Increased Thrombopoiesis and Platelet Activation in Hantavirus-Infected Patients

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Background. Thrombocytopenia is a common finding during viral hemorrhagic fever, which includes hemorrhagic fever with renal syndrome (HFRS). The 2 main causes for thrombocytopenia are impaired thrombopoiesis and/or increased peripheral destruction of platelets. In addition, there is an increased intravascular coagulation risk during HFRS, which could be due to platelet activation.

Methods. Thrombopoiesis was determined by quantification of platelet counts, thrombopoietin, immature platelet fraction, and mean platelet volume during HFRS. The in vivo platelet activation was determined by quantification of soluble P-selectin (sP-selectin) and glycoprotein VI (sGPVI). The function of circulating platelets was determined by ex vivo stimulation followed by flow cytometry analysis of platelet surface-bound fibrinogen and P-selectin exposure. Intravascular coagulation during disease was determined by scoring for disseminated intravascular coagulation (DIC) and recording thromboembolic complications.

Results. The levels of thrombopoietin, immature platelet fraction, and mean platelet volume all indicate increased thrombopoiesis during HFRS. Circulating platelets had reduced ex vivo function during disease compared to follow-up. Most interestingly, we observed significantly increased in vivo platelet activation in HFRS patients with intravascular coagulation (DIC and thromboembolic complications) as shown by sP-selectin and sGPVI levels.

Conclusions. HFRS patients have increased thrombopoiesis and platelet activation, which contributes to intravascular coagulation.

Keywords. disseminated intravascular coagulation; hantavirus; hemorrhagic fever with renal syndrome; platelets; thrombosis; viral hemorrhagic fever.

Viral hemorrhagic fevers (VHFs) are characterized by thrombocytopenia, vascular dysfunction, and disseminated intravascular coagulation (DIC). Some of the most known VHFs include Ebola (Filoviridae), Dengue (Flaviviridae), Lassa fever ( Arenaviridae), Crimean-Congo hemorrhagic fever, Rift Valley Fever, and hantaviral disease (Bunyaviridae) [1, 2]. Pathogenic hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome in the Americas [3]. The European Puumala virus (PUUV) causes HFRS and is considered a mild VHF [1].

Thrombocytopenia is one of the main determinants for clinical outcome in hantaviral diseases [4, 5]. The causes of thrombocytopenia during HFRS are largely unknown; however, the 2 main causes are impaired thrombopoiesis and/or peripheral platelet destruction [2]. There is a current gap of knowledge regarding platelet regeneration and function, which is crucial for understanding HFRS pathogenesis and increased coagulation risk.

Thrombopoiesis impairment could be due to decreased thrombopoietin (TPO) levels, which is the primary regulator of thrombopoiesis [6]. As far as we know, there is no information on hantavirus infections and TPO. Although some VHFs impair platelet regeneration by megakaryocyte dysfunction [7, 8], this seems...
Peripheral platelet destruction can be caused by platelet activation and consumption, which occur when platelets adhere to activated endothelial cells or sites of blood vessel injury with exposure of underlying extracellular matrix [2]. This is followed by platelet activation, secretion of storage organelles, and aggregation to form thrombi [12]. Platelet activation leads to integrin receptor glycoprotein (GP) IIb/IIIa conformation changes, enabling it to bind fibrinogen [13]. Additionally, activation leads to α-granule release, resulting in increased platelet surface P-selectin, where it mediates leukocyte binding [2]. The collagen receptor glycoprotein VI (GPVI) is specific for platelets and megakaryocytes, and induces platelet activation following stimulation [14]. Soluble levels of the platelet receptors P-selectin and GPVI indicate in vivo platelet activation and can thereby function as an indirect marker for peripheral platelet destruction [14–16]. Previously, the levels of soluble P-selectin (sP-selectin) and soluble GPVI (sGPVI) were shown to associate with DIC, acute myocardial infarction (AMI) and stroke [14, 17–19]. Patients with HFRS have increased coagulation risk as shown by the increased occurrence of DIC, AMI, and stroke [20, 21]. This indicates that increased peripheral platelet destruction could be one of the causes for thrombocytopenia. Our second objective in this study was therefore to study platelet activation (sP-selectin and sGPVI) during HFRS, specifically in relation to intravascular coagulation (DIC and thromboembolic complications). Furthermore, we aimed to study the function of remaining circulating platelets during HFRS using a novel flow cytometry method.

MATERIALS AND METHODS

Study Group
Patients (n = 35) were enrolled in the study following verification of PUUV infection. The diagnosis was confirmed by clinical manifestations typical of HFRS followed by detection of immunoglobulin (Ig)–G and IgM antibodies to PUUV using an immunofluorescence assay [22]. The clinical symptoms and routine laboratory tests were obtained for each patient consecutively. The Regional Ethical Review Board in Umeå approved the study, and all patients gave oral and written informed consent.

Peripheral Venous Blood Samples
Platelet poor plasma was obtained by centrifugation of patient blood in vacuum tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, New Jersey) for 20 minutes at 1500g. The plasma samples were then stored at −80°C until use for analysis of specific markers. Blood samples for platelet regeneration analysis were drawn into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). All samples were analyzed within 4 hours of collection.

Platelet Regeneration
Platelet counts were performed using an automated hematology analyzer, Sysmex XE-2100, upgraded with the IPF Master Software (Sysmex Corporation, Kobe, Japan). MPV and reticulated platelets, measured as an IPF percentage (IPF %), were obtained on the same hematology analyzer as previously described [23, 24].

TPO levels in patient plasma was analyzed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (catalog no. DTP00B) according to the instructions of the manufacturer (R&D Systems, Minneapolis, Minnesota). Where TPO values were below the lowest level of detection, the value for detection limit was inserted (31 pg/mL).

Platelet and Endothelial Activation
Soluble P-selectin levels were quantified using a commercially available ELISA kit according to the instructions of the manufacturer (catalog no. BBE6, R&D System). sGPVI levels were measured by a newly developed ELISA, which is described in detail in Supplementary Methods. Von Willebrand factor (vWF) levels were quantified by ELISA performed with reagents purchased from DAKO (Copenhagen, Denmark). The flow cytometry analysis of ex vivo platelet activation is described in detail in Supplementary Methods.

DIC Scoring and Thrombosis
The patients were scored into DIC versus no DIC groups according to a modified score taking into account the impact of infection [20]. The HFRS patients were categorized into groups of “no thrombosis” versus “thrombosis,” depending on whether they had had a radiologically verified thromboembolic event during disease (within 30 days post disease onset [DPDO]).

Statistical Analysis
Statistical analyses were performed in SPSS for Windows (version 22, IBM, New York, New York). Longitudinal changes in platelet production parameters (platelet count, MPV, IPF, TPO), platelet activation in vivo (sP-selectin and sGPVI plasma levels) and ex vivo (percentage activated platelets), and endothelial activation (vWF plasma levels) were analyzed using generalized estimation equations (GEEs), assuming an exchangeable correlation structure between repeated observations. Outcome is presented by pairwise comparisons of means at...
different time points with follow-up (at least 31 DPDO) with corresponding standard errors of the mean. The means for platelet production and platelet activation markers were tested for association using the GEE method for all samples within 16 DPDO. Pairwise comparison of the groups: DIC versus no DIC and thrombosis versus no thrombosis, for the platelet production and activation markers were presented within each specified time point using GEE. The association between the maximum level of TPO, IPF, MPV, sP-selectin, and sGPVI and the maximum DIC score during HFRS for each patient was determined using Spearman rank correlation coefficient. Only patients with 2 or more values for each specified analyte were included in this analysis. Wilcoxon-signed rank test was used for pairwise comparison of related variables. The level of significance was set at $P < .05$.

**RESULTS**

**Clinical Findings**
A total of 35 patients were included at the Clinic of Infectious Diseases at Umeå University Hospital either after hospitalization or seen by the on-call doctor and later followed as outpatients in this study during October 2007 to May 2013. The demography, clinical characteristics, and laboratory values of all patients are shown in Table 1.

**Platelet Kinetics**
During the early acute phase of HFRS, all but 1 patient had a platelet count lower than $150 \times 10^9/L$ (Figure 1 and Supplementary Figure 1A and 1B). This was followed by intensive platelet production where 31% of the patients had thrombocytosis ($>400 \times 10^9/L$) (Supplementary Figure 1C). The platelet count stabilized for most patients after 30 DPDO (Supplementary Figure 1A). Platelet counts during 31–90 compared to >90 DPDO were not significantly different (data not shown).

**Platelet Regeneration**
The primary regulator of thrombopoiesis is TPO, and ongoing thrombopoiesis markers IPF and MPV were quantified at specified time points in samples from HFRS patients. TPO values

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**Table 1. Characteristics of Hemorrhagic Fever With Renal Syndrome Patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median and IQR)</td>
<td>55 (36–57)</td>
<td>NA</td>
</tr>
<tr>
<td>Sex, n female/male (%)</td>
<td>22/13 (63/57)</td>
<td>NA</td>
</tr>
<tr>
<td>Hospital care, n (%)</td>
<td>29 (83)</td>
<td>NA</td>
</tr>
<tr>
<td>Laboratory data&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min platelet count, $10^9/L$</td>
<td>70 (47–88)</td>
<td>145–387</td>
</tr>
<tr>
<td>Max platelet count, $10^9/L$</td>
<td>315 (256–412)</td>
<td>145–387</td>
</tr>
<tr>
<td>Thrombocytopenia, n (%)</td>
<td>33 (94)</td>
<td>&lt;100 $\times 10^9/L$</td>
</tr>
<tr>
<td>Severe thrombocytopenia, n (%)</td>
<td>9 (26)</td>
<td>&lt;50 $\times 10^9/L$</td>
</tr>
<tr>
<td>Thrombocytosis, n (%)</td>
<td>11 (31)</td>
<td>&gt;400 $\times 10^9/L$</td>
</tr>
<tr>
<td>Serum max creatinine, μmol/L</td>
<td>170 (106–276)</td>
<td>50–100</td>
</tr>
<tr>
<td>Creatinine, highest fold difference&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.2 (1.5–3.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Min serum albumin, g/L</td>
<td>28.5 (24.75–31.25)</td>
<td>36–48</td>
</tr>
<tr>
<td>Albumin, lowest fold difference&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.62 (0.57–0.72)</td>
<td>NA</td>
</tr>
<tr>
<td>Max lactate dehydrogenase, μkat/L</td>
<td>4.9 (4.4–5.8)</td>
<td>&lt;4.2</td>
</tr>
<tr>
<td>Max C-reactive protein, mg/L</td>
<td>115 (53–170)</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Hemostasis data&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max PT/INR</td>
<td>1.1 (1–1.2)</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>Max PTT, sec</td>
<td>30.9 (29.9–34.3)</td>
<td>23–39</td>
</tr>
<tr>
<td>Min fibrinogen, g/L</td>
<td>4 (3.2–4.6)</td>
<td>2.0–4.5</td>
</tr>
<tr>
<td>Max D-dimer, mg/L</td>
<td>1.3 (0.7–1.8)</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>DIC, yes/no (%)</td>
<td>10/25 (29/71)</td>
<td>NA</td>
</tr>
<tr>
<td>Thrombosis, yes/no (%)</td>
<td>2/33 (6/94)</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Abbreviations:** DIC, disseminated intravascular coagulation; DPDO, days post disease onset; IQR, interquartile range; Max, maximum; Min, minimum; NA, not applicable; PT/INR, prothrombin/international normalized ratio; PTT, partial thromboplastin time.

<sup>a</sup> Values obtained within 30 DPDO for all study participants (n = 35).

<sup>b</sup> The values are shown as median with interquartile range in brackets apart from where frequencies are indicated.

<sup>c</sup> The creatinine level at follow-up was set as baseline for each patient, and all other creatinine levels were compared against this. The value shown is the highest fold difference observed for the patient within 30 DPDO.

<sup>d</sup> The albumin level at follow-up was set as baseline for each patient and all other albumin values were standardized against this value. The value shown is the lowest fold difference for the patient within 30 DPDO.

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**Figure 1.** Platelet kinetics in patients with HFRS. The estimated marginal means for platelet levels were calculated using the GEE method, and error bars indicate the standard error of the mean. The numbers underneath each time point represents the number of study individuals with 1 or more samples included in that time point. Statistical analysis for each time point was calculated using GEE for each time point versus samples obtained after 31 days post disease onset (shown as #). *$P < .05$; **$P < .01$; ***$P < .001$. Abbreviations: GEE, generalized estimating equation; HFRS, hemorrhagic fever with renal syndrome.
peak at the earliest phase of HFRS, and was significantly elevated up to 12 DPDO compared to follow-up (>31 DPDO) (Figure 2A). IPF was significantly higher in all time points up to and including 12 DPDO compared to follow-up (Figure 2B). MPV levels were significantly higher during 1–8 DPDO and significantly lower during 13–16 DPDO compared to follow-up (Figure 2C). When analyzing the association between platelet count, IPF, MPV, and TPO levels in the time points preceding and including platelet count peak (≤16 DPDO), all associated significantly with each other (Supplementary Table 1).

Platelet Activation

Plasma levels of sP-selectin and sGPVI were analyzed as markers for in vivo platelet activation (Figure 3A and 3B). The kinetics of these 2 markers were similar to each other. sP-selectin was significantly associated with sGPVI in samples obtained prior to 30 DPDO (data not shown). In addition, levels of sP-selectin and sGPVI associated positively with platelet count and negatively with TPO in samples obtained within 16 DPDO from HFRS patients (Supplementary Table 2).

sP-selectin is also stored in the Weibel–Palade bodies of endothelial cells [25]. vWF levels were quantified as a measure of endothelial Weibel–Palade release (Figure 3A). The kinetics differed with vWF peak preceding sP-selectin peak (Figure 3A). In addition, there was no significant association between sP-selectin and vWF obtained at the same time points during HFRS (data not shown). The maximum level of vWF and sP-selectin associated positively (ρ = 0.493; P = .005), but the maximum level sGPVI did not (data not shown). vWF did not associate with platelet count or TPO levels (Supplementary Table 2).

Platelet Function

To assess the function of the circulating platelets, we also followed the changes in ex vivo platelet activation potential during disease in a subset of the study group (n = 12) by flow cytometry. Generally, the proportion of platelets that bound fibrinogen (Figure 4) or exposed P-selectin on the platelet surface (Figure 5) following agonist stimulation was significantly lower during disease compared to follow-up. The percentage of nonstimulated platelets that expressed P-selectin was significantly higher during disease compared to follow-up, indicating platelet activation in vivo in HFRS patients (Supplementary Table 3). However, addition of the adenosine diphosphate (ADP)–cleaving enzyme apyrase significantly decreased platelet activation, demonstrating...
that circulating platelets during HFRS possess auto-activation potential and are therefore not preactivated in vivo to the extent of exhaustion (Supplementary Table 4).

**Platelet Regeneration and Activation in Relation to DIC and Thrombosis**

The levels of soluble plasma P-selectin and GPVI were generally higher for patients with DIC and was significantly higher early (Figure 6A) and late (Figure 6B) during disease, respectively. The maximum levels of IPF, MPV, and sP-selectin were positively associated with the maximum DIC score for each patient during disease (Supplementary Figure 2).

sP-selectin levels were significantly higher in patients with thromboembolic events compared to patients without events (Figure 6C). There were no significant differences between the levels of sGPVI, but the variation was large (data not shown).

**DISCUSSION**

In our study, we showed increased levels of TPO early during HFRS, which is the primary regulator of thrombopoiesis. This resulted in increased thrombopoiesis as shown by the enhanced levels of IPF and MPV. We thereby show at several levels that thrombopoiesis is increased and functional during HFRS. Increasing sP-selectin and sGPVI levels illustrate platelet activation during hantavirus infection. Most interestingly, we show that the levels of the platelet activation markers sP-selectin and sGPVI were significantly higher in patients with demonstrated intravascular coagulation (DIC and thromboembolic complications). The remaining circulating platelets in HFRS patients were dysfunctional as shown by decreased response to ex vivo stimulation compared to follow-up.

There are 2 main causes for thrombocytopenia: either decreased thrombopoiesis and/or increased peripheral destruction [2]. Our clinical study based on consecutive patient sampling establishes that thrombopoiesis is functional and increased during HFRS. We investigated the primary regulator of thrombopoiesis TPO through to the markers for ongoing thrombopoiesis IPF and MPV. Other hemorrhagic fever viruses, such as Dengue and Junin virus, infect and impair megakaryocyte production of platelets [7, 8], but this does not occur for hantaviruses [9, 10]. Though thrombocytopenia characterizes VHFs, different mechanisms precipitate platelet decrease during disease. The increased thrombopoiesis during HFRS observed in our study indicate that peripheral platelet destruction is the likely underlying cause of thrombocytopenia. This can be caused by platelet activation and consumption (eg, DIC, activated or damaged blood vessels) or by immunological destruction [2]. In our study, the platelet activation markers sP-selectin and sGPVI increase and peak during late HFRS, demonstrating in vivo platelet activation and consumption. However, platelet activation does not associate with platelet nadir in our HFRS patients, indicating that some other unknown mechanism is responsible for thrombocytopenia. For example, it is possible that hantaviral binding to platelets induces phagocytosis by macrophages as shown for another member of the Bunyaviridae [26], or sequestration by hantavirus-infected endothelial cells [27]. The exact underlying mechanism for thrombocytopenia at disease onset has yet to be clarified for HFRS.

In our study, the increased levels of TPO precede the platelet peak and, in some cases, thrombocytosis in the patients. This substantiates previous observations where TPO elevation induces thrombocytosis in patients with a bacterial or viral infection [28]. Interestingly, the inflammatory cytokine interleukin (IL)–6 was responsible for thrombocytosis in an in vivo study [29]. IL-6 induces hepatic TPO production during inflammation [6]. It seems likely that IL-6 is the cause of increased TPO production and thereby the thrombocytosis observed in our study, because both HFRS disease severity and platelet counts were...
Figure 4. Fibrinogen binding following ex vivo activation of platelets from HFRS patients. Platelets were stimulated ex vivo with the agonists ADP (10 µmol/L) (A), PAR-1-activating peptide (AP) (15 µmol/L) (B), PAR-4-AP (400 µmol/L) (C), and CRP (0.5 µg/mL) (D). The estimated marginal means were calculated using the GEE method for percentage of activated platelets as measured by flow cytometric analysis of platelet fibrinogen binding relative to the isotype control. Error bars indicate the standard error of the mean. The number of study individuals with 1 or more samples included in each time point: 1–7 DPDO n = 10; 8–14 DPDO n = 5; 15–30 DPDO n = 3; and >31 DPDO n = 12. The GEE method was used to calculate whether percentage fibrinogen-binding platelets differed between each time point compared to follow-up (>31 DPDO; shown as #). *P < .05; **P < .01; ***P < .001. Abbreviations: ADP, adenosine diphosphate; CRP, collagen-related peptide; DPDO, days post disease onset; GEE, generalized estimating equation; HFRS, hemorrhagic fever with renal syndrome; PAR, protease-activated receptor.

Figure 5. P-selectin exposure following ex vivo activation of platelets from HFRS patients. Platelets were stimulated ex vivo with the agonists ADP (10 µmol/L) (A), PAR-1-activating peptide (AP) (15 µmol/L) (B), PAR-4-AP (60 µmol/L) (C), and CRP (0.5 µg/mL) (D). The estimated marginal means were calculated using the GEE method for percentage of activated platelets as measured by flow cytometric analysis of exposure of P-selectin on the platelet surface relative to isotype control. Error bars indicate the standard error of the mean. The number of study individuals with 1 or more samples included in each time point: 1–7 DPDO n = 10; 8–14 DPDO n = 5; 15–30 DPDO n = 3; and >31 DPDO n = 12. The GEE method was used to calculate whether percentage P-selectin-positive platelets differed between each time point compared to follow-up (>31 DPDO; shown as #). *P < .05; **P < .01; ***P < .001. Abbreviations: ADP, adenosine diphosphate; CRP, collagen-related peptide; DPDO, days post disease onset; GEE, generalized estimating equation; HFRS, hemorrhagic fever with renal syndrome; PAR, protease-activated receptor.
associated with IL-6 levels in a previous study [30]. Of further note is the finding that thrombocytosis is an independent risk factor for thrombosis in patients that have predisposing risk factors [31, 32]. Whether this is the case for HFRS patients has yet to be shown, but it is worth mentioning that in the present study we observed a significantly higher level of TPO in patients with DIC and thromboembolic complications (data not shown). Further studies are warranted to clarify this issue.

Soluble P-selectin is also stored in the Weibel–Palade granules of endothelial cells and released upon activation, therefore the levels of circulating P-selectin is not solely derived from activated platelets [25]. To circumvent this issue in our study, we included vWF as a marker for endothelial activation [33], and we found no significant association between vWF and sP-selectin. In addition, the similar time course of sP-selectin and the specific platelet activation markers sGPVI during HFRS and the highly significant association between these indicates that most of the sP-selectin observed in our study is derived from platelets. The close correlation between the platelet count and sP-selectin and sGPVI levels raises the issue of whether platelets are activated in the vials following collection of blood samples. The blood samples were treated similarly at all time points, and at follow-up the platelet count had normalized, yet here the levels of sP-selectin and sGPVI had decreased. If platelets were activated in the vials in vitro, it would be expected that the levels of sP-selectin and sGPVI would remain high, especially as the platelet numbers were higher.

We illustrate a decreased function of the circulating platelets in our HFRS patients compared to follow-up. Platelets were stimulated with 4 different agonists targeting the thrombin receptors PAR1 and PAR4, the collagen receptor GPVI, or the ADP receptors P2Y1 and P2Y12, and the result was similar for all receptor-mediated activation responses. A few early studies found decreased platelet aggregation during HFRS [34, 35]. However, aggregometry as a measure of platelet function is affected by the number of platelets, and during severe thrombocytopenia the results become less conclusive [36, 37]. The method used in our study does not have this problem because

Figure 6. Platelet activation in patients with disseminated intravascular coagulation and thromboembolic complications. The estimated marginal means were calculated for sP-selectin (A and C) and sGPVI (B) using the GEE method for HFRS patients that fulfilled DIC criteria (A and B) or had radiologically verified thromboembolic complications (C). Error bars indicate the standard error of the mean. The numbers underneath each time point represents the number of study individuals with 1 or more samples included in that time point. The difference in these markers in patients that fulfilled DIC criteria or had thromboembolic complications (thrombosis) was compared to patients that did not fulfill DIC criteria (no DIC) or had thromboembolic complications (no thrombosis) within each given time point using the GEE method. *P<.05; **P<.01; ***P<.001. Abbreviations: DIC, disseminated intravascular coagulation; GEE, generalized estimating equation; HFRS, hemorrhagic fever with renal syndrome; sGPVI, soluble glycoprotein VI; sP-selectin, soluble P-selectin.
it is independent of the platelet count in the HFRS patient. Our
method, therefore, yields reliable platelet function results, even
during severe thrombocytopenia. We also show a slight in vivo
preactivation of circulating platelets, as illustrated by the small
though significant increase of spontaneous P-selectin–expressing
platelets during HFRS. Previous studies have highlighted
this phenomenon in hanta- and dengue virus–infected patients,
and suggested it to be an “exhausted platelet syndrome” [38,39].
However, in our study the circulating platelets retained their
ability to enhance the level of fibrinogen binding via autocrine
ADP-mediated stimulation early during HFRS. We therefore
show that platelets are not preactivated to the degree of exhaus-
tion that platelets during HFRS. Previous studies have highlighted
the ability to enhance the level of fibrinogen binding via autocrine
ADP-mediated stimulation early during HFRS. We therefore
show that platelets are not preactivated to the degree of exhaus-
tion as previously speculated.

The HFRS patients in our study with intravascular coagulation
(DIC and thromboembolic complications) have significantly in-
creased in vivo platelet activation as shown by sP-selectin and
sGPVI levels. Furthermore, the peak vWF and sP-selectin levels
were positively associated in our study, indicating that the
magnitude of endothelial activation can determine the level of
subsequent platelet activation, although these are separated in
time. HFRS patients have increased thrombin formation and
fibrinolysis [40], and are at risk for intravascular coagulation
such as DIC, AMI, and stroke [20,21]. The underlying mecha-
nisms have yet to be elucidated for this risk; however, it seems
likely that activation of platelets as shown in this study and
endothelial cells as shown by us in a previous study [41] are
likely contributors.

There are limitations to our study. Our study is based on ob-
servational data, thereby the associations do not necessarily
imply causation. The patients presented at different time points
post disease onset, therefore we do not have access to data for all
time points from all patients. The platelet ex vivo studies were
performed in a subgroup of the patients, therefore we could not
stratify the data for DIC. Finally, only 2 out of our study group
had thromboembolic events.

A major strength of our study is that we use 4 different ago-
nists that are mainly specific for different platelet receptors and
analyze platelet activation by 2 different activation markers.

We show that thrombocytopenia during HFRS is likely due to
peripheral platelet destruction. Most interestingly, in vivo plate-
let activation markers are higher in patients with coagulation
(DIC and thromboembolic complications), which could partial-
ly explain the increased risk for AMI and stroke during HFRS.
Markers of platelet production and activation could be used as
clinical guidelines for further treatment of HFRS and decrease
the risk for associated sequelae.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases
online (http://jid.oxfordjournals.org). Supplementary materials consist of
data provided by the author that are published to benefit the reader. The
posted materials are not copyedited. The contents of all supplementary
data are the sole responsibility of the authors. Questions or messages regard-
ing errors should be addressed to the author.

Notes

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other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential
Conflicts of Interest. Conflicts that the editors consider relevant to the con-
tent of the manuscript have been disclosed.

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