Thrombosis Research 179 (2019) 31–33

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

# Patients with severe orthohantavirus cardiopulmonary syndrome due to Sin Nombre Virus infection have increased circulating extracellular vesicle tissue factor and an activated coagulation system

The coagulation system is part of the innate immune response and is activated in response to viral infection [1]. A variety of viruses, such as HIV, Coxsackievirus, Dengue virus and Ebola virus have been shown to induce tissue factor (TF) expression in infected cells, such as monocytes and endothelial cells [1]. This leads to activation of the coagulation system and disseminated intravascular coagulation (DIC). Indeed, inhibition of TF was shown to prevent activation of coagulation in primates infected with either Ebola virus or simian immunodeficiency virus [2,3].

Orthohantaviruses are rodent-borne, negative stranded, RNA viruses that belong to the *Hantaviridae* family and *Bunyavirales* order. Orthohantavirus infection in North and South America causes hantavirus cardiopulmonary syndrome (HCPS) [4]. The pathogenesis of HCPS is poorly understood but includes thrombocytopenia, capillary leakage and non-cardiogenic edema. Sin Nombre Virus (SNV) is among the most virulent etiologic agents causing HCPS in the United States. Clinical diagnosis of HCPS is confounded by its similarity to the early symptoms of influenza, which include fever, muscle aches, and fatigue. There is no effective therapy for HCPS and treatment of severe disease is only supportive, including the use of extracorporeal membrane oxygenation (ECMO). HCPS typically progresses rapidly from febrile prodrome to non-cardiogenic pulmonary edema and cardiogenic shock that requires admission to an intensive care unit. The overall case fatality for HCPS approaches 50% [4]. However, survivor and non-survivor patients initially have clinically similar symptoms.

HCPS is associated with a dysregulated coagulation system. One study found that an elevated prothrombin time ($>/=14$ s) at the time of hospital admission was predictive of mortality [4], which suggested that these patients had a DIC. In addition, we found that non-surviving patients with HCPS had high plasma levels of plasminogen activator inhibitor 1 (PAI-1) [5]. More recently, we showed that lung macrophages from deceased HCPS patients exhibited increased PAI-1 and TF expression [6].

Orthohantavirus infection in Europe and Asia cause hemorrhagic fever with renal syndrome (HFRS). Puumala virus (PUUV) is an orthohantavirus carried by bank voles and causes mild viral hemorrhagic fever in Europe characterized by thrombocytopenia, enhanced coagulation and fibrinolysis, kidney dysfunction and DIC. Recent studies reported PUUV infection as a risk factor for acute myocardial infarction, stroke and venous thromboembolism [7,8]. In addition, PUUV infection of human umbilical vein endothelial cells induced PAI-1 expression and TF expression, which led to increased thrombin generation [9]. TF and PAI-1 may both contribute to the pro-thrombotic state observed in orthohantavirus infected patients. TF expression by infected macrophages and endothelial cells may be responsible for the activation of blood coagulation. Increased PAI-1 would inhibit activation of plasminogen resulting in reduced degradation of thrombi.

Levels of circulating extracellular vesicle TF (EVTF) activity are increased in a variety of diseases and may contribute to DIC and thrombosis. EVs (also known as microparticles or microvesicles) are small membrane vesicles that are released from activated or apoptotic cells. Recently, we found that EVTF activity was significantly increased in critically-ill patients with influenza A/H1N1 infection compared with controls [10]. Interestingly, levels of EVTF activity and interleukin 8, but not thrombin-antithrombin (TAT) complexes, D-dimer and other cytokines (including IL-1β), were significantly higher in non-survivors compared with survivors. This suggests that these biomarkers may serve as very early prognostic markers for patients with influenza A/H1N1 infection that would benefit from more intense therapy.

In this study, we measured plasma levels of EVTF activity, TAT complexes, and D-dimer in patients admitted to the hospital with primary HCPS and, for comparison, matched healthy controls. We hypothesized that similar to patients with severe influenza A/H1N1 infection EVTF activity could be used as a biomarker to distinguish survivor from non-survivor SNV patients, and who may benefit from more aggressive early interventions.

This was a retrospective study of HCPS patients ($n = 20$) admitted to the University of New Mexico hospital between 2005 and 2012 and approved by the Institutional Review Board of the University of New Mexico Health Sciences Center (UNMHSC). Samples were collected with written informed consent. Plasma was prepared from whole blood collected into EDTA and stored at $-80$ °C. Patients with serologically positive SNV infections were divided into 3 groups: class I patients ($n = 7$) had mild-moderate disease (no cardiopulmonary failure and did not require mechanical ventilation); class II patients ($n = 9$) had severe disease (pulmonary failure and hemodynamic compromise requiring mechanical ventilation and ECMO due to cardiac insufficiency) but survived; class III patients ($n = 4$) had severe disease (3 of 4 had ECMO) and all died. Two of the class II patients had elevated prothrombin time and activated partial thromboplastin time. The patients were stratified into survivors (class I and II, $n = 16$) and non-survivors (class III, $n = 4$). We collected between 1 and 6 samples from each patients with a total number of samples of 74. Samples were collected at admission to the hospital and during hospitalization for up to 5 days. Plasma samples from age- and sex-matched healthy individuals ($n = 30$) were used as controls. The patient population had a significantly higher percentage of Native Americans (45%) compared with the controls (3.3%). Plasma EVTF activity was measured using an in-house two-step FXa generation assay. Plasma TAT complexes were determined using the TAT Enzygnost Micro Kit (Siemens, Munich, Germany). D-dimer was de-

https://doi.org/10.1016/j.thromres.2019.04.032
Received 13 February 2019; Received in revised form 25 March 2019; Accepted 30 April 2019
Available online 01 May 2019
0049-3848/ 

**Table 1**
Comparison of EVTF activity, TAT complexes and D-dimer in controls and SNV patients.

| | Control individuals | SNV patients | *p*-Value |
|---|---|---|---|
| | (*N* = 30) | (*N* = 20) | |
| EVTF (pg/mL) | 0.14 (0.1–0.24) | 0.95 (0.31–2.63) | < 0.001 |
| TAT (ng/mL) | 2.14 (1.82–2.62) | 37.26 (21.12–85.61) | < 0.001 |
| D-dimer (mg/L) | 0.21 (0.15–0.32) | 1.82 (1.55–1.97) | < 0.001 |

The maximum value of each of the biomarkers was used. The median and interquartile range of continuous variables is shown. The non-parametric Mann-Whitney *U* test was used to compare the values for the controls versus patients.

termined using the Asserachrom D-dimer kit (Diagnostica Stago, Theresa, France).

The following statistical analyses were performed: Mann-Whitney *U* test was used to analyze whether there were significant differences of EV-TF, TAT and D-dimer values between SNV patients (peak value) and control individuals. The median and interquartile range of these values are shown in Table 1. Potential associations between EV-TF, TAT and D-dimer were determined using Pearson correlation analysis for all values obtained for all SNV patients. The level of significance was set at 0.05. The statistical analyses were performed in SPSS for windows (version 25, IBM).

SNV patients had significantly higher levels of plasma EVTF activity compared with controls (Table 1). We observed increased levels of plasma EVTF activity in 3/5 class I patients, 7/9 class II patients and all 4 of the class III patients (Fig. 1A). EVTF activity increased transiently after SNV infection (Fig. 1A). Patient 318 (class I) and patient 256 (class III) had the highest levels of EVTF activity (Fig. 1A). The peak level of EVTF activity was between day 0 and day 6 but many of the patients exhibited a peak at day 2 (Fig. 1B). However, levels of EVTF activity in the survivors and non-survivors was not different (data not shown). Similarly, there was no difference in EVTF activity between class I (no ECMO) and class II (ECMO) patients (data not shown).

Similar to EVTF activity, the levels of plasma TAT complexes was significantly higher in the SNV patients compared with the controls (Table 1). TAT complexes increased transiently in most of the patients (Fig. 1C). It is notable that patient 256 (non-survivor) had the highest levels of TAT complexes (Fig. 1C). This patient had one of the highest levels of EVTF activity (Fig. 1A) and had high levels of PAI-1 upon admission to the hospital [5]. The peak of TAT complexes in the SNV patients ranged between 0 and 5 days, although the majority of patients exhibited peaks at days 1 and 3 (Fig. 1D). Again, there was no difference in levels of TAT complexes between survivors and non-survivors (data not shown). There was a weak but significant correlation between EVTF activity and TAT complexes in SNV patients ($r = 0.318$, $p = 0.005$).

SNV patients had significantly higher levels of D-dimer compared with controls (Table 1). However, there were no differences between survivors and non-survivors, and D-dimer did not correlate with either EVTF activity or TAT complexes (data not shown).

Our study demonstrates that SNV patients have increased levels of EVTF activity, TAT complexes and D-dimer. However, there was no difference in these biomarkers between survivors and non-survivors. We recently analyzed EVTF activity in 88 HFRS patients caused by PUUV infection. Similar to the current study, we found a significant increase in EVTF activity in the HFRS patients (A-M Fors Connolly and N. Mackman, unpublished data).

The strengths of our study include our use of serial assessments of EVTF activity, TAT complexes and D-dimer, correlations with clinical outcomes and a matched, healthy control group. Limitations of the study include the small cohort size collected over a seven year period due to the sporadic nature of the infection, different numbers of samples at different time points, potential variability in time of symptoms

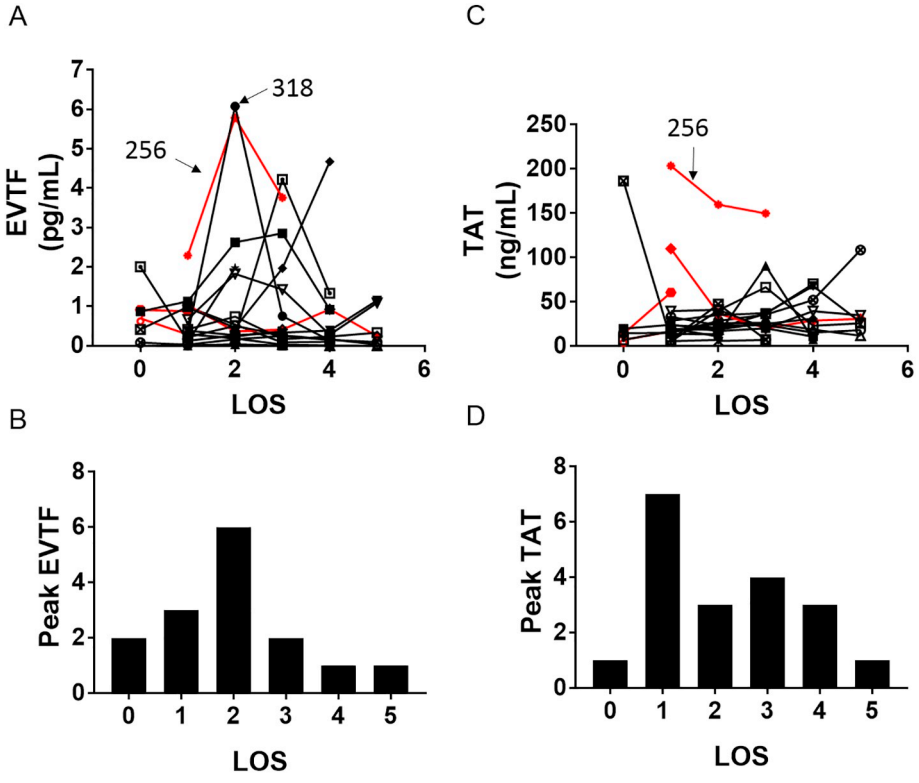


**Fig. 1.** Levels of extracellular vesicle tissue factor (EVTF) activity (A and B) and thrombin-antithrombin (TAT) complexes (C and D) in Sin Nombre Virus infected patients. In panels A and C non-survivor patients are shown in red. LOS: length of stay. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

relative to hospital admission, no information about ECMO use in the different patients, and the use of heparin in patients who went on ECMO, which would reduce the activation of coagulation.

## Acknowledgements

We would like to acknowledge Dr. Steven P. Grover and Dr. Matthew Rondina for helpful comments.

## Financial support

This work was supported by the National Institutes of Health (HL119523 NM), Scandinavian Research Foundation for varicose veins and other venous diseases (AMFC) and Swedish Society of Medicine (SLS-690711 AMFC).

## Disclosure of conflicts of interests

The authors state that they have no conflict of interests.

## References

[1] S. Antoniak, N. Mackman, Multiple roles of the coagulation protease cascade during virus infection, Blood 123 (17) (2014) 2605–2613.
[2] T.W. Geisbert, L.E. Hensley, P.B. Jahrling, T. Larsen, J.B. Geisbert, J. Paragas, H.A. Young, T.M. Fredeking, W.E. Rote, G.P. Vlasuk, Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys, Lancet 362 (9400) (2003) 1953–1958.
[3] M.E. Schechter, B.B. Andrade, T. He, G.H. Richter, K.W. Tosh, B.B. Policicchio, A. Singh, K.D. Raehtz, V. Sheikh, D. Ma, E. Brocca-Cofano, C. Apetrei, R. Tracy, R.M. Ribeiro, A. Sher, I.M.B. Francischetti, I. Pandrea, I. Sereti, Inflammatory monocytes expressing tissue factor drive SIV and HIV coagulopathy, Sci. Transl. Med. 9 (405) (2017).
[4] B. Hjelle, Epidemiology and Diagnosis of Hantavirus Infections, UpToDate Wolters Kluwer, Wellesley, MA, USA, 2018.
[5] V. Bondu, R. Schrader, M.A. Gawinowicz, P. McGuire, D.A. Lawrence, B. Hjelle, T. Buranda, Elevated cytokines, thrombin and PAI-1 in severe HCPS patients due to sin Nombre virus, Viruses 7 (2) (2015) 559–589.
[6] V. Bondu, C. Bitting, V.L. Poland, J.A. Hanson, M.S. Harkins, S. Lathrop, K.B. Nolte, D.A. Lawrence, T. Buranda, Upregulation of P2Y2R, active uPA, and PAI-1 are essential components of hantavirus cardiopulmonary syndrome, Front. Cell. Infect. Microbiol. 8 (2018) 169.
[7] A.M. Connolly-Andersen, E. Hammargren, H. Whitaker, M. Eliasson, L. Holmgren, J. Klingstrom, C. Ahlm, Increased risk of acute myocardial infarction and stroke during hemorrhagic fever with renal syndrome: a self-controlled case series study, Circulation 129 (12) (2014) 1295–1302.
[8] A.M. Connolly-Andersen, H. Whitaker, J. Klingstrom, C. Ahlm, Risk of venous thromboembolism following hemorrhagic fever with renal syndrome: a self-controlled case series study, Clin. Infect. Dis. 66 (2) (2018) 268–273.
[9] M. Goeijenbier, J.C. Meijers, F. Anfasa, J.M. Roose, C.A. van de Weg, K. Bakhtiari, H. Henttonen, A. Vaheri, A.D. Osterhaus, E.C. van Gorp, B.E. Martina, Effect of Puumala hantavirus infection on human umbilical vein endothelial cell hemostatic function: platelet interactions, increased tissue factor expression and fibrinolysis regulator release, Front. Microbiol. 6 (2015) 220.
[10] M.T. Rondina, K. Tatsumi, J.A. Bastarache, N. Mackman, Microvesicle tissue factor activity and Interleukin-8 levels are associated with mortality in patients with influenza a/H1N1 infection, Crit. Care Med. 44 (7) (2016) e574–e578.

Kohei Tatsumi[a,b], Yohei Hisada[a], Anne-Marie Fors Connolly[c], Tione Buranda[d], Nigel Mackman[a,*]

[a] *Department of Medicine, Division of Hematology and Oncology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States of America*
[b] *Department of Physiology and Regenerative Medicine, Kindai University Faculty of Medicine, Osaka, Japan*
[c] *Division of Infectious Diseases, Department of Clinical Microbiology, Umeå University, Sweden*
[d] *Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM, United States of America*
*E-mail address:* nigel_mackman@med.unc.edu (N. Mackman).

[*] Corresponding author at: Division of Hematology/Oncology, Department of Medicine, University of North Carolina at Chapel Hill, 2312C Medical Biomolecular Research Bldg CB#7126, 111 Mason Farm Road, Chapel Hill, NC 27599-7126, United States of America.