

Endothelial Activation and Repair During Hantavirus Infection: Association with Disease Outcome

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Background. Endothelial activation and dysfunction play a central role in the pathogenesis of sepsis and viral hemorrhagic fevers. Hantaviral disease is a viral hemorrhagic fever and is characterized by capillary dysfunction, although the underlying mechanisms for hantaviral disease are not fully elucidated.

Methods. The temporal course of endothelial activation and repair were analyzed during Puumala hantavirus infection and associated with disease outcome and a marker for hypoxia, insulin-like growth factor binding protein 1 (IGFBP-1). The following endothelial activation markers were studied: endothelial glycocalyx degradation (syndecan-1) and leukocyte adhesion molecules (soluble vascular cellular adhesion molecule 1, intercellular adhesion molecule 1, and endothelial selectin). Cytokines associated with vascular repair were also analyzed (vascular endothelial growth factor, erythropoietin, angiopoietin, and stromal cell-derived factor 1).

Results. Most of the markers we studied were highest during the earliest phase of hantaviral disease and associated with clinical and laboratory surrogate markers for disease outcome. In particular, the marker for glycocalyx degradation, syndecan-1, was significantly associated with levels of thrombocytes, albumin, IGFBP-1, decreased blood pressure, and disease severity.

Conclusions. Hantaviral disease outcome was associated with endothelial dysfunction. Consequently, the endothelium warrants further investigation when designing future medical interventions.

Keywords. endothelial activation; endothelial surface layer; endothelium; hantavirus; hemorrhagic fever with renal syndrome; glycocalyx; Puumala virus; vasculogenesis/angiogenesis.

Endothelial involvement has been implicated in sepsis [1] and viral hemorrhagic fevers [2]. Hantaviral disease belongs to the category of viral hemorrhagic fevers and has been divided into hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) [2]. Hemorrhagic fever with renal syndrome in Europe is caused by Puumala virus (PUUV), among others. Capillary leakage seems to be

central to hantaviral disease pathogenesis, although the exact underlying mechanisms are not fully elucidated. Hantaviruses target endothelial cells lining the microvasculature, although without any observable cytopathic effect [3–5]. Capillary leakage during hantaviral disease could be caused by released soluble mediators from, or direct interaction with, immune cells [6, 7], direct hantaviral endothelial cell infection [8, 9], or most likely a combination of both.

The endothelium is covered by the endothelial surface layer, which constitutes the first barrier to fluid exudation, and endothelial junctions form the second barrier [10]. The endothelial surface layer consists of the endothelial glycocalyx with adsorbed plasma proteins. This layer plays an important role in vascular homeostasis by inhibiting hemostasis, leukocyte and platelet adhesion, and regulating vascular permeability and tone [11–13]. The endothelial glycocalyx consists of core proteoglycans, such as transmembrane proteoglycan syndecan-1, to which glycosaminoglycans are

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attached [11, 13]. Endothelial glycocalyx degradation is one of the earliest signs of endothelial activation, and syndecan-1 is a recognized marker of glycocalyx degradation [14–16]. The endothelial glycocalyx degradation is followed by up-regulation and shedding of endothelial adhesion molecules, such as endothelial selectin (sE-selectin), vascular cell adhesion molecule (VCAM-1), and intercellular adhesion molecules (ICAM-1) [17]. However, endothelial activation need not be synonymous with endothelial dysfunction, which is defined as a state representing a liability to the host [18]. If endothelial activation leads to dysfunction and damage, the endothelium is regenerated by angiogenesis and vasculogenesis (ie, repair), which are dependent on, among others, soluble signaling factors such as vascular endothelial growth factor (VEGF), erythropoietin (EPO), angiopoietin (Ang-2), and stromal cell-derived factor 1 (SDF-1) [19, 20].

We wanted to study the implication of the first barrier consisting of the endothelial glycocalyx in the course of HFRS and relate these findings to disease outcome. Several *in vitro* studies [8, 9, 21, 22] have shown that the second endothelial barrier, which consists of intercellular junctions, plays a role in disease outcome. However, so far there are few *in vitro* models for the endothelial glycocalyx; therefore, *in vivo* studies are required to assess the involvement of the glycocalyx in pathogenesis [23].

We aimed at assessing the impact of endothelial activation and repair during HFRS, by studying the levels of endothelial glycocalyx degradation and leukocyte adhesion molecules as indicators of endothelial activation and cytokines that are implicated in vascular repair. Furthermore, we assessed whether there was a correlation between excessive endothelial activation, ie, dysfunction, and repair with hantaviral disease outcome.

MATERIALS AND METHODS

Patients

Patients ($n = 19$) were enrolled in this study after HFRS diagnosis from April 2007 to April 2010. The diagnosis was established by clinical manifestations typical of HFRS followed by detection of immunoglobulin (Ig)G and IgM antibodies to PUUV using an immunofluorescence assay. Typical clinical manifestations indicative of HFRS included fever, thrombocytopenia, malaise, and proteinuria. These patients were observed consecutively with regards to symptoms and routine laboratory tests. Blood samples were obtained at first contact with the clinic, followed by sampling approximately twice a week during the acute phase, and ending with a follow-up sample at least 60 days after disease onset. The study was approved by the Regional Ethical Review Board in Umeå. All participants gave oral and written informed consent.

Blood Samples

Peripheral venous blood samples were collected into commercially available vacutainers (Becton Dickinson, Franklin Lakes,

NJ) followed by centrifugation and plasma retrieval. The plasma samples were then stored at -80°C until use.

Clinical and Laboratory Routine Analyses of Patients

Samples from patients were assessed according to the clinical routine at an accredited laboratory at the Department of Clinical Chemistry at Umeå University Hospital for thrombocyte counts, D-dimer levels, creatinine, and albumin levels. Furthermore, clinical assessment of patient symptoms was obtained from medical records after inclusion, during acute disease, and during follow-up.

Assays for Endothelial Activation Biomarkers

Plasma samples were analyzed for the concentration levels of the following endothelial activation and repair markers according to the manufacturer's instructions: syndecan-1 (Gen-Probe Diaclone SAS, France); soluble sE-selectin, EPO, and VEGF, and insulin-like growth factor binding protein 1 (IGFBP-1) (Meso Scale Discovery, Gaithersburg, MD); soluble ICAM-1 (sICAM-1), sVCAM-1, Ang-2, and SDF-1 (R&D Systems, Minneapolis, MN).

Disease Grouping of the Patients

The patients were included in the group moderate/severe HFRS based on presence of 2 or more of the following criteria: dialysis, treatment in an intensive care unit, thrombosis-verified radiologically, platelet transfusion, moderate/severe hypotension (systolic blood pressure ≤ 90 mm Hg and requirement for intravenous fluid administration during hospitalization), and moderate/major hemorrhagic manifestations such as gastrointestinal bleeding, macroscopic hematuria, and epistaxis. If the patient did not display 2 or more of the above criteria, they were placed in the mild disease category [24].

Statistical Analysis

Statistical analyses were performed in SPSS for Windows (version 20; IBM, Armonk, NY, USA). Differences between levels of endothelial activation and repair markers in patient plasma during disease and follow-up were tested using the Wilcoxon signed-rank test. Furthermore, the kinetics of the various markers were analyzed against the follow-up time point using generalized estimating equations (GEEs) assuming an exchangeable correlation structure between repeated measurements within the same patients. The association within the endothelial activation markers and associations between endothelial activation and repair markers were analyzed using GEEs. Potential associations between endothelial activation and repair markers with laboratory and clinical parameters were assessed using GEEs. Possible associations with disease severity were assessed by choosing the maximal level of each investigated marker and associated with disease severity using the Spearman's rank correlation coefficient (two-tailed). The level of significance was set at .05.

RESULTS

Clinical Characteristics of HFRS Patients

Nineteen patients with confirmed HFRS were enrolled in our study. Of these patients, 15 were hospitalized during their disease. There were 2 patients with HFRS who fulfilled criteria for moderate/severe disease scoring, and the remaining 17 patients were categorized as having a mild disease. The main patient demography, clinical characteristics, and laboratory values are shown in Table 1.

Table 1. The HFRS Study Group Demography, Clinical Symptoms and Laboratory Data

Characteristics	Patients	Reference Values
Age (median and IQR)	62 (53–68)	NA
Sex, <i>n</i> female/male (%)	11/8 (58/42)	NA
Hospital care, <i>n</i> (%)	15 (78.9%)	NA
Days of hospital care (median and IQR)	7 (6–10)	NA
Clinical symptoms		
Temperature, maximum (median and IQR)	39 (38.2–39.7)	NA
Headache, <i>n</i> (%)	17 (89.5)	NA
Backache, <i>n</i> (%)	9 (47%)	NA
Nausea/vomiting, <i>n</i> (%)	14 (73.7)	NA
Respiratory symptoms, <i>n</i> (%)	7 (36.8%)	NA
Abdominal pains	7 (36.8%)	NA
Blurred vision	6 (31.4%)	NA
Hemorrhagic manifestations	5 (26.3%)	NA
Laboratory data		
Proteinuria, <i>n</i> (%)	19 (100%)	0
Hematuria, <i>n</i> (%)	17 (89.5%)	0
Minimum serum albumin ^a , g/L (median and IQR)	28 (25–31.5)	36–48
Serum creatinine, median μ mol/L (IQR)	177 (151–265)	50–100
Creatinine, highest fold difference ^b	2.4 (1.7–3.8)	NA
Thrombocyte count ^c , $10^9/L$	70 (42–91)	145–387
Maximum LDH, U/L (median and IQR)	311.4 (287.4–383.2)	<205.4
Maximum leukocyte, $10^9/L$ (mean and SD)	8.8 (8.2–9.6)	3.5–8.8
Disease severity: Moderate/severe/mild, <i>n</i> (%)	2/17 (11/89)	NA

Abbreviations: HFRS, hemorrhagic fever with renal syndrome; IQR, interquartile range; LDH, lactate dehydrogenase; NA, not applicable; SD, standard deviation.

^a Albumin levels were only available for 17 patients.

^b The creatinine levels at follow-up at least 60 days after disease onset was set as baseline for each patient, and all other creatinine levels were compared against this value. The value shown is the highest fold difference observed for the patient within 18 days after HFRS disease onset (IQR).

^c Thrombocyte count is based on the lowest number of thrombocytes obtained from the patient within 14 days after disease onset. Values are median (IQR).

Endothelial Activation and Repair During HFRS Compared to Follow-up

Paired plasma samples from 19 patients with HFRS were analyzed for markers of endothelial activation (syndecan-1, sE-selectin, sICAM-1, and sVCAM-1) and repair (VEGF, EPO, Ang-2, and SDF-1) during disease (within 18 days after disease onset) and follow-up. All samples tested were significantly higher during HFRS than follow-up (Table 2).

Time Course of Endothelial Activation and Repair

The average levels of syndecan-1, sE-selectin, sICAM-1, and sVCAM-1 were highest during the first week after onset of disease, and for syndecan-1 also the second week, followed by a decline (Figure 1A–D). However, VEGF remained high during HFRS and peaked at 13–18 days after disease onset (Figure 1E). Angiopoietin-2 levels were highest during the first week, dropping to lowest levels during 13–18 days after disease onset, and then increased at follow-up (Figure 1F). The levels of EPO were increased during HFRS compared to follow-up (Figure 1G). Stromal cell-derived factor 1 levels were highest during early HFRS followed by a decline (Figure 1H).

Association Between Endothelial Activation and Repair

The associations between the endothelial markers were assessed, and all associated significantly with each other apart from syndecan-1 and sICAM-1 (Supplementary Table 1).

The associations between endothelial activation and repair markers were also assessed (Supplementary Table 2). Stromal cell-derived factor 1 was significantly associated with syndecan-1 and sVCAM-1 levels, and EPO associated significantly with levels of sICAM-1 and sE-selectin. Angiopoietin was significantly associated with sVCAM-1. Vascular endothelial growth factor was not significantly associated with any of the endothelial activation markers.

Correlation of Endothelial Activation Markers With Parameters for Disease Outcome

We chose thrombocytes [25, 26], creatinine [24], and blood pressure [25] as surrogate markers because these have been implicated in hantaviral disease outcome. Low plasma protein levels correlated with leakage of plasma proteins due to endothelial activation in dengue patients; therefore, we also chose albumin [27]. Insulin-like growth factor binding protein-1 was used as a marker for hypoxia [28].

We investigated whether endothelial activation was associated with the mentioned disease outcome markers (Table 3). Endothelial glycocalyx degradation (syndecan-1) was significantly associated with levels of thrombocytes, albumin, systolic and diastolic blood pressure, and the marker for hypoxia, IGFBP-1. The adhesion molecule sVCAM-1 was significantly associated with the levels of thrombocytes, albumin, systolic blood pressure, and hypoxia (IGFBP-1). Soluble ICAM-1 was significantly associated with the levels of creatinine and the hypoxia marker

Table 2. Endothelial Activation and Repair Markers in PUUV-Infected Patients During Disease Compared to Follow-up

	Disease ^a		Follow-up ^b		P Value ^d
	Median	IQR ^c	Median	IQR ^c	
Endothelial Activation					
Endothelial glycocalyx degradation					
Syndecan 1 (ng/mL)	235.5	129.4–281.7	32.9	23.2–50.5	<.001
Endothelial adhesion molecules					
sE-selectin (ng/mL)	24.9	19.5–35.5	14.4	11.2–16.6	.004
sICAM-1 (ng/mL)	424	341.8–576.7	219.9	180–263	<.001
sVCAM-1 (ng/mL)	2572.5	1996.9–3176.3	621.7	491.3–795.2	<.001
Endothelial repair					
VEGF (pg/mL)	74.4	51.6–120.4	36.1	27.7–46.5	.002
EPO (mIU/mL)	11.9	6.5–17	7.5	5.9–8.7	.005
Angiopietin-2 (pg/mL)	473.1	369.7–866.9	295.6	252.5–657.8	.005
SDF-1 ^e (pg/mL)	102.9	30–135.3	30	30–30	.001

Abbreviations: Ang-2, angiopoietin; EPO, erythropoietin; IQR, interquartile range; PUUV, *Puumala* virus; SDF-1, stromal cell-derived factor 1; sE-selectin, endothelial selectin; sICAM-1, soluble intercellular adhesion molecules; sVCAM-1, soluble vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

^a First sample drawn from patient (0–18 days after disease onset).

^b Sample minimum 60 days after disease onset.

^c Interquartile range (25%–75%).

^d Wilcoxon signed-rank test for related samples.

^e Here values were below limit of detection, the value for half the limit of detection was inserted.

IGFBP-1, whereas sE-selectin was only significantly associated with albumin out of the tested parameters for disease outcome.

Correlation of Markers for Endothelial Repair With Parameters for Disease Outcome

We assessed the association between markers for endothelial repair with the above-mentioned clinical outcome markers using GEEs (Table 4). Stromal cell-derived factor 1, EPO, and Ang-2 were significantly associated with levels of thrombocytes, albumin, and IGFBP-1. Furthermore, EPO and Ang-2 were significantly associated with systolic blood pressure. Vascular endothelial growth factor was only associated with albumin levels.

HFRS Disease Severity and Association With Endothelial Activation and Repair

The maximal level for each endothelial activation and repair marker ($n = 19$; apart from SDF-1, $n = 15$) was investigated for association with disease severity using Spearman's rank correlation coefficient. Only the maximal level of syndecan-1 correlated with disease severity out of all the tested markers (Tables 3 and 4).

DISCUSSION

In our study of acutely ill HFRS patients, we show significant endothelial activation and repair during acute disease compared to follow-up. The level of endothelial activation was mirrored by the level of endothelial repair. Endothelial activation markers correlated with surrogate markers for disease outcome,

indicating a role of endothelial dysfunction in HFRS pathogenesis. Endothelial dysfunction was even more pronounced with regards to endothelial glycocalyx degradation, which was significantly associated with disease severity.

We showed a significant up-regulation of the endothelial activation markers syndecan-1, sVCAM-1, sICAM-1, and sE-selectin during disease compared to follow-up. The adhesion molecules (sVCAM-1, sICAM-1, and sE-selectin) were shown to be up-regulated in hantavirus-infected patients in previous studies [25, 29, 30] and also to correlate with sites of leukocyte infiltration in biopsies from kidney [31] and lung [7]. In addition, sVCAM-1 was shown to correlate with severity of HFRS disease caused by Hantaan virus [29]; however, we did not observe this effect in our PUUV-infected patients. It is possible that sVCAM-1 did not correlate with disease severity in our study because we had fewer patients and a milder form of HFRS. Hantaan virus causes a more severe form of HFRS compared with PUUV [3], and the disease categorization into mild and moderate/severe differs between previous studies and our study [29]. We observed a highly significant negative association between endothelial activation (syndecan-1 and sVCAM-1) and thrombocyte levels during the acute phase of HFRS. The endothelial glycocalyx harbors antithrombotic molecules, which are released upon glycocalyx degradation resulting in a prothrombotic and adhesive environment [11, 13, 32], indicating a possible mechanism for the observed thrombocytopenia and coagulopathy in our HFRS patients. In a recent study, we observed a strong association between HFRS and acute

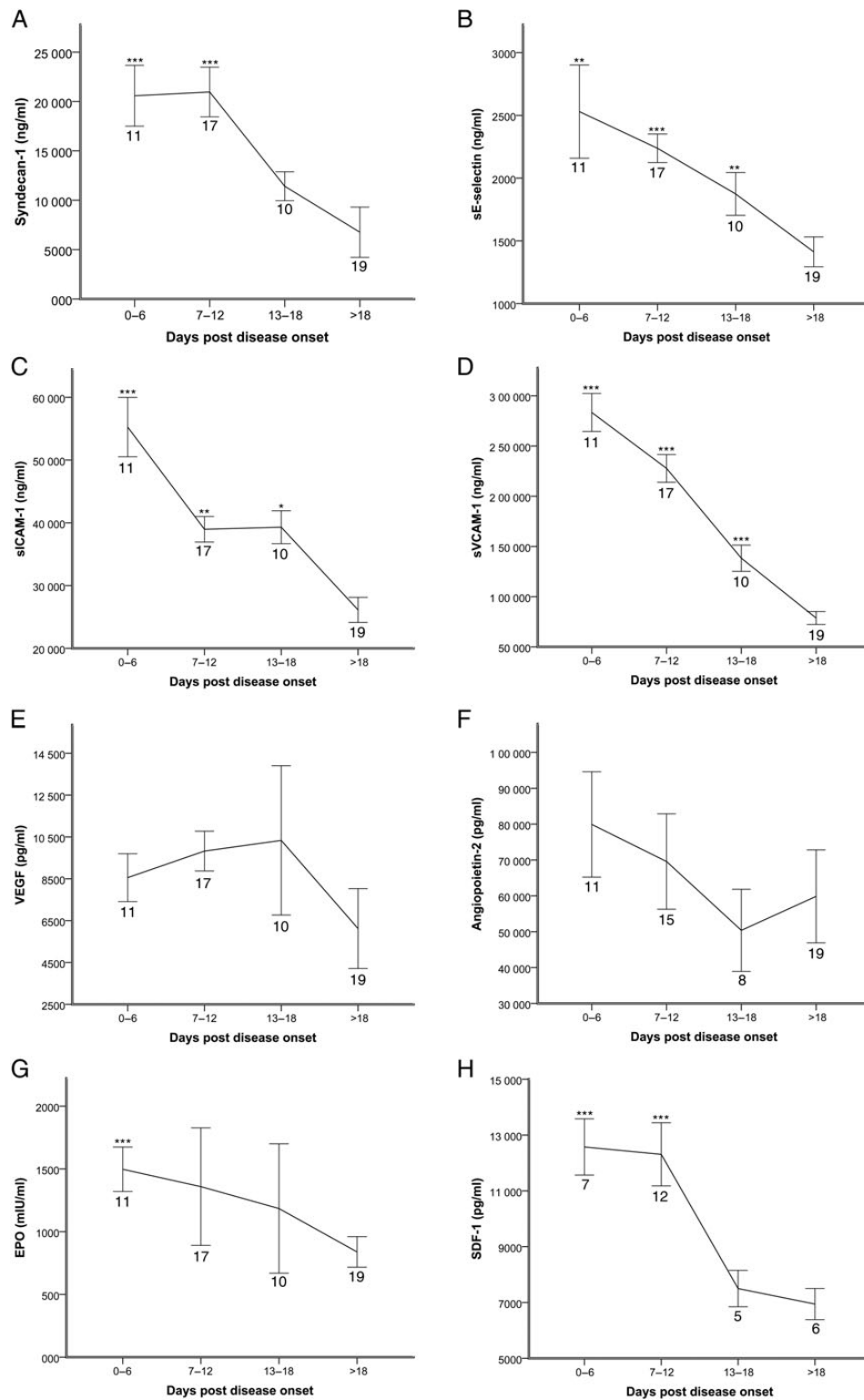


Figure 1. Time line kinetics of syndecan-1 (A), endothelial selectin (sE-selectin) (B), soluble intercellular adhesion molecules (sICAM-1) (C), soluble vascular cell adhesion molecule (sVCAM-1) (D), vascular endothelial growth factor (VEGF) (E), angiopoietin (Ang-2) (F), erythropoietin (EPO) (G), and stromal cell-derived factor 1 (SDF-1) (H) in patients with hemorrhagic fever with renal syndrome. The markers are depicted as mean values \pm standard error of the mean. Samples were obtained from 19 patients (syndecan-1, sICAM-1, sVCAM-1: 93 samples; sE-selectin and VEGF: 92 samples; EPO: 91 samples) and 17 patients (SDF-1: 44 samples). Asterisks indicate where there is a significant difference between the time points vs the follow-up (>18 days after disease onset) using generalized estimating equations GEEs (*** $P < .001$; ** $P < .01$; * $P < .05$). The number of patients included in each time point is displayed below the graph point in the respective figures.

Table 3. Association Between Endothelial Activation and Clinical Markers for Hantaviral Disease Outcome^a

	Syndecan-1 (ng/mL)	sVCAM-1 (ng/mL)	sICAM-1 (ng/mL)	sE-selectin (ng/mL)
Clinical laboratory values				
Thrombocytes, 10 ⁹ /L	$\beta = -0.487$ $P < .001$	$\beta = -0.071$ $P < .001$	$\beta = -0.311$ $P = .086$	$\beta = -3.668$ $P = 0.103$
Creatinine, $\mu\text{mol/L}$	$\beta = 0.24$ $P = .09$	$\beta = 0.026$ $P = .323$	$\beta = -0.256$ $P = .008$	$\beta = -3.032$ $P = .371$
Albumin, g/L	$\beta = -0.014$ $P < .001$	$\beta = -0.002$ $P = .001$	$\beta = -0.006$ $P = .237$	$\beta = -0.173$ $P = .03$
Blood pressure				
Systolic, mmHg	$\beta = -0.045$ $P < .001$	$\beta = -0.003$ $P = .009$	$\beta = -0.042$ $P = .052$	$\beta = -0.388$ $P = .1$
Diastolic, mmHg	$\beta = -0.015$ $P = .006$	$\beta = -0.003$ $P = .055$	$\beta = -0.017$ $P = .238$	$\beta = -0.244$ $P = .118$
Hypoxia				
IGFBP-1	$\beta = 40.283$ $P < .001$	$\beta = 6.151$ $P = .001$	$\beta = 32.754$ $P = .038$	$\beta = 659.573$ $P = .323$
Disease severity^b	$\rho = 0.47$ $P = .042$	$\rho = 0.094$ $P = .702$	$\rho = -0.188$ $P = .441$	$\rho = -0.407$ $P = .084$

Abbreviations: IGFBP-1, insulin-like growth factor binding protein 1; sE-selectin, endothelial selectin; sICAM-1, soluble intercellular adhesion molecules; sVCAM-1, soluble vascular cell adhesion molecule.

^a The estimated β -coefficients from the generalized estimating equations analysis is given along with the P value. This result corresponds to the change in endothelial activation marker levels for 1 unit increase for continuous covariates (slope). Significant associations are shown highlighted in bold.

^b The maximal values for each marker is associated with disease severity (mild vs moderate/severe disease groups) using Spearman's rank correlation coefficient analysis.

myocardial infarction and stroke within the first 3 weeks after disease onset [33].

There was a negative association between endothelial activation (syndecan-1, sVCAM-1, and sE-selectin) and plasma

albumin levels. The loss of the endothelial glycocalyx enables passage of albumin across the endothelium with concomitant fluid exudation [11]. Consequently, the degradation of endothelial glycocalyx manifests systemically as increased systemic

Table 4. Association Between Endothelial Repair and Clinical Markers for Hantaviral Disease Outcome^a

	SDF-1 (pg/mL)	EPO (mIU/mL)	Ang-2 (pg/mL)	VEGF (pg/mL)
Clinical laboratory values				
Thrombocytes, 10 ⁹ /L	$\beta = -0.971$ $P = .026$	$\beta = -1.314$ $P = .012$	$\beta = -0.053$ $P = .004$	$\beta = 0.228$ $P = .136$
Creatinine, $\mu\text{mol/L}$	$\beta = 0.426$ $P = .31$	$\beta = -0.525$ $P = .052$	$\beta = 0.03$ $P = .167$	$\beta = 0.32$ $P = .319$
Albumin, g/L	$\beta = -0.027$ $P = .015$	$\beta = -0.218$ $P = .017$	$\beta = -0.001$ $P < .001$	$\beta = -0.007$ $P = .02$
Blood pressure				
Systolic, mmHg	$\beta = -0.148$ $P = .096$	$\beta = -0.074$ $P = .042$	$\beta = -0.008$ $P < .001$	$\beta = 0.011$ $P = .718$
Diastolic, mmHg	$\beta = -0.032$ $P = .391$	$\beta = 0.013$ $P = .592$	$\beta = -0.001$ $P = .407$	$\beta = -0.012$ $P = .466$
Hypoxia				
IGFBP-1	$\beta = 96.009$ $P = .016$	$\beta = 257.36$ $P = .000$	$\beta = 4.968$ $P = .050$	$\beta = 16.039$ $P = .35$
Disease severity^b	$\rho = 0.454$ $P = .089$	$\rho = 0.031$ $P = .899$	$\rho = 0.344$ $P = .149$	$\rho = -0.063$ $P = .799$

Abbreviations: Ang-2, angiopoietin; EPO, erythropoietin; IGFBP-1, insulin-like growth factor binding protein 1; SDF-1, stromal cell-derived factor 1; VEGF, vascular endothelial growth factor.

^a The estimated β -coefficients from the generalized estimating equations analysis is given along with the P value. This result corresponds to the change in endothelial activation marker levels for 1 unit increase for continuous covariates (slope). Significant associations are shown highlighted in bold.

^b The maximal values for each marker is associated with disease severity (mild vs moderate/severe disease groups) using Spearman's rank correlation coefficient analysis.

microvascular permeability and albuminuria [34]. We observed proteinuria in all of our included HFRS patients, and a previous study showed increased pulmonary vascular leakage in HFRS patients [35]. A similar observation was made for dengue patients where glycocalyx degradation was linked to decreased plasma protein levels and proteinuria [27].

In our study, we observed a negative association between excessive endothelial activation (syndecan-1 and sVCAM-1) with low blood pressure. These findings are supported by a previous study correlating plasma levels of the stable metabolites of nitrogen oxide with hypotension in PUUV-infected patients [36]. Furthermore, severe cases of HFRS are characterized by decreased blood pressure with some entering shock [3]. It could be speculated that endothelial activation leading to increased vascular permeability and vasodilatation may play a key role in the observed decreased blood pressure during hantaviral disease.

The capillary leakage resulting in tissue edema is central to hantaviral disease pathogenesis [3]. The endothelial glycocalyx represents the first barrier to fluid exudation, with endothelial junctions forming the second barrier [34]. Several *in vitro* studies [8, 9, 21, 22] showed that the second barrier consisting of endothelial intercellular junctions plays a role disease outcome. However, to our knowledge, this is the first study to highlight the importance of the endothelial glycocalyx in hantaviral disease pathogenesis. Removal of the endothelial glycocalyx results in tissue edema formation [10, 34, 37], which causes tissue hypoxia [38]. Hypoxia leads to transcription of many target genes such as IGFBP-1 [28]. All of the endothelial activation markers (except sE-selectin) were positively associated with IGFBP-1. In addition, the reduction of gas diffusion capacity in PUUV-infected patients was correlated to vascular pulmonary leakage [35]. Speculatively, the endothelial activation with glycocalyx degradation could cause tissue hypoxia by tissue edema formation [38] and obstruction of blood flow due to leukocyte adhesion and thrombi formation [39]. This result would consequently be reflected in HFRS disease severity, and we indeed observed a significant association between syndecan-1 levels and disease severity. In previous studies, the level of glycocalyx degradation has been linked to sepsis severity and mortality [15], Crimean-Congo hemorrhagic fever disease severity [40], mortality of trauma patients [14], and ischemia during surgery [16]. Several factors could contribute to glycocalyx degradation and thereby potentially exacerbate HFRS disease. Proteases released from infected or activated endothelial cells, as shown for dengue-infected endothelial cells [41], or infected or activated leukocytes, such as hantavirus-infected dendritic cells [6], could be some of the factors that cause glycocalyx degradation [11]. Further studies are needed to clarify the mechanisms underlying endothelial glycocalyx degradation. The study of endothelial glycocalyx is complicated by the fact that there are few *in vitro* endothelial glycocalyx models [23] and no good animal models for HFRS [3].

We highlight the role of the endothelial glycocalyx in hantaviral disease outcome in our study; therefore, it would be interesting to speculate on medical interventions targeting this structure. Corticosteroids were shown to protect the endothelial glycocalyx in an *in vivo* model [37]. Unfortunately, a recent study indicated that corticosteroids did not prove beneficial in clinical outcome of HCPS [42]. Other medical intervention strategies targeting the endothelial surface layer warrant further notice [12]. For example, in a heart transplantation model, the level of glycocalyx degradation and tissue damage was decreased by incubating the heart in an albumin-augmented solution [43]. In a study of dengue patients, the infusion of a low dextran dose was hypothesized to temporarily restore the normal function of the endothelial surface layer [44]. The supposed mechanism was that dextrans adsorbed to the endothelial glycocalyx causing a similar protection as observed for the albumin in the above-mentioned heart transplant model. Whether this process would be applicable for hantaviral disease and other viral hemorrhagic fevers is unknown.

Activation of endothelial cells determines the amplitude of inflammation and thereby also tissue damage [1]. In our study, we used VEGF, EPO, SDF-1, and Ang-2 as markers for endothelial repair [19]. These markers were higher during acute disease compared to follow-up. In general, the levels of these cytokines were higher in the first stage of disease, apart from VEGF, which had another time course peaking during the later stage of disease, which has also been observed in 2 other studies [45, 46]. The delayed presence of VEGF in plasma in patients with HCPS and HFRS were interpreted as an indicator of vascular remodeling and repair [45, 46], and we observed a similar time line kinetic in our patients. In general, the level of endothelial activation and the surrogate markers for disease outcome were mirrored by the level of repair markers. Of all the cytokines associated with endothelial repair, only VEGF did not associate with endothelial activation. The endothelial repair markers studied here have all been shown to play a role in mobilizing endothelial progenitor cells [19, 20]. A recent study showed increased circulating endothelial progenitor cells in patients with HFRS compared with healthy volunteers, which were correlated to levels of C-reactive protein, thrombocytes, and albumin [46].

As with any descriptive study, it is not possible to indicate causality in any of the markers in our study. Therefore, we do not know whether there is a direct correlation between any of the studied markers or whether there is a common underlying factor that is the cause of association. To study direct causality further, *in vitro* and *in vivo* studies are needed. We measured indirect markers for endothelial dysfunction; however, these markers may not accurately reflect the level of endothelial activation nor does it indicate the site of release. Therefore, the sources of the activation markers cannot be determined in the present study; however, it seems plausible that they originate

from the endothelium because sE-selectin is significantly associated with the other activation markers.

In this study of HFRS patients, we observe an association between endothelial dysfunction with clinical markers for disease outcome and disease severity. However, more studies are needed to further clarify the mechanisms and sequence of events.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* (<http://OpenForumInfectiousDiseases.oxfordjournals.org/>).

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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