Viral load and humoral immune response in association with disease severity in Puumala hantavirus-infected patients—implications for treatment

L. Pettersson[1]*, T. Thunberg[2]*, J. Rocklöv[3], J. Klingström[4], M. Evander[1] and C. Ahlm[2]

1) *Department of Clinical Microbiology, Virology, Umeå University,* 2) *Department of Clinical Microbiology, Infectious Diseases, Umeå University,* 3) *Public Health and Clinical Medicine, Umeå University, Umeå and* 4) *Department of Medicine, Centre for Infectious Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden*

Abstract

Hantaviruses are the causative agents of haemorrhagic fever with renal syndrome (HFRS) in Eurasia and of hantavirus cardiopulmonary syndrome (HCPS) in the Americas. The case fatality rate varies between different hantaviruses and can be up to 40%. At present, there is no specific treatment available. The hantavirus pathogenesis is not well understood, but most likely, both virus-mediated and host-mediated mechanisms are involved. The aim of the present study was to investigate the association among Puumala hantavirus (PUUV) viral RNA load, humoral immune response and disease severity in patients with HFRS. We performed a study of 105 PUUV-infected patients that were followed during the acute phase of disease and for up to 1–3 months later. Fifteen of the 105 patients (14%) were classified as having moderate/severe disease. A low PUUV-specific IgG response ($p < 0.05$) and also a higher white blood cell count ($p < 0.001$) were significantly associated with more severe disease. The PUUV RNA was detected in a majority of patient plasma samples up to 9 days after disease onset; however, PUUV RNA load or longevity of viraemia were not significantly associated with disease severity. We conclude that a low specific IgG response was associated with disease severity in patients with HFRS, whereas PUUV RNA load did not seem to affect the severity of HFRS. Our results raise the possibility of passive immunotherapy as a useful treatment for hantavirus-infected patients.

Keywords: Age, disease severity, hantavirus, haemorrhagic fever with renal syndrome, humoral immune response, immunoglobulins, neutrophil, Puumala virus, viral load, white blood cell count
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Corresponding author: T. Thunberg, Department of Clinical Microbiology, Infectious Diseases, Umeå University, 90187 Umeå, Sweden
E-mail: therese.thunberg@climi.umu.se

*These authors contributed equally to this work.

Introduction

Hantaviruses can cause two febrile diseases in humans: haemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Humans are infected by inhalation of aerosolized rodent excreta containing virus. Puumala virus (PUUV) is endemic in Central and Northern Europe and causes a relatively mild form of HFRS, also known as Nephropathia Epidemica. The most common symptoms of PUUV infection are fever, headache, nausea, vomiting, myalgia, abdominal/back pain and visual disturbances [1]. The majority of patients also have signs of renal failure and other important clinical features are mild haemorrhagic manifestations and respiratory symptoms [2]. Predictors of severe renal impairment in hantavirus-infected patients include high leucocyte counts at the time of admission [3,4].

To date, there is no approved specific treatment for hantavirus-infected patients, instead supportive care is applied [5,6]. Hantaviruses are believed to be non-cytopathic and the

mechanisms behind the capillary leakage and haemorrhage are poorly understood. Studies imply that the pathogenesis is multifactorial and includes contributions from immune responses, platelet dysfunction, dysregulation of the endothelial cell barrier and genetic host factors [1,7,8]. Among immune parameters, over-activation of CD8 T cells and natural killer cells, and induction of cytokines are thought to play important roles [9–12]. Previous studies of viraemia in HCPS and HFRS suggest an association between initial high viral load of Dobrava, Sin Nombre and Hantaan viruses, respectively, and a more severe clinical outcome [13–16]. This association implicates a potential direct role for the virus in pathogenesis and interventions with antivirals as a possible therapy for hantavirus-infected patients. However, contradictory results have been reported from studies regarding antiviral treatment of HFRS and HCPS patients and one theory is that Ribavirin may have been given too late in the course of infection [6,17,18]. More data regarding the duration of viraemia are needed to determine the window of opportunity for antiviral therapy. Another approach for specific treatment of hantavirus infection may be administration of human neutralizing antibodies. However, there are no published controlled clinical trials of immunotherapy for hantavirus-infection. Studies on humoral immune responses to hantavirus infection have shown an association between low specific IgG antibody titres and disease severity for HCPS patients, suggesting that a strong IgG response to infection might be protective [19,20]. The results on specific IgA and IgM responses are contradictory regarding an association with disease severity in HCPS [19,20]. In HFRS caused by PUUV not only IgG but also IgA is known to neutralize the virus [19–21].

In this study on 105 PUUV-infected patients we analysed the duration of viraemia and the association between disease severity and viral load, humoral immune response and different laboratory parameters. Our results address the potential use of specific treatment, i.e. antivirals and immunotherapy in hantavirus-infected patients.

Methods

Study design and patient material

Patients with acute HFRS were included at the Clinic of Infectious Diseases, Umeå University Hospital. All patients were serologically verified by immunofluorescence assay for PUUV-specific IgM and IgG. If only IgM was present in the first serum sample the patient diagnosis had to be confirmed by seroconversion of IgG in follow-up samples. In three patients only IgG was present and the acute infection was verified by RNA detection with PCR or through avidity testing of the IgG antibodies [22]. Both hospitalized patients and outpatients were followed with regards to symptoms and routine laboratory tests. Blood samples were drawn at first contact with healthcare and thereafter repeated sampling was performed approximately every other day during the acute phase. The patients were followed up after 1–3 months. Written and oral informed consent was obtained from all patients and the study was approved by the regional ethics committee of Umeå University, Umeå, Sweden.

Criteria for severe illness

Patients who met two or more of the following criteria were considered to have a moderate/severe illness: treated in the intensive care unit, dialysis, radiologically verified thrombosis, need for platelet transfusion, moderate/severe hypotension (systolic blood pressure $\leq$ 90 mmHg and intravenous fluid treatment at any given time during hospitalization), and bleeding of moderate/major importance; i.e. gastrointestinal bleeding, macroscopic haematuria and metrorrhagia [23].

Real-time reverse transcription-PCR

RNA extraction and real-time reverse transcription-PCR were performed as previously described [24]. Briefly, the RNA was reverse-transcribed followed by a real-time PCR with primers and probe detecting the PUUV S-segment.

Immunofluorescence

To detect PUUV-specific IgM, IgA and IgG serum antibodies, an immunofluorecence analysis was performed as previously described [24]. Briefly, patient serum was added to spot-slides covered with fixed Vero E6 cells infected with the local strain PUUV Umeå/hu. For IgM analysis, sera were pretreated with Reumatoid factor-absorbent (Virion\Serion GmbH, Würzburg, Germany) to eliminate possible interference by rheumatoid factor. Presence of PUUV-specific antibodies was determined by use of fluorescein-conjugated rabbit anti-human IgM, IgA and IgG (F0317, F0204 and F202, respectively by DAKO A/S Glostrup, Denmark). Sera were diluted to the end-point titre (Fig. 1).

Statistical analysis

The clinical symptoms were described using percentages, whereas laboratory findings were presented as medians and range. Mann–Whitney *U* test was used to compare laboratory findings between patients with mild versus severe illness.

Binary logistic regression was performed to analyse associations with disease severity. Levels of PUUV RNA, PUUV-specific antibody titres and white blood cell count (all first available samples) were investigated as predictors for disease