

Another case of “European hantavirus pulmonary syndrome” with severe lung, prior to kidney, involvement, and diagnosed by viral inclusions in lung macrophages

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Received: 14 March 2013 / Accepted: 17 April 2013 / Published online: 14 May 2013
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Abstract *Puumala virus* (PUUV) is considered a classic Old World etiologic agent of nephropathia epidemica (NE), or hemorrhagic fever with renal syndrome (HFRS). HFRS is considered to be distinct from hantavirus (cardio-)pulmonary syndrome (HPS or HCPS), described in the New World. Here, we report a severe case, which fulfilled most, if not all, Centers for Disease Control and Prevention (CDC) criteria for HPS, needing non-invasive ventilation and subsequent acute hemodialysis. However, the etiological agent was PUUV, as proved by serological testing, real-time polymerase chain reaction (PCR), and sequencing. Viral antigen was detected by specific anti-PUUV immunostaining, showing, for the first time, greenish intracytoplasmic inclusions in bronchoalveolar lavage (BAL) macrophages. This case definitely confirms that HPS can be encountered during PUUV infections. Interestingly, special findings could render the diagnosis easier, such as greenish homogeneous cytoplasmic inclusions, surrounded by a fine clear halo in BAL macrophages. Therefore, although

the diagnosis remains difficult before the onset of renal involvement, the occurrence of severe respiratory failure mimicking community-acquired pneumonia must alert the clinician for possible HPS, especially in endemic areas.

Introduction

Members of the genus *Hantavirus* comprise the only non-arthropod-borne viruses in the family *Bunyaviridae*. Hantaviruses are primarily transmitted by the inhalation of aerosolized viral particles shed by rodents, which serve as chronically infected but asymptomatic reservoirs. Recent overviews [1, 2] have listed up to 42 named hantaviruses, of which at least 20 were described as human pathogens, mostly in the Americas. This is in contradiction, however, with the official standpoint of the International Committee of Taxonomy of Viruses (ICTV) having recognized, so far, only 23 lineages as

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separate hantavirus species, of which not all are known pathogens [3]. Each hantavirus species is multiplied and spread by a mostly unique rodent species. The most important pathogens are *Hantaan virus* and *Seoul virus* in the Far East (>90 % of worldwide infections), *Puumala virus* (PUUV) and *Dobrava-Belgrade virus* in Europe and Russia, and, finally, *Sin Nombre virus* and *Andes virus* in the Americas [1, 2]

Until recently, two clinical syndromes were classically described: hantavirus (cardio-)pulmonary syndrome (HPS or HCPS) described since 1994 in the Americas [4] and hemorrhagic fever with renal syndrome (HFRS) or its milder form nephropathia epidemica (NE), known in Eurasia since the early 1930s. However, a considerable overlap of symptoms was noted from 1994 onwards [5], and recent evidence has shown yet more pathogenic similarities between the syndromes caused by the two groups of genetically related viruses [3, 6, 7]. The case described herein adds to the mounting evidence that HPS can be seen in Europe, and be caused by PUUV, as reported previously [5, 7–9].

Case report

A 42-year-old Belgian woman was admitted in the emergency room with fever of 39 °C, thoracic pain, and progressive dyspnea lasting for 4 days. She also complained of rhinorrhea, blurred vision, headache, unusual lumbar discomfort, and nausea, with two episodes of vomiting. Her general practitioner started ibuprofen (600 mg \times 3/d), as well as a quinolone (levofloxacin 500 mg/d) 3 days before her hospital admission. She had smoked since her youth (30 pack-years) and her medical history was characterized by mild asthma, fallopian tube ligation, and a subarachnoid hemorrhage treated by endovascular therapy, resulting in complete recovery. Carbamazepine, prescribed to prevent seizures, was her only drug. She lived with her family in Doische, a rural area in the Ardennes, the densely forested South of Belgium, a region highly endemic for NE [10]. She had not traveled recently and had no pets. Her job consisted on delivering newspapers by car or on foot. She also took a stroll through the woods near Doische about 3 weeks before the onset of symptoms.

Physical examination revealed bibasal lung crackles with bilateral lumbar pain and conjunctivitis. Her heart rate was 86/min and blood pressure 100/70 mmHg. Arterial blood gases obtained while the patient was breathing room air on admission showed hypoxemia with respiratory alkalosis (pH 7.52, PO₂ 51 mmHg, PCO₂ 30 mmHg). Other significant laboratory findings were: CRP 16 mg/dl, thrombocytes 86,000/ μ l, leukocytes 7,460/ μ l (atypical activated lymphocytes 1 %), hyponatremia 130 mEq/l, hypokalemia 3.2 mEq/l, ASAT 61 UI/l [normal value (nv) <36], ALAT 88 UI/l (nv <43), LDH 920 UI/l (nv <618), but a strictly normal renal function on admission, with creatinine 0.8 mg/dl and urea 30 mg/dl. At admission, i.e., 5 days post

onset of symptoms (POS), chest X-ray showed perihilar infiltrates. Intravenous amoxicillin/clavulanate (6 g/day) and clarithromycin (1 g/day) were started for suspected severe community-acquired pneumonia.

Due to the increase of hypoxemia and the deterioration of pulmonary infiltrates (Fig. 1), the patient was eventually admitted to the intensive care unit (ICU) 24 h later, i.e., 6 days POS. At admission, she was normotensive (100/60 mmHg) and tachypneic (respiratory rate 32/min), tachycardic (heart rate 101/min), and had an oxygen saturation of only 92 % under 15 l/min of oxygen. Arterial blood analysis with FiO₂ at 50 % showed pH: 7.43; pO₂: 58 mmHg; pCO₂: 30 mmHg. Anomalies in laboratory values progressed: at day 7 POS, neutrophils had increased to 8.497/ μ l, sodium was 130 mEq/l, osmolarity 268 mOsm/kg, LDH 1,815 UI/l, ferritin 3.493 μ g/l, total cholesterol 119 mg/dl, HDL cholesterol 11 mg/dl, and fasting triglycerides were 211 mg/dl (nv <150 mg/dl). Protein electrophoresis, FAN, ANCA, and troponin I were normal, as well as renal function. A urine spot, however, showed significant proteinuria of 12.790 g/l, with an increased urinary sodium of 68 mEq/l.

Despite antibiotics, her respiratory distress worsened and initiation of non-invasive ventilation with high oxygen level was required, but orotracheal intubation could be avoided. Blood cultures and sputum remained negative. Blood serology was negative for Epstein–Barr virus, human cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, *Chlamydia pneumoniae/trachomatis*, and *Mycoplasma pneumoniae*, as well as urinary Legionella antigen. At day 8 POS, a thoracic computed tomography (CT) scan exhibited bilateral ground glass opacities, predominantly in inferior lobes (Fig. 2) and transthoracic echocardiography showed normal left ventricular function without pericardial effusion. At day 9 POS, a bronchoalveolar lavage (BAL) was performed in the lingula. The tracheobronchial mucosa appeared slightly inflammatory. The BAL fluid was

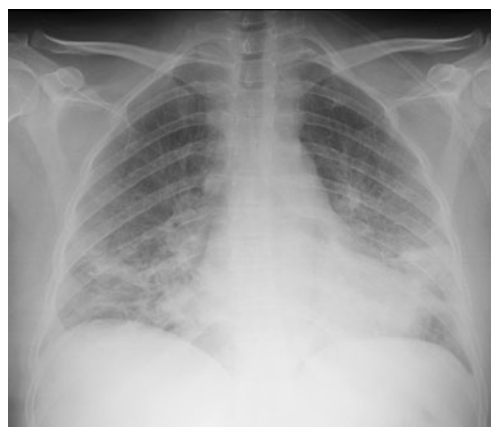


Fig. 1 Chest X-ray at hospital admission [day 5 post onset of symptoms (POS)] showing bilateral pulmonary interstitial infiltrates

macroscopically gray, and contained 393 white cells/mm³, with 75 % of them macrophages, and 19 and 15 % of neutrophils and eosinophils, respectively. Lymphocyte subtype revealed 81 % of CD8 and 17 % of CD4 T cells. Interestingly, macrophages contained isolated or multiple greenish homogeneous cytoplasmic inclusions of variable size that were surrounded by a fine clear halo (Fig. 3). This pattern suggested a viral infection, and no bacteria could be identified at direct examination. Hemosiderin was abundant in the fluid. BAL cultures were negative for bacteria, fungi, mycobacteria, and virus (including respiratory syncytial virus, adenovirus, influenza, or HCMV).

Only at day 11 POS oliguric renal failure developed, suggesting, finally, the possibility of a hantavirus infection. Serum creatinine rose to 1.2 mg/dl from 0.8 mg/dl the day before, and reaching a peak of 3.6 mg/dl at day 14 POS. PUUV infection was confirmed by serology [enzyme-linked immunosorbent assay (ELISA) technique] at day 12 POS, using PUUV strain CG18-20 antigens, with IgM optical density at 14.00 (nv below 2.00) and IgG at 1.5 (nv below 1.5), using a 200-fold diluted serum sample. Consequently, antibiotics were discontinued. The course of disease was complicated by anuria and hypotensive shock, responding well to colloid challenge and vasopressors therapy. A total of five hemodialyses were required to treat uremia (>200 mg/dl), without signs of fluid overload. Finally, the patient was discharged from the ICU 16 days POS without renal assistance, and from hospital on day 25 POS with transient oxygen support.

BAL fluid was subsequently analyzed by immunohistochemistry. Macrophage inclusions stained positively with a specific PUUV antibody (Fig. 4a, b). The acute serum at 12 days POS was also tested by real-time polymerase chain reaction (PCR) amplification, and an amplicon of 450 bp was obtained. Sequencing showed a PUUV belonging to the same clade of four other PUUV lineages, found previously in the geographically direct vicinity, i.e., the Belgian Ardennes (Fig. 5) [11].

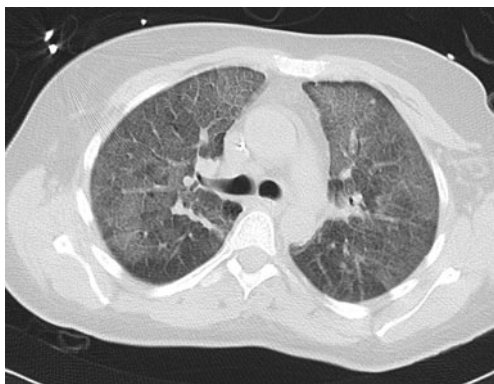


Fig. 2 Chest computed tomography (CT) scan (day 8 POS) showing bilateral ground-glass pattern

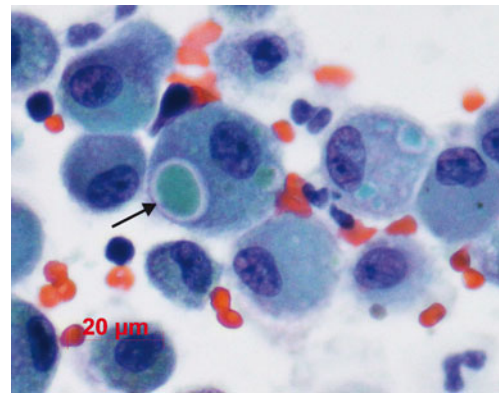


Fig. 3 Bronchoalveolar lavage (BAL) macrophages containing greenish cytoplasmic inclusions surrounded by a clear halo (arrow, Papanicolaou stain, magnification $\times 40$)

Discussion

Contrary to the paradigm that PUUV could only induce NE or HFRS, there is now increasing evidence that a clinical picture showing that most, if not all, features of HPS might also be encountered in European NE cases, as reported previously [3, 5–8, 12]. In fact, one of the very first PUUV cases in Belgium, described in 1987, presented likewise with manifest pulmonary involvement and arterial desaturation, before any renal deterioration was detected, and noteworthy, was diagnosed also in our hospital [9].

The history of our patient fulfills most of the eight clinical findings specified in the “Hantavirus Pulmonary Syndrome Case Report Form”, as issued by the Centers for Disease Control and Prevention (CDC), Atlanta, GA [13]: (1) fever, (2) thrombocytopenia, (3) elevated hematocrit, (4) elevated serum creatinine, (5) left shift leukocytosis with “atypical lymphocytes”, (6) need for supplemental oxygen, (7) need for intubation (and mechanical ventilation), and (8) chest radiograph showing unexplained bilateral infiltrates or being suggestive of adult respiratory distress syndrome (ARDS). The level of atypical lymphocytes seen in New World HPS cases is higher (rarely if ever less than 10 %) than the 1 % observed in our patient [14]. Moreover, and

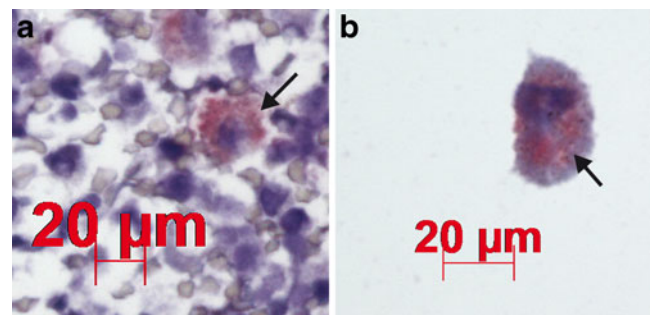


Fig. 4 a, b Macrophage inclusions stained positively with specific *Puumala virus* (PUUV) antibody [arrow, magnification $\times 60$ (a), $\times 100$ (b)]

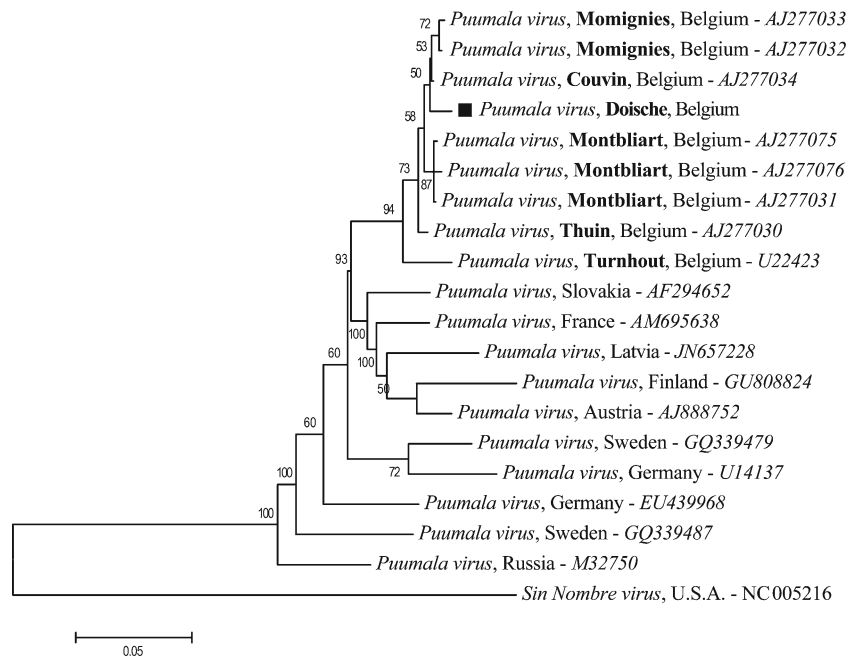


Fig. 5 Phylogenetic tree based on Belgium and European PUUV S segment sequences. *Sin Nombre virus* (SNV), the typical American etiologic agent of the hantavirus pulmonary syndrome (HPS), is used as an outgroup in the analysis. The patient lineage is marked by a *black square*, and is referred to as “Doische”, the putative place of infection in the densely forested South of Belgium, near the French border. For the Belgian strains, PUUV isolates from different regions are used. The

evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. Bootstrap values less than 50 % are not shown. GenBank accession numbers are shown in *italics*. The *scale bar* represents the number of base substitutions per site

typical for HPS, rather than for classic HFRS, is the initial clinical and radiological presentation with pronounced pulmonary involvement, but without any initial renal injury on admission, as described recently in three severe Swedish NE cases, two of which were fatal [6]. Pulmonary edema secondary to right heart decompensation after overhydration due to oliguria as a suggested mechanism in HFRS [4] could clinically and radiologically be excluded in cases described in Belgium [5, 9], France [8], and Sweden [6], as in this case with normal echocardiography. Other biological markers mainly noted so far in HFRS were also present: marked proteinuria, fasting hypertriglyceridemia contrasting with low levels of total cholesterol, and very low HDL levels [10, 11, 15]. Finally, initial blurred vision due to transient shallowing of the anterior eye chamber is an often overlooked but very specific clinical sign in NE and, to a lesser degree, HFRS cases [1, 10, 15], but have not been reported so far in HPS cases from the Americas. In the herein described HPS case, PUUV infection was confirmed serologically, and corroborated by positive blood reverse transcription (RT)-PCR, confirming the considerable clinical overlap between the New and Old World syndromes.

On the other hand, predominance of CD8 lymphocytes in BAL has been considered so far as typical for HPS [1, 2]. This case revealed the presence of greenish homogeneous cytoplasmic inclusions of variable size surrounded by a fine clear

halo in BAL macrophages, a new distinctive sign that is particularly useful. With specific immunostaining, it was possible to prove that these images corresponded to PUUV viral inclusions. PUUV viral RNA was demonstrated by real-time PCR in BAL at day 9 POS in a Swedish NE case [6], but the detection of viral antigen with immunohistochemistry has been described so far only on autopsy lung specimens [6]. Our case further supports the value of fiberoptic bronchoscopy in outpatients suffering from severe respiratory failure with bilateral pulmonary infiltrates of unknown origin.

Hantavirus pathological mechanisms are not well understood, especially the reason why some Puumala viruses had a more pronounced renal or pulmonary feature. Since direct viral tissue damage is negligible, a so-called “cytokine storm” as an immunopathological response of the human host seems to be of core importance [16]. Recent publications suggested that the increased capillary permeability observed in both HPS and HFRS might be caused by a common immunopathological mechanism, i.e., hantavirus-specific cytotoxic CD8+ T lymphocytes, attacking endothelial cells presenting viral antigens on their surfaces [17]. It is also possible that direct viral toxicities or toxicities due to mediators should be considered as well, since T cells apparently cannot be implicated in pathogenesis in an HPS animal model [18].

In conclusion, this case firmly confirms that PUUV is also an etiologic agent of HPS. Additionally, special features could help the diagnosis, such as greenish homogeneous cytoplasmic inclusions, surrounded by a fine clear halo in BAL macrophages. Therefore, diagnosis clinical suspicion of HFRS remains difficult on admission, before the onset of renal function impairment. However, the combination of an episode of fever with blurred vision, early and massive proteinuria as in this patient, followed by severe lung involvement looking like a community-acquired pneumonia, elevated LDH, hyponatremia, and thrombocytopenia must alert the clinician for a possible hantavirus infection with HPS features, especially in endemic areas.

Conflict of interest The authors declare that they have no conflict of interest.

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