ADAMTS13 Deficiency with Elevated Levels of Ultra-Large and Active von Willebrand Factor in *P. falciparum* and *P. vivax* Malaria

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**Abstract.** A deficiency in ADAMTS13 (a von Willebrand factor [VWF] cleaving protease) is associated with accumulation of prothrombogenic unusually large VWF multimers (UL-VWF) in plasma. We studied VWF release and proteolysis in patients with symptomatic *Plasmodium falciparum* or *P. vivax* malaria on the Indonesian island Sumba. Malaria patients had significantly lower platelet counts and higher VWF concentrations and VWF activation factors than healthy hospital staff controls. The latter indicates that a higher amount of circulating VWF was in a conformation enabling spontaneous platelet binding. In addition, ADAMTS13 activity and antigen levels were reduced in both malaria groups, and this was associated with the appearance of UL-VWF: The mechanism behind this reduction and the role in malaria pathogenesis needs to be further elucidated. In malaria, endothelial cell activation with increased circulating amounts of active and ultra-large VWF, together with reduced VWF inactivation by ADAMTS13, may result in intravascular platelet aggregation, thrombocytopenia, and microvascular disease.

**INTRODUCTION**

Malaria remains an important cause of morbidity and mortality in the tropics with an estimated number of 500 million cases and 1–3 million deaths each year. Although the exact pathogenesis of malaria is still incompletely understood, it is well known that thrombocytopenia and endothelial cell activation are prominent features of clinical *Plasmodium falciparum* malaria and that platelet–endothelium interactions may play an important role in its complications, such as cerebral malaria.1–4

We have previously shown in healthy volunteers participating in an experimental human *P. falciparum* infection that the decline in platelet numbers was associated with the onset of endothelial cell activation and with an increase in the amount of active von Willebrand factor (VWF)—i.e., the amount of VWF that has undergone a conformational change from a latent state to a state enabling spontaneous binding of platelets.5,6 VWF is predominantly synthesized by endothelial cells and stored in specialized granules, called Weibel-Palade bodies. VWF mediates platelet adhesion and aggregation at sites of vessel injury. Both the multimeric size and the conformation of VWF determine its activity, whereby ultra-large and elongated VWF multimers interact best with platelets. Under normal conditions, ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin-1-like domains) rapidly cleaves ultra-large and prothrombogenic VWF multimers (UL-VWF).7 UL-VWF accumulation caused by absent or markedly reduced ADAMTS13 activity is characteristic for the rare microangiopathic disease thrombotic thrombocytopenic purpura (TTP),8 highlighting the physiologic importance of ADAMTS13 cleavage of UL-VWF in humans. In recent years, an acquired ADAMTS13 deficiency has also been described in various other pathologic conditions9 such as sepsis and diffuse intravascular coagulation.10,11 Mutations or polymorphisms in the *ADAMTS13* gene may also account for reduced plasmatic ADAMTS13 activity. A gene polymorphism (*P475S; rs11575933*) that impairs ADAMTS13 activity is common in Japan (allelic frequency, 5.1%),12 but rare in China (1.7%) and Europe (0.5%).13,14 No other common functional polymorphisms have been identified thus far.

In our experimental human *P. falciparum* malaria model in healthy Dutch volunteers, we found no changes in ADAMTS13 activity levels during the pre-clinical and early clinical stages of malaria, when parasitemia levels were still below microscopy detection level.6 However, we hypothesized that secondary ADAMTS13 deficiency may occur in later stages of clinical malaria, in which they may contribute to the development of thrombocytopenia and organ dysfunction, like in other systemic microangiopathic diseases.9 Moreover, although thrombocytopenia and organ dysfunction are also observed in *P. vivax* malaria,15–17 no data are available on VWF secretion and proteolysis in *P. vivax* malaria, whereas this infection is highly prevalent in Asia and South America and is responsible for considerable morbidity and mortality.18 Therefore, the aim of this study was to determine levels of VWF, active VWF, and ADAMTS13 and its relation with thrombocytopenia in subjects with symptomatic *P. falciparum* or *P. vivax* malaria on the Indonesian island Sumba. These levels were compared with those of healthy controls. Factors associated with ADAMTS13 activity, such as the multimeric size of VWF; the presence of ADAMTS13 inhibitors, the relation with endothelial cell activation and inflammatory markers, and the occurrence of the *P475S* mutation and other mutations in the *ADAMTS13* gene on Sumba were also studied.

**MATERIALS AND METHODS**

**Study area, study population, and ethics.** This study was conducted from April to August 2007 at the Rumah Sakit Karitas Hospital in Weetabula, West Sumba, East Nusa Tenggara Province, Indonesia, an area of unstable *P. falciparum* and *P. vivax* malaria transmission.19 Consecutive subjects presenting to hospital with clinical symptoms of malaria and a *P. falciparum* or *P. vivax* parasite density of at least 2,500 and 500 parasites/µL, respectively, were enrolled. Healthy, asymptomatic Sumbanese controls with a negative blood slide were

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recruited among hospital staff from Weetabula, which can be characterized as a low malaria transmission area. In addition, a group of asymptomatic Sumbanese subjects with a negative malaria blood slide were selected from a remote village, located in a high malaria transmission area. To screen for the occurrence of common polymorphisms in the ADAMTS13 gene in the Sumbanese population, DNA from 71 Sumbanese individuals was used. This group consisted of villagers participating in a cross-section malariometric survey and of the above-mentioned asymptomatic Sumbanese controls and the hospital staff controls. The whole ADAMTS13 gene was sequenced in five subjects from this group. Healthy Dutch controls were recruited among laboratory staff and students. Finally, plasma from five Dutch TTP patients was used. Diagnostic criteria for TTP were the presence of thrombocytopenia with microangiopathic hemolysis and no detectable ADAMTS13 activity. This study received ethical approval for the use of human subjects from the Eijkman Institute for Molecular Biology Research Ethics Committees (Jakarta, Indonesia) and of the medical ethical committee of the University Medical Center Utrecht for use of TTP patient plasma for research purposes. All study participants—or parent or guardian in case of children—gave written informed consent to participate in this study.

**Sample collection.** Venous blood was collected before administration of antimalarial drugs and/or any other treatment. Blood collected in EDTA tubes (Becton-Dickinson Vacutainer Systems, Rutherford, NJ) was used for determination of a full blood count; blood collected in CTAD tubes (Becton-Dickinson Vacutainer Systems; tubes containing citrate and the platelet stabilizing agents theophylline, adenosine, and dipyridamole) was used for coagulation and endothelial cell activation marker assays and determination of interleukin (IL)-6 concentration. Blood collected in heparin tubes was used for detection of anti-ADAMTS13 autoantibodies. All samples collected in the hospital were centrifuged at 3,500 rpm for 10 minutes and frozen at −20°C until further analysis. Double centrifugation to obtain platelet-poor plasma is not necessary with the CTAD system. For normal pooled plasma, platelet-depleted plasma of 40 healthy Dutch volunteers was pooled and stored in aliquots at −80°C.

**Laboratory procedures.** Determination of parasitemia and full blood count. Thick and thin blood smears were stained with Giemsa, and the number of parasites was quantified against 200 white blood cells. Parasite density was calculated assuming a white blood cell count of 8,000/µL. A full blood count was determined by a standard hematology analyzer assuming a white blood cell count of 8,000/µL. A full blood count against 200 white blood cells. Parasite density was calculated with Giemsa, and the number of parasites was quantified with a full blood count. ADAMTS13 activity was also determined by a VWF proteolysis assay as described previously. Briefly, proteolysis of recombinant VWF (HbQ) by study participant plasma samples was compared with normal pool plasma in the absence and presence of EDTA, which is known to inactivate ADAMTS13. In this assay, ADAMTS-13 activity is taken to be absent in case ultra-large VWF multimers remain visible after proteolysis. ADAMTS13 antigen levels were measured by a commercially available ELISA according to the instructions of the manufacturer (American Diagnostica, Stamford, CA).

**ADAMTS13 inhibitors assays.** Presence of ADAMTS13 inhibitors was determined by measuring the residual ADAMTS13 activity of normal pool plasma after incubation with plasma of study participants (volume ratio 1:1) at 37°C for up to 3 hours. Presence of anti-ADAMTS13 IgG antibodies in plasma was measured using the Technozym inhibitor ELISA (Technoclone, Vienna, Austria), according to the manufacturer’s instructions. In our laboratory, the upper limit of normal of IgG or IgA anti-ADAMTS autoantibodies. Anti-IgM and anti-IgA were added, instead of anti-IgG, as secondary detection antibody, and their OD values were compared with healthy hospital staff controls. Mutations in the ADAMTS13 gene. DNA was extracted from whole blood using the Chelex-100 ion exchanger (BioRad Laboratories, Hercules, CA). Presence of the P475S genotype was determined using fluorescence-based genotyping (Assay on Demand; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands), as described in detail previously. From five subjects, the exons of the ADAMTS13 gene, including part of the intronic boundaries, were amplified by PCR on an ICycler PCR machine (Biorad Laboratories, Venendaal, The Netherlands). Primers and PCR conditions are available on request. PCR products were purified using the High Pure PCR product purification kit (Roche Applied Science, Mannheim, Germany) and sequenced in both directions using the BigDye Terminator kit version 3 (Applied Biosystems) and a 3730 or 3100 DNA Analyzer (Applied Biosystems).

**Measurement of sICAM-1 and IL-6.** Concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and IL-6 were determined by sandwich ELISA technique using anti-human sICAM-1 (R&D DuoSet ELISA Development Systems) and antihuman IL-6 antibodies (ImmunoTools, Freiburg, Germany).

**Statistical analysis.** Data are presented as median followed by interquartile range in parentheses unless otherwise stated. Differences between more than two groups were assessed by Kruskal-Wallis test for quantitative variables with the Dunn procedure for pairwise comparisons. Mann-Whitney U test was used for comparisons between two groups. Relationships between laboratory parameters were assessed using the Pearson or Spearman correlation coefficient, depending on whether parameters were normally distributed. All analyses were performed with SPSS version 15.0 for Windows.

**RESULTS**

**Demographic and laboratory characteristics.** Characteristics of the patients presenting to hospital with P. falciparum malaria or P. vivax malaria and the healthy hospital staff controls are shown in Table 1. Two patients had severe P. falciparum malaria according to World Health Organization...
criteria (hyperparasitemia). All hospital staff controls were adults, whereas 52% of the malaria patients were < 18 years of age. Thrombocytopenia was common in both *P. falciparum* and *P. vivax* malaria patients, and platelet numbers were significantly lower in the malaria patients than in hospital staff controls. Levels of the proinflammatory cytokine IL-6 and the endothelial cell activation marker sICAM-1 were significantly higher in both malaria groups than in hospital staff controls. In the malaria groups, no significant differences were present between children and adults in platelet number or in concentrations of measured mediators (data not shown).

**VWF antigen concentrations and VWF activation factors.** VWF concentrations were highest in patients with *P. falciparum* malaria (Figure 1A). However, compared with hospital staff controls, VWF concentrations in the *P. vivax* group were also significantly elevated. The mean VWF concentration in hospital staff controls was comparable to the concentration present in normal pool plasma obtained from healthy Dutch volunteers (10.1 versus 12.4 µg/mL, respectively). VWF activation factors were also significantly higher in both malaria groups than in hospital staff controls. In the malaria groups, no significant differences were present between children and adults in platelet number or in concentrations of measured mediators (data not shown).

**ADAMTS13 activity and antigen levels.** ADAMTS13 activity levels were reduced in all patients with symptomatic *P. falciparum* or *P. vivax* malaria with median (IQR) levels of 3.5% (2.8–24.5%) and 1.3% (0.4–12.6%), respectively (Figure 2A). Levels in healthy Sumbanese hospital staff were comparable to levels found in healthy Dutch volunteers, whereas Dutch TTP patients typically had no demonstrable ADAMTS13 activity. ADAMTS13 antigen concentrations were also low in nearly all malaria patients (Figure 2B), and concentrations correlated well with ADAMTS13 activity levels (Pearson R = 0.78; P < 0.001). Additionally, the findings of low plasmatic ADAMTS13 activity by the FRETS assay were confirmed in a proteolytic VWF multimer assay. Figure 3A shows the results of this assay for three patients with a moderate decrease in ADAMTS13 activity and one with severely reduced activity (4%). Especially in the latter, large VWF multimers remained visible after incubation of synthetic VWF with patient plasma, confirming a strong reduction of plasmatic ADAMTS13 activity. Further confirmation was obtained by analysis of the VWF multimer pattern in plasma of malaria patients. As shown for two patients with *P. falciparum* in Figure 3B, UL-VWF could be detected in the plasma of these patients. Finally, we determined ADAMTS13 activity and antigen levels in Sumbanese with a negative malaria slide, living in remote villages, and found very low ADAMTS13 activity and antigen levels. Compared with the hospital staff controls, these villagers had significantly lower platelet counts (222 versus 331

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**TABLE 1**

Demographic and laboratory characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th><em>P. falciparum malaria</em></th>
<th><em>P. vivax malaria</em></th>
<th>Hospital staff controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (N)</td>
<td>26</td>
<td>16</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>50.0</td>
<td>50.0</td>
<td>90.9</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.0 (3.8–37.3)</td>
<td>11.0 (2.1–25.3)</td>
<td>25.5 (21.2–30.8)</td>
<td>0.066*</td>
</tr>
<tr>
<td>Parasitemia (parasites/µL)</td>
<td>3,240 (2,420–8,200)</td>
<td>880 (800–1,240)</td>
<td>0</td>
<td>&lt; 0.001†</td>
</tr>
<tr>
<td>Hemoglobin level (g/dL)</td>
<td>9.3 (7.9–12.2)</td>
<td>11.6 (9.9–13.2)</td>
<td>13.2 (11.3–13.4)</td>
<td>0.009†</td>
</tr>
<tr>
<td>Platelet count (&lt;10^9/L)</td>
<td>122 (70–154)</td>
<td>117 (77–220)</td>
<td>237 (172–258)</td>
<td>&lt; 0.002**§</td>
</tr>
<tr>
<td>White blood cell count (&lt;10^9/L)</td>
<td>6.3 (5.0–8.0)</td>
<td>7.6 (6.2–8.6)</td>
<td>7.7 (6.6–9.4)</td>
<td>0.114*</td>
</tr>
<tr>
<td>Lymphocytes (&lt;10^9/L)</td>
<td>6.3 (5.0–8.0)</td>
<td>1.8 (1.7–3.5)</td>
<td>2.1 (1.6–2.7)</td>
<td>0.011**</td>
</tr>
<tr>
<td>Granulocytes (&lt;10^9/L)</td>
<td>4.4 (3.4–6.3)</td>
<td>4.3 (3.4–5.8)</td>
<td>4.0 (3.6–5.6)</td>
<td>0.985*</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>28.1 (20.8–87.5)</td>
<td>9.2 (2.0–45.6)</td>
<td>NA**</td>
<td>0.659†</td>
</tr>
<tr>
<td>ADAMTS13 activity (%)</td>
<td>50.0</td>
<td>90.9</td>
<td>58.6</td>
<td></td>
</tr>
</tbody>
</table>

Data are median (interquartile range) unless otherwise specified. NA, not applicable; sICAM-1, soluble intercellular adhesion molecule-1.

* Differences between three groups by Kruskal-Wallis test, whereas pairwise comparisons were done using the Dunn procedure.
† Mann-Whitney U test.
‡ P < 0.05 between *P. falciparum* and control group.
§ P < 0.05 between *P. vivax* and control group.
¶ Thrombocytopenia defined as platelet number < 150 x 10^9/L.
** Below assay's detection limit in all.

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**FIGURE 1.** Scatter dot plots with line at median showing VWF antigen concentrations (A) and VWF activation factors (B) in patients with symptomatic *P. falciparum* malaria (N = 26), *P. vivax* malaria (N = 16), and healthy Sumbanese hospital staff controls (N = 11). Group comparisons were done using Kruskal-Wallis test, whereas pairwise comparisons were done using the Dunn procedure (∗P < 0.05; ∗∗P < 0.001; ∗∗∗P > 0.05).

**FIGURE 2.** Scatter dot plots with line at median showing ADAMTS13 activity (A) and ADAMTS13 antigen concentrations (B) in patients with symptomatic *P. falciparum* malaria (N = 26), *P. vivax* malaria (N = 16), healthy Sumbanese hospital staff controls (N = 11), healthy Dutch controls (N = 9), and thrombotic thrombocytopenic purpura (TTP) patients (N = 5). ADAMTS13 activity is expressed as percentage of normal pool plasma. Group comparisons were done using Kruskal-Wallis test, whereas pairwise comparisons were done using the Dunn procedure (∗P < 0.05; ∗∗P < 0.001; ∗∗∗P > 0.05).
the ADAMTS13 gene may also influence ADAMTS13 activity and antigen levels in the Sumbanese population, we screened a group of 71 Sumbanese for the presence of the P475S polymorphism, which is common in Japan.12 This mutation was, however, not found in any of these subjects. Subsequent sequencing of the ADAMTS13 gene of five Sumbanese to screen for the occurrence of other common polymorphisms yielded multiple silent exonic mutations in all five Sumbanese and one missense mutation in one subject (Table 2). The silent mutations have all been previously reported as single nucleotide polymorphisms (SNPs),25 whereas the missense mutation has not been described before. However, although the silent mutations were highly prevalent in the remaining 66 Sumbanese, including our hospital staff controls with normal ADAMTS13 levels, the missense mutation was not found in others. Unfortunately, no ADAMTS13 level was available for the subject with the missense mutation.

**Correlations between laboratory parameters.** Figure 4 depicts correlations between VWF activation factor, platelet count, and ADAMTS13 activity and antigen concentrations in patients with *P. falciparum* or *P. vivax* infection. Higher VWF activation factors were associated with lower platelet counts (Figure 4A). Furthermore, ADAMTS13 antigen concentrations, but not ADAMTS13 activity levels, were inversely correlated with VWF activation factors (Figure 4B and C). ADAMTS13 antigen and VWF antigen concentrations were also inversely associated (Spearman R = -0.413; P = 0.007). Finally, there was an inverse correlation of IL-6 concentrations with ADAMTS13 activity levels (Spearman R = -0.417; P = 0.007) and with platelet count (Spearman R = -0.442; P = 0.006). No significant correlation was present between platelet numbers and either ADAMTS13 activity levels (Spearman R = 0.081; P = 0.626) or ADAMTS13 antigen concentrations (Spearman R = 0.273; P = 0.097).

**DISCUSSION**

In this study, we reported that symptomatic *P. falciparum* or *P. vivax* infections are associated with a significant increase in VWF and active VWF levels and a decrease in ADAMTS13 activity and antigen levels, resulting in the presence of circulating UL-VWF multimers.

The high VWF activation factors with circulating UL-VWF multimers indicated that an increased amount of the circulating VWF was in an active platelet binding conformation, which was supported by our findings of an inverse correlation between platelet numbers and VWF activation factors. We have previously found a similar association in our experimental human *P. falciparum* malaria model,1 and our current study expands these observations to naturally acquired *P. falciparum* and *P. vivax* infections. Both sICAM-1 and VWF are known

**Table 2**

Mutations in the ADAMTS13 gene identified in five Sumbanese individuals with the prevalence in 66 other Sumbanese individuals

<table>
<thead>
<tr>
<th>Subject</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>WT</th>
<th>Het</th>
<th>Ho</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 + 2 + 4 + 5</td>
<td>5</td>
<td>420 T &gt; C</td>
<td>Silent</td>
<td>6%</td>
<td>31%</td>
<td>63%</td>
</tr>
<tr>
<td>1 + 2 + 3 + 4 + 5</td>
<td>15</td>
<td>1716 G &gt; A</td>
<td>Silent</td>
<td>7%</td>
<td>36%</td>
<td>58%</td>
</tr>
<tr>
<td>1 + 2 + 4 + 5</td>
<td>19</td>
<td>2280 C &gt; T</td>
<td>Silent</td>
<td>25%</td>
<td>23%</td>
<td>51%</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>4221 C &gt; A</td>
<td>Silent</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>2814 G &gt; T</td>
<td>K938N</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

WT = wildtype; Het = heterozygous; Ho = homozygous; ND = not determined.
markers of endothelial cell perturbation, and these findings therefore suggest that endothelial cell perturbation is not a phenomenon restricted to *P. falciparum* but also occurs in *P. vivax* malaria, an infection in which thrombocytopenia is also a common observation. This is in line with a previous study, which also found an almost similar increase in sICAM-1 concentrations in patients with either *P. vivax* or uncomplicated *P. falciparum* malaria. We speculate that, in addition to providing a mechanistic explanation for the thrombocytopenia in *P. vivax* malaria, relapsing *P. vivax* blood infections, which arise from dormant liver stages, may induce repeated episodes of endothelial cell activation with excessive VWF release. In high-income countries, elevated VWF levels have been associated with adverse clinical consequences, such as increased risk for cardiovascular diseases. However, the possible clinical consequences of high VWF levels and decreased ADAMTS13 activity need to be determined for low-income countries where malaria is endemic.

Recent evidence suggests that severe disturbances in the interplay of endothelial cells, platelets, VWF, and ADAMTS13 may result in secondary microangiopathy and thrombocytopenia-associated multi-organ failure in severely ill patients. Our study included patients with symptomatic, but uncomplicated *P. falciparum* or *P. vivax* malaria. Although the occurrence of microvascular dysfunction was not routinely classified, the alterations in the balance between VWF secretion and ADAMTS13 activity in our patients probably did not seem to result in clinically relevant complications. However, at this moment, we cannot exclude with certainty that more severe disturbances in the VWF/ADAMTS13 system may contribute to the development of complications in severe malaria, as reported for severe sepsis. Future studies are needed in patients with severe malaria to test this hypothesis, as well as studies determining the functional threshold levels of ADAMTS13 activity below which complications may ensue. In addition, recent data have proposed a role for angiopoietin-2 in the pathogenesis of severe falciparum malaria. Both angiopoietin-2 and VWF are constituents of endothelial cell Weibel-Palade bodies, highlighting the possible importance of Weibel-Palade body exocytosis in malaria pathogenesis.

At this time, the exact pathogenic mechanism behind the low ADAMTS13 activity levels in our malaria patients remains elusive. Induction of ADAMTS13 autoantibodies is the usual underlying pathogenic mechanism in TTP. Malaria may also induce various autoantibodies, as suggested by their high prevalence in malaria-endemic regions. However, inhibitor assays could not show the presence of inhibitors or ADAMTS13 autoantibodies in our study population, although the used assays may not have been sufficiently sensitive to detect weak inhibitors or low ADAMTS13 autoantibody titers.

Alternatively, various non-immune mechanisms may be involved. First, consumption of ADAMTS13 is observed in situations with release of large amounts of VWF, as previously shown by desmopressin or endotoxin administration, whereas the proinflammatory cytokine IL-6 at high concentrations can inhibit, at least partially, ADAMTS13 activity. In our study, VWF and IL-6 concentrations indeed correlated inversely with ADAMTS13 activity. In patients with *P. falciparum* and *P. vivax* malaria, despite the parasite density being much lower in vivax malaria, high concentrations of free hemoglobin, as can be found in massive intravascular hemolysis or *in vitro* hemolysis, and of the coagulation proteins plasmin and thrombin have been reported to reduce ADAMTS13 activity. However, none of the plasma samples in our study were macroscopically hemolytic and a marked procoagulant state with diffuse intravascular coagulation is rare in malaria. Fourth, reduced ADAMTS13

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**FIGURE 4.** Correlation of VWF activation factor with platelet count (A), ADAMTS13 activity levels (B), and ADAMTS13 antigen concentrations (C) in patients with symptomatic *P. falciparum* (*N* = 26) or *P. vivax* (*N* = 16) malaria. Shown is the Spearman correlation coefficient.
synthesis by liver stellate cells and/or endothelial cells may also result in ADAMTS13 deficiency. Recently, inflammatory cytokines were shown to reduce ADAMTS13 synthesis in vitro. Finally, genetic mutations may influence ADAMTS13 activity and antigen levels, and malaria may have resulted in selection of certain polymorphisms in malaria-endemic regions. However, the P475S polymorphism, which is common in Japan, was not found in 71 Sumbanese individuals. In addition, sequencing of the ADAMTS13 gene in a small number of Sumbanese subjects did show three highly prevalent SNPs, but these were also present in hospital staff controls with normal ADAMTS13 activity and antigen levels, suggesting that these SNPs are not associated with altered ADAMTS13 expression.

In conclusion, we showed that symptomatic P. falciparum and P. vivax infections are associated with endothelial cell perturbation, ADAMTS13 deficiency, and increased concentrations of active and ultra-large VWF. These combined mechanisms may contribute to malaria-induced thrombocytopenia. Future studies are needed to determine whether the disturbances in the interplay between endothelial cells, VWF, ADAMTS13, and platelets may also play a role in the complications observed in severe malaria.

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