Hantavirus infections in the South-Eastern European countries: A study of two cases and literature review

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Received January 25, 2023; Accepted June 9, 2023

DOI: 10.3892/etm.2023.12129

Abstract. Hantavirus infection is a rare zoonosis in South-Eastern Europe. Depending on the serotype involved, the virus can cause hemorrhagic fever with renal syndrome which is also known as endemic nephropathy, and cardiopulmonary syndrome. Prompt diagnosis of the disease is essential for reducing the risk of severe manifestations and complications like chronic kidney disease, secondary hypertension or even death because there is no specific treatment or vaccine approved. The present study reported two cases of hemorrhagic fever with renal syndrome diagnosed in the Department of Nephrology of The Fundeni Clinical Institute (Romania). In both patients, kidney needle biopsy played a major role in establishing the diagnosis. The difficulties encountered in diagnosing this disease were also emphasized, taking into consideration the rarity of this infection in South-Eastern Europe. The key literature data on the epidemiology, pathogenesis and management of this infection were further reviewed.

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Abbreviations: HFRS, hemorrhagic fever with renal syndrome; TMA, thrombotic microangiopathy; KB, kidney biopsy; ADAMTS 13, a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13; LDH, lactate dehydrogenase; C3, component 3 of the complement system; C4, component 4 of the complement system; ANCA, antineutrophil cytoplasmic antibody; anti-HIV, anti-human immunodeficiency virus antibody; AKI, acute kidney injury; HUS, hemolytic uremic syndrome; IF, immunofluorescence; LM, light microscopy; EM, electron microscopy; HTNV, Hantaan virus; PUUV, Puumala virus; DOBV, Dobrava virus; SEOV, Seoul virus; TULV, Tula virus; SAAV, Saaremaa virus

Key words: Hantavirus, hemorrhagic fever, acute kidney injury, interstitial hemorrhage

Introduction

Hantavirus is responsible for causing a rare zoonosis in the South-Eastern European countries, but apparently with increasing incidence and geographical spread during recent years. For 2020, 28 countries from Europe reported 1,647 cases of hantavirus infection (0.4 cases per 100,000 population), mainly caused by Puumala virus (98%) (1). Several serotypes have rodents as reservoirs (2). These are categorized as follows: Old-World Hantaviruses, which may cause hemorrhagic fever with renal syndrome (HFRS), and New-World Hantaviruses which may cause cardiopulmonary syndrome (CPS) (2).

HFRS often presents as a flu-like syndrome, with fever and myalgia, which further evolves similarly to thrombotic microangiopathy (TMA), causing anemia, severe thrombocytopenia, coagulation disorders and acute kidney injury (AKI) (3,4). Patients with renal impairment often have hematuria and proteinuria up to the nephrotic range, which may be mistaken for nephritic syndrome (4). These urine changes are most likely caused by a defect in the filtration barrier, since the histological appearance may include moderate glomerular lesions, and the most frequently seen one is acute tubulointerstitial nephritis with hemorrhage in the outer medulla.

Both cases reported in the current study had been diagnosed as TMA. A kidney biopsy (KB) was essential for the diagnosis since an infection with hantavirus is often underdiagnosed in the South-Eastern European countries due to its low incidence. Hantaan (HNTV) serotype, which is usually

found in China and Russia, was identified as the causative agent in a patient who had not traveled abroad, a trigger point for reconsidering the geographical distribution of the species involved. The current knowledge regarding the diagnosis and management of this rare zoonosis is further reviewed.

Case report

Case 1. A 26-year-old female patient was hospitalized in a Buzau Emergency Hospital for the abrupt onset of gastrointestinal symptoms (fever, nausea, vomiting and diarrhea), hypotension (blood pressure of 80/50 mmHg) and headache. After admission, the patient was diagnosed with kidney impairment (serum creatinine, 1.4 mg/dl), nephritic syndrome with nephrotic range proteinuria (microscopic hematuria with dysmorphic red cells and red cells casts; proteinuria, 8 g/day), elevated liver enzymes, severe thrombocytopenia (11,000/mm^3), anemia and leukocytosis (Table I).

The stool was collected in a transport recipient by selecting from three different places, especially from zones with mucus or blood (if these areas of interest were present). The probe was cultivated on selenite broth for Salmonella, and on ADCL agar SS (agar Shigella-Salmonella). They are cultivated for 24 h. After 24 h the probe was moved from selenite broth to MacConkey medium. The colonies which suggested intestinal pathogens were incubated for 24 h more on MacConkey medium and agar SS. Colonies which were suspected to be *E Coli* were tested with agglutination sera against enteropathogenic *E Coli*, enterotoxigenic *E Coli*, enteroinvasive *E Coli* and enterohemorrhagic *E coli*. When after 48 h one of these pathogens was present, the antibiogram was be performed according to CLSI/EUCAST. The stool samples were negative for *Escherichia coli*, *Shigella* and *Salmonella*.

The patient became oliguric, with a further increase in serum creatinine (≤6 mg/dl) and persistence of severe thrombocytopenia; therefore, 3 days after hospitalization, the patient received methylprednisolone (three intravenous injections, 500 mg each). Subsequently, the patient developed polyuria and the platelet count, proteinuria, and serum creatinine improved considerably. However, the patient remained with nephritic syndrome and kidney dysfunction, developing high blood pressure (167/94 mmHg), consequently, after 3 weeks, the patient was transferred to the Department of Nephrology of The Fundeni Clinical Institute.

When transferred, the patient had no edema, high blood pressure (170/100 mmHg), tachycardia (heart rate 90 beats/min) and polyuria (4 l/day). The blood analysis revealed moderate normocytic normochromic anemia with raised lactate dehydrogenase (LDH), normal haptoglobin (0.65 g/l), a positive direct Coombs test, normal platelet count, leukocytosis, mild kidney impairment, normal serum albumin, mild hypokalemia and elevated liver enzymes (Table I). Urinalysis showed glomerular hematuria, leukocyturia and proteinuria of 2 g/day. For urinalysis a spontaneous morning void was collected in a sterile recipient and was examined within the first hour after the collection. The urine sample was verified to have the necessary quantity and then placed in a rack in the analyzer, which was a fully automated urine chemistry analyzer using reflectance photometry and refractometry methods. The analyzer was loaded with strips. Urine sample material was dropped onto each pad of a dedicated test strip within the analyzer. The entire sequence, starting from sample aspiration, to color comparison and final output of the results was fully automatic. The sample barcode reader scanned the barcode of each tube and results were transmitted to the computer system.

Regarding the antiphospholipid antibodies, the patient was positive for the lupus anticoagulant. The screening for autoimmune diseases (lupus, ANCA-associated vasculitis, anti-glomerular basement membrane, cryoglobulinemia, C1q vasculitis, Sjögren syndrome and membranous nephropathy) was negative (including normal complement level and normal serum levels of immunoglobulin A and M, with only a slightly elevated level for immunoglobulin G). A disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13 (ADAMTS 13) activity was normal; evaluation was done according to the protocol described previously (5). The serological screening for viral infections [hepatitis B (HBV) and C, HIV, cytomegalovirus and Epstein-Barr virus] and leptospira was also negative. For the serological analysis, the blood sample was allowed to settle, then centrifuged 5 min at 1,910 x g at room temperature, separating the serum (hemolyzed samples were rejected). The sample was placed in a rack in the analyzer. The rack was inserted into the input buffer on rack trays. The sample barcode reader scans the barcode of each tube. It used the immunochemical method with electrochemiluminescence detection (ECLIA) and results were transmitted to the computer system.

The tumor markers for digestive, ovarian and breast cancer were negative. An abdominal ultrasound (a 2D convex probe with a 2.5 MHz frequency; General Electric) showed a mildly increased volume of both kidneys with hyperechoic parenchyma and no signs of urinary tract obstruction.

Based on the severe thrombocytopenia (11,000/mm^3) associated with anemia and stage III AKI, TMA as part of the hemolytic uremic syndrome (HUS) was suspected. Lactate dehydrogenase was elevated, while haptoglobin was normal and the Coombs test was positive, which mainly excluded TMA, although there have been reports of TMA with microangiopathic hemolytic anemia and a positive Coombs test (i.e., Pneumococcal infection) (6). Typical HUS was ruled out by the negative stool cultures. Autoimmune, viral or malignant causes of atypical HUS (aHUS) were also excluded. Furthermore, the patient denied any intake of illicit drugs and had had no regular treatment before being hospitalized, except for oral contraceptives, which can be very rarely associated with TMA (7,8), especially in renal graft female recipients (9). Normal ADAMTS 13 activity excluded thrombotic thrombocytopenic purpura.

Since a definite etiology for AKI could not be established, a KB was performed. Immunofluorescence was performed on sections from unfixed, fresh frozen tissue. The biopsy cores were frozen with OCT at -25˚C for 15 min and 4-μm-thick sections were cut with a Leica cryostat (Leica Microsystems GmbH). The serial sections were collected on microscopy slides and dried for 30 min. Dried sections were rehydrated for 15 min in saline phosphate buffer (PBS) at pH 7.2 and incubated for 30 min in dark staining tray with FITC labeled antibodies diluted 1:50: FITC IgG antiserum (Dako; Agilent Technologies, Inc.; cat. no. F020202-2); FITC IgA antiserum (Dako; Agilent Technologies, Inc.; cat. no. F020402-2); FITC IgM antiserum (Dako; Agilent Technologies, Inc.; cat. no. F020302-2);

Table I. Laboratory data in both reported cases.

Parameter	Case 1	Case 2	Reference values
Hemoglobin, g/dl	9	12.8	11.5-17
Hematocrit, fl	30	35	43-54
Leukocytes, /mm^3	16,000 → [a]13,810	20,140	4,000-9,000
Platelets, /mm^3	11,000 → 279,000	5,000 → 54,000 → 190,000	150,000-400,000
Iron, μg/dl	110	69	35-175
Ferritin, ng/ml	154	92	15-150
Lactate dehydrogenase, IU/l	527	812	208-378
Haptoglobin, g/l	0.65	0.5	0.35-2.5
Direct Coombs test	Positive	Positive	Negative
C-reactive protein, mg/l	5	69.3	0-5
Serum creatinine, mg/dl	1.4 → 6 → 1.35	9.6 → 1.68 → 0.96	0.5-1.3
Blood Urea Nitrogen, mg/dl	75 → 94 → 43	131 → 35 → 20	7-20
Uric acid, mg/dl	5.27	14.3	2.6-9.2
Alanine aminotransferase, IU/l	150 → 82	64	0-49
Aspartate aminotransferase, IU/l	151 → 62	103	0-34
Potassium, mmol/l	3.3	4.4	3.5-5.5
Sodium, mmol/l	145	126	132-146
Albumin, g/dl	3.45	2.5	3.2-5.2
Antinuclear antibodies	Negative	Negative	Negative
Anti-double stranded DNA	Negative	Negative	Negative
Anti-Ro	Negative	Negative	Negative
Anti-La	Negative	Negative	Negative
Anti-C1q	Negative	Negative	Negative
C3, mg/dl	99	76	83-193
C4, mg/dl	19	10	15-57
Anti-myeloperoxidase-ANCA	Negative	Negative	Negative
Anti-proteinase 3-ANCA	Negative	Negative	Negative
Anti-glomerular basement membrane antibodies	Negative	Negative	Negative
Lupus anticoagulant	Positive	Negative	Negative
Anti-β2-glycoprotein antibodies	Negative	Negative	Negative
Anti-cardiolipin antibodies	Negative	Negative	Negative
HBV surface antigen	Negative	Negative	Negative
anti-HBV surface antibody, IU/l	>1,000	Negative	0-10
Anti-HBV core antibody	Negative	Negative	Negative
Anti-hepatitis C antibody	Negative	Negative	Negative
Anti-HIV	Negative	Negative	Negative
IgG, mg/dl	2,127	695	552-1,631
IgA, mg/dl	328	259	65-421
IgM, mg/dl	251	206	33-293
Proteinuria, mg/dl	1,000 → 30	1,000	0-30
Hematuria, red blood cells/μl	100 → 150	2,400	0-1
Proteinuria, g/day	8 → 2	6.8	<0.15

[a], → is the evolution of the laboratory parameter. C3/4, component 3/4 of complement system; ANCA, antineutrophil cytoplasmic antibodies; HB, hepatitis B virus.

FITC C3c antiserum (Dako; Agilent Technologies, Inc.; cat. no. F020102-2); FITC Fibrin-Fibrinogen (Dako; Agilent Technologies, Inc.; cat. no. F011102-2); FITC C1q Complement antiserum (Dako; Agilent Technologies, Inc.; cat. no. F025402-8); FITC Kappa Light Chains (Dako; Agilent Technologies, Inc.; cat. no. F019802-2); FITC Lambda Light Chains (Dako; Agilent Technologies, Inc.; cat. no. F019902-2). Each slide was washed twice in PBS 10% for 15 min, and mounted with glycerin. Each biopsy had internal staining patterns (negative and positive internal controls). For each biopsy a tissue section incubated

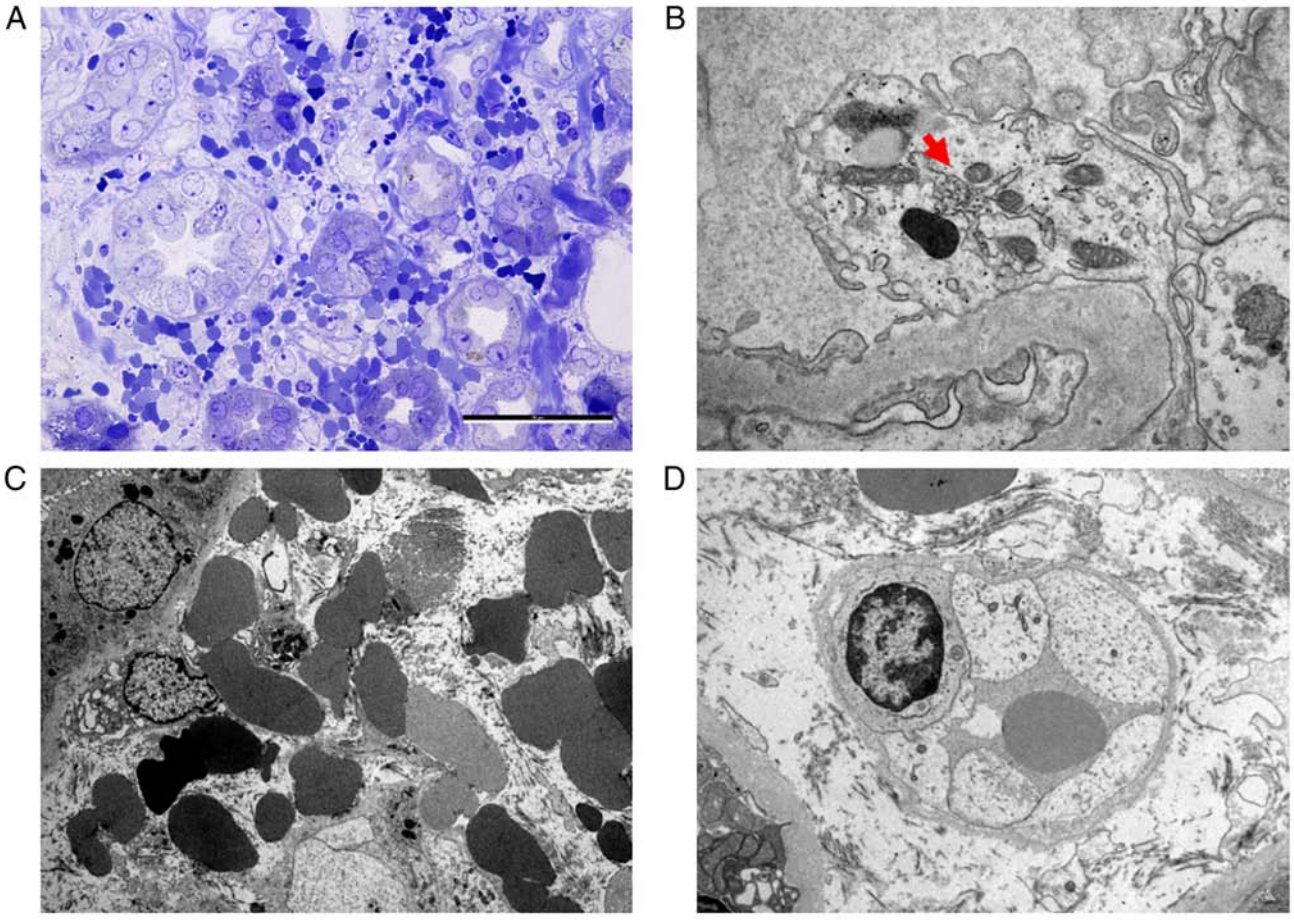

Figure 1. Kidney biopsy findings. (A) Light microscopy image showing marked interstitial hemorrhage located in the renal medulla with very few plasma cells and lymphocytes (semithin section, toluidine blue); (B) Electron microscopy image of a glomerular endothelial cell with a tubulo-reticular inclusion in the cytoplasm (red arrow; original magnification, x11,000); (C) Electron microscopy image demonstrating numerous red blood cells located in the interstitial space of the renal medulla (original magnification, x11,000); (D) Electron microscopy image showing a peritubular capillary from the renal medulla with endotheliosis (original magnification, x22,300). (The images belong to the collection of the Nephrology Department of The Fundeni Clinical Institute).

with a PBS and omitting the conjugated antibody was used as a negative staining control. The fluorescence was assessed under Leica DM6000B light microscope with epifluorescence module using the UV excitation and dedicated filters for FITC (Leica Microsystems GmbH). Representative images were recorded under a Leica DM6000B light microscope with a Leica DFC310FX digital camera using Leica LAS core-package software (Leica Microsystems GmbH). Immunofluorescence (IF) was negative. Light microscopy (LM; data not shown) revealed normal arterioles and glomeruli, dilated proximal tubules with vesicles near the apical membrane and erythrocytes in the lumen. However, the most striking finding was the significant interstitial hemorrhage in the medulla, without inflammation or interstitial fibrosis.

For electron microscopy (EM) the fragments of kidney tissue were fixed by immersion in 4% glutaraldehyde, buffered with 0.1 mol L^{-1} sodium cacodylate (pH 7.3) at 4˚C overnight. After two baths in 0.1 M cacodylate buffer, the samples were post-fixed for 1 h in 1% OsO4 with 1.5% K4Fe(CN)6 (potassium ferrocyanide-reduced osmium) in 0.1 M cacodylate buffer, at room temperature. Afterwards, the samples were dehydrated through increasing ethanol and further processed for Epon-embedding (Agar100 resin, Agar Scientific) at 60˚C. for 24 h. Epon-embedded kidney tissue fragments were sectioned using a Leica EM UC7 ultramicrotome (Leica Microsystems GmbH). Light microscopy was performed on 1-μm-thick sections stained with 1% toluidine blue for 1 min at 80˚C. Representative images were recorded under a Leica DM6000B light microscope with a Leica DFC310FX digital camera using Leica LAS core-package software (Leica Microsystems GmbH). Selected areas from Epon-embedded blocks were sectioned for TEM with a Leica EM UC7 ultramicrotome and mounted on 50-mesh copper grids (Agar Scientific). Electron microscopy imaging was performed on 60-80 nm ultra-thin sections counterstained with uranyl acetate for 15 min at room temperature and Reynolds lead citrate (Agar Scientific) for 5 min at room temperature. The ultrastructural analysis was performed at 80 kV on a Morgagni 268 TEM (FEI Company), equipped with a MegaView III CCD (Olympus Soft Imaging Solutions GmbH) and running iTEM-SIS software (Olympus Soft Imaging Solutions GmbH). EM showed glomeruli with mild glomerular endotheliosis, rare tubulo-reticular inclusions in the endothelial cells and segmental foot process effacement. In addition to the severe hemorrhage in the medulla, EM also showed extensive endotheliosis of the peritubular capillaries (Fig. 1). The presence of severe hemorrhagic interstitial nephritis suggested a Hantavirus infection, which was further confirmed by the positive serology for both IgM and IgG antibodies. The patient

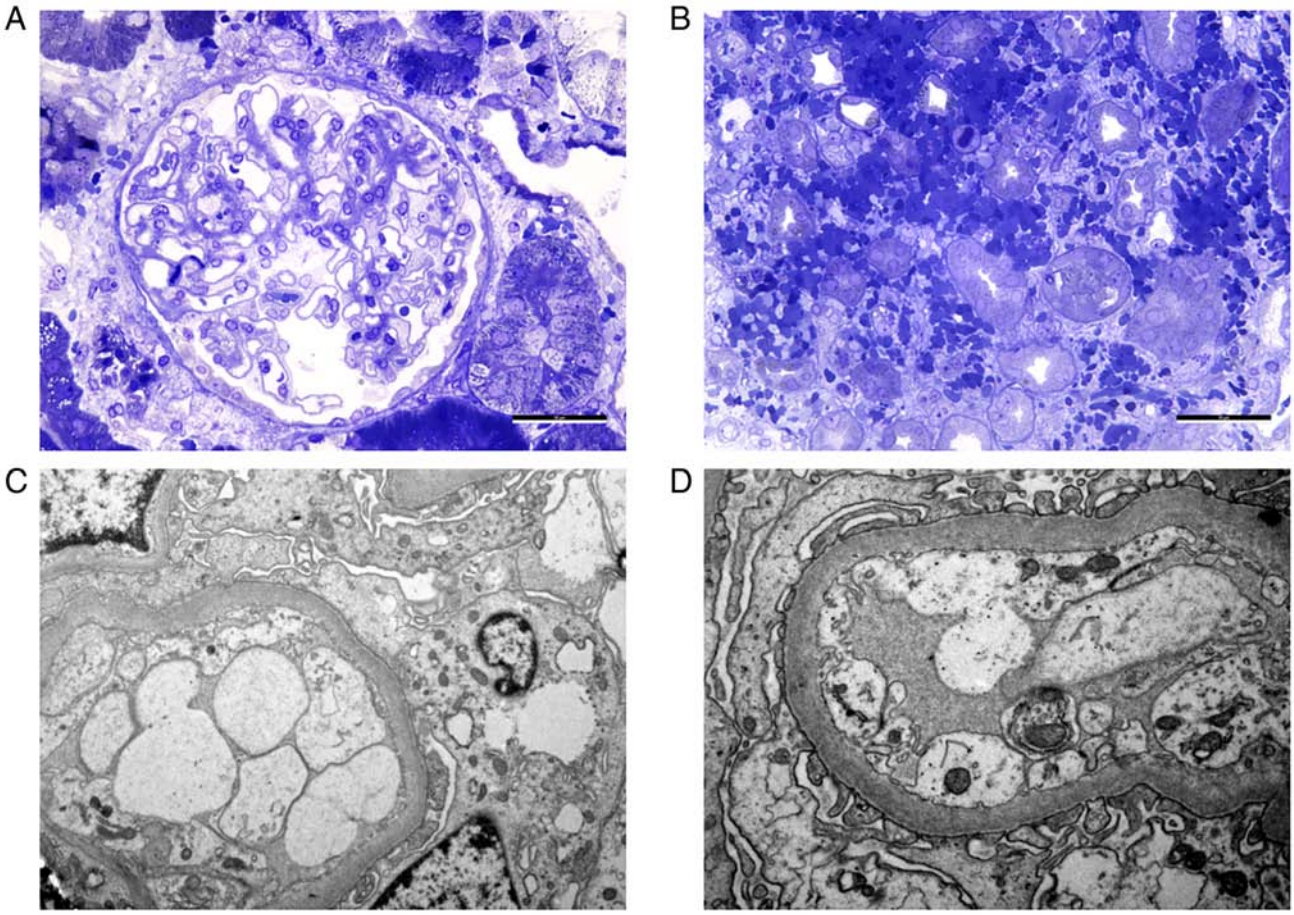

Figure 2. Kidney biopsy findings. (A) Light microscopy image showing a normal glomerulus (semithin section, toluidine blue); (B) Light microscopy image showing severe interstitial hemorrhage located in the renal medulla (semithin section, toluidine blue); (C,D) Electron microscopy images showing two glomerular capillaries with marked endotheliosis and loss of normal fenestrations with associated podocyte foot process effacement (original magnification x12,000). (the images belong to the collection of the Nephrology Department of The Fundeni Clinical Institute).

received angiotensin-converting enzyme inhibitor in maximal dose (ramipril 10 mg/day) and oral steroid methylprednisolone 16 mg/day for 2 weeks, with gradual tapering during the next 2 months. The evolution was favorable, with the normalization of kidney and liver function, but with the persistence of the changes suggesting interstitial nephritis (sterile leukocyturia, mild proteinuria and hypokalemia). At discharge, the patient also received spironolactone (25 mg/day) for hypokalemia and the prevention of kidney fibrosis. At the 12-week follow-up, the patient presented with normal blood and urine values.

Case 2. A 30-year-old male patient with no medical history was hospitalized for oliguria (400 ml/day) for >48 h and altered general status. The symptoms started 8 days before with fever (39˚C), nausea, vomiting and diarrhea, for which the patient self-medicated with antibiotics (levofloxacin 500 mg/day), with a slight improvement of the fever but with the persistence of the gastrointestinal discomfort and a progressive drop in diuresis.

The physical examination revealed a patient in obvious distress, with slightly pale skin, no edema, bilateral pleural rub, high blood pressure (147/92 mmHg) and oliguria with gross hematuria.

The laboratory evaluation (Table I) showed mild normocytic normochromic anemia with raised LDH, normal haptoglobin and positive Coombs test, severe thrombocytopenia (5,000 elements/mmc), positive D-dimers, inflammation (leukocytosis with neutrophilia and raised C-reactive protein), severe kidney impairment and liver cytolysis. Urinalysis revealed proteinuria, hematuria and glycosuria, and 24-h proteinuria was 6.8 g with hypoalbuminemia. The immunological tests revealed complement consumption, normal immunoglobulins level, negative screening for autoimmune diseases with potential kidney involvement, negative antiphospholipid antibodies and negative serology for viruses (hepatitis B and C, HIV, cytomegalovirus and Epstein-Barr virus) and *Leptospira*. The ADAMTS 13 enzymatic activity was normal. An abdominal ultrasound showed normal kidneys with no signs of obstruction, mild ascites and mild bilateral pleural effusion.

Similarly to the previous case, the diagnosis was stage III AKI. The patient also had severe thrombocytopenia, anemia with normal haptoglobin and a positive Coombs test. A KB was performed. The biopsy specimen showed macroscopic features of hemorrhage in the renal medulla. IF was negative. LM revealed normal glomeruli and massive interstitial hemorrhage in the medulla, with no associated interstitial inflammation. The EM showed a glomerulus with segmental narrowing of the lumen due to endothelial swelling and segmental foot process effacement (Fig. 2). A diagnosis of hemorrhagic interstitial nephritis with mild glomerular abnormalities was made and Hantavirus infection was considered

as a differential diagnosis. Serology was positive for both IgM and IgG antibodies against Dobrava virus (DOBV) strain. The patient received fluid replacement therapy and was started on intravenous methylprednisolone (500 mg/day for 3 days), followed by oral methylprednisolone (32 mg/day). The patient also required renal replacement therapy consisting of four hemodialysis sessions at 2 days apart. The evolution was good, with the recovery of the kidney function but with polyuria during the first days, normalization of the liver enzymes and resolution of the thrombocytopenia.

Discussion

Hantaviruses are a member of the Bunyaviridae family, including over 28 serotypes which cause infections in humans. Wild rodents are the main hosts. It is considered a rare infectious disease but with an increasing incidence during the recent years (10). The virus was first identified on pulmonary tissue from the striped field mouse *Apodemus Agrarius* (11). The serotypes which are found in Europe and Asia belong to the Old-World Hantaviruses group; Hantaan (HNTV), Puumala (PUUV), Seul, Dobrava (DOBV), the Tula virus (TULV) and the Saaremaa virus (SAAV) (3). The DOBV is mostly found in the Balkan Peninsula and it is responsible for a 12% mortality rate (4,12). These serotypes use several species of wild rodents as hosts, such as *Apodemus agrarius*, *Rattus rattus*, *Microtus arvalis* and *Myodes glareolus* (13,14), but they can also spread to other wild or domestic animals, including moose, bat, fox, cat and dog (15-18).

The European Centre for Disease Prevention and Control reported 1,826 cases of Hantavirus infection in 2018, most of the cases (97%) involving PUUV (19). In the same year, the Activity report of The National Public Health Institute from Romania included 40 cases of suspected Hantavirus infection in patients from the region and only one confirmed case (20). Several mild and moderate forms of HFRS remain undiagnosed and so the incidence of the disease in Romania may be underestimated. Regarding the current two cases, the patients were infected by HNTV (case 1) and DOBV (case 2); they both live in Buzau County, in a rural area, at a small distance from each other (30 km). DOBV presence was confirmed in Central and South-Eastern Europe, but not in Romania. It was also surprising to identify HNTV, which is usually found in China and Russia, in case 1. It raises the question of whether the infection is not underdiagnosed in Romania and whether the HNTV is not already present in the Balkan peninsula. Both DOBV and HNTV have the same reservoir, the mouse *Apodemus Agrarius*, which is found in Romania.

Hantavirus is transmitted to humans through aerosolized particles containing the virus from saliva and droppings shed by rodents or through bite (3,4,21). Individuals from rural areas or areas that are natural reservoirs for rodents (farmers and hunters) are more prone to infection.

Hantaviruses cause tubular lesions by altering the tight junctions between the tubular epithelial cells, disrupting the podocyte cytoskeleton, and also causing an endothelial dysfunction with increased vascular permeability. The virus penetrates the target cells via $\alpha v\beta 1$ and $\alpha v\beta 3$ receptors (22). The immature dendritic cells can serve both as viral carriers to the lymph nodes and the epithelial cells and as antigen-presenting cells that will stimulate the T lymphocytes (23,24). The endothelial lesions are mostly caused by the cytokine storm caused by T lymphocytes. The complement pathway is activated and cytokines such as IL-10 and IL-6, IFN, TGF and TNF-α are secreted (4,25). The role of the podocyte injury is supported by the increased serum levels of the mediators involved in the regulation of podocyte function, such as urokinase-type plasminogen activator receptor, VEGF and IL-6 (26).

The manifestations of HFRS can be grouped into several stages. The disease begins with fever (3-7 days) and flu-like symptoms (myalgia and headache), then it evolves to a hypotensive phase (maximum 2 days) which can be accompanied by thrombocytopenia, leukocytosis, petechial rash, gastrointestinal hemorrhage and hematuria. These manifestations are followed by the oliguric phase (3-7 days) with AKI development, then the polyuric phase with the recovery of the renal function (up to a few weeks). There are cases which develop long-term complications, such as arterial hypertension and chronic kidney disease (27-29).

Thrombocytopenia is the result of increased peripheral consumption and is regarded as a marker of severity. The virus facilitates the adhesion of the platelets to the surface of infected endothelial cells through the $\beta 3$-integrin receptor, resulting in the alteration of platelet activation and thrombocytopenia. Furthermore, endothelial lesions can promote coagulation activation and fibrinolysis, resulting in increased prothrombin and D-dimer levels (30,31). The second patient developed more severe thrombocytopenia and positive D-dimer values. The patient also had a more severe clinical evolution with AKI requiring dialysis, in agreement with the literature data that state that platelet count is a predictor for disease severity and progression (32,33). PUUV infection causes complement alternative pathway activation, resulting in reduced component 3 of the complement system (C3) and increased C5b-9 serum levels in the acute phase of the disease in patients who have chest X-ray abnormalities (34-36). The second case, infected with DOBV, developed bilateral pleural effusions and low complement levels (C3 and component 4 of the complement system).

Hantavirus infection can result in other acute extra-renal complications, such as meningoencephalitis, pituitary gland hemorrhage, pericarditis, myocarditis, pulmonary edema, and disseminated intravascular coagulation.

The most frequent chronic complications are hypothyroidism, arterial hypertension, membranoproliferative glomerulonephritis and chronic tubulointerstitial nephritis (37-42). The first patient developed hypertension during the polyuric phase of AKI, which showed that the pathogenesis was not the hypervolemia of the oliguric phase. One of the proposed mechanisms for the development of hypertension is the injury of the small vessels of the kidneys, accompanied by significant interstitial and tubular inflammation. Any factors that cause vasoconstriction in the renal medulla and adjacent cortex can induce hypertension. This theory is supported by histology (congestion and hemorrhage around the vessels in the outer medulla and the cortico-medullary junction) and by the endothelial injury caused by the virus (38). At 4 weeks after discharge, the first patient developed mild proteinuria and leukocyturia with sterile urine culture, indicating persistent tubulointerstitial nephritis.

Kidney biopsies usually show lesions of acute tubulointerstitial nephritis. The pathognomonic lesion is the interstitial hemorrhage in the outer medulla (42,43). IF is usually negative. EM reveals diffuse foot processes effacement, cells with enlarged Golgi apparatus, denudation of the tubular epithelial cells and endothelial swelling (43). The KB specimens obtained during the acute phase of the disease from the present patients raised the suspicion of Hantavirus infection immediately after tissue removal, as they revealed macroscopic features of hemorrhage in the medulla. The first patient, with higher proteinuria, had resorption droplets at the apical pole of the proximal tubular epithelial cells. EM showed extensive foot process effacement and detachment of the podocytes from the glomerular basement membrane, which explained the nephrotic range proteinuria seen in both cases and emphasized the role of the alterations of the cytoskeleton of the podocytes in the pathogenesis of the disease.

The infection with Hantavirus has to be suspected in all cases with fever together with AKI and thrombocytopenia, and confirmed by serology testing. The most frequently used laboratory methods for the detection of antibodies are indirect ELISA for IgM and IgG and the immunochromatographic test.

The treatment consists mostly of conservative measures and symptomatic therapy. A series of studies supported the use of Ribavirin, but only when administered in the early phases (44-46). Corticosteroids can be used, as they target the secretion of cytokines and complement activation (47). In the present study, both patients received steroids. Their evolution was eventually favorable. DOBV and HNTV serotypes usually have more severe manifestations. Only the second case evolved as a severe form. The different evolution could have been determined by the earlier administration of corticosteroids in the first case (5 days after onset), compared to the second one (9 days after the onset and after starting dialysis).

Hantavirus endemic nephropathy belongs to a group of rare zoonoses in Romania and South-Eastern Europe. Dobrava and Puumala are the most frequent serotypes that cause the disease in this geographical area. The first case had positive serology for the Hantaan strain, which draws attention to a possible underdiagnosis of the disease in these regions and also to the need for more epidemiological studies, since they may pose major risks to public health. The disease manifests with fever and evolves with AKI, anemia, severe thrombocytopenia and coagulation disorders. The symptoms are similar to TMA secondary to the hemolytic uremic syndrome, this being one of the major differential diagnoses. Due to the rarity of infection in the South-Eastern European countries, we face multiple challenges, one being the absence of any initial suspicion. Furthermore, it is necessary to include this infection in the algorithm of differential diagnosis of disorders characterized by thrombocytopenia, anemia, liver cytolysis and acute kidney injury.

Acknowledgements

The authors wish to thank to Professor Mihaela Gherghiceanu (Victor Babes National Institute of Pathology, Bucharest, Romania), Dr Ilinca Radu (Carol Davila University of Medicine and Pharmacy, Bucharest, Romania) and Mrs Emilia Gheoda (Principal Biochemist, Synevo Romania Srl, Romania), for all the support in writing this manuscript.

Funding

Publication of this study was supported by the University of Medicine and Pharmacy Carol Davila, through the institutional program Publish not Perish.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

GL, IV and ML conceived and designed the present study. RS was responsible for methodology. CA, IA and MB were responsible for data curation. IA, GI and GL were responsible for analysis and interpretation of data. AA, MB and RS were responsible for writing, review and editing. GL was responsible for supervision. GI, AA and ML were responsible for project administration. GL and AA confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Fundeni Hospital (Bucharest, Romania; approval no. 21713).

Patient consent for publication

Written informed consent was obtained from each patient for the publication of the present study.

Competing interests

The authors declare that they have no competing interests.

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