Brief report

Von Willebrand factor–cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura

Valentina Bianchi, Rodolfo Robles, Lorenzo Alberio, Miha Furlan, and Bernhard Lämmle

A severe deficiency in von Willebrand factor–cleaving protease (ADAMTS13) activity (<5% that in normal plasma) has been observed in most patients with a diagnosis of thrombotic thrombocytopenic purpura (TTP) but not in those with a diagnosis of hemolytic uremic syndrome. However, ADAMTS13 deficiency has been claimed not to be specific for TTP, since it was observed in various thrombocytopenic and other conditions. We studied 68 patients with thrombocytopenia due to severe sepsis or septic shock (n = 17), heparin-induced thrombocytopenia (n = 16), idiopathic thrombocytopenic purpura (n = 10), or other hematologic (n = 15) or miscellaneous conditions (n = 10). Twelve of the 68 patients had subnormal levels of ADAMTS13 activity (≤30%), but none had less than 10%. Thus, the study showed that ADAMTS13 activity is decreased in a substantial proportion of patients with thrombocytopenia of various causes. A severe deficiency of ADAMTS13 (<5%), identified in more than 120 patients during 1996 to 2001 in our laboratory, is specific for a thrombotic microangiopathy commonly labeled TTP.

© 2002 by The American Society of Hematology

Study design

Patients

We recruited 68 patients with thrombocytopenia (platelet count, <140 x 10⁹/L), including 17 patients from a previous study with severe sepsis or septic shock with or without DIC; 16 patients with heparin-induced thrombocytopenia type 2 (HIT) studied between 1995 and 2001 (all with a
Table 1. Characteristics of different groups of patients with thrombocytopenia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Severe sepsis (n = 17)</th>
<th>HIT (n = 16)</th>
<th>OMF (n = 3)</th>
<th>MDS (n = 4)</th>
<th>ITP (n = 10)</th>
<th>AL (n = 6)</th>
<th>SAA (n = 2)</th>
<th>Misc (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: M/F</td>
<td>14/3</td>
<td>10/6</td>
<td>3/0</td>
<td>3/1</td>
<td>4/6</td>
<td>5/1</td>
<td>2/0</td>
<td>6/4</td>
</tr>
<tr>
<td>Median age, y</td>
<td>68 (32-77)</td>
<td>73 (27-83)</td>
<td>70 (54-79)</td>
<td>65 (61-71)</td>
<td>46 (22-64)</td>
<td>62 (46-71)</td>
<td>58 (49-67)</td>
<td>47 (24-72)</td>
</tr>
<tr>
<td>Median Hb, g/L</td>
<td>97 (81-131)</td>
<td>98 (74-135)</td>
<td>94 (67-140)</td>
<td>81 (67-88)</td>
<td>138 (112-153)</td>
<td>87 (76-91)</td>
<td>111 (82-140)</td>
<td>116 (75-157)</td>
</tr>
<tr>
<td>Median WBCs, ×10^9</td>
<td>40 (15-80)</td>
<td>70 (10-120)</td>
<td>75 (50-100)</td>
<td>87.5 (25-100)</td>
<td>≥100 (≥100)</td>
<td>≥100 (≥100)</td>
<td>≥100 (≥100)</td>
<td>≥100 (≥100)</td>
</tr>
<tr>
<td>ADAMTS13, %</td>
<td>ADAMTS13 30% or lower, no.</td>
<td>6 (5)</td>
<td>10 (10, 15)</td>
<td>30 (35, 30)</td>
<td>25 (20, 25)</td>
<td>30 (35)</td>
<td>25 (0)</td>
<td>25 (0)</td>
</tr>
</tbody>
</table>

Values in parentheses are ranges unless otherwise indicated.

OMF indicates osteomyelofibrosis; AL, acute leukemia; SAA, severe aplastic anemia; Misc, miscellaneous; Hb, hemoglobin; WBCs, white blood cells; and Plts, platelets.

*All plasma samples with ADAMTS13 activity of 30% or less were reassayed several times on different days; individual results (%) of repeated ADAMTS13 assays are in parentheses.

Table 1 shows the characteristics of different groups of patients with thrombocytopenia, including the number of patients in each group and specific details such as age, hemoglobin levels, WBC counts, and ADAMTS13 activity levels.

The data indicate that the high clinical probability of HIT and the diagnosis confirmed by a high titer of antiheparin–platelet factor 4 antibodies; L. Alberio, et al, manuscript submitted); and 35 patients, prospectively enrolled from July 2001 to December 2001, with thrombocytopenic states with the following causes: ITP (n = 10), idiopathic osteomyelofibrosis (n = 3), myelodysplastic syndrome (MDS; n = 4), acute leukemia (n = 6), severe aplastic anemia (n = 2), and miscellaneous (n = 10) (Table 1). In none of the 68 patients with thrombocytopenia had TTP or HUS been considered as an alternative diagnostic possibility. Platelet count, hemoglobin level, and leukocyte count were determined for each patient. VWF-cleaving protease activity was measured in citrated plasma samples stored at −20°C until assay. The study was approved by the responsible ethical committee (Kantonale Ethische Kommission, Bern, Switzerland).

ADAMTS13 activity

We determined the activity of ADAMTS13 by using a previously described immunoblotting test.1,2 Briefly, plasma samples diluted (1:20) in 0.01 M Tris and 0.15 M sodium chloride (pH 7.4) containing 1 mM Pefabloc SC (Boehringer Mannheim, Germany) were incubated with 10 mM barium chloride at 37°C for 5 minutes and then added to purified protease-free VWF substrate. The reaction mixture was dialyzed on the surface of a hydrophilic filter membrane for 16 to 20 hours at 37°C against a buffer containing 1.5 M urea and 5 mM Tris (pH 8.0). The reaction was stopped by adding EDTA, and the extent of VWF degradation was analyzed by multimer analysis on 1.4% sodium dodecyl sulfate–agarose gels and immunoblotting using peroxidase-conjugated rabbit antihuman VWF antibodies.1,2 Dilutions of a pool of normal human plasma (NHP) from 42 healthy male donors were used for calibration of the protease assay in plasma samples from patients. This technique allows accurate determination in the range below 25% of normal activity, permitting discrimination of activity levels of 3% of normal plasma from those below 1% (Figure 1). All plasma samples from patients were tested at least twice, and samples with protease activity of 30% or less were reasayed several times on different days. Each patient’s pattern of VWF degradation was compared with the standard curve (Figure 1).

Figure 1. Activity of VWF-cleaving protease (ADAMTS13) in 68 patients with thrombocytopenia. Multimer analysis of VWF substrate digested by diluted (1:20) plasma samples from patients. On each gel (A-H), a calibration curve using dilutions of pooled plasma from healthy donors (1:20 dilution corresponding to 100%) is included. Seventeen patients with severe sepsis (gels A,B), 16 with HIT (gels C,D) and 35 with thrombocytopenia due to various causes (gels E-H) were analyzed. Plasma samples were applied on top of the gel. OMF indicates osteomyelofibrosis; AL, acute leukemia; SAA, severe aplastic anemia; and Misc, miscellaneous.
Results and discussion

Among the 68 patients with thrombocytopenia, 12 (18%) had ADAMTS13 activity of 30% or less, the lowest value being 10% (Figure 1, gel C, no. 1). Patients with thrombocytopenia associated with severe sepsis or septic shock, often accompanied by DIC (Figure 1, gels A and B), had a median ADAMTS13 activity level of 40% (range, 15%-80%) and 6 of 17 had levels between 15% and 30%. This is in agreement with findings by Loof et al,16 who reported ADAMTS13 activity of 36% ± 24% in 14 patients with DIC.

The 16 patients with confirmed HIT (Figure 1, gels C and D) had a median ADAMTS13 activity level of 70% (range, 10%-100%). Among the 35 prospectively enrolled patients with thrombocytopenia due to various hematologic or other conditions (Figure 1, gels E-H), one patient with MDS had a 25% level of ADAMTS13 activity (Figure 1, gel H, no. 31), whereas all others had levels of 40% or higher (Table 1). All plasma samples with an activity level of 30% or less were reanalyzed at least once on different days; essentially concordant results were obtained (Table 1), thus confirming reproducibility of the immunoblotting assay.

The ADAMTS13 values in this series of patients with thrombocytopenia contrast sharply with those obtained in our earlier multicenter study4 of patients with TTP or HUS: 20 of 24 patients with acute nonfamilial TTP had ADAMTS13 activity below 5% of normal levels, due to a circulating inhibitor detected in 20 of the 24 patients. All 6 patients with constitutional TTP had ADAMTS13 activity levels below 5% as well, and no inhibitor was detected in their plasma.

By December 2001, we had identified more than 120 patients with ADAMTS13 activity levels below 5% in our laboratory. According to the (sometimes incomplete) clinical information available to us, all had clinical and laboratory findings compatible with TTP except 2 apparently asymptomatic siblings of patients with hereditary TTP11 and one child with a diagnosis of Escherichia coli–associated HUS who had a transient acquired severe deficiency of ADAMTS13 activity.19

Therefore, because of the results of the current study in thrombocytopenic patients—all with ADAMTS13 levels of at least 10% that in NHP—as well as the previous analyses of 120 healthy subjects4 and 74 hospitalized or healthy controls5—all with ADAMTS13 levels of at least 45% of NHP—we conclude that a severe ADAMTS13 deficiency (<5% of the activity in NHP) is a specific finding for a form of thrombotic microangiopathy most often diagnosed as TTP. Our data contradict the findings of Moore et al,15 who reported severe ADAMTS13 deficiency not only in some patients with TTP but also in several patients with thrombocytopenia due to other conditions. Nevertheless, it is also evident from our study that slightly decreased (25%-50%) or moderately decreased ADAMTS13 activity (10%-25%) is rather common in thrombocytopenic patients with severe sepsis or HIT, a finding that is compatible with the observation of mild ADAMTS13 deficiency in various inflammatory disease states17 and in patients with metastasizing neoplasia20 or neoplasia-associated thrombotic microangiopathy.21

Even though very low ADAMTS13 activity (<5% of the activity in NHP) is a specific feature of the clinical condition labeled TTP, the sensitivity of this laboratory finding for the diagnosis of TTP remains questionable. Although, in retrospective studies, Tsai and Lian13 observed severe ADAMTS13 deficiency in 37 of 37 patients and Furlan et al4 in 26 of 30 patients with a diagnosis of acute TTP, the prospective study by Veyradier et al7 found a severe deficiency in only 47 of 66 patients presenting with idiopathic or secondary TTP (sensitivity, 71%). Therefore, besides severe acquired or hereditary ADAMTS13 deficiency, other pathogenetically relevant factors may cause a thrombotic microangiopathy clinically indistinguishable from TTP.

Further prospective studies are needed to delineate whether specific clinical or laboratory features (including response to therapy) in patients with thrombotic microangiopathy with severe ADAMTS13 deficiency are different from those in patients without such a deficiency. If such differences are observed, a new classification scheme for thrombotic microangiopathies might be justified, with severe acquired ADAMTS13 deficiency and severe constitutional ADAMTS13 deficiency categorized as 2 distinct entities.14

Finally, it is important to distinguish ADAMTS13 activity levels of 10% or higher from those under 5%. Patients with chronic recurring TTP caused by severe hereditary ADAMTS13 deficiency are kept in remission by regular plasma infusions (every 2 to 3 weeks) that raise ADAMTS13 activity to just above 10% to 15%, and 5% of activity may be sufficient to degrade the unusually large VWF multimers and prevent intravascular platelet clumping.14,22

Acknowledgments

We thank Drs W. A. Wüllemin, S. Zeerleder, and C. Caliez for providing plasma samples from patients with severe sepsis or septic shock.

References

13. Upshaw JD. Congenital deficiency of a factor in normal plasma that reverses microangiopathic


Von Willebrand factor–cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura

Valentina Bianchi, Rodolfo Robles, Lorenzo Alberio, Miha Furlan and Bernhard Læmmle