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 prothrombogenic VWF multimers (UL-VWF) together with reduced VWF inactivation by ADAMTS13, may result in intravascular platelet aggregation, thrombocytopenia, and microvascular disease.7

Plasmodium *falciparum* infection that the
distribution 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.

**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 enrolled.

**E-mail:** q.demast@aig.umcn.nl

**Address correspondence to Quirijn de Mast, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, Room 456, PO Box 9101, 6500 HB, Nijmegen, The Netherlands.**
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, adrenaline, and diprydiamole) 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.

**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.

**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 activity was also determined by a VWF proteolysis assay as described previously.23 Briefly, proteolysis of recombinant VWF (HbO) 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 is a titer of 25 U/mL. A modification of this assay was used to detect the presence of IgM or IgA anti-ADAMTS13 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 *P. falciparum* genotype was determined using fluorescence-based genotyping (Assay on Demand; Applied Biosystems, Nieuwkerk 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, Veenendaal, 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 activity was also determined by a VWF proteolysis assay as described previously. Briefly, proteolysis of recombinant VWF (HbO) 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 is a titer of 25 U/mL. A modification of this assay was used to detect the presence of IgM or IgA anti-ADAMTS13 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 *P. falciparum* genotype was determined using fluorescence-based genotyping (Assay on Demand; Applied Biosystems, Nieuwkerk 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, Veenendaal, 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).
Thrombocytopenia (%)<sup>¶</sup>  69.2  50.0  9.1

Platelet count (×10<sup>9</sup>/L) <sup>§</sup>  122 (77–220) <sup>‡</sup>  237 (172–258) <sup>‡</sup>  0.002*<sup>‡</sup>

Hemoglobin level (g/dL)  9.3 (7.9–12.2)  11.6 (9.9–13.2)  13.2 (11.3–13.4) <sup>‡</sup>  0.009*<sup>‡</sup>

Parasitemia (parasites/µL)  3,240 (2,420–8,200)  880 (800–1,240)  0 < sup>†</sup>  <sup>†</sup>  0.001†

Sex (% female)  50.0  50.0  90.9

Table 1

<table>
<thead>
<tr>
<th></th>
<th>P. falciparum malaria</th>
<th>P. vivax malaria</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>69.2</td>
<td>50.0</td>
<td></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></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></td>
</tr>
<tr>
<td>Platelet count (×10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>122 (77–220)</td>
<td>237 (172–258)</td>
<td>0.002*</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia (%)&lt;sup&gt;¶&lt;/sup&gt;</td>
<td>69.2</td>
<td>50.0</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>White blood cell count (×10&lt;sup&gt;9&lt;/sup&gt;/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></td>
</tr>
<tr>
<td>Lymphocytes (×10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>0.9 (0.6–1.7)</td>
<td>1.8 (1.4–3.7)</td>
<td>2.1 (1.9–2.7)</td>
<td></td>
</tr>
<tr>
<td>Granulocytes (×10&lt;sup&gt;9&lt;/sup&gt;/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></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></td>
</tr>
<tr>
<td>sICAM-1 (pg/mL)</td>
<td>245.7 (213.7–283.4)</td>
<td>190.5 (141.8–402.5)</td>
<td>123.3 (94.6–140.4)</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, with post hoc testing by 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 × 10<sup>9</sup>/L.
** Below assay’s detection limit in all.

...of ADAMS13 activity. ADAMS13 antigen concentrations were also low in nearly all malaria patients (Figure 2B), and concentrations correlated well with ADAMS13 activity levels (Pearson R = 0.78; P < 0.001). Additionally, the findings of low plasmatic ADAMS13 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 ADAMS13 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 ADAMS13 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 ADAMS13 activity and antigen levels in Sumbanese with a negative malaria slide, living in remote villages, and found very low ADAMS13 activity and antigen levels. Compared with the hospital staff controls, these villagers had significantly lower platelet counts (222 versus 331...
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),23 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 to expand these observations to naturally acquired P. falciparum and P. vivax malaria infections. Both sICAM-1 and VWF are known to

<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 \textit{P. falciparum} but also occurs in \textit{P. vivax} malaria, an infection in which thrombocytopenia is also a common observation.\textsuperscript{15} This is in line with a previous study, which also found an almost similar increase in sICAM-1 concentrations in patients with either \textit{P. vivax} or uncomplicated \textit{P. falciparum} malaria.\textsuperscript{26} We speculate that, in addition to providing a mechanistic explanation for the thrombocytopenia in \textit{P. vivax} malaria, relapsing \textit{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.\textsuperscript{37} 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.\textsuperscript{26–30} Our study included patients with symptomatic, but uncomplicated \textit{P. falciparum} or \textit{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.\textsuperscript{31} 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.\textsuperscript{32} 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,\textsuperscript{33,34} whereas the proinflammatory cytokine IL-6 at high concentrations can inhibit, at least partially, ADAMTS13 activity.\textsuperscript{35} In our study, VWF and IL-6 concentrations indeed correlated inversely with ADAMTS13 activity levels.\textsuperscript{33,34} Regrettably, the limited sample size of our study did not allow a multivariate analysis. Second, parasites express various proteases, and these might theoretically inactivate ADAMTS13, as has recently been shown for the bacterium \textit{Bacillus anthracis}.\textsuperscript{36} ADAMTS13 activity levels were, however, equally low in patients with \textit{P. falciparum} and \textit{P. vivax} malaria, despite the parasite density being much lower in vivax malaria. Third, high concentrations of free hemoglobin,\textsuperscript{37} as can be found in massive intravascular hemolysis or \textit{in vitro} hemolysis, and of the coagulation proteins plasmin and thrombin have been reported to reduce ADAMTS13 activity.\textsuperscript{38} 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.\textsuperscript{39} Fourth, reduced ADAMTS13...
perturbation, ADAMTS13 deficiency, and increased cytokines. However, the in selection of certain polymorphisms in malaria-endemic SNPs are not associated with altered ADAMTS13 expression. 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.

Received October 3, 2008. Accepted for publication November 17, 2008.

Acknowledgments: The authors thank all patients and staff at the Kantitas Hospital in Wectabula, Sumba, Indonesia, for their support and participation. We also thank Dr. M. Roest and A. Barendrecht for determination of the P475S mutation and the ICAM-1 and IL-6 ELISAs.

Fiscal support: This study was supported in part by the Noaber and Hope Dennen foundations. PBA is supported by NWO-WOTRO (WIZ93-465) through PRIOR. MGN is supported by a Vidi Grant of the Netherlands Organization for Scientific Research (NWO). The sponsors of this study are public or nonprofit organizations that support science in general. They had no role in gathering, analyzing, or interpreting the data.

Authors’ addresses: Quirijn de Mast, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: q.demast@aiig.umcn.nl. Evelyn Groot, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887557769, Fax: +31887555418, E-mail: e.groot6@umcutrecht.nl. Puji B. Asih, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: puji@eijkman.go.id. Din Syafrudden, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: din@eijkman.go.id. Marije Oosting, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: m.oosting@aiig.umcn.nl. Silvie Sebastian, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887557769, Fax: +31887555418, E-mail: s.a.e.sebastian@umcutrecht.nl. Bart Ferwerda, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: b.ferwerda@aiig.umcn.nl. Mihai G. Netea, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: m.netea@aiig.umcn.nl. Philip G. de Groot, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887555418, E-mail: Ph.G.deGroot@umcutrecht.nl. Andre J. A. M. van der Ven, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: a.vanderven@aiig.umcn.nl. Rob Fijnheer, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887557769, Fax: +31887555418, E-mail: Ph.G.deGroot@umcutrecht.nl.

REFERENCES
5. de Mast Q, Groot E, Lenting P, de Groot PG, McCall M, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: b.ferwerda@aiig.umcn.nl. Puji B. Asih, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: din@eijkman.go.id. Din Syafrudden, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: din@eijkman.go.id.

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.

Authors’ addresses: Quirijn de Mast, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: q.demast@aiig.umcn.nl. Evelyn Groot, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887557769, Fax: +31887555418, E-mail: e.groot6@umcutrecht.nl. Puji B. Asih, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: puji@eijkman.go.id. Din Syafrudden, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, E-mail: din@eijkman.go.id. Marije Oosting, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: m.oosting@aiig.umcn.nl. Silvie Sebastian, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887557769, Fax: +31887555418, E-mail: s.a.e.sebastian@umcutrecht.nl. Bart Ferwerda, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: b.ferwerda@aiig.umcn.nl. Mihai G. Netea, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: m.netea@aiig.umcn.nl. Philip G. de Groot, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887555418, E-mail: Ph.G.deGroot@umcutrecht.nl. Andre J. A. M. van der Ven, Department of General Internal Medicine, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands, Tel: +31243618822, Fax: +31243566336, E-mail: a.vanderven@aiig.umcn.nl. Rob Fijnheer, Laboratory for Thrombosis and Haemostasis, Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands, Tel: +31887557769, Fax: +31887555418, E-mail: Ph.G.deGroot@umcutrecht.nl.


23. van der Plas RM, Schiphorst ME, Huizinga EG, Hene RJ, Verdonck LF, Sixma JJ, Fijnheer R, 1999. von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplanta-


cytopenia-associated multiple organ failure—a newly appreci-


34. Mannucci PM, Capoferri C, Canciani MT, 2004. Plasma levels of von Willebrand factor regulate ADAMTS-13, its major cleav-


immune patients with falciparum malaria: no evidence of dif-