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Enhanced thrombin formation and fibrinolysis during acute Puumala hantavirus infection

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ABSTRACT

Introduction: Nephropathia epidemica (NE) is a viral hemorrhagic fever with renal syndrome associated with thrombocytopenia and mild bleeding. We assessed activation of coagulation and fibrinolysis during the acute phase of NE.

Materials and methods: 19 hospital-treated patients were involved. Plasma levels of D-dimer, prothrombin fragments 1 + 2 (F1 + 2), activated partial thromboplastin time (APTT), prothrombin time (PT%), thrombin time (TT), fibrinogen, antithrombin (AT), protein S free antigen (PS), protein C (PC) and complete blood count (CBC) were measured three times during the acute phase and once at 32-54 days after the onset of fever (recovery phase). Laboratory abnormalities were evaluated by the disseminated intravascular coagulation (DIC) scoring advocated by the International Society of Thrombosis and Haemostasis (ISTH).

Results: APTT was prolonged and D-dimer and F1 + 2 increased during the acute phase of NE. AT, PC and PS decreased, and TT was shortened, all implying increased thrombin generation. Acutely F1 + 2 was 3.4-fold and D-dimer even 24-fold higher compared with the recovery phase (median 726 vs 213 pmol/l, and median 4.8 vs 0.2 mg/l, respectively, $p<0.001$ for both). Platelet count correlated with AT, PC, and PS ($r=0.73$, $r=0.81$, and $r=0.71$, respectively, $p<0.001$ for all) as well as with fibrinogen ($r=0.72$, $p<0.001$). Only five patients fulfilled the ISTH diagnosis of DIC.

Conclusions: During acute NE thrombocytopenia was associated with decreased natural anticoagulants, shortened thrombin time and enhanced fibrinolysis. Augmented thrombin formation and fibrinolysis characterize this hantavirus infection.

Introduction

Puumala virus (PUUV), a member of the *Hantavirus* genus in the *Bunyaviridae* family, is an enveloped RNA virus transmitted to humans by inhalation of aerosolized excreta of infected rodents. The natural host of PUUV is the bank vole, *Myodes glareolus,* which can be found throughout Europe with the exception of Mediterranean coastal regions and most of the Iberian Peninsula and Greece [1].

In humans hantaviruses cause a spectrum of illnesses which are often divided into two clinical diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Acute PUUV infection, i.e. nephropathia epidemica (NE), is a mild form of HFRS. The disease may, however, in rare cases be fatal. In Finland most infections probably remain subclinical or undiagnosed, since only about 1000-2000 serological diagnoses are made annually despite a seroprevalence of 6% [2,3]. The clinical onset of the disease is characterized by high fever, headache, nausea and vomiting with abdominal and/or lower back pains [2]. Transient proteinuria, hematuria and rising serum creatinine levels indicate renal involvement and about 5% of hospital-treated patients require transient hemodialysis [4]. Pulmonary manifestation is also common in NE [5]. Although clear thrombocytopenia is encountered in the majority of NE patients, bleeding manifestations are usually mild. Petechiae, epistaxis, macroscopic hematuria and conjunctival bleeding have been reported [6]. Nuutinen and coworkers demonstrated mild gastrointestinal bleeding by gastroscopy in nearly all NE patients [7]. In

Abbreviations: NE, nephropathia epidemica; F1 + 2, prothrombin fragments; APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; AT, antithrombin; PS, protein S free antigen; PC, protein C; CBC, complete blood count; DIC, disseminated intravascular coagulopathy; ISTH, International Society of Thrombosis and Haemostasis; PUUV, Puumala virus; HFRS, hemorrhagic fever with renal syndrome; HCPS, hantavirus cardiopulmonary syndrome; CRP, C-reactive protein, and HPA, human platelet alloantigen.

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fatal cases of NE hemorrhage of the pituitary gland and other organs has been reported [8–10].

Thrombocytopenia and bleeding tendency in NE are commonly considered signs of disseminated intravascular coagulopathy (DIC) [11,12]. However, so far data published on the activity and regulation of coagulation and fibrinolysis in NE remain sparse.

We aimed to evaluate different laboratory markers of coagulation and fibrinolysis during the acute phase of NE in association with the clinical severity of the disease. Further, we sought to grade the possible DIC during the acute phase of NE with the ISTH score.

Materials and methods

The study was carried out in Tampere University Hospital, University of Tampere Medical School, and Helsinki University Central Hospital, Finland. The patients were selected from those who participated in a previous larger prospective study basing on the availability of the laboratory samples. All patients came from the Pirkanmaa region and were hospitalized in Tampere University Hospital with serologically confirmed acute PUUV infection during the period from September 2000 to December 2002. The study group consisted of 19 patients (17 males), median age 38 years (range 30-64 years). Regarding medical history, two of the patients had a neurological disease (1 multiple sclerosis, 1 epilepsy) and one a chronic inflammatory bowel disease treated with mesalazine medication. There were patients with dyslipidemia (n = 2), coronary heart disease (n = 1), arterial hypertension (n = 1) and paroxysmal atrial fibrillation (n = 1). No patient was under immunosuppression or anticoagulation. Two patients used anti-platelet therapy (aspirin).

The patient charts were retrospectively reviewed to establish the length of hospital stay (days), daily urinary output (ml), need of transient hemodialysis treatment (no/yes) and signs of bleeding (no/yes). The treatment with platelet or plasma transfusions to correct the possible coagulopathy as well as the treatment with heparin were noted.

To assess the activation of coagulation and fibrinolysis, three blood samples per patient were collected during the acute phase of the disease. The first sample was obtained on admission as early as possible, 2-9 (median 6) days after the onset of fever. The second sample was obtained 3-10 (median 7) days, and the third 6-13 (median 10) days after the onset of fever. The last sample of the study, the fourth sample, was drawn at full recovery (ranging 32-54 days after the onset of fever, median 43 days), this representing a control sample. Citrate-anticoagulated (109 mM sodium citrate) samples were centrifuged at 1500 g for 20 min and separated plasma samples were frozen at -70 °C. Once defrosted the samples were recentrifuged at 2500 g for 15 min prior to analysis.

Laboratory analysis included plasma prothrombin time expressed as % (PT%, Nycotest PT®, Axis-Shield PoC As, Oslo, Norway), activated partial thromboplastin time (APTT, Actin FSL®, Siemens Healthcare Diagnostics, Marburg, Germany) and thrombin time (TT, Siemens Healthcare Diagnostics). Fibrinogen was measured with a modification of the Clauss method (Multifibren® U, Siemens Healthcare Diagnostics) and D-dimer with an immunoturbidimetric assay (Tina-quant D-Dimer®, Roche Diagnostics, Mannheim, Germany). Prothrombin fragments (F1 + 2) were measured by an enzyme immunoassay (Enzygnost® F1 + 2, monoclonal, Siemens Healthcare Diagnostics). The reference values for PT% were 70-130%, APTT 23-33 s, TT 17-25 s, fibrinogen 1.7-4.0 g/l, D-dimer ≤ 0.5 mg/l and F1 + 2 69-229 pmol/l. Protein S free antigen (PS) level was determined by an automated latex ligand immunoassay (Instrumentation Laboratory, Lexington, MA), reference values being 66-158% for males and 50-177% for females. Antithrombin (AT) and protein C (PC) activities were both determined by chromogenic assays (Berichrom®Antithrombin III and Berichrom®Protein C, Siemens Healthcare Diagnostics), reference values being 84-108% and 74-141%, respectively.

During the hospital stay, complete blood count (CBC), plasma C-reactive protein (CRP) and serum creatinine were taken according to the clinical needs of the patients, and determined at the Laboratory Centre of the Pirkanmaa Hospital District using standard methods. The reference values for serum creatinine were <105 μmol/l for males and <95 μmol/l for females and for CRP<10 mg/l. For platelet and leukocyte counts the reference values were 150-360 and 3.4-8.2 × 10^9/l, respectively. The reference values for blood hemoglobin were 134-167 g/l for males and 117-155 g/l for females.

The DIC scoring system by the International Society of Thrombosis and Haemostasis (ISTH) was used to evaluate individual patients [13]. The most abnormal value for each variable was chosen for scoring based on the three acute assessments.

Statistics

To analyse the data, the most abnormal value of each continuous variable measured during the acute phase of NE was designated the maximum or the minimum value. The change between the maximum or the minimum and the control value (the fourth sample) was calculated. Means (± standard deviations) and medians (ranges) were provided. To evaluate changes for each variable between the acute and the control phase, paired samples *t*-test or Wilcoxon's test was used. Relationships between continuous variables were examined using Pearson's or the Spearman rank correlation coefficient. Comparisons between the groups were based on the Mann-Whitney U test for the numerical and χ^2 test for the categorical data. The limit of significance was set at 0.05 (2-tailed). SPSS 7.5 was used for computation.

Results

Clinical and laboratory findings

All 19 patients presented with clinical characteristics typical of NE. The most prominent symptoms on admission were: fever 100% (n = 19), nausea and/or vomiting 63% (n = 12), headache 47% (n = 9), blurred vision 32% (n = 6) and abdominal or back pain 16% (n = 3). The median duration of hospitalization was 7 days (range 4-15 days). During the hospital stay only minor bleeding events occurred: two patients suffered from epistaxis and one from minor conjunctival bleed. The median lowest daily urinary output was 880 ml (range 40-3740 ml). Three of the 19 patients needed transient hemodialysis treatment; one of them showed oozing at the base of the central venous catheter for several days and received a platelet transfusion of two units. Five patients were treated with low molecular weight heparin for a few days either for thromboprophylaxis or maintenance of hemodialysis. All patients recovered.

During the acute phase of NE the lowest platelet count ranged from 13 to 238 × 10^9/l (median 75 × 10^9/l), and thrombocytopenia was found in 15 (79%) patients. However, 17 patients (89%) had their platelet count within normal values by the time of discharge. Seventeen patients had a leukocyte count higher than 10.0 × 10^9/l (median maximum 11.8 × 10^9/l, range 7.3 - 23.2 × 10^9/l), and 15 patients were anemic (median minimum hemoglobin 126 g/l, range 98 - 157 g/l). CRP was elevated in 17 patients (median maximum 61 mg/l, range 6 - 198 mg/l), and creatinine concentration was elevated in 18 (95%) patients (median maximum 321 μmol/l, range 74 - 1258 μmol/l).

Laboratory markers of coagulation and fibrinolysis

The values for coagulation variables during the acute phase and at recovery are shown in Table 1.