Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Loss of von Willebrand factor high-molecular-weight multimers at acute phase is associated with detectable anti-ADAMTS13 IgG and neurological symptoms in acquired thrombotic thrombocytopenic purpura

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ABSTRACT

Introduction: Thrombotic thrombocytopenic purpura (TTP) is a rare life threatening thrombotic microangiopathy caused by a severe functional deficiency of ADAMTS13, most frequently due to autoantibodies to ADAMTS13, thus termed acquired autoimmune TTP. ADAMTS13 specifically regulates the adhesive activity of von Willebrand factor (VWF) by cleaving its high-molecular-weight multimers (HMWM). We investigated whether VWF-HMWM level at acute phase of TTP could be a predictive factor for morbidity.

Material and methods: We gathered clinical and biological data from a cohort of 114 patients with acquired TTP at acute phase. VWF-HMWM were assessed by electrophoretic analysis and by an ELISA measuring the capacity of VWF to bind to collagen (VWF:CB), and linear correlation between these two methods was carried out. We cross-referenced clinical and biological data with VWF-HMWM levels.

Results: VWF-HMWM levels were heterogeneous, but half of our patients were below normal range (50% if assessed by electrophoresis; 47.4% if assessed by ELISA). The correlation study between electrophoresis and ELISA reached statistical significance ($r^2 = 0.5979$; $p < 0.0001$). Statistical analysis showed that loss of VWF-HMWM as assessed by VWF:CB < 70 IU/dL is associated with detectable anti-ADAMTS13 antibodies, severe neurological symptoms and thrombocytopenia ($p < 0.05$).

Conclusion: Our results confirm that VWF-HMWM can be satisfactorily assessed by VWF:CB, much easier to perform than electrophoresis. The association highlighted between loss of VWF-HMWM, detectable anti-ADAMTS13 IgG and neurological symptoms may offer new insights to understanding the pathophysiology of acquired auto-immune TTP.

1. Introduction

Thrombotic microangiopathies (TMA) are rare, life threatening diseases defined by the association of mechanical hemolytic anemia with erythrocyte fragmentation, consumption thrombocytopenia and organ failure of variable severity. Thrombotic thrombocytopenic purpura (TTP) is a form of TMA specifically caused by a severe functional deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats, member 13), the specific von Willebrand factor (VWF)-cleaving protease [1]. At the acute phase of TTP, the severe functional deficiency of ADAMTS13 activity (below

10% of normal) leads to the accumulation of ultralarge hyper-adhesive VWF multimers in plasma, which spontaneously bind to platelets, hence consumption thrombocytopenia, and form VWF-rich platelet microthrombi on the surface of which red blood cells are fractured into schistocytes [2]. These microthrombi obstruct blood flow in the microcirculation, thus causing multivisceral ischemia. After effective therapy, the ischemia symptoms and the bicytopenia disappear, defining remission phase, during which patients still require periodic blood cell count and ADAMTS13 activity monitoring to predict any relapse [3]. TTP is an acquired disease in most cases and can also be hereditary in a few cases, due to recessively inherited mutations of the

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ADAMTS13 gene (Upshaw-Schulman Syndrome, USS) [4]. TTP is termed acquired autoimmune when due to autoantibodies against ADAMTS13 [5].

Acquired TTP is a medical emergency requiring rapid management. Frequent clinical features include fever, neurological symptoms (such as headache, confusion, convulsions, coma or brain ischemia) and cardiac ischemia, but clinical presentation is widely heterogeneous and TTP diagnosis can be challenging [6,7].

Finding new parameters that could explain the variability of clinical presentation at acute phase of TTP could be useful, either as part of new diagnosis algorithms or as prognostic tools. In this study, we focused on VWF multimeric analysis and hypothesize that clinical or biological features exhibited at acute phase of TTP could partially depend on VWF-HMWM levels. VWF-HMWM are known to be the most adhesive platelet-binding fraction of all VWF multimers, and they are crucial to maintain functionality of VWF [8]. This is highlighted in von Willebrand Disease (VWD) type 2A, a subtype of VWD characterized by decreased VWF-dependent platelet adhesion due to selective loss of VWF-HMWM usually resulting from an increased sensitivity to cleavage by ADAMTS13 [9]. VWF multimeric analysis is usually performed using electrophoresis as the reference method, and it can be helpful to diagnose VWD2A. However, electrophoresis is time consuming and technical to carry out [10]. Rather than this complex in-house method, we chose to assess HMWM through two approaches: i) semi-automated electrophoresis using SDS-agarose gel and immunochemical revelation and ii) ELISA method based on the capacity of VWF to bind to human type III collagen (VWF:CB) as VWF:CB not only assesses the integrity of VWF collagen binding domains (A1 and A3) but also shows preferential binding to VWF-HMWM [11].

While TTP is characterized by an excess of ultralarge hyperadhesive VWF-HMWM, a previous study showed that plasma VWF multimeric analysis in TTP patients at acute phase was very heterogeneous [12], with roughly a third each of selective loss, excess or normal fraction of VWF-HMWM and no apparent connection to clinical or biological features. Moreover, VWF antigen (VWF:Ag) levels are usually high but vary greatly amongst TTP patients, probably in accordance with the level of endothelial lesion.

In this study, we chose to rely on VWF-HMWM levels (expressed in IU/dL), as the product of HMWM fraction assessed by electrophoresis and VWF:Ag levels, and look into whether a specific apparent decreased VWF-HMWM level could be associated with higher morbidity. To support that hypothesis, we also performed VWF:CB as the reflect of VWF-HMWM levels and cross-referenced both parameters to clinical and biological data gathered from a cohort of 114 TTP patients.

2. Material and methods

2.1. Patients

Since 2000, all patients in France with a presumptive diagnosis of TMA (defined as the presence of microangiopathic hemolytic anemia with hemoglobin level < 12 g/dL with or without presence of schistocytes, and thrombocytopenia $< 150 \times 10^9$/L with or without ischaemic manifestations) have been enrolled in the registry of the National Reference Center for TMA (Health Ministry National Plan for Rare Diseases) and have been investigated for ADAMTS13 by the French reference laboratory for TMA.

We studied 114 patients (109 adults and 5 children, 4 < 15 years old and 1 > 15 years old) with well documented acquired TTP at acute phase prospectively enrolled from 2006 to 2010 as part of a clinical research program. Nine patients with USS at remission phase, thus presenting a constant excess of VWF-HMWM through genetic deficiency of ADAMTS13 were used as controls. Inclusion criteria comprise clinical diagnosis of TTP, severe deficiency of ADAMTS13 activity (< 10%), hemoglobin level < 10 g/dL and/or platelet count $< 150 \times 10^9$/L, while exclusion criteria include cancer, HIV and organ graft. Clinical

and biological data were collected after extensive analysis of medical records, including 8 to 14 years of follow-up (relapse and/or death). Neurological symptoms were particularly looked into: symptoms such as headaches or confusion were considered mild neurological symptoms, while seizures, coma, focal deficiency or two (or more) neurological symptoms were labelled severe. Cerebral ischemia was assessed by clinical presentation and magnetic resonance imaging. Written informed consent was obtained from all patients according to the Declaration of Helsinki. The study was approved by the Ethics Committee of Hospital Pitié-Salpêtrière (Paris. France). The study is registered at www.clinicaltrials.gov as number NCT00426686, at the Health Authority and by the French Ministry of Health as number P051064/PHRC AOM05012.

Venous blood was collected at the time of enrollment (acute phase) before any treatment, into 1:10 final volume of 3.8% sodium citrate. A normal pooled plasma (14 healthy volunteers) was used as normal control for all biologic assays.

2.2. ADAMTS13 activity assay

ADAMTS13 activity was assessed using the method of Kokame et al. [13] using commercial recombinant FRETS-VWF73 peptide (Peptide Institute, Osaka, Japan) according to the manufacturer's instructions. Normal range is between 50% and 150%.

2.3. VWF:Ag measurement

Plasma VWF:Ag was measured using the ASSERACHROM® VWF:Ag ELISA kit (Stago, Asnières-sur-Seine, France) according to the manufacturer's instructions. The normal range is 50 IU/dL–150 IU/dL.

2.4. Multimeric distribution of VWF

The assessment of multimeric distribution of VWF was carried out using the HYDRAGEL von WILLEBRAND MULTIMERS® agarose gel on the semi-automated system HYDRASIS 2® and using the software PHORESIS version 8.6.3 for densitometry analysis (Sebia, Lisses, France) according to the manufacturer's instructions. High molecular weight multimers are defined as all peaks beyond the 10th peak (10th mer). Given the technical performances of the electrophoretic assay we used, we lowered the cut-off value to the 8th peak and analyzed collectively all peaks beyond as high molecular weight multimers. VWF-HMWM (expressed in IU/dL) were then calculated as the product of VWF:Ag and HMWM percentage. The mean HMWM fraction was validated from 24 assessments of a normal pool carried out over 20 electrophoretic assays.

2.5. VWF:CB measurement

Plasma VWF:CB measurement was assessed using the ASSERACHROM® VWF:CB ELISA kit (Stago, Asnières-sur-Seine, France) according to the manufacturer's instructions. The normal range, as stated by the French National Reference Center for von Willebrand Disease, is 70 IU/dL–100 IU/dL [10].

2.6. Statistical analysis

Categorical variables were compared with χ^2 or Fisher's exact tests as appropriate. Continuous variables were compared with the nonparametric Mann-Whitney *U* test. Linear regression was performed using Pearson's formula. All tests were two-sided and statistical significance was set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism® version 7.00 (GraphPad Software, La Jolla, California, United States).

Table 1

Demographic, clinical and biological characteristics of the cohort of 114 patients with acquired TTP.

Continuous variables	114 patients
Age (years)[*]	35.5 (1.5–71.3)
Sex	77 females–37 males; sex ratio 2F/1M
Hemoglobin level (g/dL)	7.8 (3.2–15.8)
Platelet count ($\times 10^9$/L)	15 (2–251)
LDH (U/L)	2028 (269–8358)

Non-continuous variables	N patients/total (%)
Idiopathic TTP	80/114 (70.2%)
Non-idiopathic TTP	34/114 (29.8%)
Autoimmune disease	17/114 (14.9%)
Infection	9/114 (7.9%)
Pregnancy[**]	6/55 (10.9%)
Drugs[***]	2/114 (1.7%)
Positive anti-ADAMTS13 IgG	95/112 (84.8%)
Schistocytes	96/107 (89.7%)
Neurological disorders	65/109 (59.6%)
Headache	37/109 (33.9%)
Confusion	22/109 (20.2%)
Seizure	2/109 (1.8%)
Coma	7/109 (6.4%)
Focal deficiency	8/109 (7.3%)
Proteinuria	23/109 (21.1%)
Hemoglobinuria	16/109 (14.7%)
At least one relapse	31/114 (27.2%)
TTP related death	1/114 (0.9%)

Continuous variables data are presented as median (min–max); categorical variables are presented as n/number of patients where data was available, and percentage. LDH: lactate dehydrogenase. TTP: thrombotic thrombocytopenic purpura. ADAMTS13: a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13.

[*] One hundred and nine adults and 5 children (4 < 15 years old and 1 > 15 years old).

[**] Fifty-five women aged 15 to 45.

[***] Two patients had non-idiopathic TTP associated with drugs: interferon-beta (n = 1) and cocaine (n = 1).

3. Results

3.1. Characteristics of the cohort at presentation

The demographic, clinical and biological features of our 114 patients with acquired TTP are presented in Table 1. Median age in our cohort was 35.5 years (range 1.5–71.3) with 67.5% of female patients. More than two third of our patients had idiopathic TTP (69.3%) while non-idiopathic TTP (30.7%) were mostly associated with specific clinical contexts such as auto-immune disease (14.9%), non-HIV infection (7.9%) or pregnancy (10.9% of women aged 15 to 45). Clinical presentation was heterogeneous, but neurological abnormalities were present in 59.6% of patients, ranging from minor such as headaches (33.9%) or confusion (20.2%) to severe symptoms like seizure (1.8%), coma (6.4%) or focal deficiency (7.3%), with 10.0% of patients presenting at least two neurological symptoms. Most adult patient did not exhibit renal failure, with median creatinine level at 114 μmol/L (min. 66–max. 312 μmol/L), however 21.1% of patients had proteinuria and 14.7% hemoglobinuria. Out of the 5 pediatric patients studied, only three records contained creatinine level: two were within normal range for the age of the patient, and only one pediatric patient exhibited high creatinine level (192 μmol/L). The biological data revealed that a vast majority of patients had detectable anti-ADAMTS13 IgG (84.8%), consistent with auto-immune acquired TTP. Microangiopathic hemolytic anemia and/or thrombocytopenia were found in all patients with median hemoglobin at 7.8 g/dL (min. 3.2–max. 15.8 g/dL) and median platelet count at 15 × 10^9/L (min. 2–max. 251 × 10^9/L). Only 2 patients did not have thrombocytopenia, but both had microangiopathic

Table 2

Levels of VWF:Ag and VWF:CB in 123 patients with thrombotic thrombocytopenic purpura (TTP) (114 acquired TTP (aTTP) and 9 Upshaw-Schulman Syndrome (USS)).

	VWF:Ag (IU/dL)		VWF:CB (IU/dL)	
	A-TTP (n = 114)	USS (n = 9)	A-TTP (n = 114)	USS (n = 9)
Median	146	100	74	116
Minimum	48	56	7	69
Maximum	526	174	299	139
Interquartile range	122	49	74	24

hemolytic anemia. Schistocytes were detected on blood smears in 89.7% of patients. Thirty-one patients (27.2%) had at least one relapse reported during follow-up, and only one patient (0.9%) died of TTP.

3.2. VWF multimeric analysis — VWF:CB

VWF:Ag levels measured in our TTP patients ranged from subnormal values (min: 48 IU/dL) to frank excess (max: 526 IU/dL) with a median value of 146 IU/dL. The 9 USS patients however showed levels roughly within normal range, with median level measured at 100 IU/dL (range 56–174 IU/dL) (Table 2).

VWF multimeric analysis was performed on normal pooled plasma and mean HMWM fraction was estimated at 37.3% ± 5.6%) while VWF:Ag was measured at 100 IU/dL, thus mean VWF-HMWM was assessed at 37.3 IU/dL. We used this value as our cut-off value for the loss of VWF-HMWM. Cut-off value of VWF:CB was fixed at 70 IU/dL, in accordance with the recommendations of the French National Center for von Willebrand Disease [10].

Our TTP patients showed wide heterogeneity, ranging from severe loss to pronounced excess of VWF-HMWM. The median level was 36.9 IU/dL (range 3.2–206.8 IU/dL). Fifty-seven (57/114, 50%) of them showed levels below 37.3 IU/dL. As expected, remission USS patients showed marked excess of VWF-HMWM levels with median level calculated at 57.4 IU/dL (range 34.5–71.7 IU/dL) (Fig. 1).

These results are supported by similar findings as to VWF:CB levels: median level in the 114 TTP patients was 74 IU/dL, ranging from 7 IU/dL to 299 IU/dL, and 54 patients (54/114, 47.4%) showed VWF:CB < 70 IU/dL, thus indicating a loss of VWF-HMWM. Median VWF:CB level was 116 IU/dL in the 9 USS patients (range 69 IU/dL–139 IU/dL), indicating an overall excess of VWF-HMWM consistent with the congenital deficiency in ADAMTS13 (Table 2).

Linear correlation performed between VWF-HMWM levels assessed by electrophoresis and VWF:CB measured by ELISA reached statistical significance (r^2 = 0.5979, p < 0.0001), reinforcing the concept of overlap between those two parameters (Fig. 2).

3.3. Clinical comparison

We cross-referenced clinical and biological data from the medical records of the 114 acquired TTP patients, to VWF:CB and VWF-HMWM levels. We considered age, sex, neurological symptoms (defined as mild if comprising headache and/or confusion and severe if the patient exhibit at least one of the following: convulsions, coma, cerebral ischemia), acute kidney failure, hemoglobin level, platelet count, detectable anti-ADAMTS13 IgG and the occurrence of relapse.

VWF:CB cut-off value was set at 70 IU/dL, with 54 TTP patients exhibiting VWF:CB < 70 IU/dL and 60 patients exhibiting VWF:CB > 70 IU/dL. Neurological symptoms were associated with lower VWF:CB (p < 0.05) (Fig. 3). The same association is brought to light when splitting patients into no neurological symptoms, mild neurological symptoms and severe neurological symptoms, the latter
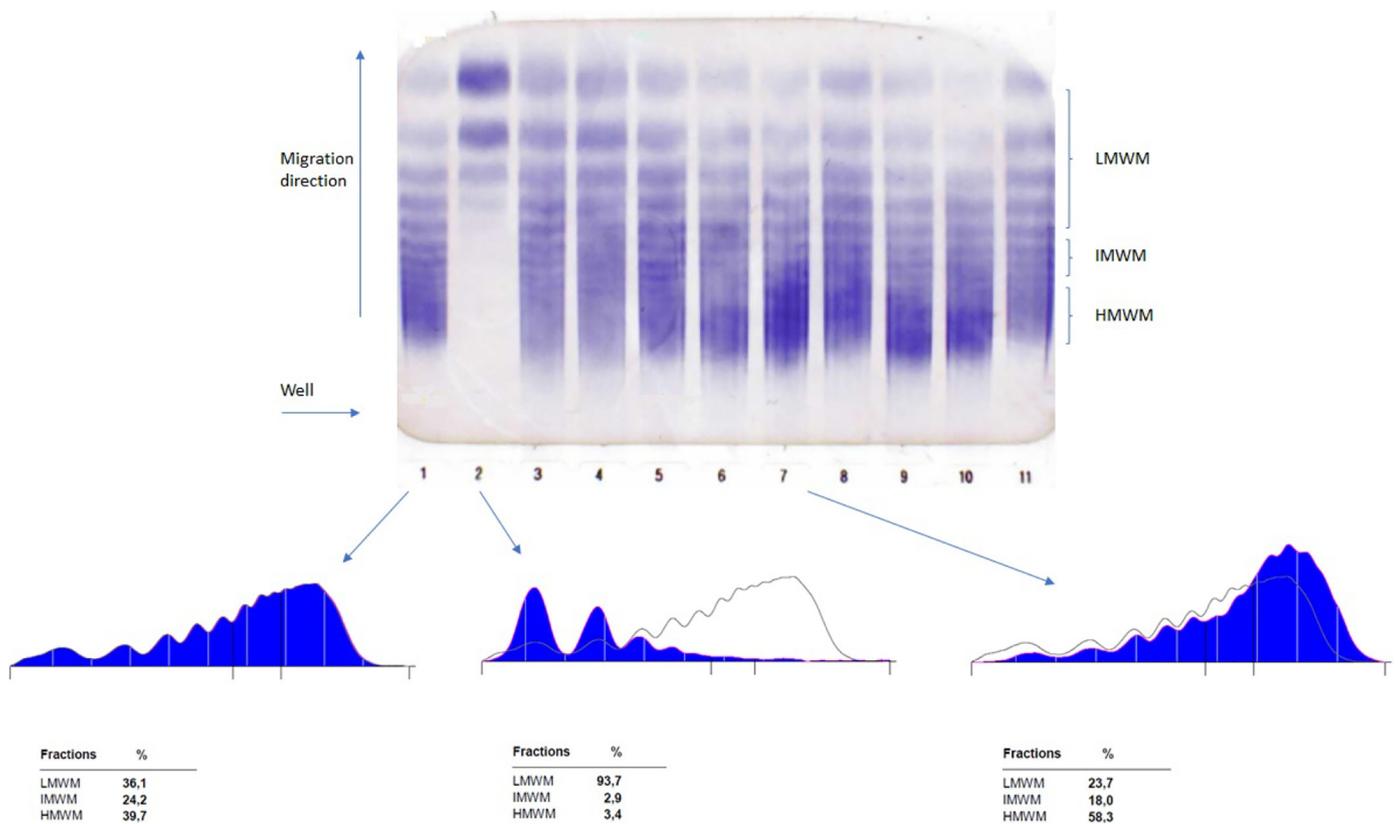
Fig. 1. Multimeric distribution of VWF multimers in 8 patients with TTP.
LMWM: low molecular weight multimers; IMWM: intermediate molecular weight multimers; HMWM: high molecular weight multimers. Lanes 1 and 11: Normal pooled plasma showing normal VWF multimeric distribution. Lane 2: Patient with von Willebrand disease type 2A used as control for a significant defect of VWF-HMWM. Lanes 3 to 10: Patients with TTP. To show the densitometry analysis, the TTP patient from lane 7, showed as an example, exhibits an excess of VWF-HMWM.
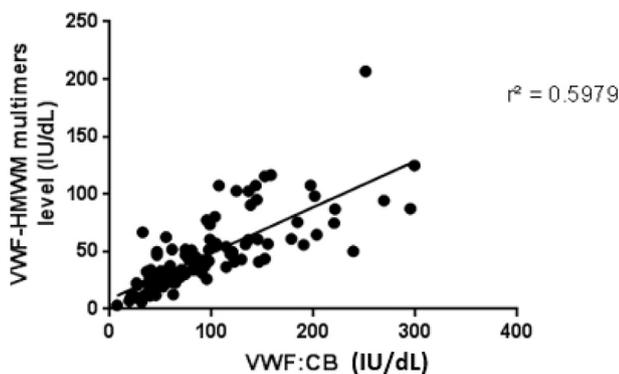

Fig. 2. Linear correlation between VWF:CB and VWF-HMWM levels performed in 123 patients with TTP (114 acquired TTP and 9 USS).
VWF-HMWM: high molecular weight multimers of von Willebrand Factor; VWF:CB: von Willebrand Factor collagen binding capacity.
Correlation coefficient calculated with Pearson's formula was $r = 0.5979$, $p < 0.0001$.

being markedly more frequent within the VWF:CB $<$ 70 IU/dL group ($p < 0.05$) (Fig. 3A). Neurological symptoms data were available in 109 of our 114 patients, and within the 51 who had VWF:CB $<$ 70 IU/dL, 36 (36/51 = 70.6%) exhibited such sign (13/51 = 25.6% only had minor symptoms while 23/51 = 45.0% had at least one severe neurological symptom). However, amongst the 58 patients showing VWF:CB $>$ 70 IU/dL, only 29 (29/58 = 50%) exhibited any form of neurological symptoms (15/58, 25.9% only experienced mild symptoms while 14/58, 24.1% were diagnosed with at least one severe neurological symptom).

This association with neurological features is even more marked if the cut-off value for VWF:CB is set at 100 IU/dL (VWF:CB $<$ 100 IU/dL, n = 77; VWF:CB $>$ 100 IU/dL, n = 37), with lowered p values (respectively $p < 0.001$ if considering neurological symptoms as opposed to no neurological symptoms; $p < 0.01$ if considering the severity of neurological symptoms) (Fig. 3B). VWF-HMWM level cut-off value was set at 37.3 IU/dL, according to our experiments on normal pooled plasma, with 56 patients having HMW-VWF multimers level $<$ 37.3 IU/dL and 58 patients HMW-VWF multimers level $>$ 37.3 IU/dL. Similarly than with the VWF:CB cut off set at 70 IU/dL, our study shows an association between the loss of VWF-HMWM and neurological impairment. Out of the 109 patients for which neurological symptoms data were available, 51 had VWF-HMWM level $<$ 37.3 IU/dL, 36 of which had neurological symptoms (36/51: 70.5%): 13 exhibited mild symptoms (13/51: 25.5%) while 23 (23/51: 45.0%) experienced severe neurological symptoms. In the 58 remaining patients with VWF-HMWM level $>$ 37.3 IU/dL, only 29 experienced neurological symptoms (29/58: 50%) with roughly half with mild and half with severe symptoms (respectively 15/58 = 29.9% and 14/58 = 24.1%) ($p < 0.05$) (Fig. 3C).

VWF:CB $<$ 70 IU/dL was also associated with deeper thrombocytopenia: mean platelet count in the VWF:CB $<$ 70 IU/dL group was 17×10^9/L (standard deviation 15×10^9/L) versus 32×10^9/L (standard deviation 45×10^9/L) in the VWF:CB $>$ 70 IU/dL group ($p < 0.05$) (data not shown). Unexpectedly, we found an association between VWF:CB $<$ 70 IU/dL and positive anti-ADAMTS13 IgG. Amongst the 112 patients on 114 where this data was available, anti-ADAMTS13 IgG were found positive in 49 out of 53 patients with VWF:CB $<$ 70 IU/dL (49/53 = 92.5%) while they were only found positive in 46 of the remaining 59 patients (46/59 = 78%) with VWF:CB $>$ 70 IU/dL ($p < 0.05$) (Fig. 4A).
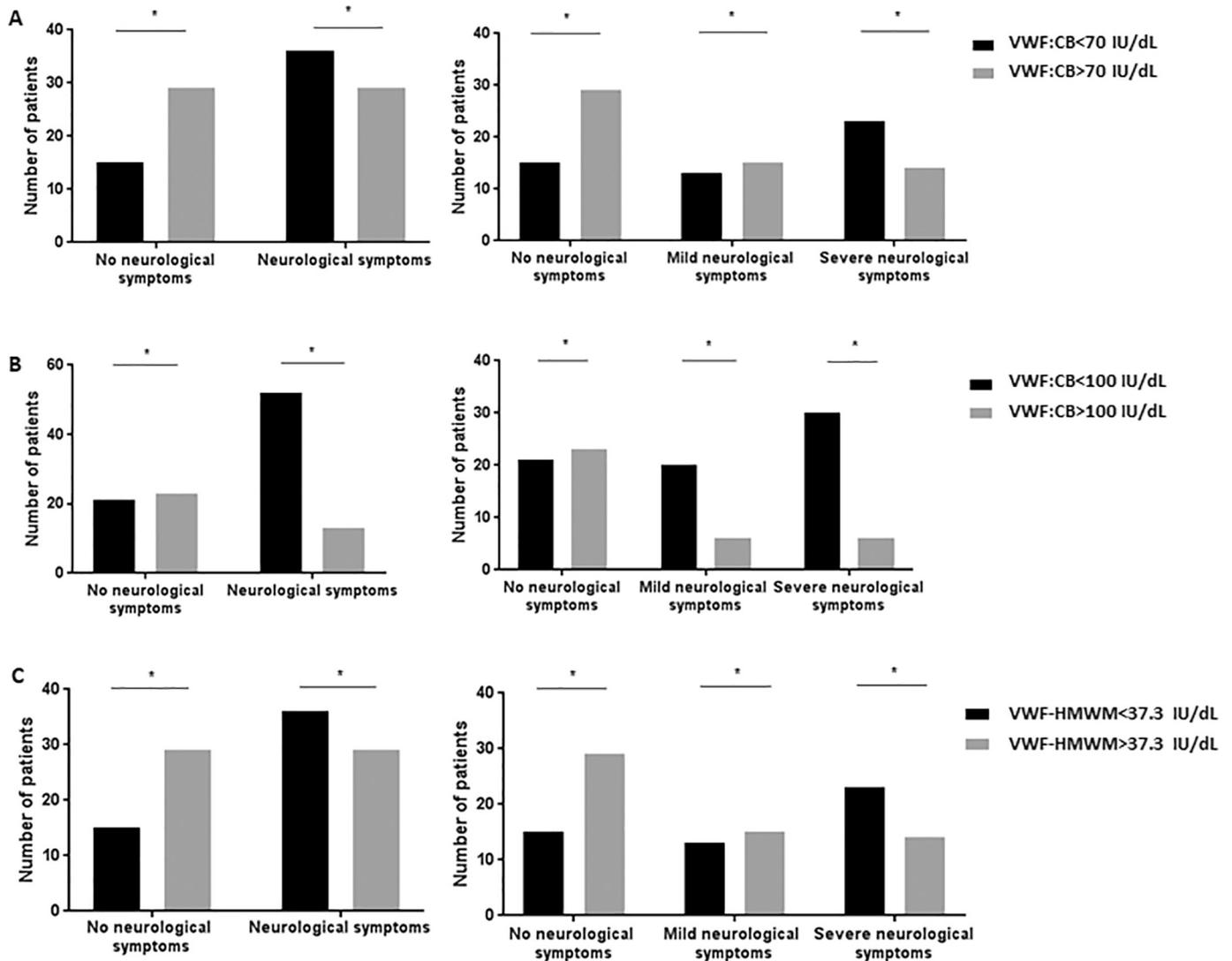
Fig. 3. Correlation of neurological symptoms with VWF:CB and VWF-HMWM level in 114 acquired TTP patients.
Neurological symptoms in 114 acquired TTP patients are more frequent in lowered VWF:CB group compared to normal or elevated VWF:CB group, (A) VWF:CB cut-off set at 70 IU/dL and (B) VWF:CB cut-off set at 100 IU/dL (lowest and highest values for VWF-CB assay as stated by the French National Reference Center for von Willebrand Disease [10]). Neurological symptoms in 114 acquired TTP patients are more frequent in lowered VWF-HMWM level group compared to elevated VWF-HMWM levels (C) (cut-off value: 37.3 IU/dL). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Mild neurological symptoms: headache and/or confusion. Severe neurological symptoms: seizure, coma, cerebral ischemia.

As with VWF:CB, an association with positive anti-ADAMTS13 IgG and HMW-VWF multimers < 37.3 IU/dL was highlighted in this study: 52 out of 56 patients with HMW-VWF multimers < 37.3 IU/dL had positive anti-ADAMTS13 IgG (52/56: 92.9%) while only 43 of the remaining 56 patients with HMW-VWF multimers > 37.3 IU/dL had such profile (46/56 = 76.8%) ($p < 0.05$) (Fig. 4B).

No association was found between platelet count and VWF-HMWM levels, and no association was found between sex, age, hemoglobin,
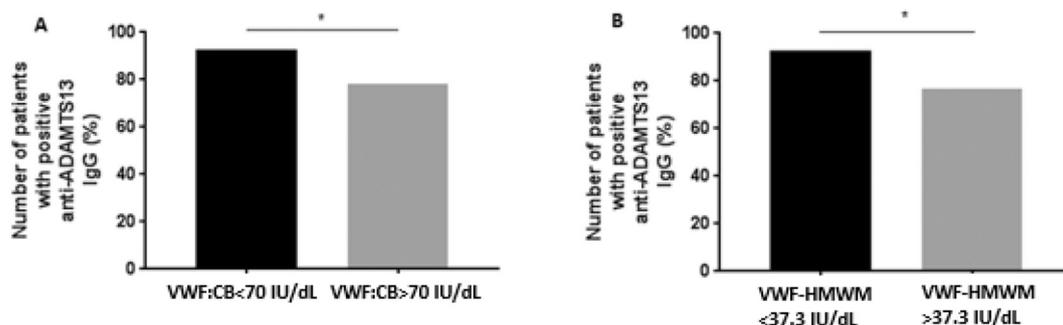

Fig. 4. Correlation of detectable anti-ADAMTS13 IgG with VWF:CB and VWF-HMWM in 114 acquired TTP patients.
VWF:CB < 70 IU/dL (A) and VWF-HMWM < 37.3 IU/dL (B) are associated with more frequent positive anti-ADAMTS13 IgG. *: $p < 0.05$.

acute kidney failure, the occurrence of a relapse and both VWF:CB and VWF-HMWM (data not shown).

4. Discussion

This study focusing on acquired TTP shows that VWF-HMWM could partially explain the clinical variability exhibited by TTP patients, and that VWF-HMWM can satisfactorily be assessed by VWF:CB, much easier to carry out than electrophoresis.

Demographic, biological and clinical data are consistent with previous descriptions of large TTP cohorts [14–17], with however an apparent over-representation of idiopathic TTP, probably related to the exclusion criteria of our study.

USS patients exhibited as expected high levels of VWF-HMWM and higher than normal VWF:CB, consistent with the constant excess of HMW due to the genetic deficiency of ADAMTS13. These profiles were used as internal control to evaluate the performances of both electrophoresis and ELISA to detect and quantify VWF-HMWM excess. However, these USS patients (well documented and monitored) regularly undergo prophylactic plasmapheresis, which could explain why some of them appear to have normal levels of both VWF:CB and VWF-HMWM.

The first aim of this work was to establish a correlation between VWF:CB and VWF-HMWM levels as methods to detect and quantify either a loss or an excess of VWF-HMWM. Our study suggests that VWF:CB is indeed a good reflect of the multimeric distribution of VWF, and as it is both much easier to carry-out and less time consuming than electrophoresis, it could replace satisfactorily the latter reference method.

Clinical and biological comparisons showed that decreased VWF-HMWM levels were associated with the presence and the severity of neurological symptoms and positive anti-ADAMTS13 IgG; decreased VWF:CB showed similar results with the addition of an association with low platelet count. It is surprising that this result is only found with VWF:CB and not with VWF-HMWM levels, and we can assume that the relatively small number of patients enrolled could not reach statistical significance. We do not however find an association between VWF-HMWM and the occurrence of a relapse, suggesting that VWF-HMWM may only be associated with the ongoing acute phase and not predict future evolution.

Studies on multimeric distribution of VWF in acquired TTP are very scarce, notably because of the technical complexity inherent in VWF multimer analysis. Moake and colleagues reached a milestone in deciphering the pathophysiology of TTP in 1982 when they reported the presence of unusually large VWF-HMWM in 4 probable USS patients and demonstrated the loss of VWF-HMWM during acute TTP episodes, thus paving the way for the discovery of ADAMTS13 [18]. This study did not, however, look into the relationship between VWF-HMWM and clinical features. The prospective study conducted by Veyradier and colleagues in 2001 on the role of ADAMTS13 in 111 cases of TMA (including 66 TTP) highlighted the vast heterogeneity of VWF multimeric distribution in TTP [12] (with roughly similar results to ours), without focusing on the potential clinical impact of VWF-HMWM. Both studies used complex in house-methods, with superior resolution and certainly a better assessment of the VWF-HMWM than the semi-automated method we used. Nonetheless, the relative ease of our method allowed us to study a quite large number of patients.

Our results suggest that acquired auto-immune TTP lead to more decreased levels of VWF-HMWM compared to non-autoimmune acquired TTP. This apparent decrease can probably be explained by a sequestration of these VWF-HMWM within the microthrombi, which are therefore not free in plasma. We can hypothesize that acquired auto-immune TTP could have a different development kinetic compared to other etiologies. Rapid formation of microthrombi leading to both deeper consumption thrombocytopenia and decreased VWF-HMWM with faster advent of neurological symptoms in acquired auto-immune

TTP could explain our results. This is further supported by a previous study on acquired TTP suggesting that TTP patients with detectable anti-ADAMTS13 IgG display a worse prognosis [19].

Although our results appear to demonstrate an association between VWF-HMWM and the clinical presentation of acquired TTP, we do not believe that the assessment of the multimeric distribution of VWF is relevant as a diagnostic or prognostic tool. It was merely used in our study as a proof of concept to understand underlying mechanisms in acquired TTP.

To our knowledge, this is the first study to focus on VWF-HMWM levels rather than percentage, and to focus on the relevance of VWF-HMWM on the clinical presentation of acquired TTP. A further study looking into a potential link between VWF-HMWM and cardiac symptoms, troponin levels and treatment could substantiate this work. Our results may offer new insights to understanding the pathophysiology of acquired auto-immune TTP.

Declaration of Competing Interest

None.

Appendix A

The members of the Reference Center for Thrombotic Microangiopathies (CNR-MAT) are: Augusto Jean-François (Service de Néphrologie, dialyse et transplantation; CHU Larrey, Angers); Azoulay Elie (Service de Réanimation Médicale, Hôpital Saint-Louis, Paris); Barbay Virginie (Laboratoire d'Hématologie, CHU Charles Nicolle, Rouen); Benhamou Ygal (Service de Médecine Interne, CHU Charles Nicolle, Rouen); Bordessoule Dominique (Service d'Hématologie, Hôpital Dupuytren, Limoges); Boyer Olivia (Service de Néphrologie Pédiatrique, Hôpital Necker); Charasse Christophe (Service de Néphrologie, Centre Hospitalier de Saint-Brieuc); Charvet-Rumpler Anne (Service d'Hématologie, CHU de Dijon); Chauveau Dominique (Service de Néphrologie et Immunologie Clinique, CHU Rangueil, Toulouse); Choukroun Gabriel (Service de Néphrologie, Hôpital Sud, Amiens); Coindre Jean-Philippe (Service de Néphrologie, CH Le Mans); Coppo Paul (Service d'Hématologie, Hôpital Saint-Antoine, Paris); Corre Elise (Service d'Hématologie, Hôpital Saint-Antoine, Paris); Delmas Yahsou (Service de Néphrologie, Hôpital Pellegrin, Bordeaux); Deschenes Georges (Service de Néphrologie Pédiatrique, Hôpital Robert Debré, Paris); Devidas Alain (Service d'Hématologie, Hôpital Sud-Francilien, Corbeil-Essonnes); Dossier Antoine (Service de Néphrologie, Hôpital Bichat, Paris); Dossier Claire (Service de Néphrologie Pédiatrique, Hôpital Robert Debré, Paris); Fain Olivier (Service de Médecine Interne, Hôpital Saint-Antoine, Paris); Fakhouri Fadi (Service de Néphrologie, CHU Hôtel-Dieu, Nantes); Frémeaux-Bacchi Véronique (Laboratoire d'Immunologie, Hôpital Européen Georges Pompidou, Paris); Galicier Lionel (Service d'Immunopathologie, Hôpital Saint-Louis, Paris); Grangé Steven (Service de Réanimation Médicale, CHU Charles Nicolle, Rouen); Guidet Bertrand (Service de Réanimation Médicale, Hôpital Saint-Antoine, Paris); Halimi Jean-Michel (Service de Néphrologie Pédiatrique, Hôpital Bretonneau, Tours); Hamidou Mohamed (Service de Médecine Interne, Hôtel-Dieu, Nantes); Herbrecht Raoul (service d'Oncologie et d'Hématologie, Hôpital de Hautepierre, Strasbourg); Hié Miguel (Service de Médecine Interne, Groupe Hospitalier Pitié-Salpêtrière, Paris); Jacobs Frédéric (Service de Réanimation Médicale, Hôpital Antoine Béclère, Clamart); Joly Bérangère (Service d'Hématologie Biologique, Hôpital Lariboisière, Paris); Kanouni Tarik (Unité d'Hémaphrèse, Service d'Hématologie,

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References

[1] J.E. Sadler, Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura, Blood 112 (1) (2008) 11–18.

[2] M. Furlan, R. Robles, M. Galbusera, et al., Von Willebrand factor-cleaving protease in thrombotic thrombocytopenis purpura and the hemolytic-uremic syndrome, N. Engl. J. Med. 339 (22) (1998) 1578–1584.

[3] F. Peyvandi, S. Lavoretano, R. Palla, et al., ADAMTS13 and anti-ADAMTS13 anti-bodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission, Haematologica 93 (2) (2008) 232–239.

[4] G.G. Levy, W.C. Nichols, E.C. Lian, et al., Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura, Nature 413 (6855) (2001) 488–494.

[5] H.-M. Tsai, E.C. Lian, Antibodies to Von Willebrand factor-cleaving protease in acute TTP, N. Engl. J. Med. 339 (22) (1998) 1585–1594.

[6] B.S. Joly, P. Coppo, A. Veyradier, Thrombotic thrombocytopenic purpura, Blood 129 (21) (2017) 2836–2846.

[7] J.A. Kremer Hovinga, P. Coppo, B. Lämmle, et al., Thrombotic thrombocytopenic purpura, Nat. Rev. Dis. Prime. 3 (2017) 17020.

[8] M. Stockschlaeder, R. Schneppenheim, U. Budde, Update on von Willebrand factor multimers: focus on high- molecular-weight multimers and their role in hemostasis, Blood Coagul. Fibrinolysis 25 (3) (2014) 206–216.

[9] J.E. Sadler, U. Budde, C. Eikenboom, et al., Update on the pathophysiology and classification of von Willebrand disease : a report of the Subcommittee on von Willebrand Factor, J. Thromb. Haemost. 4 (10) (2006) 2103–2114.

[10] 10. A. Veyradier, P. Boisseau, E. Fressinaud, et al., A laboratory phenotype/gen-otype correlation of 1167 French patients from 670 families with von Willebrand disease, Medicine 95 (11) (2016) 1–11.

[11] E.J. Favaloro, Utility of the von Willebrand factor collagen binding assay in the diagnosis of von Willebrand disease, Am. J. Hematol. 92 (1) (2017) 114–118.

[12] A. Veyradier, B. Obert, A. Houllier, et al., Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases, Blood 98 (6) (2001) 1765–1772.

[13] K. Kokame, Y. Nobe, Y. Kokubo, et al., FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay, Br. J. Haematol. 129 (1) (2005) 93–100.

[14] E. Mariotte, E. Azoulay, L. Galicier, et al., Epidemiology and pathophysiology of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency (thrombotic thrombocytopenic purpura): a cross-sectional analysis of the French national registry for thrombotic microangiopathy, Lancet Haematol. 3 (5) (2016) 237–245.

[15] M. Scully, H. Yarranton, J. Cavenagh, et al., Regional UK TTP Registry: correlation with laboratory ADAMTS 13 analysis and clinical features, *Br. J. Haematol.* 142 (5) (2008) 819–826.

[16] Y. Fujimura, M. Matsumoto, Registry of 919 patients with thrombotic micro-angiopathies across Japan: database of Nara Medical University during 1998–2008, Intern. Med. 49 (1) (2009) 7–15.

[17] J.N. George, J.A.K. Hovinga, D.R. Terrell, et al., The Oklahoma Thrombotic Thrombocytopenic Purpura–Hemolytic Uremic Syndrome Registry: the Swiss con-nection, *Eur. J. Haematol.* 80 (4) (2008) 277–286.

[18] J.L. Moake, C.K. Rudy, J.H. Troll, et al., Unusually large plasma factor VIII: Von Willebrand Factor multimers in chronic relapsing thrombotic thrombocytopenic purpura, N. Engl. J. Med. 307 (23) (1982) 1432–1435.

[19] P. Coppo, M. Wolf, A. Veyradier, et al., Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura, Br. J. Haematol. 132 (1) (2006) 66–74.