S.10.C.6 Hantavirus-induced acute renal failure

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Introduction

In the past, acute renal failure from Hantavirus infection was regarded as a curiosity of the far East (hemorrhagic fever with renal syndrome, HFRS) and Northern Europe (nephropathia epidemica). In the far East, hemorrhagic fever had been known for decades, but attracted considerable attention only during the Korean war. It is a severe disease which carried then a mortality of 30–40% (now approximately 5%). It is characterized by mucosal hemorrhage, acute renal failure and cardiac involvement. A more benign disease, nephropathia epidemica, has long been known in certain regions of Northern Europe. It is characterized by low mortality, but a high rate of renal involvement. Both diseases do not cause major residual renal damage.

Hantavirus infection has only recently been identified as an important cause of primary acute renal failure in patients from Western Europe. Using virological methodology, Lee *et al.* [1] identified the virus, a member of the Bunyaviridae viruses.

In the following chapter we describe the virological background, clinical presentation, diagnosis, and management of such patients.

Virological background

Hantaviruses cause a variety of human diseases referred to as hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS), respectively [2, 3]. As members of the Bunyaviridae family Hantaviruses possess a negative-strand RNA genome consisting of three segments (S = small, M = medium and L = large) which encode three structural proteins, i.e. the nucleocapsid protein (N, encoded by the S segment), and two envelope glycoproteins (Gl and G2, encoded by the M segment) as well as a single nonstructural protein, the viral polymerase (encoded by the L segment), which transcribes the viral sense RNA into messenger RNA [4]. Genomes of Hantavirus isolates and genome fragments identified by polymerase chain reaction, respectively, reveal considerable nuclear acid sequence variability. On this basis, several genomic groups can be distinguished which correspond to immunologically defined serotypes (Table 1) [5, 6, 7]. The viruses are associated with various rodent species as their principal reservoirs, and with various disease manifestations in man. The variety of viruses and host associations is explained by coevolution of Hantaviruses and their rodent hosts. In man, serotypes Hantaan, Seoul and Belgrade cause the severe type of HFRS, which is prevalent in South East Asia. It is also termed Korean hemonhagic fever (KHF). This fever is characterized by a high rate of hemorrhagic manifestations. In contrast, the serotype Puumula is the causative agent of a mild variant of HFRS which is also known as nephropathia epidemica. In May 1993, in the Southwest of the United States [3], a previously unknown clinical manifestation of Hantavirus infection was

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Table 1. Hantavirus serotypes causing disease in man.

serotype	clinical manifestations	principal host reservoir	geographic distribution
Hantaan	HFRS, Korean hemorrhagic fever (KHF)	*Apodemus agrarius* (striped field mouse) *Apdemus flavicollis* (yellow-necked mouse)	South East Asia Southern Europe
Puumala	HFRS, Nephropathia epidemica	*Clethrionomys glareolus* (bank vole)	Central and Northern Europe
Seoul	HFRS, milder variant of KHF	rats	worldwide
Belgrade/Dosrava	HFRS, severe variant (similar to KHF)	*Apodemus flavicollis* (yellow-necked mouse)	Balkan States
Four Corners	Hantavirus pulmonary syndrome	*Peromyscus maniculatus* (deer mouse)	United States (with the exception of East Coast), Canada
Bayou	Hantavirus pulmonary syndrome	unknown, not *P. maniculatus*	United States, East Coast
Black Creek Canal	Hantavirus pulmonary syndrome	*Sigmodon hispidus* (cotton rat)	United States, Florida, Southeast

recognized which was later called Hantavirus pulmonary syndrome (HPS). The first cases appeared amongst Navajo Indians living in the Four Corners region (border region of the Federal States of Arizona, New Mexico, Colorado and Utah), but soon further cases were diagnosed in other states as well. In the sera of HPS patients, antibodies against Hantaviruses could be detected by means of serodiagnostic assays. Nucleotide sequence analysis of PCR products amplified from patient materials identified a new genomic group of Hantaviruses, which is now termed Four Corners virus. The virus is associated with *Peromyscus maniculatus* (deer mouse) as its main rodent reservoir. In the meantime, additional serotypes (Bayou virus, Black Creek Canal virus) and subtypes have been described which cause HPS. They are associated with different rodent species [8, 9].

Epidemiology

HFRS and HPS are zoonotic infections that occur in geographical foci. Hantaviruses induce persistent infections in their rodent hosts, which shed the virus in high quantity in urine and feces [10]. Transmission to man occurs by aerosolized rodent excretions or by direct contact with the rodents. In proteinaceous materials Hantaviruses are able to remain viable for several days. Transmission does apparently not occur from man to man. Even in the 1993 HPS outbreak no direct (e.g. nosocomial) transmission could be observed. In Central Europe, seasonal population peaks of rodents in spring and autumn correlate with the incidence of Hantavirus infections in man. Such surges in the rodent population are as well observed periodically every three to four years. They occur also in relation to climatological and environmental changes [11].

The *Apodemus*-associated Hantaan serotype predominates in South East Asia as well as in Russia and Greece, whereas the Puumala serotype is endemic in Central and Northern Europe. Its principal reservoir host is the bank vole, *Clethrionomys glareolus*, whose preferred habitat is beech forests, but Puumala virus has also been detected in *Microtus spp.*, *Mus musculus* and even in insectivora such as shrews, although it is currently unclear which role these hosts play in the maintenance of the virus in nature. Viruses belonging to serotype Belgrade are endemic in the Balkan States. Apparently, they coexist in this geographical area with serotypes Hantaan and Puumala. The serotype Seoul has been detected worldwide in rat populations, and presumably, has spread via the international waterways. This serotype has also been found in laboratory animals and can induce HFRS among laboratory workers. The deer mouse *Peromyscus maniculatus*, which is the principal reservoir host of Four Corners virus (FCV), occurs on the entire territory of the United States with the exception of the East coast. Investigations of rodent populations by means of PCR have shown that FCV is prevalent in up to 30% of deer mice [12]. Virus variants which are locally endemic in mice are homologous to the variants found in HPS patients of the corresponding area. It can therefore be concluded that the HPS outbreak in 1993 was not induced by the spread of a new virus. Rather,

FCV had already existed in the rodent populations for a long time. Presumably, a surge in the reservoir host population has led to a cluster of cases in 1993 and subsequently to the discovery of this new Hantavirus strain. The number of cases of HPS has continuously increased ever since. An endemic disease pattern was noted with sporadic occurrence of HPS in the entire endemic area. By May 1995 a total of 107 cases had been recorded by the Centers for Disease Control (CDC, Atlanta), with an overall lethality rate of 55% at that time. Besides the serotypes mentioned above, others exist which so far have not been associated with disease in man (Prospect Hill virus, Tula virus, Thottapalayam virus, Thailand virus).

The annual incidence of Hantavirus infections is up to 200.000 cases worldwide [13, 14]. In China alone up to 150.000 cases per year have been observed. Up to 2,000 cases occur annually in Korea and other countries in the Far East, and about 10,000 cases are reported from Russia. A good estimate of the cases occuring annually in Central and Northern Europe would be several hundred.

In Germany, a total of about 400 clinical cases have so far been documented [15, 16, 17]. Serosurveys revealed an average antibody prevalence of 1.8% in West Germany, with a range of 1.2% to 3.1% [18]. In East Germany the seroprevalence was 1.2%. Endemic areas with a significantly elevated antibody prevalence of the local population (up to 3.1%) were identified, as well as risk groups with a presumed occupational exposition to the reservoir hosts (up to 26%). Soldiers have been identified as a high risk group. In World War I thousands fell ill with "war nephritis" which, in retrospect, was probably Hantavirus infection. In the Korean War more than 3,000 American and Korean soldiers suffered from Korean Hemorrhagic Fever and about 10% died [19]. During world war II 10,000 German soldiers fell ill with nephropathia epidemica in Finland. In 1990, a small outbreak of HFRS occurred among American soldiers during a manoeuvre near Ulm (Southern Germany) [20].

Clinical manifestations

Prognosis

Course and prognosis of Hantavirus-induced acute renal failure vary according to geography. Prognosis is grave in East Asia [2], Eastern Russia and some Balkan countries. In the Balkans both Puumula and Hantaan subtypes are found. This is of special interest, since increasing tourism and extensive travelling expose Western citizens to the severe type of infection when travelling in Balkan countries. Mortality from Hantavirus infection acquired in Balkan countries may reach 20% [21], as reported from Greece by Siamopoulos [21]. Prognosis is much better in Central and Northern Europe where Hantavirus subtype Puumula prevails. One fatal case has been reported [22]. Approximately one third of the patients must be admitted to intensive care units. Renal function recovers in almost all patients within a few weeks.

There is a slight but definite possibility of long-term renal damage. In 1989, two Greek patients were described who developed chronic renal failure. In our own series 2 out of 30 patients developed hypertension, another 2 patients exhibited mild persistent reduction of GFR (40 and 55 ml/min) and 1 patient showed persistent proteinuria [23]. Chronic hematuria, proteinuria and renal impairment were also reported by Yugoslav authors [24]. Hypertension in a patient who had recovered from Hantavirus infection was described by Kleinknecht [25]. In contrast, not a single case of chronic renal failure has been observed amongst 74 Swedish patients who had recovered from Hantavirus infection [26].

Clinical presentation

Fig. 1 schematically depicts the clinical course of nephropathia epidemica (Puumula subtype infection), i.e. the most common infection in Central and Northern Europe and in the Western part of Russia. The typical clinical signs which should alert the clinician to consider the diagnosis are summarized in Table 2 [27].

One can schematically subdivide the evolution of Puumula virus infection into three stages [28].

After an incubation time of 5–35 days, a first phase lasting from 3–4 days is characterized by high fever, myalgia, headache, photophobia and pharyngeal enanthema. A discrepancy is noted between high fever and presence of bradycardia, pointing to latent or overt myocarditis. Occasionally, transient visual impairment is found. In this prodromal period, analgesics, antipyretics and antibiotics are often prescribed. In the past, interstitial nephritis had erroneously been ascribed to such medication.

In a second phase, usually 3–6 days after the beginning of fever, severe lumbalgia and abdominal pain is observed. This may lead to unnecessary diagnostic and/or surgical interventions, e.g. i.v. pyelogram because of nephrolithiasis, or appendectomy because an acute abdomen is suspected. Administration of contrast media, or surgical trauma,

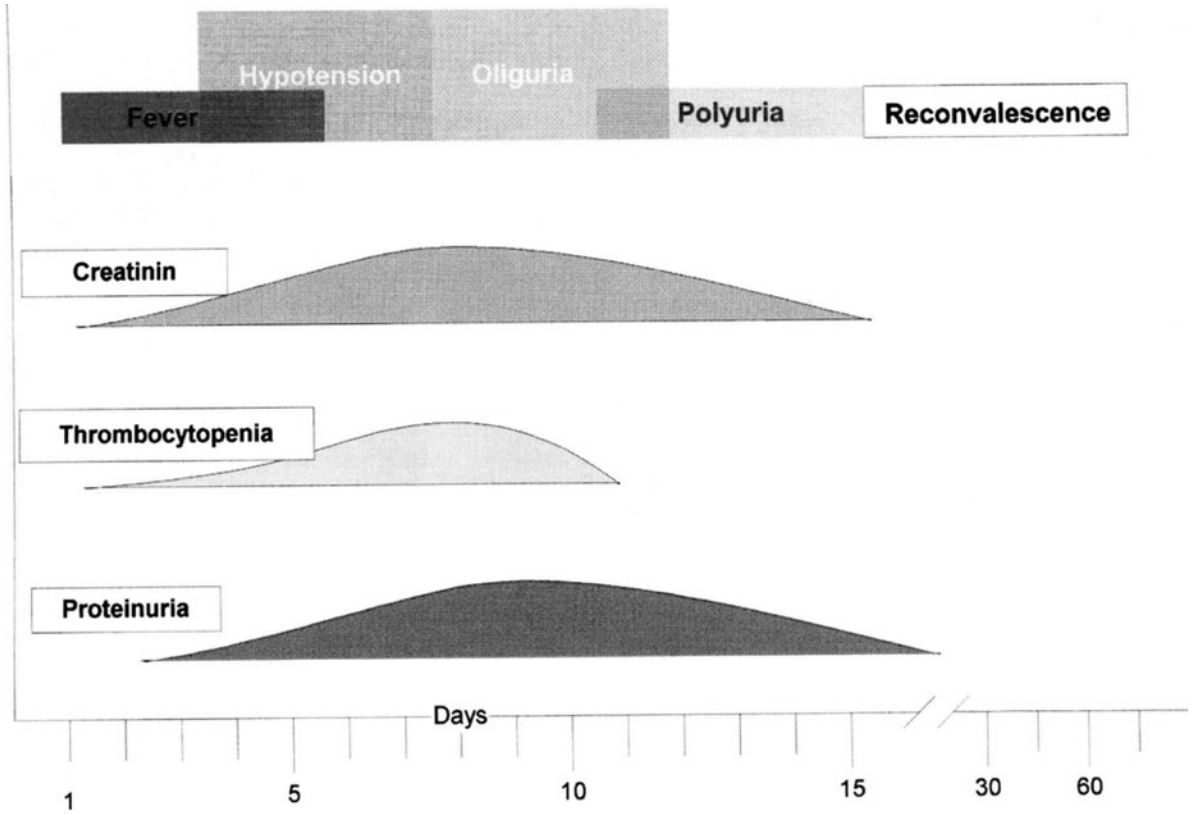

Figure 1. Schema of the evolution of nephropathia epidemica.

certainly aggravates acute renal failure. Table 3 summarizes common diagnostic errors.

A third phase, approximately 4–10 days after the acute onset of fever, is dominated by renal involvement. Renal failure, i.e. azotemia and oliguria, develop. Proteinuria may be quite intense and microhematuria is almost obligatory. The climax of acute loss of renal function is usually reached after approximately 10 days. A considerable proportion of such patients require temporary hemodialysis.

Table 2. Clinical presentation of hemorrhagic fever with renal syndrome.

	%
sudden onset	95
fever (>39°C)	95
lower back pain	81
abdominal pain or colic-like pain	50
diarrhea	31
epistaxis	12
hematemesis	5
meningism	7

Renal manifestations

Renal involvement is characterized by interstitial nephritis (Fig. 2), with prominent interstitial edema

Table 3. Diagnostic errors in disease caused by Hantavirus infection (admission diagnosis in 42 cases of HFRS).

	(n)
acute abdomen	3
lumbago	2
diarrhea salmonellosis	5
sepsis	3
pyelonephritis	3
nephrolithiasis	3
analgesics-induced renal failure	3
acute pancreatitis	2
circulatory collapse	1
meningitis	1
leptospirosis	1
thrombotic-thrombocytopenic purpura	1
other	14

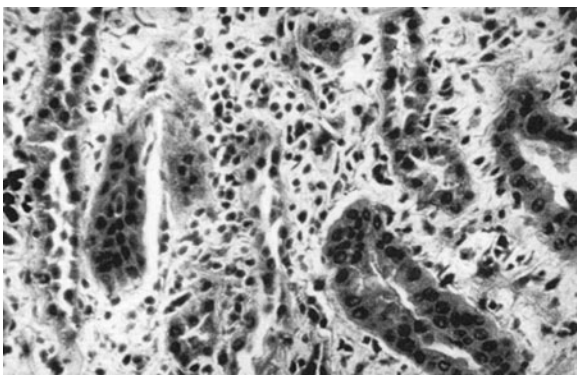

Figure 2. Renal biopsy in a case with acute renal failure resulting from nephropathia epidemica. Hematoxylin-eosine stain, ×240.

and capillary congestion at the border zone, between cortex and medulla. These features presumably reflect endothelial cell damage from viral infection [29, 30]. In contrast to previous opinion, glomerular lesions are also common, i.e. mesangial hypercellularity and mesangial expansion. The severity of glomerular lesions is disproportionately mild in relation to the potentially marked proteinuria, i.e. up to 6 g/24 h [31]. Early on in the disease, atypical uroepithelial cells are found in the urine. They are characterized by large nuclei, increased nucleus/cytoplasm ratio and nuclear hyperchromasia (Fig. 3). Such transformed cells may be mistaken for malignant uroepithelial cells. Such cells disappear spontaneously in the further course of the disease.

Extrarenal manifestations

Hantavirus infections cause generalized specific involvement of endothelial cells, as clinically reflected

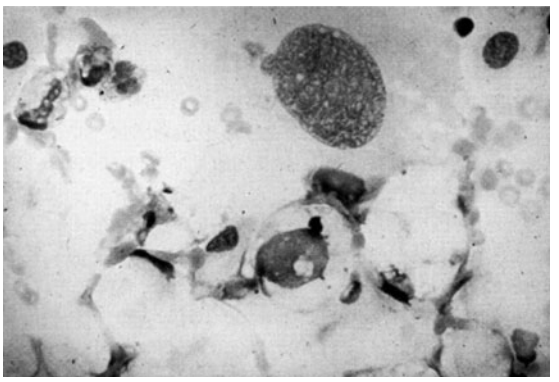

Figure 3. Urinary sediment exhibiting atypical uroepithial cells. Papanicolau stain.

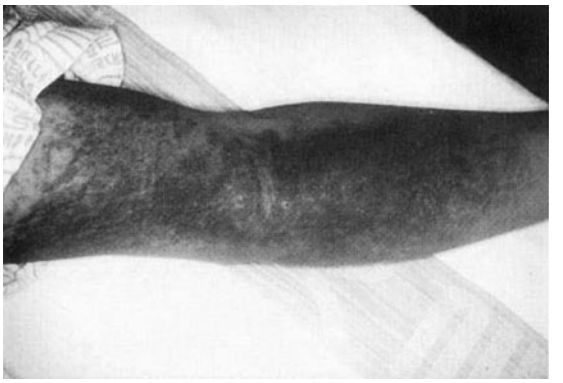

Figure 4. Severe cutaneous hemorrhage in a patient with nephropathia epidemica.

by subtle or profuse cutaneous (Fig. 4) or gastrointestinal hemorrhage. Gastrointestinal bleeding is seen in severe cases and may require multiple blood transfusions [35].

Table 4 summarizes the clinically most important extrarenal manifestations. Involvement of the central nervous system is characterized by generalized seizures and acute hypophyseal failure (Sheehan syndrome), resulting from hemorrhage into the hypophysis (Fig. 5). This condition causes severe hypotension and carries a grave prognosis [32]. Myocardial involvement is common. As early as 1954 [3] changes in the ECG were recognized in soldiers during the Korean war. Characteristic is an inappropriately low pulse rate in the presence of high body temperature [31]. Severe cardiac complications include hemorrhage into the right atrium and left ventricle [34].

Mild hepatitis is frequent and explains elevation of liver enzymes. Histology shows periportal infiltration [36].

Some cases show pancreatic involvement with increased amylase.

Hantavirus pulmonary syndrome

The Hantavirus pulmonary syndrome (HPS) is a form of ARDS. In HPS the duration of prodromi is considerably shorter, being 2–3 days. Interstitial lung

Table 4. Clinically most important extrarenal manifestations

CNS, eyes, hypophyses
myocard
GI

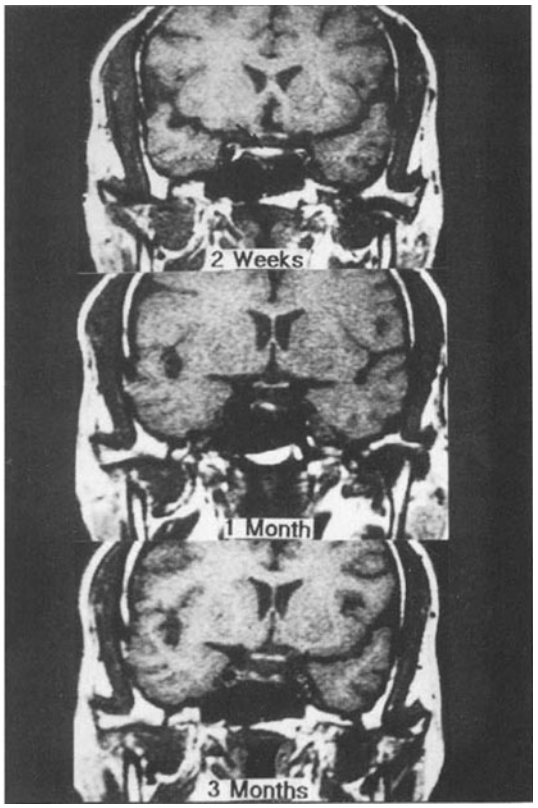

Figure 5. Hypophyseal hemorrhage leading to Sheehan syndrome; in a Korean patient with HFRS (courtesy Professor Lee, Seoul, University Hospital).

edema may develop within hours and lead to ARDS. The first cases in the South-West of the US, the so-called Four-Corners-Region, had no renal involvement [37]. More recently however, cases with both ARDS and renal manifestations have been described [38]. Although this had not been appreciated previously, pulmonary involvement is also common in Puumula virus infection, particularly in cases with hypoproteinemia and leucocytosis [39].

A case presenting solely with lung manifestations has recently been reported from Northern Germany, caused by a Puumala subtype called Berkel virus (40), but this observation has not been universally accepted.

Diagnosis

The clinical diagnosis of HFRS or HPS must usually be confirmed by a specific laboratory assay. Early in the course of infection, patients develop antibodies of the IgM and IgG type. It could be shown that the initial humoral immune response is primarily directed against the nucleocapsid protein, which also induces the strongest response [41, 42]. In most patients antibodies can be detected at the time of onset of symptoms, if sensitive methods are used. The IgG response reaches its maximum within a few weeks and persists over years, probably for life. In the past, the indirect immunofluorescence assay was extensively used for serodiagnosis of HFRS [43, 44, 45]. In this assay, virus-infected Vero E6 cells serve as antigens. The IgM-immunofluorescence assay, however, was reported to have low sensitivity by some authors.

In our own experience, almost all cases diagnosed in Germany were positive by IgG and IgM specific immunofluorescence assays within a few days after the onset of clinical manifestations. However, IgM antibodies remain detectable only for a period of about two weeks. If IgM specific assays are negative, the diagnosis can be made on the basis of a significant rise of the IgG antibody titer. In nonendemic areas, a single IgG titer of >128 is also a strong indicator of acute infection.

A highly sensitive assay, which is now widely used for the detection of IgM antibodies, is the μ-capture enzyme immunoassay. Native viral or recombinant antigens are used to detect specific IgM antibodies [46, 47]. A suitable recombinant antigen for this purpose is the full-length nucleocapsid protein, which has been expressed in *E. coli* [46, 48, 49]. Most patients are positive in the assay within the first few days after onset of symptoms, or even before. Maximum extinctions are reached between days 8 and 25 and most patients have lost detectable IgM after two to three months, although in a few cases IgM persisted up to one to three years after the acute phase.

Further IgG and IgM enzyme immunoassays, designed as classical solid-phase assays, have been developed, and are commercially available. They employ recombinant nucleocapsid proteins of Hantaviruses as antigens. Recent results revealed that the immunodominant antigenic epitopes are located on the aminoterminus of the protein, which allows the use of a truncated protein that is easier to purify as antigen source [50]. These ELISAs are suitable screening assays. Positive results should be confirmed by immunofluorescence assay.

A number of other serological assays, e.g. the high density particle agglutination assay (IPA), the hemagglutination inhibition assay (HIA), the Western immunoblot (WB) and the plaque reduction neutralization assay (PRNT) have been described for the immunodiagnosis of Hantavirus infections. They are used by specialized laboratories.

In all assays used for routine diagnosis of Han-

tavirus infections, one should use the antigens of at least two serotypes (Puumula, Hantaan) in order to recognize all infections. Cross-reactions between the different serotypes may be found. Predominant reaction with one of the antigens points to infection with this particular virus serotype, and may thus be of prognostic, epidemiologic and therapeutic importance. For the diagnosis of HPS the use of FCV antigen is mandatory. In most infections by Puumula serotype viruses, cross-reactive antibodies against the Hantaan serotype are found, but the Hantaan-specific assays usually show lower titers and extinctions [51]. On the other hand, in many Hantaan virus infections, no cross-reactive antibodies are detected against the Puumula serotype. Most Hantavirus infections occurring in Central Europe can be reliably diagnosed using a Puumula-specific assay. Nevertheless, epidemiological investigations and rare clinical observations documented that Hantaan-like viruses are endemic in Germany, particularly in the Eastern part of the country [18].

Polymerase chain reaction (PCR) has proved to be a valuable tool for the detection and identification of new Hantaviruses. Only limited experience exists with its practical use for diagnostic purposes. In HPS cases, viral RNA could be detected in the early clinical phase in peripheral blood monocytes, lymphocytes, plasma and clots [52]. However, only few data are available on the validity of PCR for the diagnosis of Puumula or Hantaan virus induced HFRS [53]. At present, PCR does not seem to have advantages over serology. It should be reserved for special indications (e.g. diagnosis from biopsies or, retrospectively, from autopsy materials).

Most hantaviruses can be propagated in Vero E6 cells, but other cell lines are also permissive. Virus isolation from clinical specimens is difficult, time-consuming and hazardous. Usually, animal passages are needed prior to inoculation of cell cultures. Propagation of the virus requires special L3 facilities.

Therapy

In general, Hantavirus infections are treated symptomatically. Application of the antiviral agent Ribavirin proved successful in severe HFRS cases (Korean hemorrhagic fever), and less severe disease of shorter duration was seen [53]. Ribavirin is a synthetic purine nucleoside derivative resembling guanosine. It has broad *in vitro* activity against many DNA and RNA viruses. It must be given early in the course of the disease to be effective. For HPS, no data on the effectiveness of Ribavirin are available.

So far, trials with Interferon failed to document a benefit in patients with HFRS. Intensive efforts are currently being made to develop a vaccine for Hantaviruses [55]. In South East Asia, inactivated virus vaccines are currently being evaluated in clinical trials. Rapid progress in genome-, protein- and epitope-mapping of the viruses provide new perspectives for the development of recombinant vaccines. The investigation of antiviral drugs is hampered by the fact that suitable animal models for hantavirus disease are lacking.

Summary

In Western Europe, interstitial nephritis, secondary to Hantavirus infection, accounts for 5–10% of all cases of primary acute renal failure in which no obvious cause (e.g. surgery, trauma etc.) can be found. Subjects with outdoor activities are at particular risk. Viruses are transmitted via rodents. Hantaviruses belong to the *Bunyaviridae* family, which are single-stranded RNA viruses. Several serogroups have been identified (Hantaan, Puumula, Prospect Hill, Seoul, Four Corners). A characteristic feature is tropism for endothelial cells. The disease is characterized by a biphasic course. A prodrome of fever, myalgia and particularly severe back pain, conjunctivitis with photophobia is followed by a stage which is characterized by thrombocytopenia, moderately elevated liver enzymes, relative bradycardia (as evidence of subclinical myocarditis) and acute renal failure. In contrast to other types of acute renal failure, marked microhematuria and proteinuria are observed. Histology shows hemorrhagic interstitial nephritis which is particularly pronounced in the border zone between cortex and medulla. Extrarenal complications comprise severe hemorrhagic complications, cardiac problems, CNS complications, ARDS, hepatitis and rarely, pancreatitis. The diagnosis is made serologically, i.e. by IgM-specific ELISA.

Management of the patient comprises supportive measures, hemodialysis and infusion of fresh frozen plasma thrombocytes in patients with severe hemorrhagic complications. A novel approach is early treatment with ribavirin. Prevention using recombinant vaccine is on the horizon.

References

1. Lee HW, Lee PW, Johnson KM. Isolation of the etiologic agent of Koran hemorrhagic fever. J Infect Dis 1978; 137: 298–308.

2. Lee HW, van der Groen G. Hemorrhagic fever with renal syndrome. Progress in Medical Virology 1989; 36: 62–102.
3. Nichol ST, Spiropoulou CF, Morzunov S *et al.* Genetic identification of a Hantavirus associated outbreak of acute respiratory illness. Science 1993; 262: 914–7.
4. Schmaljohn CS, Hasty SE, Dalrymple JP *et al.* Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome Science 1985; 227: 1041–4.
5. Gligic A, Dimkovic N, Yiao S-Y *et al.* Belgrade virus: a new Hantavirus causing severe hemorrhagic fever with renal syndrome in Yugoslavia. J Infect Dis 1992; 166: 113–20.
6. Lee PW, Gibbs CJ, Gajdusek D, Yanagihra R. Serotypic classification of hantaviruses by indirect immunofluorescent antibody and plaque reduction neutralization tests. J Clin Microbiology 1985; 22: 940–4.
7. Niklasson B, Leduc J. Isolation of the nephropathia epidemica agent in Sweden. Lancet. 1984; 1012–3.
8. Morzunov SP, Feldmann H, Spiropoulou CF *et al.* A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Lousiana. J Virol 1995; 69: 1980–3.
9. Ravkov EV, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST. Genetic and serologic analysis of Black Creek Canal virus and its association with human disease and *Sigmodon hispidus* infection. Virology 1985; 210: 482–9.
10. Yanagihara R, Amyx HL, Gajdusek DC. Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica in bank voles (*Clethrionomys glareolus*). J Virol 1985; 55: 34–8.
11. Lundkvist A, Niklasson B. Hemorrhagic fever with renal syndrome and other hantavirus infections. Rev Med Virol 1994; 4: 177–84.
12. Simpson GL. Hantaviral infection in the Southwest: clinical and epidemiological features. ASM-Meeting, 1994.
13. Le Duc JW. Epidemiology of Hantaan and related viruses. Lab Anim Sci 1987; 37: 413–8.
14. Lee HW, Dalrymple JL. Manual of hemorrhagic fever with renal syndrome. WHO Collaborating Center for Virus Reference and Research, Seoul 1989.
15. Gärtner L, Emmerich P, Schmitz H. Hantavirusinfektionen als Ursache von akutem Nierenversagen. Deutsch Med Wschr 1988; 113: 937.
16. Pilaski J, Ellerich C, Kreutzer T, Lang A. Hemorrhagic fever with renal syndrome (HFRS) in Germany. Int Symposium Hemorrhagic Fever with Renal Syndrome, Leningrad, USSR 1991; 2.
17. Zeier M, Andrassy K, Waldherr R, Ritz E. Akutes Nierenversagen durch Hantavirus. Dtsch Med Wschr 1986; 111: 207–10.
18. Zöller L, Faulde M, Meisel H *et al.* Seroprevalence of Hantavirus antibodies in Germany as determined by a new recombinant enzyme immunoassay. Eur J Clin Microbiol Infect Dis 1995; 14: 305–13.
19. Gajdusek DC, Goldgaber D, Millard E. Bibliography of hemorrhagic fever with renal syndrome (muroid virus nephropathy) US Dept. of Health and Human Services, N.I.H. Bethesda, Maryland, 1983.
20. Clement J, Underwood P, Ward D, Pilaski J, Le Duc J. Hantavirus outbreak during military manoeuvres in Germany. Lancet 1996; 347: 336.
21. Siamopoulos KC, Elisaf M, Antoniadis A, Moutsopoulos HM. Hemorrhagic fever with renal syndrome in an endemic area of Greece. Am J Nephrol 1992; 12: 170–3.
22. Linderholm M, Settergren B, Ahlin C *et al.* A Swedish fatal case of nephropathia epidemica. Scand J Infect Dis 1991; 23: 501–2.
23. Becker C, Goubeaud G, Zeier M, Zöller L. Hantavirus-Infektionen und Chronische Niereninsuffizienz. Med Welt 1993; 44: 569–73.
24. Grcevska L, Polenacovic M, Oncevski A, Zografski DJ, Gligic A. Different histopathological presentations of acute renal involvement in Hantavirus infection: report of two cases. Clin Nephrol 1990; 34: 197–201.
25. Kleinknecht D, Rollin PE. Hypertension after hemorrhagic fever with renal syndrome. Nephron 1992; 61: 121.
26. Settergren B, Trollfors B, Fasth A, Hultberg B, Norrby SR. Glomerular filtration rate and tubular involvement during acute disease and convalescence in patients with nephropathia epidemica. J Infect Dis 1990; 161: 716–20.
27. Zöller L, Krüger DH. Hantaviren: Neue Infektionserreger mit wachsender Bedeutung. Die gelben Hefte 1996; 36: 31–41.
28. van Ypersele de Strihou, Mery JP. Hantavirus related acute interstitial nephritis in Western Europe. Q J Med 1989; 73: 941–50.
29. Lähdevirta J. Nephropathia epidemica in Finland. A clinical, histological and epidemiological study. Ann Clin Res 1971; 3 (suppl 8): S1–S154.
30. Mustonen J, Hellin H, Pietilä K *et al.* Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 1994; 41: 121–6.
31. Zeier M, Zöller L, Haußmann W, Andrassy K, Ritz E. Klinik und Therapie der Hantavirus-Infektion. Dtsch Med Wochenschr 1990; 115: 1678–81.
32. Park JE, Pyo H-J. Delayed onset of diuresis in a patient with acute renal failure due to hemorrhagic fever with renal syndrome who also developed anterior hypopituitarism. Clin Nephrol (in press).
33. Pearson CM, Hall RJ. Electrocardiographic findings in epidemic hemorrhagic fever. Circulation 1954; 10: 855–8.
34. Chun PKC, Godfrey LJ. Unique selective right atrial hemorrhage with epidemic (Korean) hemorrhagic fever. Am Heart J 1984; 108: 410–2.
35. Zeier M, Zöller L, Weinreich T *et al.* Severe hemorrhagic complications with nephropathia epidemica strain of Hantavirus. Clin Nephrol 1992; 38: 190–2.
36. Smorodintsev AA, Chudakov VG, Churilor AV. Hemorrhagic nephroso-nephritis. Tr. from the Russian by Catherine Matthews. Pergamon Press, London 1959.
37. Duchin JS, Koster FT, Peters CJ *et al.* Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. N Engl J Med 1994; 330: 949–55.
38. Chu YK, Jennings G, Schmaljohn A *et al.* Cross-neutralisation of Hantaviruses sera from experimentally infected animals and from hemorrhagic fever with renal syndrome and Hantavirus pulmonary syndrome patients. J Infect Dis 1995; 172: 1581–4.

39. Linderholm M, Billström A, Settergren B, Tärnvik A. Pulmonary involvement in nephropathia epidemica as demonstrated by computed tomography. Infection 1992; 5: 263–6.
40. Schreiber M, Laue T, Wolff C. Hantavirus pulmonary syndrome in Germany. Lancet 1986; 347: 336–7.
41. Groen J, Dalrymple J, Fisher-Hoch S *et al.* Serum antibodies of structural proteins of Hantavirus arise at different times after infection. J Med Virol 1992; 37: 283–7.
42. Zöller L, Scholz J, Stohwasser R *et al.* Immunblot analysis of the serological response in Hantavirus infections. J Med Virol 1989; 27: 231–7.
43. Groen J, Jordans HGM, Clement JP *et al.* Identification of Hantavirus subtypes by testing of post-infection sera in immunofluorescence and enzyme-linked immunosorbent assays. J Med Virol 1991; 33: 26–32.
44. Lähdevirta J, Savola J, Brummer-Korvenkuntio M. Clinical and serological diagnosis of nephropathia epidemica, the mild type of hemorrhagic fever with renal syndrome. J Infect 1984; 9: 230–8.
45. Settergren B, Juto P, Wadell G. Detection of specific serum immunoglobulin M in nephropathia epidemica (Scandinavian epidemic nephropathy) by a biotin-avidin-amplified immunofluorescence method. J Clin Microbiol 1987; 25: 1134–6.
46. Niklasson B, Kjellson T. Detection of nephropathia epidemica (Puumala virus) specific immunoglobulin M by enzyme linked immunosorbent assay. J Clin Microbiol 1988; 26: 1519–23.
47. Zöller L, Yang S, Gött P, Bautz EKF, Darai G. A novel μ-capture ELISA based on recombinant proteins for sensitive and specific diagnosis of hemorrhagic fever with renal syndrome. J Clin Microbiol 1993; 31: 1194–9.
48. Gött P, Zöller L, Yang S *et al.* Antigenicity of Hantavirus nucleocapsid proteins expressed in *E. coli.* Virus Research 1991; 16: 1-16.
49. Zöller L, Yang S, Gött P, Bautz EKF, Darai G. Use of recombinant nucleocapsid proteins of the Hantaan and nephropathia epidemica serotypes of Hantaviruses as immunodiagnostic antigens. J Med Virol 1993; 39: 200–7.
50. Gött P, Zöller L, Yang S, Bautz EKF, Darai G. A major antigenic domain of hantaviruses is located on the amino proximal site of the viral uncleocapsid protein. Virus Genes 1997; 14: 31–40.
51. Zöller L, Scholz J, Stohwasser R, Giebel L, Sethi KK, Bautz EKF, Darai G. Immunoblot analysis of the serological response in hantavirus infections. J Med Virol 1989; 27: 231–237.
52. Hjelle B, Spiropoulou CF, Torrez-Martinez N *et al.* Detection of Muerto Canyon virus RNA in peripheral blood mononuclear cells from patients with Hantavirus pulmonary syndrome. J Inf Dis 1994; 170: 1013–7.
53. Sibold C, Meisel H, Krüger DH, Darai G. Detection of Hantavirus infection by polymerase chain reaction. In: Darai G, Becker Y (eds). PCR: Protocols for diagnosis of human and animal virus diseases. Springer, Berlin 1995: 392–402.
54. Huggins JW, Hsiang CM, Cosgriff TM. Prospective, double blind, concurrent placebo-controlled clinical trial of intravenous therapy of hemorrhagic fever with renal syndrome. J Inf Dis 1991; 164: 1119–27.
55. Schmaljohn C. Prospects for vaccines to control viruses in the family of Bunyaviridae. Rev Med Virol 1994; 4: 185–96.