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**Author:** Lotta, Luca Andrea

**Title:** Pathophysiology of thrombotic thrombocytopenic purpura : pathophysiology of thrombotic thrombocytopenic purpura : the "two-hit" paradigm

Date: 2012-11-13

## **CHAPTER 7**

**Platelet-reactive conformation and multimeric pattern of von Willebrand factor in acquired thrombotic thrombocytopenic purpura during acute disease and remission**

**Luca A Lotta**, Rossana Lombardi, Mariagabriella Mariani, Stefano Lancellotti, Raimondo De Cristofaro, Martine J Hollestelle, Maria T Canciani, Pier M Mannucci, Flora Peyvandi.

Adapted from Journal of Thrombosis and Haemostasis 2011;9:1744-51.

## **Abstract**

Binding of von Willebrand factor (VWF) multimers of ultralarge size to platelets is considered the triggering mechanism of microvascular thrombosis in thrombotic thrombocytopenic purpura (TTP). We assessed the potential of VWF-related measurements as markers of disease activity and severity in TTP. VWF antigen (VWF:Ag), platelet glycoprotein-Ib- $\alpha$  binding-conformation (GPIb- $\alpha$ /BC) and multimeric pattern were investigated in 74 patients with acquired TTP and 73 healthy controls. VWF ristocetin co-factor activity (VWF:RCo) and collagen binding (VWF:CB) were also measured in a subgroup of patients. VWF:Ag and VWF-GPIb- $\alpha$ /BC were higher in TTP patients than controls. However, there was no appreciable difference in VWF-GPIb- $\alpha$ /BC between samples obtained during acute TTP and remission. Larger VWF multimers were frequently lacking in acute TTP patients, who displayed ultralarge multimers at remission. The degree of loss of larger VWF multimers was associated with the degree of abnormality of hemoglobin, platelet counts and serum LDH and was also associated with low levels of both VWF:RCo/Ag and VWF:CB/Ag ratios. In TTP, the platelet-binding conformation of VWF is not exclusively present in acute disease, nor is it associated with its clinical and laboratory severity. The loss of larger VWF multimers, accompanied by low VWF:RCo/Ag and VWF:CB/Ag ratio values, represents an index of disease activity and severity of acute TTP in patients with severe ADAMTS13 deficiency.

## Introduction

Thrombotic thrombocytopenic purpura (TTP), a rare disease characterized by single or multiple episodes of disseminated microvascular thrombosis, is associated with the severe plasmatic deficiency of the von Willebrand factor (VWF)-cleaving metalloprotease ADAMTS13.<sup>1</sup> In patients with TTP the presence in plasma of highly platelet-reactive VWF forms of ultralarge (UL) molecular weight (ULVWF), which in physiological conditions are cleaved by ADAMTS13 into regular-sized multimers,<sup>2</sup> is the main mechanism of intravascular platelet adhesion/aggregation and disseminated thrombosis in the microcirculation.<sup>3-6</sup> A nanobody (i.e., a single domain antibody) with specificity towards the platelet glycoprotein Ib- $\alpha$  binding conformation of VWF (GPIb- $\alpha$ /BC) enables to measure by immunoassay the proportion of plasma VWF which is highly reactive with this platelet glycoprotein.<sup>7</sup> In TTP, VWF-GPIb- $\alpha$ /BC (measured as the ratio of VWF in its platelet-binding conformation to the whole circulating mass of the protein) was 2-fold (acquired TTP) to 8-fold (congenital TTP) higher than normal in 17 patients with acute disease,<sup>7</sup> but only 1.5-fold higher in 22 patients in clinical remission.<sup>8</sup> The higher amounts of VWF-GPIb- $\alpha$ /BC suggest that a change in the conformation of circulating VWF is responsible for the heightened platelet reactivity that leads to disseminated microvascular thrombosis.

In this study, we sought to replicate these findings and to evaluate in a large cohort of patients the relationship between the amount of platelet-reactive VWF and different clinical and laboratory presentations of acquired TTP (acute disease/remission, with and without ADAMTS13 deficiency). Antigen

(VWF:Ag) levels, ristocetin cofactor activity (VWF:RCo), collagen binding (VWF:CB) and the multimeric pattern of VWF were also investigated.

## **Patients and Methods**

### *Patient and sample selection*

The 74 patients investigated in the study were selected from a larger group of 136 patients with TTP included in the Milan TTP registry (URL: [www.ttpdatabase.org](http://www.ttpdatabase.org)). The features of the registry and the clinical definitions adopted have been described elsewhere.<sup>9, 10</sup> A patient selection flow-chart is in Figure 1. Selected patients with acute TTP were those with available plasma samples, after excluding those transfused with blood products during the 10 days preceding sampling. Plasma samples were also available from patients during disease remission. Patients included in the study with acute phase samples were excluded from the choice of remission samples in order to respect the independence of observations. A summary of the study design is in Figure 2. Study measurements were investigated in four primary subgroups (a-d) chosen according to the following criteria (see below “Rationale for subgroup selection”): (a) 18 patients with a first episode of acute TTP and severe ADAMTS13 deficiency (<6%) at presentation; (b) 16 patients with a recurrent episode of acute TTP (second through eighth episode) and severe ADAMTS13 deficiency at the time of recurrence; (c) 20 patients with previous acute TTP currently in remission, with severe ADAMTS13 deficiency at the time of remission; (d) 20 patients with previous acute TTP, currently in remission with normal plasma levels of ADAMTS13 (46-160%). Each patient was included in

only one of the four primary subgroups. Additional analyses of the study results were carried out in subgroups formed by combining in various ways primary subgroups a-d: (e) 34 patients with acute TTP (group a + b, i. e., patients with first acute episodes plus patients during acute recurrence, all with severe ADAMTS13 deficiency); (f) 40 patients with previous acute TTP currently in remission (group c + d, i. e., those with severe deficiency (<6%) plus those with normal ADAMTS13 (>46%); (g) 54 patients with severe ADAMTS13 deficiency (group a + b + c, i. e., those with first acute episodes plus those with recurrences plus those in remission, all with ADAMTS13 levels <6%). For 17 of the 34 patients with acute TTP (group e) samples collected both during acute disease and remission were also available and used for paired comparisons of the study measurements at different times of the clinical course in the same individuals. In this subgroup, VWF:RCo and VWF:CB were also measured and the VWF:RCo/Ag and VWF:BC/Ag ratios calculated.

#### *Rationale for subgroup selection*

Patients with first acute episodes and those with acute recurrences (n=34) were included in two distinct primary subgroups (a and b) because recurrences are usually diagnosed earlier and are milder than first acute episodes of TTP in terms of clinical and laboratory abnormalities, creating a possible source of confounding.<sup>10</sup> We chose to analyze separately 54 patients with ADAMTS13 <6%, whether in the acute phase or during disease remission, in order to investigate patients with a similar disease mechanism, i.e., the severe deficiency of the VWF-cleaving protease. The subgroup of 20 patients with normal ADAMTS13 during remission was chosen in order to allow comparison of the

VWF-related measurements in patients with and without severe ADAMTS13 deficiency.

#### *Control group selection*

Controls for VWF:Ag and VWF-GPIIb- $\alpha$ /BC measurements were 73 blood donors at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, similar in age and sex with the whole group of TTP patients and with each study subgroup.

#### *Study measurements*

The multimeric pattern of plasma VWF was analyzed by sodium dodecyl sulfate agarose gel electrophoresis followed by luminographic visualization of multimers. EDTA plasma samples were diluted in order to obtain final VWF:Ag concentrations of 10%. Gel systems of 1% high gelling temperature agarose (HGT-Seakem) were chosen for low-resolution multimeric analysis. After electrophoresis, proteins were electrotransferred to an immobilon polyvinylidene fluoride membrane (Millipore, Billerica, MA) overnight and multimers visualized using a rabbit polyclonal anti-human VWF antibody (Dako Cytomation, Glostrup, Denmark) and a goat anti-rabbit IgG peroxidase conjugated antibody (BioRad Laboratories, Hercules, CA). The filters were stained with a luminol-iodophenol solution, covered with transparent film, and densitometry of multimers was then carried out with Typhoon 8600 Variable Mode Imager (Image Quant TM Software; Amersham Biosciences, Uppsala, Sweden). The multimeric patterns of patient and control samples were compared with that of a reference pooled plasma run on the same gel. Densitometric analysis was performed on the blotted membrane using the graph line obtained by the single

lane of the gel and plasma samples with similar VWF concentrations in order to obtain comparable area values. According to Budde and Scheneppenheim,<sup>11</sup> the reference plasma lane was divided into small (1-5), intermediate (6-10) and larger (>10) multimers of regular size. The proportion of larger multimers in the sample was calculated by dividing the area corresponding to larger multimers by the total area of the lane. The ultralarge VWF (ULVWF) ratio was calculated as the ratio of (i) the proportion of large multimers in the test sample to (ii) the corresponding area in the reference plasma within the same gel. A normal range was established by calculating the ULVWF ratio in 36 healthy subjects. VWF-GPIb- $\alpha$ /BC was measured using an immunoadsorbent assay,<sup>7</sup> based upon the nanobody AU/VWF-a11 that specifically recognizes the VWF-GPIb- $\alpha$ /BC. Antibodies were provided by P. G. de Groot and P. J. Lenting. Before incubation, serial dilutions of VWF:Ag were obtained (4 dilutions with concentrations ranging from 250 ng/ml to 31 ng/ml). The same pooled normal plasma used for the VWF:Ag assay was used as reference in each experiment. The VWF-GPIb- $\alpha$ /BC was calculated as the ratio of the slope of the close response curve of plasma samples to the slope of normal plasma pool (set to a value of 1). Plasma from a patient with type 2B von Willebrand disease was included in all the experiments as a positive control, because these patients have higher amounts of VWF-GPIb- $\alpha$ /BC in their plasma.<sup>12</sup> ADAMTS13 activity was measured using the collagen binding assay, anti-ADAMTS13 antibodies were searched for by western blotting.<sup>9</sup> The lower laboratory limit of sensitivity of the collagen binding assay is 6%, so that ADAMTS13 deficiency was arbitrarily defined as severe when plasma levels were lower than 6%. VWF:Ag was quantified using the ACL TOP Analyzer and



pooled normal plasma as reference. VWF:RCo was measured by the Siemens Healthcare Diagnostics BC von Willebrand Reagent assay using lyophilized platelets in the presence of ristocetin. The test was performed in an automatic coagulometer (BCS, Siemens Healthcare, Milano, Italy). VWF:CB was measured on plasma using a solution of 95% type I and 5% type III collagens (Horm®, Nycomed Austria GmbH, Linz, Austria) as described previously.<sup>13</sup>

### *Statistical analysis*

The chi-square or Fisher's exact tests and Mann-Whitney U- or Student t-tests were used when appropriate to compare categorical and continuous variables in different primary and secondary study groups. The Wilcoxon signed-ranks test was used for paired comparisons of continuous variables. Pearson's correlation was used to assess the relationships between the VWF-related measurements and the clinical and laboratory features of TTP patients at the time of acute disease presentation.

## **Results**

The general features and results of VWF and ADAMTS13 measurements in the whole group of TTP patients and controls are shown in Table 1. Median values of VWF:Ag and VWF-GPIIb- $\alpha$ /BC were higher in TTP patients than controls (Mann-Whitney U test,  $p < 0.001$  and  $p = 0.004$ ), whereas there was no statistically significant difference pertaining to ULVWF multimers, which, however, showed a much wider range of ratio values in cases than in controls.

Among study subgroups, VWF:Ag was higher during acute TTP (first and recurrent episodes combined) than during remission (median value in 34 acute

cases 202% vs 130% in 40 remission cases,  $p=0.002$ ). VWF-GPIb- $\alpha$ /BC values were not significantly different in 34 cases with acute TTP (first and recurrent episode combined) compared with 40 cases in remission (median values 1.20 vs 1.31,  $p=0.28$ ). There was also no difference in the paired analysis of the 17 cases with both acute and remission samples available (Table 2). In patients with first acute disease episode and remission with severe ADAMTS13 deficiency, VWF-GPIb $\alpha$ /BC values were higher than in those with recurrent episodes, those with normal protease levels and controls (see Table 1). Owing to the lack of large VWF multimers, the ULVWF multimer ratio was markedly decreased in patients with first acute episodes, whereas it was higher than normal (due to the presence in plasma of ultralarge multimers) during remission in patients with severe ADAMTS13 deficiency (Table 1 and Figure 3A). The marked changes of the multimeric pattern of VWF from defective to ultralarge are shown in Figure 3B, which compares the ratio values during acute episodes and remission obtained in paired samples from 17 TTP cases. These changes occurred particularly in patients who had low ULVWF ratio at the time of their acute episode and severe ADAMTS13 deficiency at remission (Figure 3B). The multimeric structure was not different from normal in patients during acute recurrences and during remission in patients with normal ADAMTS13 activity (Table 1 and Figure 3A). Figure 4 shows representative examples of VWF multimer patterns in the aforementioned patient subgroups. In the 17 patients with both acute and remission samples available, antigen-normalized values of VWF:RCo and, particularly, VWF:CB correlated with ULVWF ratio (VWF:RCo/Ag:  $r=0.71$ ,  $p=0.01$ ; VWF:CB/Ag:  $r=0.88$ ,  $p=0.0006$ ). Similarly to what observed for

ULVWF ratio, the VWF:CB/Ag ratio was reduced in acute TTP compared to remission (Table 2). The VWF:RCo/Ag ratio was reduced in the first acute episodes of TTP compared to the corresponding remissions (0.47 vs 0.78;  $n=10$ ;  $p=0.01$ ), but not in recurrent episodes compared to their remissions (1.03 vs 0.75;  $n=7$ ;  $p=0.13$ ). ABO blood groups influenced both VWF:Ag and VWF-GPIb- $\alpha$ /BC, because both in patient and controls plasma levels were higher in carriers of non-O blood groups (Table 3). However, the associations of high levels of VWF:Ag and VWF-GPIb- $\alpha$ /BC with TTP remained statistically significant after stratification for O/non-O blood group (Table 3). The ULVWF multimer ratio was independent from blood groups (not shown). In patients, VWF:Ag and VWF-GPIb- $\alpha$ /BC levels did not correlate with the ULVWF ratio. Finally, we chose to evaluate whether or not during acute TTP there was a correlation between the aforementioned VWF-related measurements, the degree of abnormality of laboratory markers of disease activity and severity (platelet count, hemoglobin, serum lactate dehydrogenase) and the prevalence of clinical symptoms (neurological, renal, cardiovascular, hemorrhagic). While there was no correlation between these laboratory measurements of TTP activity and VWF:Ag, VWF:RCo and VWF-GPIb- $\alpha$ /BC levels (data not shown), the ULVWF multimer ratio was positively correlated with the degree of anemia ( $r=0.48$ ;  $p=0.007$ ), thrombocytopenia ( $r=0.59$ ;  $p<0.001$ ) and negatively correlated with serum LDH levels ( $r=-0.54$ ;  $p=0.003$ ). VWF:CB/Ag and VWF:RCo/Ag ratio were similarly correlated with TTP-related laboratory measurements at presentation. VWF:CB/Ag displayed statistically significant correlations with platelet counts ( $r=0.65$ ,  $p=0.01$ ) and LDH (negative correlation with  $r=-0.57$  and  $p=0.05$ ) and a

trend towards correlation with hemoglobin ( $r=0.47$ ,  $p=0.09$ ). VWF:RCo/Ag showed a statistically significant correlation with platelet counts ( $r=0.74$ ;  $p=0.003$ ) and a trend towards negative correlation with LDH ( $r=-0.53$ ;  $p=0.07$ ). There was no statistically significant association with hemoglobin ( $r=0.37$ ;  $p=0.19$ ), perhaps due to the smaller sample size and lower correlation of VWF:RCo/Ag with ULVWF ratios compared with VWF:CB/Ag (see above). None of the study measurements was associated with the prevalence and type of clinical symptoms (data not shown).

## **Discussion**

This study aimed to investigate the relationships between VWF-related properties and different clinical and laboratory presentations of acquired TTP (acute disease or remission with or without ADAMTS13 deficiency). In previous studies,<sup>7, 8</sup> plasma levels of VWF in its platelet-binding conformation (VWF-GPIb- $\alpha$ /BC) were high during acute TTP,<sup>7</sup> suggesting that this rise might be associated with, or even be responsible of, the formation of platelet-rich thrombi in the microcirculation, and hence of disease activity. In our cohort, platelet-reactive VWF was indeed increased in the acute phase of the first disease episode (subgroup a) in comparison to controls, but during remission the plasma levels of this conformation of VWF were not different from those measured during the first acute episode. Moreover, even in patients during the acute phase there was a large overlap of values between them and controls. No correlation was found with the degree of abnormalities of such laboratory markers of TTP activity and severity as platelet count, hemoglobin and LDH levels. Hence, platelet-reactive

VWF appears to be a weak index of disease activity in these patients. Its presence in plasma was closely associated with the presence of severe ADAMTS13 deficiency, indicating that the rise in the platelet-reactive conformation of VWF is among the prothrombotic changes induced by the deficiency of the VWF-cleaving protease.

There were striking differences in the multimeric structure of VWF between patients in the acute phase of the first disease and those in remission. The largest multimeric fraction of VWF was markedly reduced on electrophoresis in patients with a first acute episode of TTP, and this reduction was correlated with the degree of impairment of laboratory markers of disease activity and severity. The defect of larger VWF multimers was pronounced in patients during their first acute episode but not in those with recurrences, consistent with the views that recurrences are generally less severe than first disease episodes in terms of clinical and laboratory measurements, perhaps because they are diagnosed and managed earlier.<sup>4</sup> During remission, the multimeric pattern changed dramatically, with the fresh appearance in plasma of ultralarge VWF multimers similar to those present in vascular endothelial cells and platelets, in agreement with the pioneering observations made by Moake et al in 1982.<sup>14</sup> The lack of larger VWF multimers in patients with acute TTP was previously described in the frame of case reports and small case series.<sup>15-17</sup> In this study the correlation between the degree of defect of larger multimers of regular size and laboratory markers of TTP activity and severity suggests that the electrophoretic measurement of the ULVWF ratio is an index of disease activity and severity at presentation, at least in patients with ADAMTS13 deficiency. Lack of larger VWF multimers strongly

correlated with low VWF:CB/Ag and VWF:RCo/Ag ratios, suggesting that VWF:CB/Ag and VWF:RCo/Ag ratios, which are more easily measurable than the ULVWF ratio, may be candidate markers of acute disease severity in TTP. Pertaining to the striking differences in the multimeric structure of VWF observed during the first acute episode of TTP in comparison with the same patients during remission, we offer the following mechanistic explanations. During acute disease the plasmatic deficiency of the metalloprotease impairs the cleavage of the ultralarge multimeric forms of VWF secreted in plasma from markedly activated endothelial cells. However, these forms and the largest regular sized multimers are not seen in patient plasma because they avidly bind to platelets, thereby causing a multimeric defect that resembles that seen in acquired and type 2 von Willebrand disease. Perhaps this VWF defect adds to that of thrombocytopenia in causing the bleeding tendency sometimes observed in the acute phase of TTP. Indeed no association was found in this study between hemorrhagic symptoms and degree of multimeric effect, perhaps owing to the low prevalence in our patients of these symptoms, that tend to be overlooked and reported less frequently in TTP cases than the more striking ischemic symptoms. In contrast, during TTP remission, the degree of endothelial cell activation and the associated secretion of ultralarge multimers are likely to be much smaller, so that the balance between secretion of ultralarge VWF and platelet uptake is less turned towards the latter than during acute disease. The same situation of relative balance does perhaps take place at the time of recurrence, because recurrent episodes are diagnosed earlier, before massive platelet uptake of VWF is large enough to cause the loss of larger multimers in plasma. In TTP cases

characterized by normal ADAMTS13 levels during remission, the normal multimeric pattern uniformly observed in this study is likely to be explained by the restoration of a physiological degree of secretion of ultralarge VWF from endothelial cells that are no longer abnormally activated, as well as by the capacity of plasma ADAMTS13 to process ultralarge multimers into smaller regular sized multimers. This study has a number of limitations. The selection of the case material is based upon a registry for information on clinical data, symptoms and routine laboratory parameters such as platelet count, hemoglobin and serum LDH. However, the ADAMTS13 and VWF-related measurements, i.e., the core of this study, were centralized in one laboratory. The criteria for the diagnosis of TTP in the acute phase and remission are those based upon the exclusion of other thrombotic microangiopathies usually adopted in the medical literature. The criteria chosen for the analysis of primary and secondary subgroups are arbitrary, but the rationale for their choice is explained.

In conclusion, this study confirms that the platelet-reactive form of VWF as measured with the nanobody-based immunoassay is sometime present at increased concentrations during TTP. However, it also demonstrates that this measurement is not an accurate and sensitive index of disease activity and severity, being detectable also in several patients at the time of disease remission. The most striking and consistent finding was a defect of the large VWF multimers during the acute phase of TTP, accompanied by low VWF:CB/Ag and VWF:RCO/Ag ratios. The defect of VWF multimers was associated with disease activity and severity, as indicated by the degree of thrombocytopenia, anemia and organ damage measured by serum LDH.

## Acknowledgments

The authors thank Dr. Luigi Flaminio Ghilardini for the help in the preparation of manuscript images and Dr. Roberta Palla for the help with study procedure organization.

F.P. was supported by Italian Ministry of University and Research (PRIN 2007, N°2007T9HTFB and FIRST EX-60% 2006) . FP and PMM were also founded by Italo Monzino Foundation.

We gratefully acknowledge the following participating centers that provided patient samples and information:

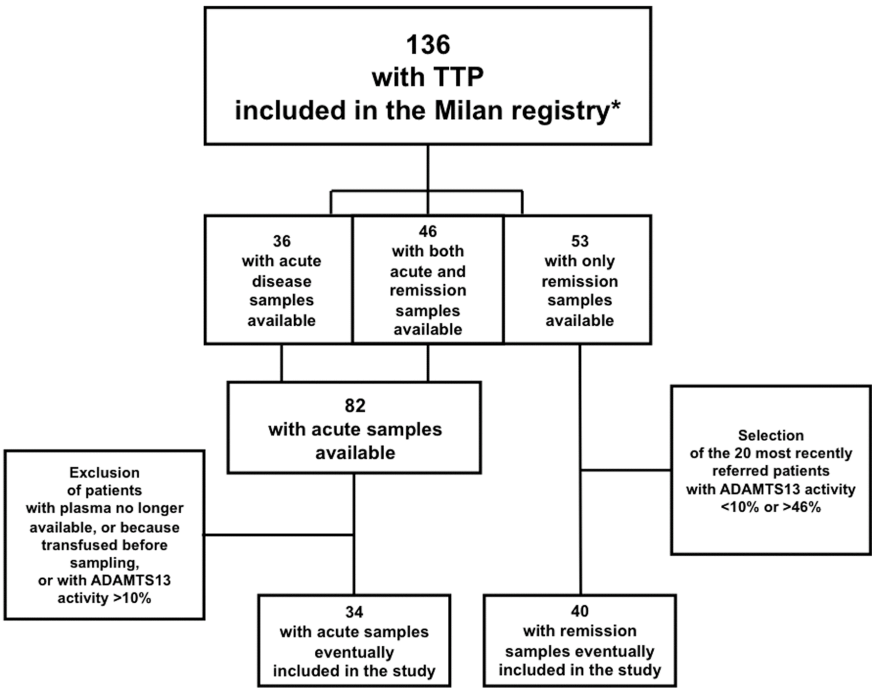
Italian Centres: *C. Balduini and P. Noris*, IRCCS San Matteo Hospital, Pavia; *D. Grisillo, M. Caremani and U. Occhini*, S. Donato Hospital, Arezzo; *M. Benucci*, Nuovo Ospedale San Giovanni di Dio, Florence; *V. Santini*, AO Careggi, Florence; *R. Vallone*, AO G. Rummo, Benevento; *Zatoni and Spinzi*, Valduce Hospital, Como; *C. Cristofalo*, A. Perrino Hospital, Brindisi; *M. G. Mazzucconi and C. Santoro*, La Sapienza University, Rome; *P. Spedini and M. Tajana*, AIO Cremona; *L. Zighetti and G. Gerli*, S. Paolo Hospital, Milan; *L. Gatti, M. Agnelli, M. Colombi and A. Zanella*, IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan; *S. Morandi*, Istituti Ospedalieri, Cremona; *G. Rossi*, Ospedali Civili, Brescia; *S. Sammassimo*, AO Senese, Siena; *M. C. Refe and A. Gabrielli*, Ospedali Riuniti, Ancona; *M. Simoncelli*, Infermi Hospital, Rimini; *E. Venegoni and G. Fornaroli*, Magenta Hospital, Magenta; *M. C. Bertoncelli, S. Colombi, D. Rossi and G. Gaidano*, AO Maggiore della Carità, Novara; *M. Pizzuti*, S. Carlo Hospital, Potenza; *R. De Cristofaro*, Catholic University School of Medicine, Rome; *S. Bulgarelli*, AO Arcispedale S.Maria Nuova, Reggio Emilia; *A. Caddori*, G. Brotzu Hospital, Cagliari; *F. Marongiu and A. Solinas*, Policlinico Universitario, Cagliari; *L. Sottile*, Policlinico Universitario, Messina; *G Leopardi and C. Cecchini*, S.Salvatore Hospital, Pesaro.

International Centres: *B. Andjelic and D. Vucelic*, Clinical Centre of Serbia, Belgrade, Serbia; *C. Mavragani*, Medical School of Athens, Greece; *M. B. Dolnicar*, University Children Hospital Ljubljana; *M. Tombuloglu and O. Taylan*, Ege Universitesi Tip Fakultesi Dahiliye AD Hematoloji BD Bornova, Izmir, Turkey; *G. Huseyin*, Trakya University, Erdine, Turkey, *Mehran Karimi*, University of Medical Sciences, Shiraz, Iran.



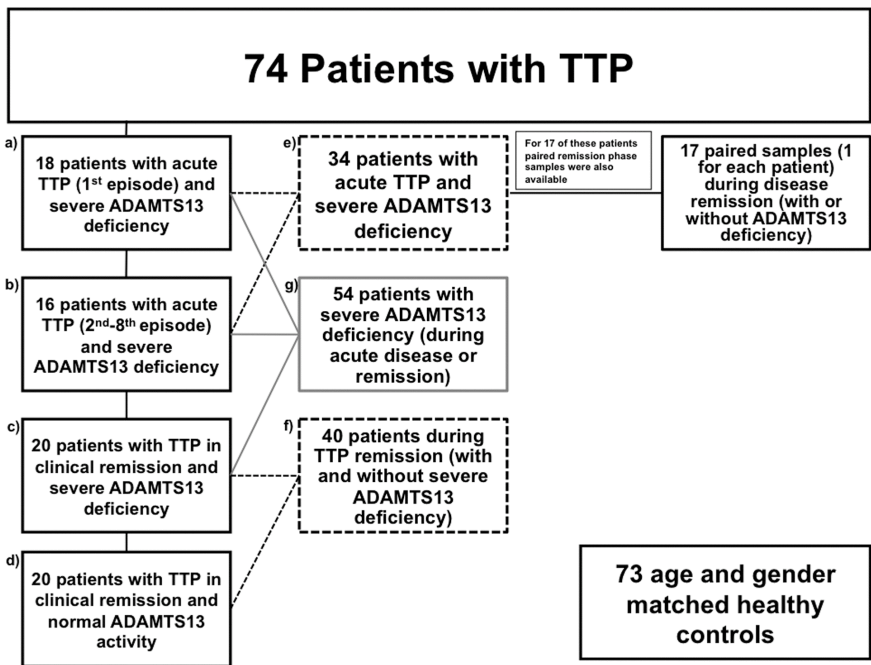
**Figures**

**Figure 1.** This flow-chart depicts the criteria for patient inclusion in the study.

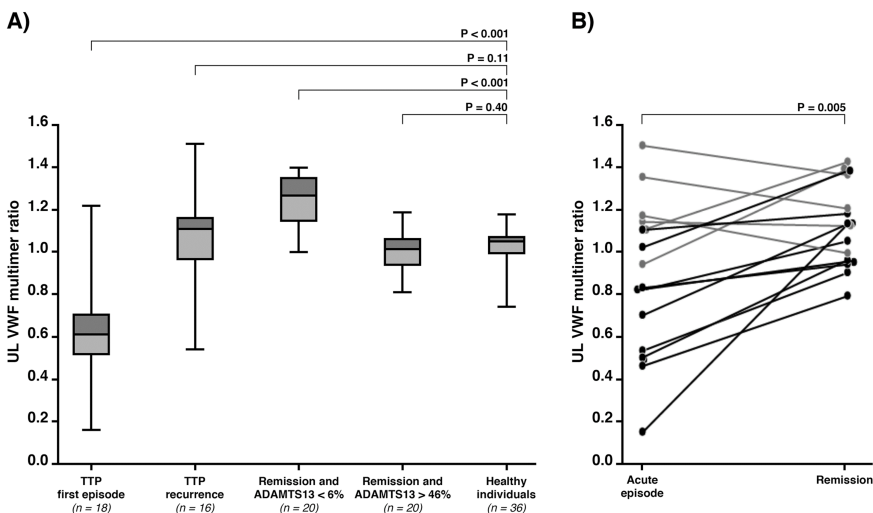


\* Number updated at the beginning of the study (July 2009). One patient had no available samples.

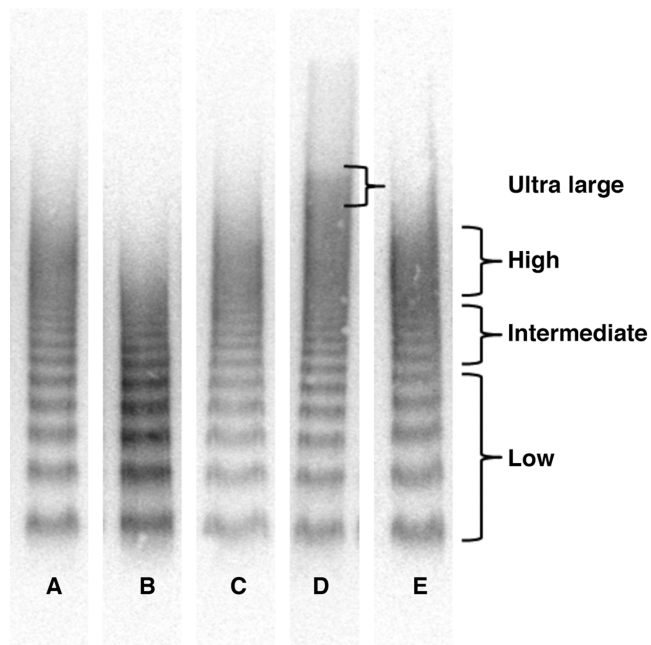
**Figure 2.** This flow chart shows the criteria followed in the selection of the primary and secondary subgroups from the 74 patients selected from the Milan TTP registry (see Figure 1).



**Figure 3.** ULVWF multimer ratio in study subgroups (A) and according to paired samples analysis (B). In (A) box plots were used to describe the distribution of ULVWF ratio across study groups. Each box plot represents median, quartiles and range of ULVWF ratio. Panel (B) represents paired comparisons of ULVWF during acute disease (left) and remission (right). In (B) closed circles denote patients investigated at the time of their first TTP episode, open circles those investigated at the time of recurrences. All patients represented in (B) had severely deficient ADAMTS13 activity at the time of acute disease. During remission, only four of the patients represented in (B) had severe ADAMTS13 deficiency, displaying ultralarge VWF multimers in plasma with an ULVWF ratio above 1.20. The other patients investigated during remission in paired comparisons had normal ADAMTS13 activity.



**Figure 4.** Multimeric analysis in pooled normal plasma (A), patient at first TTP episode (B), patient at recurrent (third) episode (C), patient during remission with severe ADAMTS13 deficiency (D), patient during remission with normal ADAMTS13 activity (E). Low-, intermediate-, high and ultralarge molecular weight VWF multimers are highlighted by parentheses at the right side of the figure.



## Tables

**Table 1.** General features and study results in TTP (all patients and primary subgroups a-d) and controls.

| Groups and subgroups                              | Controls            | All TTP             | Group (a):<br>first acute episode,<br>ADAMTS13 <6% | Group (b):<br>recurrent acute episode,<br>ADAMTS13 <6% | Group (c):<br>remission,<br>ADAMTS13 <6% | Group (d):<br>remission<br>ADAMTS13, normal |
|---------------------------------------------------|---------------------|---------------------|----------------------------------------------------|--------------------------------------------------------|------------------------------------------|---------------------------------------------|
| Number of cases (males/females)                   | 73<br>(18/55)       | 74<br>(18/56)       | 18<br>(5/13)                                       | 16<br>(3/13)                                           | 20<br>(7/13)                             | 20<br>(3/17)                                |
| Age at first episode, years                       | /                   | 40<br>(14-71)       | 45<br>(18-64)                                      | 36<br>(14-69)                                          | 38<br>(19-54)                            | 36<br>(23-71)                               |
| Age at sampling, years                            | 46<br>(18-72)       | 46<br>(18-72)       | 45<br>(18-64)                                      | 43<br>(14-70)                                          | 47<br>(33-63)                            | 44<br>(27-72)                               |
| O blood group, n (%)                              | 30<br>(41)          | 32<br>(43)          | 6<br>(33)                                          | 7<br>(43)                                              | 8<br>(40)                                | 11<br>(55)                                  |
| Hemoglobin at first episode, g/dL                 | /                   | 8.3<br>(4.1-13.5)   | 8.7<br>(4.1-11.4)                                  | 7.2<br>(5.7-13.5)                                      | 7.7<br>(5.5-11.2)                        | 8.3<br>(4.7-12)                             |
| Platelets at first episode, x1000/mm <sup>3</sup> | /                   | 15<br>(1-89)        | 15<br>(5-24)                                       | 17<br>(3-44)                                           | 12<br>(1-67)                             | 17<br>(2-89)                                |
| Platelets at sampling, x1000/mm <sup>3</sup>      | /                   | 145<br>(2-410)      | 15<br>(5-24)                                       | 36<br>(2-117)                                          | 240<br>(150-410)                         | 250<br>(152-366)                            |
| VWF antigen <sup>a</sup> , %                      | 105<br>(84-124)     | 147<br>(104-234)    | 200<br>(125-268)                                   | 202<br>(124-290)                                       | 134<br>(99-194)                          | 110<br>(89-152)                             |
| VWF-gpIb- $\alpha$ /BC ratio <sup>a</sup>         | 0.92<br>(0.74-1.3)  | 1.30<br>(0.83-1.73) | 1.32<br>(0.98-1.96)                                | 1.07<br>(0.85-1.89)                                    | 1.41<br>(0.75-1.55)                      | 1.17<br>(0.69-1.51)                         |
| ULVWF multimer ratio <sup>a</sup>                 | 1.05<br>(0.97-1.07) | 1.04<br>(0.85-1.17) | 0.61<br>(0.50-0.74)                                | 1.11<br>(0.95-1.17)                                    | 1.26<br>(1.14-1.35)                      | 1.01<br>(0.94-1.06)                         |
| ADAMTS13 activity %                               | 93<br>(46-160)      | <6                  | <6                                                 | <6                                                     | <6                                       | 89<br>(55-170)                              |

All continuous variables were expressed as median (with ranges between parentheses) unless specified.

a Expressed as medians (interquartile ranges).

**Table 2.** Study measurements in 17 patients with available acute disease and remission samples (paired comparison).

|                               | <b>Acute disease</b> | <b>Remission</b> | <b>P value</b> |
|-------------------------------|----------------------|------------------|----------------|
| VWF antigen, %                | 234 (144-311)        | 184 (150-263)    | 0.35           |
| ULVWF multimer ratio          | 0.85 (0.54-1.14)     | 1.14 (0.98-1.29) | 0.005          |
| VWF-GPIIb- $\alpha$ /BC ratio | 1.05 (0.98-1.68)     | 1.46 (0.81-1.94) | 0.30           |
| VWF:Rco, %                    | 109 (77-180)         | 129 (92-119)     | 0.20           |
| VWF:Rco/Ag ratio              | 0.65 (0.38-0.93)     | 0.77 (0.60-0.92) | 0.37           |
| VWF:CB, IU/dL                 | 112 (74-250)         | 166 (136-220)    | 0.15           |
| VWF:CB/Ag ratio               | 0.75 (0.40-0.90)     | 1.01 (0.78-1.12) | 0.005          |

Acute vs remission values were compared by Wilcoxon signed ranks test. All values expressed as median (interquartile range).

**Table 3.** Results of VWF:Ag and VWF-GPIIb- $\alpha$ /BC according to O/non-O blood groups.

| Blood group | Variable | VWF:Ag, % |          | P=     |
|-------------|----------|-----------|----------|--------|
|             |          | Cases     | Controls |        |
| O           | N=       | 32        | 30       | <0.001 |
|             | Median   | 118       | 85       |        |
| non-O       | N=       | 42        | 43       | <0.001 |
|             | Median   | 194       | 115      |        |

| Blood group | Variable | VWF-GPIIb- $\alpha$ /BC ratio |          | P=   |
|-------------|----------|-------------------------------|----------|------|
|             |          | Cases                         | Controls |      |
| O           | N=       | 32                            | 30       | 0.06 |
|             | Median   | 1.01                          | 0.79     |      |
| non-O       | N=       | 42                            | 43       | 0.03 |
|             | Median   | 1.41                          | 1.15     |      |

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