

nonrandom abnormalities characterizing the megakaryoblastic proliferations.

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To the editor:

Deficiency of ADAMTS13 and thrombotic thrombocytopenic purpura

Bianchi et al¹ report that a severe deficiency of the von Willebrand factor (VWF)-cleaving protease ADAMTS13 is specific for thrombotic thrombocytopenic purpura (TTP). This is in agreement with the results of our studies, demonstrating that a severe deficiency of ADAMTS13 is detected only in patients with either the acquired² or congenital form of TTP³ but not in patients with other pathologic conditions.^{2,4,5} On the other hand, Remuzzi et al⁶ report contradictory results. Discrepancy in the case criteria clearly contributes to the variant results observed by Remuzzi et al. The syndrome of thrombocytopenia and microangiopathic hemolysis consists of a heterogeneous group of disorders with overlapping clinical manifestations but different pathogenesis. In the study by Remuzzi et al,⁶ criteria for distinguishing TTP from hemolytic uremic syndrome (HUS), based on the presence of neurologic or renal abnormalities, are unfortunately arbitrary. The use of a decreased high-molecular-weight (HMW) multimers to low-molecular-weight (LMW) multimers ratio (HMW/LMW) as an indicator of increased cleavage of VWF is particularly misleading because VWF multimer size distribution is determined by a kinetic balance among secretion, proteolysis, and VWF-platelet binding. At the advanced stage of TTP, VWF-platelet binding predominates, resulting in a depletion of the ultralarge and large multimers; hence, a normal or even decreased HMW/LMW-multimer ratio is common, as observed by Remuzzi et al. Now that the molecular mechanism of TTP is elucidated,³ it would be of little value and counterproductive to apply the diagnosis of TTP to patients who do not have severe ADAMTS13 deficiency. A deficiency of ADAMTS13 as the cause of the manifestations of TTP offers an explanation of why plasma infusion or exchange is effective. The role of plasma therapy in patients without deficiency of ADAMTS13 is questionable. Since plasma exchange is costly and associated with potentially serious complications, efforts should be directed toward delineating the role, if any, of plasma therapy in patients without TTP.

Bianchi et al also report that ADAMTS13 activity is very low in some cases with heparin-induced thrombocytopenia or severe sepsis, and they raise doubt that all cases of TTP are associated with a severe deficiency of ADAMTS13 activity. First, in patients with heparin-induced thrombocytopenia, the ADAMTS13 activity is not as low as the authors report.² Previously, we investigated the level

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of ADAMTS13 activity in 18 patients with heparin-induced thrombocytopenia.² Table 1 compares the result of that study with that described by Bianchi et al.¹ While 4 of our cases had ADAMTS13 activity below the normal range, none had a level below 30%. The discrepancy is obviously due to the difference in the normal ranges of the assays. Notably, the narrow normal range of our assay is critical for correctly identifying the members in kindreds with genetic deficiency of the protease, leading to the positional cloning of the *ADAMTS13* gene.³

Furthermore, the low levels of ADAMTS13 detected by Bianchi et al in patients without TTP are not accompanied by evidence that cleavage of VWF is diminished. Since ADAMTS13 regulates the size of VWF in the circulation, a low ADAMTS13 level that is not associated with evidence of diminished VWF cleavage raises doubt on the validity of the test result. Alternative explanations of low laboratory values, such as plasma factors interfering the assays or instability of ADAMTS13 activity in vitro, have not been explored. Hence, unless the result is supported by evidence of diminished VWF proteolysis or the presence of inhibitors, we urge caution before inferring that a low laboratory value indicates that the ADAMTS13 activity is decreased in the patient.

On the other hand, clinical observations and laboratory studies suggest that in conditions with severe ADAMTS13 deficiency, the propensity of VWF to bind platelets is likely to be affected by the genetic composition of the individuals and by environmental factors such as fever, infection, surgery, or pregnancy. Just as patients with von Willebrand disease have variable severity of bleeding manifestations, in patients with severe ADAMTS13 deficiency, the absence of apparent thrombocytopenia or microangiopathic hemolysis is not evidence against the diagnosis of TTP, provided the low level of ADAMTS13 is supported by evidence of decreased VWF proteolysis.

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Table 1. ADAMTS13 levels in patients with heparin-induced thrombocytopenia

Reference	No. of cases	ADAMTS13 activity	
		Range in patients, % (normal range, %)	No. of cases no more than 30%
Bianchi et al ¹	16	10-100 (> 50)	5
Tsai et al ²	18	48-160 (78-127)	0

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Response:

ADAMTS13 and thrombotic thrombocytopenic purpura

We thank Dr Tsai for his letter commenting on our studies¹ and Remuzzi et al's² studies. The criticisms concerning our paper are addressed as follows.

1. Tsai states that our main conclusion (a severe deficiency of ADAMTS13 activity is a specific finding for a thrombotic microangiopathy commonly labeled thrombotic thrombocytopenic purpura [TTP]) is in agreement with his own findings.

In fact, during the past 6 years there were several instances when Tsai's and our groups independently and almost simultaneously published articles on similar topics. These topics included the isolation and partial characterization of von Willebrand factor (VWF)-cleaving protease,^{3,4} the discovery of its severe acquired deficiency in patients with acute TTP,^{5,6} as well as the finding of always,⁷ or most often,⁸ normal protease levels in children with enterohemorrhagic *Escherichia coli*-associated hemolytic uremic syndrome (HUS). Thus, our and Tsai's data largely confirmed and sometimes complemented each other, increasing the likelihood that these findings are true.

2. Tsai correctly mentions that the syndrome of thrombocytopenia and microangiopathic hemolysis consists of a heterogeneous group of disorders with overlapping clinical manifestations but different pathogenesis, and he believes that TTP should no longer be diagnosed in patients without severe ADAMTS13 deficiency. It is exactly because of the overlapping clinical manifestations between TTP and HUS that no clear-cut clinical criteria for distinction can be provided. Thus, some experts in the field refrain from clinically distinguishing, at least in adults, between TTP and HUS as 2 separate entities.⁹ Rather surprisingly, in our multicenter study on patients with TTP-HUS,⁵ 21 of 23 patients diagnosed with HUS at the respective participating centers had normal VWF-cleaving protease activity, whereas 26 of 30 diagnosed with TTP had a severe VWF-cleaving protease deficiency. Similarly, Veyradier et al¹⁰ found a severe protease deficiency in all 25 patients diagnosed with idiopathic TTP, whereas all 17 with idiopathic HUS had normal levels. Among the 69 patients with secondary thrombotic microangiopathy, the distinction was somewhat less clear-cut; 7 of 41 patients with secondary TTP had normal ADAMTS13 activity, whereas 6 of the 28 patients with secondary HUS had partial or complete protease deficiency. Therefore, the laboratory finding of severe ADAMTS13 deficiency delineates a group of patients more likely to be clinically diagnosed with TTP than with HUS, and the finding of normal or moderately decreased ADAMTS13 activity delineates a group more likely to be diagnosed with HUS than with TTP. The sensitivity of severe ADAMTS13 deficiency for the clinical diagnosis of TTP varied between 66% and 100% in 3 retrospective studies^{5,6,11} and 1 prospective study.¹⁰ This suggests that many but not all patients with a clinical diagnosis of TTP have severe protease deficiency, but that in some patients other pathogenetic factors may lead to a

clinical picture indistinguishable from TTP. A new classification scheme of the thrombotic microangiopathies, based on their pathophysiology, might be needed with severe hereditary and acquired ADAMTS13 deficiency as 2 distinct entities.¹²

3. Tsai suggests that the role of plasmatherapy in patients without ADAMTS13 deficiency is questionable. We agree with Tsai that the therapeutic and prophylactic effectiveness of fresh frozen plasma (FFP) infusion in patients with Upshaw-Schulman syndrome¹³ (constitutional TTP) associated with severe hereditary ADAMTS13 deficiency is likely to be explained by the replacement of the missing protease.^{12,14-16} The effectiveness of plasma exchange and replacement of FFP in acquired TTP^{17,18} can nowadays be explained by removal of autoantibodies inhibiting ADAMTS13 activity and replacement of the enzyme. Mori et al¹¹ observed that patients diagnosed with TTP having acquired severe ADAMTS13 deficiency showed a better response to plasma exchange and higher survival than those diagnosed with TTP without severe deficiency. This may, as suggested by Tsai, indicate that plasma exchange and FFP replacement may not be the optimal therapy for these patients. Still, in the absence of knowledge on the underlying pathogenesis of the thrombotic microangiopathy in these patients, it is probably not justified to withhold plasma exchange until better pathophysiology-based therapeutic measures become available.

4. Tsai then criticizes the fact that in our report¹ several patients with heparin-induced thrombocytopenia type 2 or sepsis had "very low levels" of ADAMTS13 activity. He found fewer patients with heparin-induced thrombocytopenia showing decreased protease activity and the extent of the decrease of ADAMTS13 activity was less pronounced⁶ than in our patients.¹ Tsai believes that this is related to the narrower normal range of ADAMTS13 activity as assessed by his method^{4,6} compared with our method.^{1,3,5}

Different assay techniques for ADAMTS13 may show some discrepancies in the measured activity levels. This is the very reason why we initiated a multisite study in June 2002. We invited several researchers (including Dr Tsai), having reported different methods for assaying ADAMTS13 activity, to measure the VWF-cleaving protease activity in some 30 plasma samples that vary in ADAMTS13 activity. The results of this study are expected later this year.

Tsai's method was helpful in identifying clinically healthy heterozygous carriers of hereditary TTP and thereby allowed Levy et al¹⁹ to identify, using a genome-wide positional cloning approach, the ADAMTS13 gene as the gene responsible for hereditary TTP in 4 families. Nevertheless, as of July 2002, using our method,¹ we too were able to identify 30 patients with hereditary TTP having ADAMTS13 activity lower than 3% and more than 100 patients with acquired TTP lacking protease

activity due to inhibiting autoantibodies (Furlan and Lämmle¹² and B.L. et al, unpublished observations, July 2002). The healthy parents of patients with constitutional TTP most often had values around 50%,¹⁴ but they were normal (> 50%) in one family.¹⁵ Kinoshita et al,¹⁶ using a modification of our assay, also identified 3 children with hereditary TTP and VWF-cleaving protease activity lower than 3%, their 6 parents having values between 5.6% and 60%. This broad range of ADAMTS13 activity levels in the presumably heterozygous parents of the 3 patients¹⁶ should not be taken as evidence of imprecision of the assay used. Instead, different genetic mutations of the ADAMTS13 gene might account for differing activity levels in these heterozygous carriers.²⁰ Thus, in our opinion, there is no good reason to believe that Tsai's assay is superior or inferior to our method.

5. Tsai further states that the low levels of VWF-cleaving protease in some of our thrombocytopenic patients¹ are not accompanied by evidence that cleavage of VWF is diminished, and he argues that this raises doubt on the validity of the test results. It is true that we did not show VWF multimer analyses in our 68 patients with various thrombocytopenic disorders other than TTP.¹ Twelve of the 68 patients had moderately decreased ADAMTS13 activity between 10% and 30%. In 1997, following earlier observations²¹ of unusually large (UL) VWF multimers in patients with chronic relapsing TTP in remission, we reported 4 such patients showing ULVWF multimers in their plasma.²² It was exactly this finding of ULVWF multimers that led to the identification of a severe VWF-cleaving protease deficiency in these patients.²² Nevertheless, patients with congenital TTP rapidly become symptom-free and their platelet count normalizes after infusion of only small amounts of FFP that will arithmetically lead to an increase of ADAMTS13 activity to just about 10% to 15% of normal human plasma (NHP).^{12,14-16} Therefore, ADAMTS13 activity levels as low as 10% are sufficient to degrade the ULVWF multimers leading to in vivo platelet clumping, and we strongly oppose Tsai's reasoning that a protease activity level of 10% to 30% is invalid if no evidence of diminished VWF cleavage is provided.

Whether plasma factors may interfere with our and/or Tsai's ADAMTS13 activity assays remains to be tested (eg, by using recombinant ADAMTS13²³ tested with and without addition of plasma). Stability of ADAMTS13 activity in plasma samples stored at -20°C for several months is excellent and the in vitro half-life of its activity in plasma or serum incubated at 37°C is more than 1 week.²⁴

6. Finally, Tsai suggests that the absence of thrombocytopenia or microangiopathic hemolysis is not evidence against the diagnosis of TTP in the presence of severe ADAMTS13 deficiency, provided that the latter is supported by decreased VWF proteolysis. At present, the diagnosis of TTP is still used to characterize a clinical condition and not a laboratory finding strongly predisposing to the disease. This may be a mere semantic problem, however, as long as the new pathophysiology-based classification scheme is not generally used.

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Response:

ADAMTS13 in thrombotic microangiopathies

We disagree with Dr Tsai that severe deficiency of the von Willebrand factor (VWF)-cleaving protease ADAMTS13 is found only in patients with the acquired or congenital forms of thrombotic thrombocytopenic purpura (TTP) but not in other pathologic conditions. Our argument is based on the following evidence.

1. Complete deficiency of ADAMTS13 activity was found in children with a diagnosis of hemolytic uremic syndrome (HUS). Three of these patients, who never had any neurologic signs, were reported in our study¹; Loirat et al² reported 5 patients with a diagnosis of diarrhea-negative (D⁻) atypical HUS and 1 patient with D⁺ typical HUS related to *Escherichia coli* 0157:H7; and another case of D⁺ HUS related to *E. coli* 0157 infection was reported by Hunt et al.³

2. Undetectable ADAMTS13 activity was also found in 2 adults with atypical HUS without neurologic signs and in 4 adults with secondary HUS.⁴

3. Severe deficiency of the protease activity was also found in diseases other than thrombotic microangiopathies such as immune thrombocytopenic purpura, disseminated intravascular coagulation, systemic lupus erythematosus,⁵ metastatic malignancies,⁶ liver cirrhosis, and chronic inflammation.⁷

Dr Tsai states that the clinical and laboratory criteria we used to distinguish TTP from HUS, based on the prevalent presence of neurologic or renal abnormalities, are arbitrary. In the current absence of other criteria, the same critical argument does apply to anybody who tries to make a differential diagnosis among thrombotic microangiopathies, including Dr Tsai. Maybe it is time to think about a new classification of thrombotic microangiopathies. A committee of experts should take in account the clinical history and the identification of genetic abnormalities and perhaps employ the term "thrombotic microangiopathy" (TMA)⁸ with ADAMTS13 deficiency in infants or children who have clinical features of HUS and undetectable ADAMTS13 activity. For consistency, such a term should also apply to adult patients with TTP or HUS and ADAMTS13 deficiency.

Dr Tsai also criticizes the use of the high-molecular-weight (HMW) multimers–low-molecular-weight (LMW) multimers ratio (HMW/LMW) as an index of VWF proteolysis. We agree that this is an indirect index; however, Dr Tsai should consider that the increased cleavage of VWF in TMA patients during the acute phase of the disease is supported by the analysis of VWF subunit and fragments, showing a decreased native 225-kDa subunit.^{9,10} During the acute phase, increased levels of normal VWF fragments were also present in patients with undetectable ADAMTS13 activity.¹⁰ We agree that VWF multimer size is determined by a kinetic balance among secretion, proteolysis, and VWF-platelet binding. However, there is no direct evidence to prove that during the acute phase of the disease in vivo unusually large (UL) VWF multimers disappear from the circulation because they are bound to platelets because, as stated also by Chow et al,¹¹ it is not possible to evaluate the size of VWF multimers bound to platelets. In this respect, in our patients with familial forms of TMA and congenital deficiency of ADAMTS13, no ULVWF multimers were found in remission at the time when platelet count was normal. Yet in these patients, there was an increased proportion of VWF fragments.¹⁰ Moreover, patients with the recurrent form of TMA had ULVWF multimers in the circulation independently of disease activity.¹⁰

Dr Tsai also raises doubts on the validity of test results when low ADAMTS13 levels are not associated with diminished VWF cleavage. There are no data supporting the views that deficient ADAMTS13

activity is associated with decreased VWF proteolysis. It has been clearly documented that VWF can be cleaved by a number of proteases (ie, calpain, elastase, plasmin) other than ADAMTS13.¹²⁻¹⁴ In addition, it has recently been shown that plasma from TMA patients with complete deficiency of ADAMTS13 activity has the same capacity as control plasma to proteolyze VWF, leading to the generation of normal fragments, and that this proteolytic activity was inhibited by a serine protease inhibitor.¹⁵ Altogether these evidences challenge the views of Dr Tsai that low ADAMTS13 levels must be associated with diminished VWF cleavage.

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