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Full Length Article

Hantavirus infection-induced thrombocytopenia triggers increased production but associates with impaired aggregation of platelets except for collagen☆

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ABSTRACT

Introduction: We evaluated the mechanisms of thrombocytopenia encountered in hantavirus disease by studying platelet production together with platelet aggregation and deposition to collagen surface.
Patients and methods: The study group consisted of 31 prospectively recruited, consecutive, hospitalized patients having acute Puumala hantavirus infection. Blood samples were collected acutely and at the control visit and subjected to analysis in Sysmex® XE-5000 to capture mean platelet volume (MPV) and immature platelet fraction (IPF%). Platelet aggregation under low shear rate conditions was assessed with impedance aggregometry Multiplate®, whereas platelet function analyzer (PFA)-100® was applied under blood flow of high shear forces.
Results: IPF% was 3.1-fold higher acutely compared with the control (median 7.4%, range 2.0–23.8% vs. median 2.4%, range 1.4%–5.2%, $p < 0.001$) tightly associating with the low platelet count ($r = -0.76$, $p < 0.001$). Accordingly, acute MPV was high (median 11.4 fl, range 9.4–13.1 fl vs. median 10.5 fl, range 9.0–12.0 fl, $p = 0.003$). Acute platelet aggregation in Multiplate® was decreased to all agonists compared with the later control ($p < 0.05$ for all agonists). Aggregation capacity associated with thrombocytopenia (for all agonists $r \geq 0.81$, $p < 0.001$), but impaired aggregation occurred also among patients with a nearly normal platelet count. Triggered by collagen, 20% of values were below reference range, while 73% of responses were low with thrombin receptor activating peptide. Significantly, under high shear platelet deposition to collagen surface was normal despite thrombocytopenia.
Conclusions: During acute hantavirus disease, platelet aggregation is impaired especially when induced with thrombin. Platelet adhesive mechanisms on collagen are intact despite thrombocytopenia while thrombopoiesis is active.

1. Introduction

Hantaviruses are enveloped RNA viruses with a diameter of 120 nm belonging to the family of Bunyaviridiae. In Europe and Asia over 50,000 cases of hemorrhagic fever with renal syndrome (HFRS) are recorded annually, and the mortality rises up to 15% in the most severe forms of the disease [1]. In Americas the disease is called hantavirus cardiopulmonary syndrome (HCPS) and has a much lower incidence (4000

Abbreviations: HCPS, hantavirus cardiopulmonary syndrome; PUUV, Puumala virus; NE, nephropathia epidemica; AKI, acute kidney injury; ADAMTS13, a thrombospondin type 1 domain 13; PAI-1, plasminogen activator inhibitor 1; HTNV, Hantaan virus; MPV, mean platelet volume; IPF%, immature platelet fraction; PFA-100, platelet function analyzer 100; CRP, C-reactive protein; ADP, adenosine diphosphate; ARA, arachinodic acid; COLL, collagen; TRAP, thrombin receptor activating peptide; RISTO, ristocetin; ADR, adrenalin; PF4, platelet factor 4; ITP, immune thrombocytopenia.

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reported cases since 1993), but carries a high fatality rate of approximately 35–50% [2]. Currently there is no approved prophylaxis or therapy available for hantavirus infections [3].

The most common European hantavirus is Puumala virus (PUUV) and most reports come from Finland with thousands of cases recognized annually [4]. The clinical course of PUUV infection (also called nephropathia epidemica, NE) is usually mild and favorable with a low case-fatality rate of 0.1–0.4% [5,6]. However, approximately half of the reported patients need hospital care on the average for 7 days and up-to 5% need transient hemodialysis, and some individuals also prolonged intensive-care treatment [7]. Acute kidney injury (AKI) and increased vascular permeability along with thrombocytopenia characterize PUUV infection. Typical symptoms include fever, headache, visual disturbances, abdominal or back pain and nausea or vomiting. Hemorrhagic manifestations usually remain mild (petechiae, epistaxis, conjunctival bleeding, hematuria) but can be clinically noted in approximately one third of the patients [8]. Mild gastrointestinal bleeds have been demonstrated by gastroscopy in all NE patients [9]. Hemorrhage of pituitary gland, kidneys, heart, liver, lungs and peritoneal cavity has been documented [10–12].

Decreased platelet count characterizes the acute phase of hantavirus disease. Enhanced thrombin formation and fibrinolysis [13] occur together with altered platelet ligands and decreased ADAMTS13 activity [14]. D-dimer is markedly elevated and polymorphism of plasminogen activator inhibitor (PAI-1) is reported to associate with the severity of AKI in NE [13,15]. High plasma levels of PAI-1 have been observed among patients with extremely severe HCPS [16]. Spleen is enlarged in NE, but the spleen size does not associate with minimum platelet count [17]. Platelet production has been addressed by obtaining bone marrow aspirates of the patients with acute Hantaan virus (HTNV) or PUUV infection without any evidence of decreased production in the two small series [18,19]. Recently, high mean platelet volume (MPV) and immature platelet fraction (IPF%) together with increased thrombopoietin levels were reported in patients with acute PUUV infection, some also diagnosed with disseminated intravascular coagulopathy and thromboembolic complications [20].

The mechanisms of thrombocytopenia and bleeding disorder encountered in hantavirus disease are still obscure. We set out to study patients with acute PUUV infection severe enough to cause hospital care with easily accessible, minimally invasive methods. To assess platelet production, platelet indices MPV and IPF% were derived from routine platelet count. Moreover, we evaluated hemostatic capacity of platelets by studying whole blood aggregation with Multiplate® and platelet deposition to collagen under high shear rate with platelet function analyzer (PFA)-100®. All variables were compared at acute and later recovery phase.

2. Materials and methods

2.1. Patients

The study was carried out in Tampere University Hospital, University of Tampere School of Medicine and Helsinki University Central Hospital. All patients came from the Pirkanmaa region and were hospitalized at Tampere University Hospital due to serologically confirmed acute PUUV infection [21] during the period from October 2010 to November 2014. Written informed consent was obtained from all patients, and the Ethics Committee of Tampere University Hospital approved the study protocol.

The study group was comprised of 31 prospectively collected, consecutive patients (19 males), median age 31 years (ranging from 21 to 67 years). Concomitant diseases included arterial hypertension (n = 4), diabetes mellitus (n = 3), coronary heart disease (n = 3), hypercholesterolemia (n = 2) and sleep apnea (n = 2). The other co-morbidities included polycystic kidney disease, celiac disease and chronic obstructive pulmonary disease (n = 1 for each). No patient used immunosuppressive or antithrombotic medication or other medication affecting platelet functions.

2.2. Clinical and basic laboratory data

The following variables were recorded: smoking (yes/no), body mass index (kg/m^2), the number of days from the onset of fever before the first study sample was collected, the length of hospital stay (days), clinical diagnosis of shock (yes/no), infusion of platelets (yes/no), signs of bleeds (yes/no), thromboembolic complications (yes/no), need for transient hemodialysis treatment (yes/no), the lowest and highest systolic and diastolic blood pressure (mm Hg), the lowest and highest daily urinary output (ml) and maximum gain in weight (kg). The last variable reflects fluid retention during the hospital stay in the oliguric phase of the disease. Complete blood count, plasma C-reactive protein (CRP) and plasma creatinine were measured according to clinical need. The laboratory analyses were carried out at the Laboratory Centre of Pirkanmaa Hospital District using standard methods.

2.3. Assessment of platelet formation and functions

Blood samples were obtained at three different time points for study purposes. The first acute-phase samples (n = 27) were drawn median 7 days (range 4–12 days) from the beginning of the fever. The second acute phase samples (n = 30) were taken median 10 days (range 6–12 days) from the beginning of the fever. The control samples (n = 24) were collected at the recovery visit, median 43 days (range 38–76 days) from the beginning of the fever.

To determinate IPF% (reference range 1–5%) and MPV (reference range 9–12 fl) the blood samples were collected in EDTA and subjected to analysis in Sysmex® XE-5000 (Sysmex Corporation, Kobe, Japan) within 4 h.

Platelet functions were studied with three methods. Multiplate® analyzer (impedance aggregometry, Roche Diagnostics International AG, Rotkreuz, Switzerland) used hirudin-anticoagulated whole blood samples and adenoside diphosphate (ADP; reference range 53–122 U), arachidonic acid (ARA; reference range 75–136 U), collagen (COLL; reference range 46–117 U), thrombin receptor activating peptide (TRAP; reference range 94–156 U) and ristocetin (RISTO; reference range 90–201 U) as agonists. PFA-100® (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) used citrate-anticoagulated whole-blood samples and collagen/adrenalin (COLL/ADR, reference range 82–150 s) and collagen/ADP (COLL/ADP, reference range 62–100 s) coated membranes under high shear blood flow (4000 l/s) to study the closure time. Samples for platelet factor 4 (PF4) measurements were collected into CTAD tubes on ice, centrifuged at 2500 *g* for 30 min, and separated plasma samples were frozen at −80 °C. Once defrosted the samples were analyzed by an enzyme-linked immunosorbent assay (ELISA; Diagnostica Stago, Paris, France). The upper reference limit for the assay was 10 IU/ml.

Plasma fibrinogen levels were assessed from citrate-anticoagulated samples after centrifugation at 2000 *g* for 10 min at room temperature using a viscosity-based detection system (Diagnostica Stago; reference range 2.0–4.0 g/l).

2.4. Statistics

Since all continuous variables were skewed, medians and ranges were calculated to describe the data. Percentages were used for categorical variables. Comparisons between the groups are based on Mann–Whitney U-test or Kruskal–Wallis test for numerical data and Fisher's test for categorical data. To evaluate changes between the acute and the recovery phase, Wilcoxon test was used. Relationships between the continuous variables were examined using Spearman rank correlation coefficient. The limit of significance was set at 0.05 (2-tailed). IBM