a power 0.8. We assumed a probability of exposure among controls of 0.2, and a correlation coefficient for exposure between matched cases and controls of 0.2. It is estimated that a minimum sample size of 212 cases and 212 controls was required to detect a true OR of 2.0.

3. Results

The characteristics of the enrolled DVT cases ($n = 365$) and controls ($n = 292$) are reported in Table 1. The mean age was 48 years for cases and 47 years for controls. Both patients and controls had a higher percentage of non-O blood group than O blood group. FVIII:C levels were higher in patients than in controls (median, 148 vs. 113%), whereas the median of plasma ADAMTS13 activity was slightly lower in patients (94%; IQR 81–108) than in controls (98%; IQR, 86–112). Two out of 365

Table 1
Characteristics of the study population.

	Patients(n = 365)	Controls(n = 292)
Age (*years*), median (IQR)	48 (38–61)	47 (37–54)
Female sex, n (%)	184 (50.4%)	153 (52.4%)
ABO, n (%)		
O Group	50 (13.7)	95 (32.5)
Non O Group	147 (40.3)	113 (38.7)
Missing	168 (46.0)	84 (28.8)
FVIII:C (%),[a] median (IQR)	148 (124–173)	113 (93–137)
VWF:Ag (%),[b] median (IQR)	169 (136–209)	115 (87–148)
ADAMTS13 Activity (%), median (IQR)	94 (81–108)	98 (86–112)
Anticoagulant therapy,[c] n (%)	173 (47%)	–
Progestogens, n (%)	1 (0.2)	–
Time from acute event (*months*), median (IQR)	9 (3–32)	–
Major illness, n (%)		
Liver disease	2 (0.01)	–
Kidney disease	2 (0.01)	–

ABO, blood group system; FVIII:C, FVIII coagulant activity, VWF:Ag von Willebrand factor antigen. IQR, interquartile range.

[a] Available for 330 cases and 292 controls.

[b] Available in 342 cases and 247 controls.

[c] Warfarin ($n = 81$), low molecular weight heparin ($n = 54$), rivaroxaban ($n = 25$), subcutaneous heparin ($n = 6$), fondaparinux (n = 5), apixaban ($n = 1$).

patients showed ADAMTS13 activity levels slightly below the normal range (42%; normal range 45–138%). Patients had increased VWF:Ag levels (169%; IQR, 136–209) than controls (115%; IQR, 87–148). The 47% of patients were receiving anticoagulant therapy at the time of blood sampling. The median time between DVT event and sample collection was about 9 months (IQR, 3–32).

The association between ADAMTS13 activity levels and DVT was initially calculated using as arbitrary cut-off the 1st and 5th percentile of ADAMTS13 distribution of the pooled control group. ADAMTS13 levels ≤5th percentile, i.e. ≤67.6%, were associated with an almost 2-fold increased risk for DVT (age- and sex-adjusted OR 1.63, 95% CI 0.84–3.12). Similar results were found for ADAMTS13 activity levels ≤1st percentile, i.e. ≤59% (age- and sex-adjusted OR 2.52, 95% CI 0.81–7.87). Dividing ADAMTS13 activity levels into quartiles, ADAMTS13 activity levels ≤86% were associated to a 1.6-fold increased risk of DVT (age- and sex-adjusted OR 1.64, 95% CI 1.05–2.55), while intermediate quartiles showed only a small association with DVT, thus excluding a clear dose-response relationship (Table 2).

This trend was confirmed when the relationship between ADAMTS13 levels (kept continuous) and DVT risk was evaluated with a restricted cubic spline function: a slightly non-linear component in the log-odds of DVT was visible for ADAMTS13 activity levels below 90% even after adjustment for age and sex (Fig. 1).

Since several previous studies have shown the association of FVIII and VWF with DVT, we performed a quartile analysis to confirm those results in our population. We found that both factors were independently associated with DVT with a dose-response effect, as showed in Table 2. The highest VWF:Ag levels (above the 4th quartile) conferred a 23-fold increased risk for DVT (95% CI 11.2–48.3). Similarly, FVIII:C levels above the 4th quartile were associated with a 13-fold increased risk for DVT (95% CI 7.22–25.0).

The combined effect of low ADAMTS13 activity (below the 1st quartile, i.e., ≤86%) and high VWF:Ag levels (above the 4th quartile, i.e., >148%) was evaluated using binary variables and results are reported in Table 3. When low ADAMTS13 levels were considered alone, the estimated risk was similar to that of the main analysis (OR 1.75, 95% CI 1.04–2.96 and OR 1.64, 95% CI 1.05–2.55, respectively). High VWF:Ag levels with ADAMTS13 levels above 1st quartile were associated with an increased risk for DVT (age- and sex-adjusted OR 5.52, 95% CI 3.60–8.54). The estimated risk conferred by the combination of low ADAMTS13 and high VWF was much higher than that obtained by the sum of the two separated risks (combined estimated risk: OR 15.45, 95% CI 7.80–33.80 vs. expected OR 1 + [1.75 − 1] + [5.52 − 1] = 6.27),

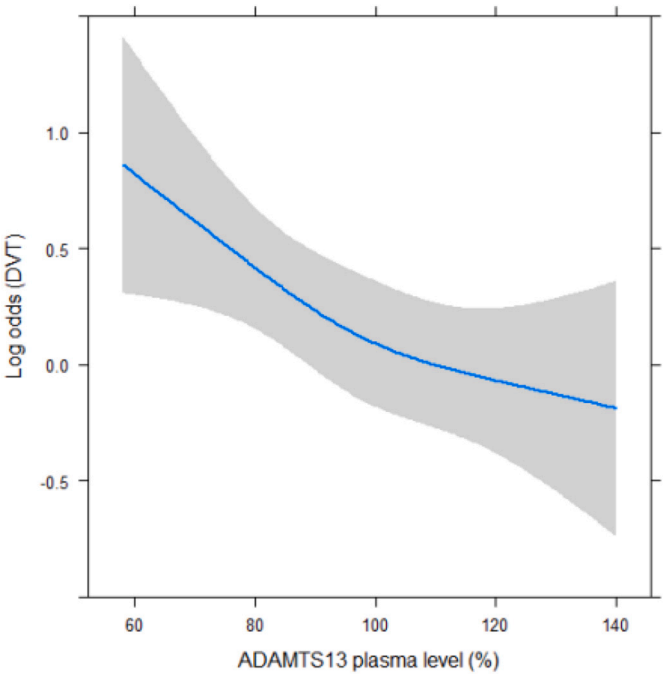

Fig. 1. Restricted cubic spline curve showing the model-predicted log odds of DVT against ADAMTS13 activity levels, adjusted for age and sex (solid line). The grey area represents 95% confidence intervals.

Table 2
Risk of DVT according to various plasma levels of ADAMTS13, VWF and FVIII.

	Patients	Controls	OR (95% CI)	OR[1] (95% CI)
ADAMTS13 activity	**(n = 365)**	**(n = 292)**		
≤5th percentile (≤67.6)	29	14	1.74 (0.89–3.31)	1.63 (0.84–3.12)
>5th percentile (>67.6)	336	278	1 (reference)	1 (reference)
≤1st percentile (≤59)	13	4	2.66 (0.93–9.52)	2.52 (0.81–7.87)
>1st percentile (>59)	352	288	1 (reference)	1 (reference)
1st quartile (≤86%)	130	76	1.73 (1.13–2.68)	1.64 (1.05–2.55)
2nd quartile (87–98%)	91	78	1.18 (0.76–1.85)	1.15 (0.74–1.80)
3rd quartile (99–111%)	72	65	1.12 (0.70–1.79)	1.10 (0.69–1.75)
4th quartile (>111%)	72	73	1 (reference)	1 (reference)
VWF:Ag	**(n = 342)**	**(n = 247)**		
1st quartile (≤87%)	10	62	1 (reference)	1 (reference)
2nd quartile (88–115%)	37	62	3.70 (1.69–8.10)	3.70 (1.69–8.10)
3rd quartile (116–148%)	69	62	6.90 (3.26–14.62)	6.92 (3.26–14.67)
4th quartile (>148%)	226	61	23.0 (11.1–47.4)	23.2 (11.2–48.3)
FVIII:C	**(n = 330)**	**(n = 292)**		
1st quartile (≤93%)	190	75	1 (reference)	1 (reference)
2nd quartile (94–113%)	79	73	2.89 (1.50–5.56)	2.95 (1.53–5.69)
3rd quartile (114–137%)	45	73	5.07 (2.71–9.49)	5.27 (2.80–9.92)
4th quartile (>137%)	16	71	12.54 (6.85–22.96)	13.43 (7.22–25.00)

VWF:Ag, von Willebrand factor antigen; FVIII:C, FVIII coagulant activity. Predefined cut-off points set at the 5th and 1st percentiles were created using the ADAMTS13 distribution of the pooled control group. ADAMTS13 activity, VWF:Ag and FVIII:C were categorized into quartiles using the control group distribution. OR, odds ratios were reported as measures of relative risk; OR[1], values adjusted for age and sex; 95% CI, 95% confidence intervals.

Table 3
Risk of DVT in relation to the combination of low ADAMTS13 and high von Willebrand factor plasma levels.

Low ADAMTS13 (< 1st quartile)	High VWF (> 4th quartile)	Patients, n	Controls, n	OR[1] (95% CI)
–	–	75	142	1 (reference)
+	–	39	43	1.75 (1.04–2.96)
–	+	149	52	5.52 (3.60–8.54)
+	+	79	10	15.45 (7.80–33.80)

OR, odds ratios were reported as measures of relative risk; OR[1], values adjusted for age and sex; 95% CI, 95% confidence intervals. Binary variables were created to evaluate: low ADAMTS13 plasma levels, below the 1st quartile and low (+/−); high VWF plasma levels, above the 4th quartile (+/−), or both (+/+). ADAMTS13 activity VWF:Ag levels were available in 342 cases and 247 controls.

indicating a synergistic effect of the two risk factors.

Finally, we performed a sensitivity analysis restricting our study population to the 167 patients whose blood samples were collected at least three months after the DVT event or not during anticoagulant therapy (Table 4). The association between ADAMTS13 levels < 86% and DVT was similar to that of the main analysis (age- and sex-adjusted OR 1.69, 95% CI 0.98–2.91). VWF:Ag and FVIII:C still showing a clear increasing trend of DVT risk, which reached the maximum association for the highest quartiles (Table 4). Most importantly, the sensitivity analysis performed to evaluate the combined effect of low ADAMTS13 activity levels and high VWF levels did confirm the main analysis results with a 13-fold increased risk for DVT (95%CI 5.80–29.22, Table 5).

Table 5
Sensitivity analysis of DVT risk in relation to the combination of low ADAMTS13 and high von Willebrand factor plasma levels.

Low ADAMTS13 (< 1st quartile)	High VWF (>4th quartile)	Patients, n	Controls, n	OR[1] (95% CI)
–	–	40	142	1 (reference)
+	–	19	43	1.66 (0.86–3.18)
–	+	59	52	4.14 (2.45–6.99)
+	+	34	10	13.01 (5.80–29.22)

Odds ratios were reported as measures of relative risk; OR[1], values adjusted for age and sex; 95% CI, 95% confidence intervals. Binary variables were created to evaluate: low ADAMTS13 plasma levels, below the 1st quartile and low (+/−); high VWF plasma levels, above the 4th quartile (+/−), or both (+/+). ADAMTS13 activity VWF:Ag levels were available in 152 cases and 247 controls. Samples were collected at least 3 months after DVT event and in the absence of any oral anticoagulant treatment.

4. Discussion

In this study, we evaluated the role of low ADAMTS13 activity levels as possible new risk factor of DVT. ADAMTS13 cleaving activity of ultra large VWF multimers is fundamental to maintain a proper hemostasis. Furthermore, the role of this metalloprotease has been previously described in the pathogenesis of other thrombotic disorders such as myocardial infarction and ischemic stokes [12–14]. Based on this background, we decided to evaluate the role of ADAMTS13 activity levels in 365 DVT patients referred to our Center and 292 controls with the following aims: (i) to evaluate the association between ADAMTS13 activity and risk for DVT (ii) to evaluate the possible synergistic effect of low ADAMTS13 and high VWF levels as potential novel mechanism in the pathogenesis of the disease; and (iii) to confirm the association between DVT risk and high levels of FVIII and VWF, as previously reported by other authors [22–24].

We found that DVT patients had a moderately lower median ADAMTS13 activity levels than controls. First evidence of ADAMTS13 association with DVT was showed for levels below the 5th and 1st percentile (OR 1.63, 95% CI 0.84–3.12 and OR 2.52, 95% CI 0.81–7.87, respectively). When ADAMTS13 activity were categorized into quartiles, we showed that even a modest reduction of ADAMTS13 activity (below 86%) was associated with an increased risk of DVT (OR 1.64, 95% CI 1.05–2.55). Interesting results were obtained by the evaluation of the combined effect of low ADAMTS13 activity and high VWF antigen levels, which resulted in a 15-fold increased risk for DVT. As the combined estimated risk was clearly higher than the sum of the separated risks (15-fold vs. 6-fold, respectively), a synergistic effect of these two factors was plausible. Therefore, these data showed that a modest reduction of ADAMTS13 activity, especially when combined with high VWF levels (ADAMTS13-VWF equilibrium alteration) plays a role in the onset of DVT events.

Interestingly, highest VWF levels showed a higher association to DVT than highest FVIII levels (23-fold vs. 13-fold, referred to the main analyses). This finding led us to speculate that VWF association with DVT is not related to its protective role of FVIII [22], but it acts as independent risk factor [23].

To date, the role of ADAMTS13 levels was evaluated in few studies referred to VTE patients. Our results were in accordance with those reported by Karakaya *et al*, who described low ADAMTS13 antigen and high VWF levels, although referred to a small study population of 30 VTE patients and 30 controls [15]. Low ADAMTS13 antigen levels, below the 5th and 10th percentile, have also been associated with VTE event in Spanish women, but not in men, who had a first VTE event before 70 years of age [16]. Our results, were in contrast with those of Mazzetto *et al*, who described a high ADAMTS13 and VWF levels in VTE patients, in presence of high inflammatory markers long time after the VTE event. These authors speculated that high ADAMTS13 levels could be due to a kind of compensatory mechanism against increased VWF levels following thrombotic events [17]. However, the lack of information about inflammatory markers in our study population did not allow a proper comparison with that study.

The mechanism involving ADAMTS13 in DVT pathogenesis is not fully understood. Because of the nature of the study design, we cannot establish whether the slightly reduced ADAMTS13 activity levels and the increased VWF are the cause or the consequence of DVT event. We can speculate that the reduction of ADAMTS13 activity levels are due to increased VWF antigen levels (i.e. excessive ADAMTS13 consumption), thus resulting in an excess of ultra-large VWF, which is more prone to

Table 4
Sensitivity analyses for DVT risk according to various plasma levels of ADAMTS13, VWF and FVIII.

	Patients	Controls	OR (95% CI)	OR[1] (95% CI)
ADAMTS13 activity	**(n = 167)**	**(n = 292)**		
1st quartile (≤86%)	59	76	1.67 (0.98–2.83)	1.69 (0.98–2.91)
2nd quartile (87–98%)	40	78	1.10 (0.63–1.92)	1.01 (0.63–1.93)
3rd quartile (99–111%)	34	65	1.12 (0.63–2.01)	1.11 (0.62–2.00)
4th quartile (>111%)	34	73	1 (reference)	1 (reference)
VWF:Ag	**(n = 152)**	**(n = 247)**		
1st quartile (≤87%)	5	62	1 (reference)	1 (reference)
2nd quartile (88–115%)	21	62	4.2 (1.49–11.85)	4.18 (1.48–11.80)
3rd quartile (116–148%)	35	62	7.00 (2.57–19.05)	7.01 (2.57–19.10)
4th quartile (>148%)	91	61	18.50 (7.03–48.66)	18.88 (7.12–50.02)
FVIII:C	**(n = 167)**	**(n = 292)**		
1st quartile (≤93%)	86	75	1 (reference)	1 (reference)
2nd quartile (94–113%)	40	73	3.08 (1.41–6.76)	3.05 (1.39–6.71)
3rd quartile (114–137%)	30	73	4.11 (1.91–8.26)	4.16 (1.92–9.02)
4th quartile (>137%)	10	71	9.10 (4.37–48.87)	9.15 (4.67–19.87)

Samples were collected at least 3 months after DVT event and in the absence of any anticoagulant treatment. VWF:Ag, von Willebrand factor antigen; FVIII:C, FVIII coagulant activity. ADAMTS13 activity, VWF:Ag and FVIII:C were categorized into quartiles using the control group distribution. OR, odds ratios were reported as measures of relative risk; OR[1], values adjusted for age and sex; 95%CI, 95% confidence intervals.

bind platelets. Furthermore, the genetic component should also play a part in the alteration of VWF-ADAMTS13 balance. Indeed, our group previously described that carriers of one rare single nucleotide of *ADAMTS13* had lower activity levels than non-carriers [25].

This study could suffer of some limitations that require to be mentioned. First, blood sampling was not performed at the same time point (distance from the acute event) in all patients. This aspect may have introduced some variability on VWF, FVIII and ADAMTS13 levels. On the other hand, the sampling procedure was performed at a median of 9 months after the DVT event, leading us to exclude that the proteins levels were still a consequence of the DVT event, at least for VWF and FVIII levels. Indeed, different authors have previously reported that both FVIII [24,26] and VWF levels [24] remain stable for years after the DVT event, whereas this information is still to be clarified for ADAMTS13. Second, the continuation of anticoagulant therapy at the time of blood collection for half of patients could have influenced the results, although an effect on FVIII, VWF:Ag and ADAMTS13 levels is unlikely [24]. However, to rule out these hypotheses, analyses were repeated after excluding patients whose samples were collected less than three months from DVT event and/or during anticoagulant therapy. Neither the quartile analyses of ADAMTS13, VWF and FVIII nor the combined effect of ADAMTS13 and VWF seemed to be affected, thus confirming our results. Third, the lack of consecutive samples did not allow us to directly evaluate the potential variation over time of ADAMTS13, VWF and FVIII levels in our population or to assess whether ADAMTS13 and VWF levels may be helpful to predict a recurrence of DVT, as already described for FVIII levels [27]. Lastly, because the levels of inflammatory markers were not determined, we cannot exclude that inflammatory events other than acute DVT may have contributed to the increased VWF/FVIII levels and decreased ADAMTS13 levels. However, our study aimed to establish the association of slightly decreased ADAMTS13 levels (alone or combined with VWF levels) with DVT and to confirm that with high VWF and FVIII levels with DVT, rather than explaining the mechanisms which cause them.

5. Conclusions

We evaluated the effect of ADAMTS13 activity levels as adjunctive risk factor for DVT. We showed that a modest reduction of ADAMTS13 activity is enough to have a 1.6-fold increased risk of DVT. Furthermore, we found that the combination of low ADAMTS13 levels and high VWF levels resulted in a clearly increased risk. This led us to hypothesize that a synergistic effect between these two factors plays an important role in the DVT pathogenesis, although the causative mechanism which involves them still needs to be clarified.

Authors' contributions

M.T. Pagliari designed the research, carried out part of the measurements, interpreted the results and wrote the manuscript. F. Peyvandi designed the research and interpreted the results. M. Boscarino carried out the analysis. I. Martinelli and P. Bucciarelli recruited subjects and collected data. I. Mancini and A. Cairo participated in the design of the research and interpreted the results. F. Rossi carried out part of the measurements. F.R. Rosendaal interpreted the results and critically reviewed the manuscript. All authors reviewed and approved the final version of the manuscript.

Founding information

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Declaration of competing interest

F. Peyvandi has received honoraria for participating as a speaker at satellite symposia organized by Bioverativ, Grifols, Roche, Sanofi, Sobi, Spark and Takeda. F. Peyvandi reports participation at advisory board of Roche, Sanofi and Sobi. I. Mancini received honoraria for participating as a speaker at educational meetings organized by Instrumentation Laboratory and Sanofi-Genzyme. The other authors state that they have no conflict of interest.

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