ANTTI PAAKKALA

Radiological Findings in Nephropathia Epidemica

Analysis of Chest Radiography, Renal Ultrasonography and Renal Magnetic Resonance Imaging

■

ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
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ACADEMIC DISSERTATION

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To my family

ABSTRACT

Nephropathia epidemica (NE) is a mild form of hemorrhagic fever with renal syndrome (HFRS). Its course varies from asymptomatic to fatal. The etiologic agent, Puumala virus (PUUV), belongs to the Hantavirus genus of the Bunyaviridae family. The pathogenesis of PUUV infection in humans is in many respects unclear.

Respiratory symptoms, from common cold to respiratory distress, occur in NE. This series investigated radiologic pulmonary involvements in NE patients. In the first study 125 and in the second 344 hospital-treated acute-phase NE patients were assessed retrospectively and their clinical course compared to chest radiograph findings (I,II). In study I 28% and in study II 35% of patients had disease-related changes in their chest radiographs. In both studies pleural effusion, atelectasis and interstitial infiltrates were the most common radiographic findings and pulmonary edema was observed in 4% of patients. The occurrence and severity of findings were associated with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, leukocytosis and thrombocytopenia.

Acute renal failure (ARF) is evident in over 90% of hospital-treated NE patients. In order to evaluate renal ultrasonography (US) findings and their pathogenesis, 250 hospital-treated acute-phase NE patients were studied and qualitative renal US findings analyzed retrospectively (II); 47% showed pathological qualitative findings in their renal US, increased cortical echogenicity in 36% and cortical swelling in 28% being the most common findings.

Quantitative renal US findings were prospectively analyzed (III) and qualitative renal US findings were retrospectively analyzed (IV) in 23 NE patients. The patients were used as their own control and qualitative US findings were analyzed in two different ways: without and with comparison image.

The renal resistive index (RI) was abnormal in 12 and fluid collections (perirenal, pleural, pericardial, ascites) were found in 13 patients during the acute phase of disease. Renal length decreased in every patient, cortical parenchymal thickness in 19 and RI in 18 from the primary to the repeat studies (III).

Qualitative findings were recorded in 87% of the patients in renal US, assuming that change between two studies is a sign of abnormality (IV); 91% yielded qualitative findings in renal US where a

comparison study was available (IV). Parenchymal swelling decreased in 20/21 patients (without/with comparison image), the overall rating of kidney status improved in 20/20, corticomedullary border differentiation improved in 15/21, the patchy pattern in parenchymal echo-texture decreased in 18/15 and echogenicity decreased in 14/18 patients from the primary to the repeat studies.

Clinical course was compared to quantitative and qualitative renal US findings. The severity of the findings in US were to some extent associated with clinical fluid volume overload, degree of clinical renal insufficiency and leukocytosis (II,III,IV).

Magnetic resonance imaging (MRI) findings have not previously been studied in NE. Here morphologic renal MRI findings in 20 NE patients were analyzed and compared to the clinical course (V). The patients were used as their own control. Renal parenchymal volume, renal length and parenchymal thickness were decreased in all patients from the primary to the repeat study. Edema/fluid collections were found bilaterally in 16 patients in the primary study. The severity of the findings in MRI evinced a mild association with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, inflammation and thrombocytopenia.

In conclusion, capillary leakage and inflammation play a role in the pathogenesis of NE lung involvement, and fluid volume overload associated with renal insufficiency appeared to contribute markedly to the chest radiograph findings in NE. Capillary leakage, inflammation and fluid volume overload associated with renal insufficiency also play a role in the renal US and MRI findings and in the pathogenesis of NE renal involvement.

Chest and renal involvement in acute disease were regarded as transient. Chest radiograph, renal US and MRI findings are not disease-specific in NE. Chest radiography is nonetheless useful if patients have respiratory symptoms; it can rule out severe findings of NE, other inflammatory diseases and heart failure during NE. Qualitative US findings are as sensitive as quantitative US findings in the assessment of clinical course and recovery, assuming that a comparison study is available. US and MRI are rather limited in evaluating NE patients´ clinical situation. MRI is more accurate than US in evaluating quantitative renal findings and superior to US in evaluating edema/fluid collections in NE patients. US is nevertheless more appropriate than MRI in assessing clinical course and renal findings in NE patients by reason of its low cost and good availability. The main reason for performing US during the acute phase of NE is to rule out other causes of ARF.

CONTENTS

ABBREVIATIONS

ARDS	adult respiratory distress syndrome
ARF	acute renal failure
C	complement factor
CRP	C-reactive protein
CT	computed tomography
ESR	erythrocyte sedimentation rate
HFRS	hemorrhagic fever with renal syndrome
HLA	human leukocyte antigen
HPS	hantavirus pulmonary syndrome
Ig	immunoglobulin
IL	interleukin
KHF	Korean hemorrhagic fever
MRI	magnetic resonance imaging
NE	nephropathia epidemica
NO	nitric oxide
PUUV	Puumala virus
RI	resistive index
RNA	ribonucleic acid
SNV	Sin Nombre virus
TNF	tumor necrosis factor
US	ultrasonography

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals I-V.

I Kanerva M, Paakkala A, Mustonen J, Paakkala T, Lahtela J, Pasternack A (1996): Pulmonary involvement in nephropathia epidemica: radiological findings and their clinical correlations. Clin Nephrol 46: 369-378.

II Paakkala A, Lempinen L, Paakkala T, Huhtala H, Mustonen J (2004): Medical imaging in nephropathia epidemica and their clinical correlations. Eur J Intern Med 15: 284-290.

III Paakkala A, Kallio T, Huhtala H, Apuli P, Paakkala T, Pasternack A, Mustonen J (2002): Renal ultrasound findings and their clinical associations in nephropathia epidemica. Analysis of quantitative parameters. Acta Radiol 43: 320-325.

IV Paakkala A, Kallio T, Huhtala H, Apuli P, Paakkala T, Mustonen J (2004): Value of ultrasonography in acute renal failure: analysis of qualitative features in patients with nephropathia epidemica. Acta Radiol 45: 785-790.

V Paakkala A, Dastidar P, Ryymin P, Huhtala H, Mustonen J (2004): Renal MRI findings and their clinical associations in nephropathia epidemica: analysis of quantitative findings. Eur Radiol, in press, published online since June 5.

INTRODUCTION

Nephropathia epidemica (NE) was first described independently by two authors in Sweden (Myhrman 1934, Zetterholm 1934). In Finland the disease became known through Lähdevirta´s comprehensive thesis, "Nephropathia epidemica in Finland. A clinical, histological and epidemiological study" (1971). The causative agent in NE, Puumala virus (PUUV), belonging to the group of hantaviruses, was isolated from the bank vole (*Clethrionomys glareolus*) in the village of Puumala at the end of the 1970s (Brummer-Korvenkontio et al. 1980). A number of different hantaviral zoonoses causing hemorrhagic fevers have since been detected nearly world-wide.

Respiratory symptoms and lung involvement are not infrequent in NE. Changes in the chest radiograph and computed tomography (CT) have previously been reported in 16-53% of patients (Lähdevirta 1971, Linderholm et al. 1992, Mustonen et al. 1994a). Chest radiograph findings, on the other hand, have not hitherto been studied and compared to the clinical course in a large NE patient material.

Acute renal failure (ARF) is evident in over 90% of hospital-treated patients (Mustonen et al. 1994a), and tubulointerstitial nephritis is the typical renal histopathological lesion in NE (Collan et al. 1991, Mustonen et al. 1994b). Isotope nephrography has been reported to be more sensitive than US in identifying renal functional impairment in NE patients (Paul et al. 1991). Renal ultrasonography (US) findings in NE patients have not previously been fully studied or compared to the clinical course. Magnetic resonance imaging (MRI) findings have likewise not been studied in NE.

The present work was carried out in Tampere University Hospital. Special attention was paid to lung and renal involvement to investigate the occurrence and pathogenesis of manifestations during the acute phase of infection and during recovery. Participants in study I were selected from two groups. Firstly, 97 consecutive hospital-treated acutely ill NE patients during the years 1982-1989 were included and secondly, 28 NE patients with pulmonary changes from the years 1990-1994 in order to enlarge the number of pathologic chest radiographs. Study II involved 380 NE patients in whom PUUV infection was confirmed during the years 1982-1998 and in whom radiological examinations had been performed. Chest radiograph and renal US findings were evaluated. In study III quantitative renal US findings were studied prospectively and in study IV qualitative renal US findings were retrospectively assessed in 23 consecutive NE patients treated from January 1997 to February 1998. In study V renal MRI in 20 consecutive NE patients from October 2000 to October 2002 were prospectively studied. All these radiological findings were correlated to the clinical course of NE.

REVIEW OF THE LITERATURE

Virological aspects of Puumala and other hantaviruses

Hantavirus genus and Bunyaviridae family

Hantaviruses are found in most regions of the world (Zhao and Hay 1997), and they belong to the group of enveloped ribonucleic acid (RNA) viruses causing two clinical disease syndromes, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) (Schmaljohn et al. 1985). This includes agents in four taxonomic families: the Bynyaviridae, the Filoviridae, the Arenaviridae, and the Flaviviridae (Table 1).

Bunyaviridae is a family of over 300 viruses divided into five genera, namely, Bynyavirus, Hantavirus, Nairovirus, Phlebovirus and Tospovirus (Murphy et al. 1995). The genus Hantavirus comprises different serotypes, e.g. Dobrava, Hantaan, Khabarovsk, Prospect Hill, Puumala (PUUV), Seoul, Sin Nombre (SNV), Thailand, Thottapalayam and Topografov. Numerous novel hantaviruses have been characterized during recent years, the most prominent new comer being SNV, the causative agent of HPS. Of these viruses, PUUV is genetically, antigenically and serologically most closely related to Prospect Hill, Khabarovsk, Topografov and Tula viruses and secondly to other American hantaviruses, but is more distantly related to Dobrava, Hantaan and Seoul viruses (Shesberadaran et al. 1988, Vapalahti et al. 1992, Chu et al. 1994, Hjelle et al. 1994, Plyusnin et al. 1994, Xiao et al. 1994, Schmaljohn et al. 1995, Hörling et al. 1996, Plyusnin et al. 1996).

Puumala virus

PUUV, like other hantaviruses, is an enveloped, single-stranded RNA virus with a trisegmented genome and negative polarity (Elliot 1990). Hantaviruses are typically named after the place they were first detected. PUUV, the etiologic agent of nephropathia epidemica (NE), was detected in the lungs of a bank vole (*Clethrionomys glareolus*) trapped in Puumala, Finland (Brummer-Korvenkontio et al. 1980) and isolated and adapted in cell culture from the lungs of a bank vole trapped in Sotkamo,

Table 1. Taxonomic classification of viruses causing hemorrhagic fevers (Murphy et al. 1995, Hörling et al. 1996, Plyusnin et al. 1996, Vapalahti 1996)

Bunyaviridae
Genus Bunyavirus
Genus Hantavirus
Bayou virus
Black Creek Canal virus
Dobrava virus
El Moro Canyon virus*
Hantaan virus
Isla Vista virus*
Khabarovsk virus
New York virus
Prospect Hill virus
Puumala virus
Rio Mamore virus*
Rio Segundo virus*
Seoul virus
Sin Nombre virus
Shelter Island virus
Thailand virus
Thottapalayam virus
Topografov virus
Tula virus
Genus Nairovirus
Genus Phlebovirus
Genus Tospovirus
Filoviridae
Ebola virus
Marburg virus
Arenaviridae
Junin virus
Lassa virus
Machupo virus
Flaviviridae
Dengue virus
Omsk virus

*These are not so far known to cause disease in man

Northern Finland (Schmaljohn et al. 1985). This is the prototype virus of the PUUV serotype (Karabatsos 1985). There are various strains of PUUV, e.g. Sotkamo in Finland and Hällnäs in Sweden. These differ from each other by about 5% in sequence analysis, whereas the Seoul virus differs from PUUV by about 20% and the Hantaan virus by about 40% at amino acid level (Vapalahti et al. 1992, Plyusnin et al. 1996). Sera from NE patients react specifically with PUUV nucleocapsid protein (Vapalahti et al. 1996). Antibodies against PUUV and other hantaviruses persist for decades after infection (Lee and Dalrymple 1989, Settergren et al. 1991a, Lundkvist et al. 1993, Ahlm et al. 1997).

Serodiagnostics of Puumala virus infection

From 1979 to 1983 serodiagnosis of NE was made by antibody assays using sections of acetone-fixed PUUV-infected bank vole lungs as antigen in indirect immunofluorescence (Brummer-Korvenkontio et al. 1980), from 1984 to 1988 by antibody assays using acetone-fixed PUUV-infected cultured Vero E6 cells as antigen in indirect immunofluorescence. From 1989 to 1990 the diagnosis of acute infection was made by immunoglobulin (Ig) G avidity assays using acetone-fixed PUUV-infected cultured Vero E6 cells as antigen in indirect immunofluorescence (Hedman et al. 1991). In addition, the finding that granular fluorescence is associated with acute NE and diffuse during later convalescence was used as a screening criterion in diagnostics (Vapalahti et al. 1995a, Kallio-Kokko et al. 2001). Since 1996 the IgM enzyme immunoassay using recombinant baculovirus-expressed PUUV nucleocapsid protein as antigen has been used in the serodiagnosis of PUUV infection (Vapalahti et al. 1996).

Pathogenesis of nephropathia epidemica

The pathogenesis of HFRS and NE is poorly known. The original Russian hypothesis suggested direct viral damage to capillary endothelial cells (Smorodintsev et al. 1959), and increased capillary permeability would appear to be an essential feature in hantavirus infections. Possible causes of impaired hemostasis are vascular injury, platelet dysfunction, thrombocytopenia and disseminated intravascular coagulation (Cosgriff 1991). Endothelial cells have been suggested as important targets of hantavirus replication (Yanagihara and Silverman 1990, Temonen et al. 1993). As PUUV infects endothelial cells in vitro with no visible changes in the cell morphology, it is possible that

immunological mechanisms also contribute to endothelial damage or functional changes, leading to capillary leakage (Temonen et al. 1993). Elevated concentrations of endothelin-1 (a potent vasoconstrictor polypeptide containing 21 amino acids) in the plasma of patients with NE might be of pathophysiological importance in this disease (Forslund et al. 1993). Increased levels of cytokines have been encountered during the acute phase, e.g. tumor necrosis factor (TNF)-α and interleukin (IL)-6 (Linderholm et al. 1996a). TNF-α is known to induce pathophysiologic and clinical changes similar to those seen in NE and diseases caused by other hantaviruses. Excessive amounts of nitric oxide (NO) have also been found in the sera of patients with acute PUUV infection (Groeneveld et al. 1995, Groeneveld et al. 1996, Linderholm et al. 1996b).

Immunological mechanisms may also be involved in the pathogenesis of HFRS, as suggested by changes observed in T-lymphocyte subsets during the acute phase of the infection (Park et al. 1996). Certain human leukocyte antigen (HLA) haplotypes, namely the HLA B8 and DRB1*0301 alleles, are associated with a severe clinical course of NE (Mustonen et al. 1996), and one, namely HLA B27, with benign clinical course (Mustonen et al. 1998), which would suggest genetic susceptibility. In pediatric patients no significant differences in clinical picture have emerged between patients with and without HLA B8 and DRB1*0301 alleles (Mustonen et al. 2004).

PUUV is pantropic, i.e. it can infect a wide variety of human cells, including cells originating from the brain, heart, lung, spleen, liver and kidney tissue in fatal HFRS (Kanerva et al. 1998). The infection in these cells is non-cytopathic and proceeds in all cell types at almost the same rate in vitro and in vivo (Temonen et al. 1993). This suggests that PUUV is able to cause systemic infections. No specific target for PUUV can be pinpointed. Also peripheral blood monocyte/macrophages are susceptible to PUUV, suggesting that these cells could contribute to the spread of the virus from the primary site of infection to other organs during viremia (Temonen et al. 1993). During hematogenous dissemination, the virus most probably infects first the endothelial cells, which further permits its entry into parenchymal organs. Type I interferons inhibit PUUV replication in these cells. Vascular leakage is the central feature of all human pathogenic hantavirus infections. It is however likely that other factors in addition to endothelial cell infection contribute to capillary leakage. Infected and activated macrophages may mediate significant cytotoxic effects on the infected endothelium (Kanerva 1996).

In one study where pathogenetic mechanisms underlying pulmonary involvement in NE were investigated, the findings suggested that pulmonary involvement may be an inherent trait of NE (Linderholm 1997). The authors suggested a role for soluble inflammatory mediators in the disease. In

particular, the protracted rise in levels of TNF-α and the increase in end-products of NO may be pathophysiologically relevant, in that these mediators are known to evince several activities mimicking signs and symptoms of HFRS, including NE (Linderholm 1997). Increase in capillary permeability, direct effect of virus replication, fluid reabsorption and redistribution during oliguria/anuria and myocardial depression may be important in the pathogenesis of pulmonary involvement in NE (Kanerva 1996).

The fact that hemorrhages are not typically seen in HPS suggests that the endothelial involvements in HPS and HFRS may be in some extent functionally different or involve different anatomical locations. Temonen and associates (1996) found no evidence of PUUV in renal biopsies, but group under Groen (1996) detected PUUV viral antigen in biopsied kidney cells by immunoperoxidase techniques. Tubular cells are directly damaged via viral infection (Temonen et al. 1993). This causes peritubular and interstitial inflammation and tubular injury. On the other hand, capillary permeability causes plasma leakage in the kidneys, this causing perivascular edema and congestion leading to medullary ischemia and tubular injury. Both inflammation and medullary ischemia may play a role in the pathogenesis of tubular injury (Kanerva 1996). Plasma IL-6 concentrations and urinary IL-6 excretion have been seen to be markedly increased in patients with acute NE, but there was no correlation between plasma and urinary IL-6 levels. The high urinary IL-6 levels might reflect local production of this proinflammatory cytokine in the kidneys during acute infection (Mäkelä et al. 2004).

By combining the data from the study of the pathogenesis of PUUV infection and the literature covering other hantaviruses, suggestive conclusions as to the transmission route in humans, the mechanisms of vascular leakage and pulmonary and renal involvement in hantavirus infections are presented in Figures 1-4 (Kanerva 1996).

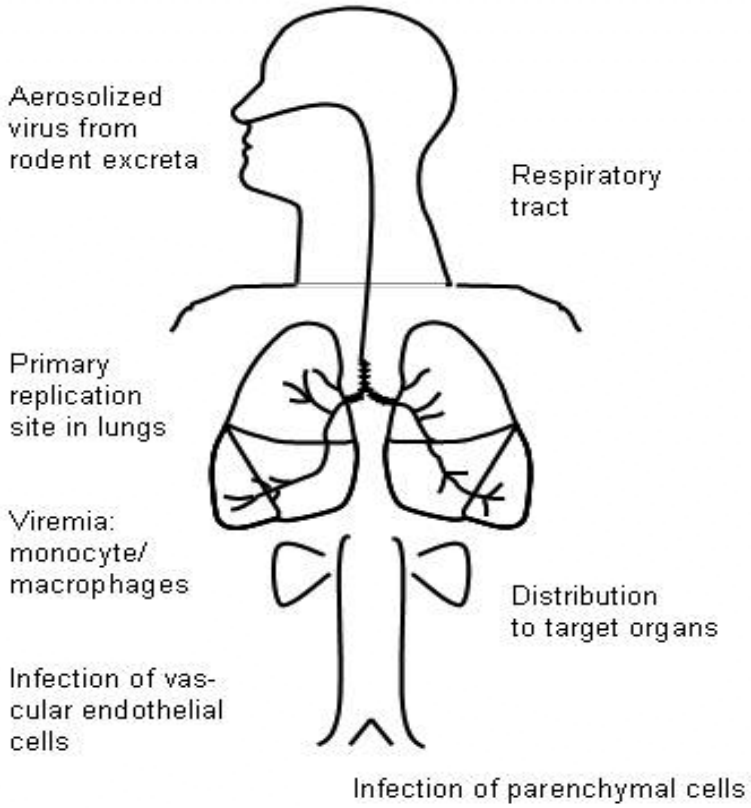

Figure 1. Hypothetical transmission route of hantavirus infections in humans

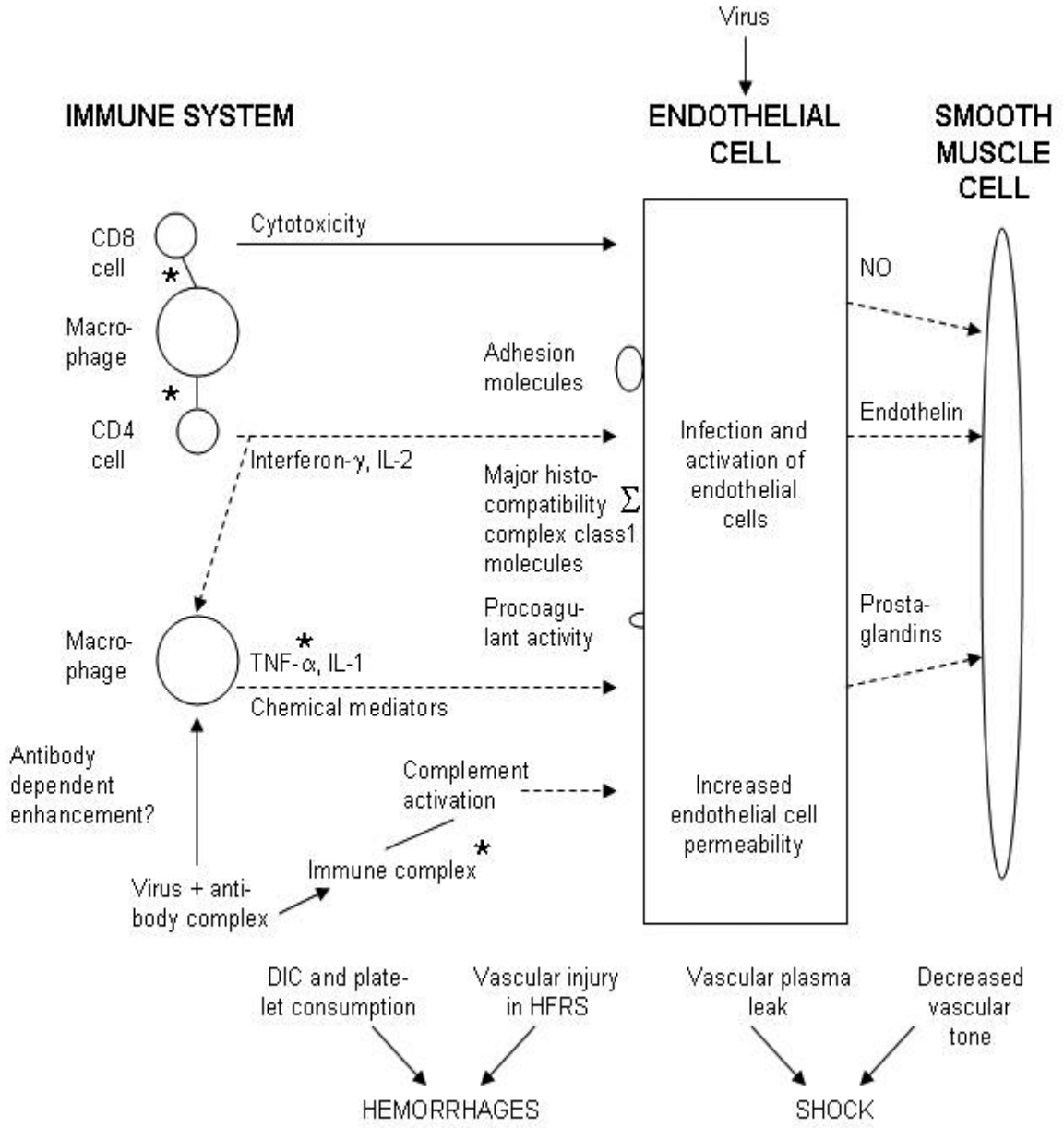

Figure 2. Hypothesis of the pathogenesis of shock and hemorrhages in hantavirus infections (Kurane and Ennis 1992). Asterisk (*) shows the mediators possibly regulated by genetic predisposition (association to HLA B8 DRB1*0301 haplotype) in severe NE. DIC=disseminated intravascular coagulation

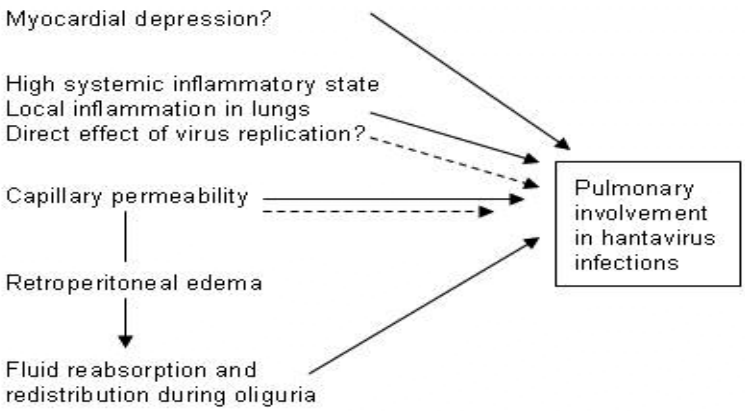

Figure 3. Hypothesis for the pathogenesis of pulmonary involvement in NE (→) and HPS (- -►)

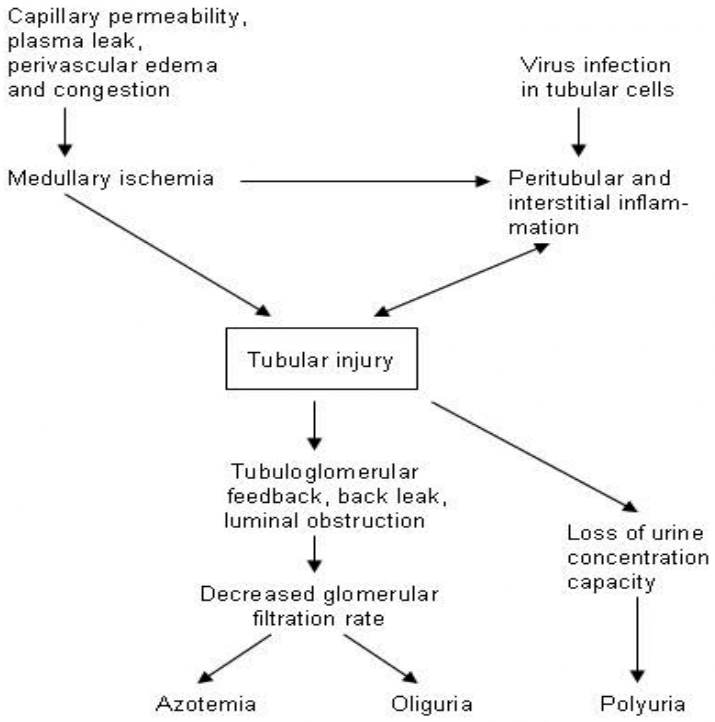

Figure 4. Suggested pathogenesis of renal failure in HFRS (adapted from Cosgriff 1991)

Epidemiology of nephropathia epidemica

Geographical distribution

The disease known as NE was formerly thought to occur only in Finland and Scandinavia. More recently, however, seropositivity to PUUV has been found in Russia, central and southern Europe, and even in Japan (Lee et al. 1982), Argentina (Weissenbacher et al. 1996) and the United States (Auwaerter et al. 1996). Clinical cases of NE have also been identified in most of these countries. However, northern Europe, especially central and eastern Finland, Norrland in Sweden and the corresponding part of Norway are considered endemic areas for PUUV and NE (Lähdevirta 1989, Vapalahti 1996). In the northern part of Europe the following seroprevalences of PUUV have been reported: 4-19% in Sweden (Niklasson et al. 1983, Niklasson and LeDuc 1987, Niklasson et al. 1987, Ahlm et al. 1994), 24% in Norway (Sommer et al. 1988) and over 20% in endemic areas of Finland (Brummer-Korvenkontio et al. 1982). In the European part of Russia, seroprevalences of 3% in the Samara region (Alexeyev et al. 1996) and 15% in Bashkirtostan (Niklasson et al. 1993) have been reported. In Germany, Clement and associates (1996) reported a PUUV outbreak in 16 patients during military maneuvers in 1990. In France, NE has been known since 1983 (Rollin et al. 1995), and extensive sero-surveys among blood donors have shown a <1% prevalence of antibodies in the epidemic foci in the northeastern part of the country (Le Guenno and Coudrier 1995).

In Finland, the mean annual number of serologically confirmed clinical cases of NE has been about 1000, with a variation of 800-1200 from 1991 to 1995 (Hedman et al. 1991, Vapalahti et al. 1995b, Orion diagnostica 1996). However, the maintenance of the 5% average PUUV seroprevalence in the Finnish population would require about 4000 PUUV infections each year, which would suggest that only 25% of cases are either so severe or so typical that the patient seeks medical care and clinicians suspect PUUV infection (Vapalahti 1996). Sweden has reported from 100 to 300 annual cases (Swedish institute for infectious disease control 1993, 1995) and Norway significantly fewer (Sommer et al. 1988). However, the number of subclinical cases has been estimated to be up to 14-20 times higher (Niklasson et al. 1987). PUUV infection is the most frequent serologically diagnosed viral disease in Finland (Vapalahti et al. 1996).

Ecology

The host of PUUV is a mouse-like small rodent, the bank vole (*Clethrionomys glareolus*). The annual incidence of NE depends on the abundance of bank voles, which have strong population cycles, with numbers peaking at 3- to 4-year intervals (Hansson and Henttonen 1985, Ahlm et al. 1997). Niklasson and colleagues (1995) have identified PUUV antibodies in 19% of bank voles, the antibody prevalence increasing with increasing weight (age) of the animal, reaching more than 50% in the heaviest group. Infected voles develop a chronic, apparently subclinical infection and excrete the virus in the urine, feces and saliva (Lee et al. 1981, Tsai 1987, Bernshtein et al. 1999). Thereafter, the virus is apparently transmitted by inhalation or ingestion of contaminated rodent excreta (Lee et al. 1981, Tsai 1987). Transmission from human to human has not been reported (Vitek et al. 1996). However, NE has been described in one 60-day-old breastfed baby whose mother had had the disease 25 days before the baby fell ill, and here person-to-person transmission is a possible explanation (Brummer-Korvenkontio et al. 1999). Cases of NE occur year-round with a typical seasonal epidemic peak in Finland from October to January (Lähdevirta 1971, Lähdevirta and Elo 1975, Brummer-Korvenkontio et al. 1982, Sommer et al. 1988). In central Europe, due to the warmer climate, the seasonal variability of incidence is milder than in Scandinavia and peaks rather in summer than in autumn (van Ypersele de Strihou 1991).

Demographic characteristics

The risk of contracting NE varies greatly between different countries and localities (Lähdevirta 1971, Lähdevirta and Korpela 1977, Ahlm 1997). In Finland, NE is more widely encountered in the lake area in eastern and central Finland than e.g. in the surroundings of Helsinki. In Sweden, almost all cases of NE are concentrated in the northern part of the country, north of the 59th parallel (Settergren et al. 1988b, Ahlm 1997). Rural people are more prone to contract NE than urban (Lähdevirta 1971, Lähdevirta and Elo 1975, Ahlm et al. 1994), and the disease is most common in 25-45 year old males, with a 2.1:1 male to female ratio (Lähdevirta 1971, Settergren et al. 1988b, Niklasson et al. 1993). However, there is a report of no gender difference in the prevalence of PUUV antibodies (Ahlm 1997). The highest occurrence of NE (Lähdevirta 1971, Lähdevirta and Elo 1975) and the highest PUUV seroprevalences (16%, 26%) have been reported among farmers and forestry workers (Pilaski et al. 1991, Ahlm et al. 1994, Moll van Charante et al. 1994). Furthermore, soldiers (Stuhlfauth 1943,

Markotic et al. 1996) and mammalogists (Vapalahti et al. 1995b) are at major risk of hantaviral infections. An age-related increase in seroprevalence has also been reported (Ahlm 1997).

Clinical features of nephropathia epidemica

Clinical findings

PUUV infection may be either subclinical or associated with mild to severe manifestations. The clinical picture of NE infection in children is similar to that found in adult cases (Mustonen et al. 1994c, Ahlm 1997). The disease begins after an incubation period estimated to be about 2-3 weeks, with a range of 3 days to 2 months (Stuhlfauth 1943, Myhrman 1957, Lähdevirta and Korpela 1977). A typical case of NE sets in with acute high fever lasting 3-5 days, followed by frontal or retroorbital headache, abdominal pain, backache, nausea and myalgia lasting 2-7 days (Lähdevirta 1971, Lähdevirta et al. 1984, Mustonen et al. 1994a). Simultaneously urinary output diminishes to oliguria, rather seldom to anuria, and serum creatinine begins to rise. From 7 to 10 days after the onset of fever, the polyuric phase begins, and the clinical syndrome begins to subside rapidly. Polyuria (peak average 3000ml/day) lasts from a few days to 2-3 weeks, and simultaneously the serum creatinine returns to normal levels (Lähdevirta 1971, Settergren et al. 1988a, Settergren et al. 1989, Mustonen et al. 1994a). Recovery usually takes several weeks, but general weakness can continue for up to 2-4 months, depending on the severity of the disease. According to a study by Lähdevirta (1971), the mean length of hospitalization for NE was 16.6 days, whereas in Settergren`s study (1988a) the mean duration was 8.3 days. Only 44% of the patients concerned were hospitalized for more than 1 week.

PUUV causes a systemic infection leading to a general increase in capillary permeability. This is well in accord with the observation of clinical manifestations in multiple organ systems. NE is most often defined by acute nephritis, but e.g. carditis, hepatitis or meningoencephalitis may also complicate the course (Lähdevirta 1971, Launes and Hautanen 1988, Mustonen et al. 1994a). Mortality in NE is estimated to be 0.1% (Lee et al. 1990, Mustonen et al. 1994a, Valtonen et al. 1995). Autopsies of NE patients have revealed venous congestion and hemorrhage of the kidneys, myocarditis, pulmonary edema and hemorrhage, as well as hypophyseal hemorrhage and necrotic areas of the pituitary gland associated with panhypopituitarism (Linderholm et al. 1991, Forslund et al. 1992, Settergren et al.

1992, Alexeyev and Morozov 1995, Valtonen et al. 1995, Hautala et al. 2002). Central nervous system-related symptoms occur frequently in NE. In one study involving 26 patients with acute NE, the most common symptoms were headache, insomnia, vertigo and nausea (Ahlm et al. 1998).

Ophthalmic symptoms and signs are commonly encountered in patients hospitalized for NE. Transient myopia has been reported in 50% and 53% of patients (Saari et al. 1977, Saari and Luoto 1984), myopic shift in 19% (Pärssinen et al. 1993) as well as decrease in intraocular pressure. Judging from US measurements the anterior chamber is narrower, the crystalline lens thicker and the axial length of the eye shorter during the acute phase of the disease, as compared to measurements obtained after recovery from the systemic infection. The mechanism of myopic shift is mainly a combination of two factors: forward movement of the anterior diaphragm and thickening of the crystalline lens. Ophthalmic manifestations resulting from increased capillary permeability and impaired hemostasis in NE include lid edema, conjunctival chemosis and hemorrhage, as well as retinal edema and hemorrhage (Kontkanen 1998).

Abnormalities in the electrocardiogram are reported in 20% of hospital-treated patients, these including non-specific T-wave and ST-segment abnormalities (Mustonen et al. 1995). Regional left ventricular contraction abnormalities have been detected in echocardiography in 7 out of 20 patients during the acute phase of NE. PUUV infection may affect almost every organ, and some anecdotally reported complications seen in NE patients are shown in Table 2.

The prevalence of clinical symptoms and findings in NE patients is shown in Table 3.

Table 2. Rare complications in NE patients

Complication	**Reference**
Acute disseminated encephalomyelitis	(Toivanen et al. 2002, Krause et al. 2003)
Cerebral hemorrhage	(Settergren et al. 1991b, Zeier et al. 1992, Alexeyev and Morozov 1995)
Meningoencephalitis	(Lähdevirta 1971, Launes and Hautanen 1988, Mustonen et al. 1994a, Bergmann et al. 2002)
Guillain-Barré syndrome	(Forslund et al. 1992, Esselink et al. 1994)
Pancreatitis	(Settergren et al. 1992)
Panhypopituitarism	(Forslund et al. 1992, Settergren et al. 1992, Valtonen et al. 1995, Hautala et al. 2002)
Perimyocarditis	(Mustonen et al. 1994a, Bergmann et al. 2002)
Urinary bladder paralysis	(Alexeyev and Morozov 1995)
Disseminated intravascular coagulation	(Settergren et al. 1989, Linderholm et al. 1991, Clement et al. 1994)

Table 3. Clinical symptoms and findings in NE patients

	Studies		
	Lähdevirta 1971	Settergren et al. 1989	Mustonen et al. 1994a
Number of patients	76	74	126
Symptoms/findings (%)			
Fever	100	99	98
Nausea	78	84	58
Back pain	82	82	54
Abdominal pain	67	65	43
Headache	90	85	62
Myalgia	na	69	27
Visual disturbances	12	31	36
Petechiae	12	1	2
Hemorrhagic manifestations	epistaxis 11 macroscopic hematuria 3 conjuctival bleeding 6	epistaxis 28 macroscopic hematuria 1 conjuctival bleeding 16	10
Diarrhea	12	20	18
Cough	6	32	14
Hypotension (SBP<90mmHg)	2	1	11 (<100mmHg)
Dialysis treatment	1	0	6
Oliguria (<400ml/day)	54	70	na
Polyuria (>2000ml/day)	97	97	na

Abbreviations: SBP=systolic blood pressure, na=not available

Laboratory findings

Abnormal values in the erythrocyte sedimentation rate (ESR) are common (Lähdevirta 1971). Elevated levels of C-reactive protein (CRP) are seen in almost all cases (Settergren et al. 1988a, Settergren et al. 1989, Mustonen et al. 1994a). Thrombocytopenia is found in 52-75% (Settergren et al. 1988a, Settergren et al. 1989, Mustonen et al. 1994a) and leukocytosis in 23-57% of patients with acute NE (Lähdevirta 1971, Settergren et al. 1989, Mustonen et al. 1994a). A shift to the left is fairly common, and a case has been reported with pronounced monocytosis (Ala-Houhala et al. 2000). During the febrile phase there is typically hemoconcentration, followed by hemodilution and slight anemia due to hypervolemia (Lähdevirta 1971).

Transient derangements of electrolyte levels are common, but rarely serious. During the febrile phase of the disease, hyponatremia, hypokalemia, hypocalcemia and hyperphosphatemia are common. Later, however, some patients may develop hypernatremia and hyperkalemia (Lähdevirta 1971, Mustonen et al. 1994a). Low levels of serum total and HDL-cholesterol, as well as high triglyceride levels have been noted during the acute phase of the disease (Mustonen et al. 1994a).

The prevalences of the various laboratory findings in NE patients are shown in Table 4.

Table 4. Laboratory findings in NE patients

	Studies		
	Lähdevirta 1971	Settergren et al. 1989	Mustonen et al. 1994a
Number of patients	76	74	126
Findings			
Max blood leukocyte count (>10.0x10^9/l) (%)	57	23 (>12.0x10^9/l)	50
Max blood leukocyte count (10^9/l) (mean)	na	7.8	11.4
Max serum creatinine (>100µmol/l) (%)	86	96	94
Max serum creatinine (µmol/l) (mean)	na	386	439
Min blood thrombocyte count (<100x10^9/l) (%)	20	52	75
Min blood thrombocyte count (10^9/l) (mean)	na	96	117
Max CRP (mg/l) (mean)	na	60	52
Proteinuria (%)	100	100	94
Proteinuria of nephrotic range (>3.5g/l) (%)	na	na	25
Hematuria (%)	87	85	58
Hypoproteinemia/Hypoalbuminemia	S-Prot<60g/l in 24%	S-Alb<36g/l in 64%	S-Prot<30g/l in 50%

Abbreviations: S-Alb=serum albumin, S-Prot=serum protein, na=not available

Renal disease

Renal involvement in NE results in transient proteinuria, microscopic hematuria and acute renal failure (ARF) with oliguria followed by polyuria and spontaneous recovery. Dialysis treatment is seldom necessary; in 0-6% of hospitalized patients (Lähdevirta 1971, Settergren et al. 1988a, Settergren et al. 1989, Mustonen et al. 1994a). Serum creatinine concentrations can be normal even in hospital-treated patients (Settergren et al. 1989, Settergren et al. 1990, Mustonen et al. 1994a). Polyuria may be seen without preceding oliguria, reflecting tubular dysfunction (Lähdevirta 1971, Settergren et al. 1990, Lee 1991, Mustonen et al. 1994a).

Proteinuria has been observed in almost all cases; it begins abruptly and can be massive, and decreases rapidly in the polyuric phase (Lähdevirta 1971, Lähdevirta et al. 1984, Settergren et al. 1988a, Mustonen et al. 1994a). Nephrotic-range proteinuria (>3.5g/day) has been reported in 25% of hospital-treated patients (Mustonen et al. 1994a). Proteinuria is non-selective (Cosgriff 1991), indicating a defective glomerular barrier. An increased glomerular permeability associated with impairment of both the size- and charge-selective properties of the glomerular filter has been shown (Ala-Houhala et al. 2002). Concomitant urinary loss of low-molecular-weight proteins such as β_2-microglobulin (Settergren et al. 1990, Cosgriff 1991) and α_1-microglobulin (Ala-Houhala et al. 2002)

indicates that tubular injury also contributes to the proteinuria. No relation has been found between the severity of renal histological changes and protein excretion (Mustonen et al. 1994b).

The occurrence of microscopic hematuria is almost the rule, while macroscopic hematuria is rare (Lähdevirta 1971, Settergren et al. 1988a, Settergren et al. 1989, Settergren et al. 1990, Mustonen et al. 1994a, Settergren et al. 1997). Leukocyturia has been reported in 28 -67% of cases (Lähdevirta 1971, Mustonen et al. 1994a), and glycosuria has occasionally been observed (Settergren et al. 1988a, Mustonen et al. 1994a). Urine microscopy has revealed granular or hyaline casts in 25% of patients (Settergren et al. 1990).

A transient elevation in serum creatinine values was described in detail by Lähdevirta (1971). Later, in a series of 355 patients (Settergren et al. 1988a), the peak value for serum creatinine was more than 250 µmol/l in 68%, and almost one–third of patients reached levels of more than 500 µmol/l. The mean creatinine in a study by Mustonen and colleagues (1994a) was 439 µmol/l. In a group examined by Alexeyev and associates (1993) serum creatinine was elevated in 85% of patients.

The glomerular filtration rate studied either by ^{51}CrEDTA or inulin clearance, and effective renal plasma flow studied by para-aminohippurate clearance, have been shown to be significantly reduced in patients during the acute phase of NE (Settergren et al. 1990, Ala-Houhala et al. 2002). Renal involvement in the acute phase has been further characterized by a high filtration fraction (Ala-Houhala et al. 2002). The fall in systemic blood pressure does not explain these functional changes, since renal failure may occur without hypotension and blood pressure levels do not correlate with the severity of renal failure in HFRS. Probably intrarenal events play an important role in the development of renal failure in HFRS (Cosgriff 1991).

Renal biopsy findings in a NE patient were first presented in 1964 (Kuhlbäck et al. 1964). The chronological pattern of histological findings in the kidneys was described by Lähdevirta in 1971, whereafter renal biopsy came to be as a diagnostic tool. Immunohistochemical (Jokinen et al. 1978) and electron microscopy studies (Collan et al. 1978a, Collan et al. 1978b) have provided additional hints as to the pathogenesis of the acute-phase renal involvement. The characteristic histopathological finding in renal biopsy during the acute phase of NE is tubulointerstitial nephritis, and infiltrating cells include lymphocytes, plasma cells, monocytes, macrophages and polymorphonuclear leukocytes (Collan et al. 1991, Mustonen et al. 1994b, Temonen et al. 1996). Medullary hemorrhages are found in 20-60% of acute-phase biopsies (Collan et al. 1991, Mustonen et al. 1994b). Today, in the era of serodiagnosis, the renal biopsy is no longer useful for diagnosing NE, nor is it of significance in

determining the severity of renal failure (Mustonen et al. 1994b). It is, however, possible to isolate PUUV antigen from renal tissue (Groen et al. 1996), but this is of no practical benefit in clinical work.

Immunofluorescence studies have revealed occasional glomerular deposits of IgA, IgG, IgM, complement factor (C) 1q, C3 and fibrinogen, but these have not been related to any other histological findings (Collan et al. 1991, Mustonen et al. 1994b). Despite common and sometimes massive proteinuria seen in patients with NE, histological glomerular changes are surprisingly mild, and reported only in one fourth of cases (Mustonen et al. 1994b). They include slight mononuclear hypercellularity and minor mesangial proliferation (Collan et al. 1991, Mustonen et al. 1994b). In electron microscopy, only slight degenerative changes have been seen in the glomerular endothelial cells (Collan et al. 1991).

Hantavirus pulmonary syndrome

The original description of HPS came from a study of SNV-induced infections (Duchin et al. 1994). SNV is a major agent responsible for HPS in North America (Duchin et al. 1994, Simpson 1998, Enria et al. 2001, Peters and Khan 2002). Instead of hemorrhages and renal failure, HPS is typically characterized by severe pulmonary involvement (Duchin et al. 1994, Chapman et al. 2002), but the infection can also present without respiratory failure (Kitsutani et al. 1999). The clinical features of HPS were originally divided into four phases (febrile, cardiopulmonary, diuretic and convalescent), in contrast to the five for HFRS, which are febrile, hypotensive, oliguric, polyuric and convalescence phases (Lähdevirta 1971, van Ypersele de Strihou and Mery 1989, Mustonen et al. 1994a).

The prodromal symptoms include fever, myalgia and malaise. Also headache, anorexia, nausea, vomiting, diarrhea and back and abdominal pains may occur (Chapman et al. 2002). The prodrome typically lasts 3-5 days (Peters and Khan 2002). The next stage of disease is characterized by shock and pulmonary edema, which may progress in 4-24 hours. Patients often have a cough, tachypnea, tachycardia and hypotension (Peters and Khan 2002). The case fatality has decreased from more than 50% in 1993 to 20% in 1997 (Enria et al. 2001). Leukocytosis, thrombocytopenia and elevated hematocrit are typical signs of an infection by SNV or related hantaviruses (Duchin et al. 1994, Nolte et al. 1995, Zaki et al. 1995, Chapman et al. 2002). The kidneys are not usually affected in a major way as in HFRS, and elevated serum creatinine concentrations are thought to be related to shock and

hypovolemia. Proteinuria is, however, a common finding (Duchin et al. 1994, Nolte et al. 1995, Zaki et al. 1995). The diuretic phase is characterized by a rapid clinical improvement. The convalescent phase may last up to 2 months, with patients recovering apparently fully (Enria et al. 2001).

Histologically, interstitial and air-space edema, mild to moderate interstitial infiltrates of lymphocytes, epithelial necrosis, vascular thrombosis, and hyaline membranes are seen (Ketai et al. 1994). The lung disease in HPS evinces some distinct pathologic differences from diffuse alveolar damage due to other causes, namely extensive cellular debris, destruction of type I cells, prominence of type II cells, neutrophil infiltrates and fibrosing alveolitis (Ketai et al. 1994). Pulmonary edema is non-cardiogenic in origin, and pulmonary capillary wedge pressures are normal, as is the heart size on the radiograph.

There are probably also subclinical and/or mild HPS cases, since high human seroprevalence rates of antibodies against hantaviruses have been reported, ranging from 7 to 21% among asymptomatic groups and community residents (Williams et al. 1997).

Chest radiological findings in nephropathia epidemica and other hantavirus infections

Chest radiography in nephropathia epidemica

Respiratory symptoms such as cough have been reported by up to one-third of NE patients (Settergren et al. 1989). However, in that study chest radiography yielded pathological findings (slight pleural effusion) in only 1 out of 10 patients with pronounced respiratory symptoms. In another study, chest radiography was pathological in 24% of patients (Lähdevirta 1971). In a study where a chest radiograph was taken in 94 cases, pulmonary involvement was observed in 16% (Mustonen et al. 1994a). In 15 cases an abnormal finding related to the present illness was recorded: pleural effusion in 9, parenchymal infiltrations in 6, pulmonary edema in 3 and cardiac enlargement in 2. Both patients with an enlarged heart had acute myopericarditis. In no patient could bacterial pneumonia be documented (Mustonen et al. 1994a). Pulmonary edema is also sometimes seen in HFRS including NE, especially during the oliguric phase of the disease, and is often considered primarily a complication of

volume overload (Lee and Dalrymple 1989). Moreover, HPS-like clinical disease has been described in association with NE (Clement et al. 1994).

Chest radiography in hantavirus pulmonary syndrome

Chest radiographic findings have been studied in patients with HPS (Boroja et al. 2002). Bilateral parenchymal or interstitial and alveolar infiltrates with pleural effusions were noted and seen to be associated with the severity of disease. Once pulmonary edema is present, the disease proceeds rapidly, patients dying within 24-48 hours, hypoxia, circulatory collapse or both being the immediate cause of death. Boroja and associates (2002) indentified two broad categories of HPS clinically and radiographically: a) a rapidly progressive, fulminant and often fatal clinical form with radiographic features of rapidly progressive alveolar pulmonary edema, air-space consolidation, and pleural effusion; b) a limited, less severe clinical form usually associated with mild interstitial edema and minimal air-space disease. All patients with the limited form of HPS survived the illness, whereas 46% of those with the fulminant form died.

Computed tomography in nephropathia epidemica

Computed tomography (CT) of the lungs and chest radiograph were examined in the acute phase of NE in a prospective study with 19 patients (Linderholm et al. 1992). Infiltrates and/or pleural effusion were seen in 10 out of 19 patients. In two of them, abnormalities were disclosed only by CT. Patients with pathologic lung findings evinced a more pronounced inflammatory response, as measured by CRP and blood leukocyte count, than did those with normal findings. It was concluded that radiological evidence of pulmonary involvement is a common finding early in the course of NE.

Renal imaging findings in nephropathia epidemica and other diseases with acute renal failure

Ultrasonography

US plays a valuable role in the clinical evaluation of patients with ARF as it can provide information on the size of the kidneys, the thickness and echogenicity of the parenchyma, the size of the medullary pyramids and the degree of corticomedullary contrast. US findings in the kidneys in ARF are non-specific (Hricak 1982, Mittelstaed 1987, Platt et al. 1991b). Many attempts have been made to correlate the US appearance with the type of renal disease and its severity, but with little success (Rosenfield and Siegel 1981, Hricak et al. 1982). In ARF the parenchyma may swell and increase in thickness and parenchymal reflectivity may be either reduced or markedly increased as in some cases of acute glomerulonephritis and interstitial nephritis (Hricak et al. 1982).

Doppler US can provide useful information regarding the status of renal vascular resistance and may thus be helpful in the evaluation and follow-up of renal diseases (Platt et al. 1989, Rodgers et al. 1992, Chen et al. 1993). The renal vascular bed in a normal kidney is characterized by low impedance blood flow, resulting in a continuous forward flow in the diastole (Dubbins 1989). Parenchymal disease may alter the blood flow pattern to a variable degree depending on the severity of involvement of the vascular channels. The resistive index (RI) in the renal arteries is consequently relatively low, typical normal values of 0.58-0.64 being reported (Platt et al. 1991a). It should be noted that the RI shows greater variability in children (Gill et al. 1994). The RI also tends to be higher in patients over the age of 60 years (Terry et al. 1992). Care must therefore be taken when interpreting Doppler findings in these patients, as a high RI may be due to physiological factors rather than pathology. A reasonable upper limit for the normal mean intrarenal RI would appear to be 0.7 (70%) (Norris et al. 1984, Gottlieb et al. 1989, Kim et al. 1990b, Terry et al. 1992).

In a study with 10 NE patients renal US was performed in 9 cases within one day of the first nephrography (Paul et al. 1991). In two patients US was normal. The characteristic pathological changes consisted of renal edema and obliteration of the border between the renal parenchyme and medulla. In one patient there was slight hydronephrosis and in one a modest perirenal effusion. In two

patients the pathological findings were symmetrical, while in five the changes were more marked on the right, and among these the nephrographs also showed more prolonged retention values in three cases and in the remaining two the nephrographs were symmetrical. In contrast, one of the two patients with normal US showed marked asymmetry in nephrography (Paul et al. 1991). US findings have not previously been compared to the clinical course of NE.

Computed tomography

The pattern of renal enhancement and washout of contrast medium has been observed on sequential follow-up CT in 12 patients with Korean hemorrhagic fever (KHF), which is a severe type of HFRS (Lim et al. 1987). Renal contrast enhancement and washout were delayed longer in patients with severe oliguric renal failure. The delayed washout peaked at 4-5 days and did not return to normal until 8-9 days in the patients with severe oliguria. In those without severe oliguria the times were 1-2 days and 3-4 days, respectively. A characteristic "cart-wheel" pattern was observed during the washout stage in patients without severe oliguria. This pattern of washout is thought to result from relief of vasoconstriction and repair of tubular function. Multifocal "wedge-shaped" nonenhanced areas of the kidney, seen on the 2-week follow-up postcontrast CT, are thought to be ischemic zones due to persistent vasoconstriction. On the 6-week follow-up postcontrast CT in one patient, scarring of the kidney was detected in the same area which was not enhanced on the 2-week CT. This scarring was thought to be a result of permanent vasoconstriction (Lim et al. 1987).

Rebibou and associates (1997) describe a case of NE, which a few years after acute infection exhibited arterial hypertension and multiple papillary necrosis. Papillary necrosis was diagnosed by intravenous pyelography and CT scan. A complete evaluation of the patient failed to show any other etiology for medullary necrosis. Necrosis may explain severe vascular troubles engendered by Hantavirus infection in the kidney medulla (Rebibou et al. 1997).

Magnetic resonance imaging

MRI may provide additional information regarding ARF. Of particular value are the possibilities of differentiating the renal cortex from the medulla and of obtaining a contrast material-enhanced image without use of iodinated intravascular contrast medium, which is contraindicated in patients with ARF.

MRI findings have not previously been studied in NE. With the very high sensitivity to metals such as iron in hemoglobin and its related compounds, characteristic MRI findings have been reported in KHF (Kim et al. 1990a). Renal MRI of KHF patients has shown low signal intensities along the medulla, especially the outer medulla, on T2-weighted images, possibly representing medullary hemorrhage. In three cases MRI was performed in the convalescent phase of KHF. The low signal intensity in the medulla on T2-weighted images encountered in these patients may represent intertubular fibrosis (Kim et al. 1990a). Such findings have not been reported in other renal diseases (Marotti et al. 1987, Demas et al. 1988). In a study by Kim involving application of MRI in two KHF patients, the low signal intensities in the medulla on T2-weighted images were related to congestion and hemorrhage (Kim et al. 1997).

Isotopic nephrography

In a study with 10 NE patients [$^{99}Tc^{m}$] MAG 3 gamma camera nephrography was performed and followed up in 9 of them 22-68 days later when they had clinically recovered. Variables for renal clearance of [$^{99}Tc^{m}$] MAG 3 and the retention of radioactivity in the kidneys and blood were calculated. In all patients renal function was acutely impaired. There was marked reconstitution of renal function in the control studies. [$^{99}Tc^{m}$] MAG 3 clearance was inversely related to the serum creatinine level. On visual inspection the nephrograms showed no focal changes. In that study nephrography was more sensitive than US in identifying renal impairment in NE (Paul et al. 1991).

AIMS OF THE PRESENT STUDY

The main aim here was to investigate by radiological methods the occurrence and pathogenesis of lung and renal findings during the acute phase of NE. The specific or additional aims were:

– to elucidate the putative pathophysiologic cascades of NE lung involvement and establish whether they bear any resemblance to the lung disease in HPS. (I,II)

– to evaluate by radiological methods how NE patients´ lung and renal findings recover during follow up. (I-V)

– to assess the value of radiological methods when evaluating NE patients´ clinical situation and recovery. (I-V)

PATIENTS AND METHODS

Patients

All patients who participated in this series had suffered from serologically confirmed acute PUUV infection (Brummer-Korvenkontio et al. 1980, Hedman et al. 1991, Vapalahti et al. 1995a, Vapalahti et al. 1996). The studies were carried out in Tampere University Hospital and in the Medical School, University of Tampere, and all patients were from the Pirkanmaa region.

Patients were selected in two groups in study I: 97 consecutive hospital-treated acutely ill NE patients during the years 1982-1989 were included and 28 NE patients with pulmonary changes from the years 1990-1994.

In study II 380 NE patients in whom radiological examinations had been performed during the years 1982-1998 were examined; 33% of these patients were the same as in study I.

In studies III and IV 23 consecutive NE patients treated from January 1997 to February 1998 were examined. In study III findings were analyzed prospectively and in study IV retrospectively.

In study V 20 consecutive NE patients treated from October 2000 to October 2002 were prospectively examined.

A more detailed description of the participants is given in the original publications.

Methods

Clinical course

Clinical data and laboratory findings were retrospectively analyzed from patient records. Minimal and/or maximal values and differences between maximal and minimal values during hospital care were used. The radiologists were unaware of the patients´ clinical data.

Chest radiograph analysis and correlation to the clinical course

In study I one to three chest radiographs per patient were analyzed, one on admission to the hospital, another if the patient had respiratory symptoms or radiograph findings were suspected in the first chest radiograph, and the last as a control at close of treatment if needed. All radiographs were assessed by two radiologists together. The nature and location of the findings were analyzed in 6 fields of the lung: the low, middle and upper fields of the left and right lung separately. Clinical and laboratory findings were correlated to the chest radiograph findings in four different ways: 1) patients without and with pathological chest radiograph findings, 2) according to the severity of the chest radiograph findings: mild, moderate and severe (Table 5), 3) those with pulmonary edema and those with other findings than pulmonary edema, 4) those with viral pneumonia-like findings and those with other pathologic findings.

In study II all chest radiographs taken during hospital care were analyzed by two radiologists together; 51% of the patients underwent digital chest radiography and the rest conventional chest radiography. The nature and location of the findings were analyzed in 6 fields of the lung: the low, middle and upper fields of the left and right lung separately. Clinical and laboratory findings were correlated to the chest radiograph findings in two different ways: 1) patients without and with pathological chest radiograph findings, 2) according to the severity of the chest radiograph findings: mild, moderate and severe (Table 5).

Table 5. Severity grading of chest radiographs (I,II)

Mild changes	Atelectasis, slight pleural effusion, slight infiltrates in 1-4 of the 6 lung fields or intense infiltrates in 1 of the 6 lung fields, slight increase in venous stasis, slightly enlarged heart
Moderate changes	Interstitial or slight alveolar edema, slight infiltrates in 5-6 lung fields or intense infiltrates in 2-3 lung fields, marked pleural effusion, clear increase in venous stasis, clearly enlarged heart
Severe changes	Marked alveolar edema or intense infiltrates in 4-6 lung fields

Ultrasonography analysis and correlation to the clinical course

In study II all renal US images recorded during hospital care were analyzed retrospectively by two radiologists together. Other renal US images were used for comparison if available. Qualitative US findings were analyzed in both kidneys. Because findings were mostly equal between both kidneys a common statement was made. Clinical and laboratory findings were correlated to the renal US findings in two different ways: 1) patients without and with pathological renal US findings, 2) according to the severity of the renal US findings: mild, moderate and severe (Table 6).

Table 6. Severity grading of renal US findings (II)

Mild changes	Slight increase in parenchymal swelling, slight increase in cortical echogenicity (about the same as in the liver), slight disturbance in corticomedullary border differentiation, presence of slight patchy pattern in parenchymal echo texture
Moderate changes	Appreciable increase in parenchymal swelling, appreciable increase in cortical echogenicity, appreciable disturbance in corticomedullary border differentiation (partly preserved), presence of appreciable patchy pattern in parenchymal echo texture, perirenal/ascites fluid without other pathological US findings
Severe changes	Marked increase in parenchymal swelling, marked increase in cortical echogenicity, corticomedullary border differentiation impossible, perirenal/ ascites fluid with other pathological US findings

In studies III and IV two renal US examinations were made per patient, the first on admission to the hospital and the second as a follow-up examination. All US studies were made with an Acuson Sequoia 512 using a 4V2-sector transducer with a function frequency of 2.5-4 MHz. The radiologist was free to use optimum frequency and native tissue harmonic imaging mode. Other settings remained constant. Focusing was set at the level of the kidney. Patients were studied by one of the two radiologists.

In study III the following kidney variables were prospectively determined: length, cortical parenchymal thickness and RI in the Doppler spectrum. In addition, the presence of perirenal, pleural, pericardial or ascites fluid collections was registered. Initially the findings in the primary and repeat US studies were compared, whereafter the patients were divided into two groups for each of the five US criteria below. Each division except for the presence of any fluid collections in the primary study was

made according to the median values of the US findings as differentiating criteria. These findings were: 1) RI in the primary study, 2) change in renal length, 3) change in cortical parenchymal thickness, and 4) change in RI between the primary and repeat US studies. Clinical and laboratory findings between two groups of each of these five divisions were tested.

In study IV the best coronal US image from the digital data was chosen for each kidney and printed out for films to a standard size without labels or written data, each image on a separate film. The blinded images were analyzed by three radiologists retrospectively in two phases and the mean values were used. First, the films were displayed one by one for the radiologist to evaluate each independently. Second, the images of a single kidney from both the primary and repeat study were presented simultaneously for evaluation by the same investigators, two images at a time without revealing their chronological order. Five different subjective US findings were estimated: the presence of parenchymal swelling, disturbance in corticomedullary border differentiation, presence of a patchy pattern in parenchymal echo-texture, cortical echogenicity and overall subjective rating of kidney pathology. Initially the findings in the primary and repeat US studies were compared. Thereafter the patients were divided into two groups (mild and severe changes) for each of the five US criteria. For each criterion, changes between the two US studies were determined. Each division was made according to the median change as a differentiating criterion. Differences in clinical and laboratory findings were tested between these two US groups in five qualitative US divisions.

Magnetic resonance imaging analysis and correlation to the clinical course

In study V two renal MRI examinations were performed per patient, the first on admission to the hospital and second as a follow-up examination. MRI was performed using a scanner Signa Horizon Echospeed LX (General Electric, Milwaukee, WI, USA) operating at 1.5 T, using torso phase array coil. The following kidney measurements were determined: parenchymal volume, renal length and parenchymal thickness. The presence of perirenal, inside and outside Gerota fascia edema/fluid collections was registered. Initially the findings in the primary and repeat MRI studies were compared, whereafter the patients were divided into two groups for each of the four MRI criteria. Each division except for the presence of edema/fluid collections in the primary study was made according to the median values of the MRI findings as a differentiating criterion. These findings were: 1) change in parenchymal volume, 2) change in renal length, and 3) change in parenchymal thickness between the

primary and repeat study. Clinical and laboratory findings between two groups of each of these four divisions were tested.

Statistical methods

In study I means and standard error of means of the highest and lowest values of the parameters measured during hospital care were used. Differences in clinical and laboratory data between the different chest radiograph findings were tested by one-way analysis of variance and Mann-Whitney U-test. To detect possible correlations linear regression analysis was used. The data analysis was also carried out using polychotomous or binary logistic regression modelling, depending on the classes of response variables, to define explanatory models and predictative parameters for the different radiograph findings. The calculations were carried out with a Sun computer using a BMDP software package (Los Angeles, CA).

In study II frequency distributions or cross-tabulations were used. Mann-Whitney U test was used to compare radiological findings with each other. T-test or Mann-Whitney U test was used to compare clinical course to normal and abnormal radiological findings. Analysis of variance or Kruskal-Wallis test was used to compare clinical course to different severity groups of radiological findings.

In study III the Wilcoxon test was used in comparing right and left kidneys and the findings in the primary and repeat studies. The Mann-Whitney U-test was used to compare the clinical course to the US findings.

In study IV T-test or Mann-Whitney U-test was used to compare the clinical course to the US findings. Interindividual agreement between the radiologists´ assessments was analyzed by kappa value.

In study V Wilcoxon´s test was used in comparing right and left kidneys and the findings in the primary and repeat studies. The Mann-Whitney U-test was used to compare the clinical course to the MRI findings. The correlation coefficient was used to compare MRI measurements with each other. The repeatability of calculations between two time points was evaluated by the method of Bland and Altman (Bland and Altman 1986).

Data analysis in studies II-V were carried out using SPSS 9.0 statistical software and p-values <0.05 were regarded as significant in all studies.

RESULTS

Clinical course

The clinical course was typical of acute NE in most of the patients participating in studies I-V. Two patients died of NE between the years 1982-2002. Clinical and laboratory findings in 380 NE patients included in studies I-IV are shown in Table 7 and in 20 NE patients included in study V in Table 8.

Table 7. Clinical and laboratory findings in 380 NE patients

	Median	Mean	SD	Range
Clinical findings				
Duration of hospital care (days)	7	8	5	1-66
Change in body weight* (g)	2700	3454	3040	0-18500
Min daily urine excretion (ml)	1300	1422	907	0-4840
Max daily urine excretion (ml)	3980	4316	1759	800-11000
Max systolic blood pressure (mmHg)	140	141	20	90-210
Max diastolic blood pressure (mmHg)	85	87	12	55-120
Laboratory findings				
Max blood leukocyte count (10^9/l)	10.1	11.3	5.6	3.8-44.7
Max CRP (mg/l)	65	73	44	6-305
Max serum creatinine (μmol/l)	227	352	306	2-1645
Max serum urea (mmol/l)	17.8	20.6	13.6	2.1-65.0
Max daily urine protein excretion (g)	1.3	2.4	2.7	0.06-17.8
Min serum protein (g/l)	59	59	7	43-98
Min serum albumin (g/l)	30	30	5	19-45
Min blood thrombocyte count (10^9/l)	74	90	63	9-378
Min hematocrit	37	37	5	21-50
Max hematocrit	43	44	6	26-65

*Difference between highest and lowest weight during hospital care

Table 8. Clinical and laboratory findings in 20 NE patients

	Median	Mean	SD	Range
Clinical findings				
Duration of hospital care (days)	7	7	3	3-15
Change in body weight* (g)	2200	2820	2679	0-8900
Min daily urine excretion (ml)	1495	1675	1353	200-4900
Max daily urine excretion (ml)	3750	4016	1678	1490-6820
Min systolic blood pressure (mmHg)	120	122	18	86-162
Max systolic blood pressure (mmHg)	138	145	22	110-204
Min diastolic blood pressure (mmHg)	71	72	12	54-100
Max diastolic blood pressure (mmHg)	88	89	12	70-110
Laboratory findings				
Max blood leukocyte count (10^9/l)	11.4	12.2	3.6	8.0-19.3
Max CRP (mg/l)	96	112	71	13-269
Max serum creatinine (µmol/l)	294	341	217	78-950
Max serum urea (mmol/l)	20.0	20.8	10.7	3.2-36.1
Min serum albumin (g/l)	32	31	6	22-40
Min blood thrombocyte count (10^9/l)	70	84	62	14-292
Min hematocrit	36	35	5	25-43
Max hematocrit	45	45	7	29-55

*Difference between highest and lowest weight during hospital care

Chest radiograph findings

Altogether 28% (I) to 35% (II) of the patients yielded disease-related findings in their chest radiographs. Pleural effusion, atelectasis and interstitial infiltrates were the most common findings, whereas frank pulmonary edema was rare. Prevalences of pathologic chest radiograph findings are shown in Tables 9 (I) and 10 (II).

Table 9. Prevalence of pathologic chest radiograph findings in 97 NE patients (I)

Radiograph findings	Number of patients	Percentage of patients
Pleural effusion	21	22
Atelectasis	11	11
Pulmonary infiltrate	7	7
Bilateral pleural effusion	6	6
Venous congestion	6	6
Pulmonary edema	4	4
Sum of abnormalities	27	28

Table 10. Prevalence of pathologic chest radiograph findings in 344 NE patients (II)

Radiograph findings	Number of patients	Percentage of patients
Slight pleural effusion	92	27
Atelectasis	52	15
Slight (<1cm) lung infiltrate	38	11
Heart enlargement (slight/clear)	30 (26/4)	9 (8/1)
Increase in venous stasis (slight/clear)	20 (17/3)	6 (5/1)
Marked pleural effusion	15	4
Interstitial edema	13	4
Peripheral alveolar edema (slight/marked)	8 (5/3)	2 (1/1)
Central alveolar edema (slight/marked)	8 (4/4)	2 (1/1)
Slight (>1cm) lung infiltrate	8	2
Intense (<1cm) lung infiltrate	6	2
Intense (>1cm) lung infiltrate	4	1
Sum of abnormalities	121	35

Including the 28 patients with pathologic chest radiographs there were altogether 55/125 acute-phase NE patients evincing chest radiograph changes (I); 44 (80%) of these showed mild, 6 (11%) moderate and 5 (9%) severe changes.

A total of 121/344 of the patients had disease-related findings in their chest radiographs (II); 94 (78%) of these had mild, 19 (16%) moderate and 8 (6%) severe changes.

Chest radiograph findings did not vary significantly between different years (II).

In conclusion, chest radiograph findings are not disease-specific in NE. Three groups of the most typical chest radiograph findings in NE could be defined. The first and most frequent finding comprised accumulation of pleural fluid and atelectasis, the second infiltrates, interstital and peribronchovascular streaks most profusely in the lower parts of the lung resembling the features of viral pulmonary infection (Fraser et al. 1989) except for hilar adenopathy. The third and most

uncommon finding was slight interstitial edema or typical alveolar edema. The pulmonary involvement of the acute disease was regarded as transient in all cases.

Associations between chest radiograph findings and clinical course

Abnormal findings in chest radiograph in both studies (I,II) were associated with fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, leukocytosis and thrombocytopenia (Table 11).

Table 11. Associations of chest radiograph findings with clinical course (I, mean values) (II, median values)

	Chest radiograph (I)		Chest radiograph (II)	
	normal/abnormal	p-value	normal/abnormal	p-value
Clinical findings				
Duration of hospital care (days)	na		7/10	<0.001
Change in body weight* (g)	3000/6300	<0.001	2613/5037	<0.001
Min daily urine excretion (ml)	1472/1137	<0.01	1513/1204	<0.001
Max daily urine excretion (ml)	na		3998/4879	<0.001
Max systolic blood pressure (mmHg)	130/151	<0.01	138/149	<0.001
Max diastolic blood pressure (mmHg)	na		85/90	<0.001
Laboratory findings				
Max blood leukocyte count (10^9/l)	10.6/14.1	<0.001	10.4/13.4	<0.001
Max CRP (mg/l)	61/74	0.073	72/71	0.609
Max serum creatinine (µmol/l)	356/590	<0.05	284/520	<0.001
Max serum urea (mmol/l)	17.0/32.0	<0.001	16.0/28.5	<0.001
Max daily urine protein excretion (g)	2.6/2.7	0.73	2.4/2.5	0.265
Min serum protein (g/l)	62/54	<0.001	61/56	<0.001
Min serum albumin (g/l)	33/28	<0.001	32/28	<0.001
Min blood thrombocyte count (10^9/l)	113/77	<0.01	96/79	0.019
Min hematocrit	38/34	<0.001	38/35	<0.001
Min serum sodium (mmol/l)	135/129	<0.001	na	
Max serum potassium (mmol/l)	4.6/4.9	<0.05	na	

*Difference between highest and lowest weight during hospital care, na=not available

Differences in serum electrolytes were also noted accompanying normal and abnormal findings in the chest radiograph (I). The serum protein and albumin concentrations were significantly lower in patients with pathological chest radiography (I,II). Values of parameters including the highest measured CRP (I,II), ESR (I), liver transaminases (I), serum immunoglobulins (I), complement components (I), proteinuria (I,II) and lowest measured blood pressure (I) did not differ between the groups.

In both studies (I,II) the groups with normal or mild changes differed significantly from those with moderate or severe changes in respect of laboratory findings such as clinical renal insufficiency, highest blood leukocyte count, hematocrit nadir, and variables related to fluid volume overload like the smallest daily urine output (diuresis) and the greatest difference in body weight during the oliguric and recovery phases of the disease. Hypoproteinemia best predicted the occurrence of abnormal chest radiograph findings in NE (I).

Renal ultrasonography findings

Quantitative findings

Renal length, cortical parenchymal thickness and RI differed significantly between the primary and repeat studies (III). Right and left kidneys did not differ significantly from each other in these US findings. The mean renal length (right and left) was greater in the primary as compared to the repeat study in all 23 patients (range of change 5-23mm). The mean renal cortical parenchymal thickness was greater in the primary as compared to the repeat study in 19 patients (range of change 1-12mm); it remained unchanged in 3 patients and increased by 1mm in 1. The mean renal RI was higher in the primary study as compared to the repeat in 18 patients (range of change 0.5-32.5%) and lower in 5 (range of change 0.5-7%). In the primary study it was normal (47-69%) in 11 patients and abnormal (71.5-89.5%) in 12; in the repeat study it was normal in 21 patients and abnormal (72% and 76%) in 2. Fluid collections (pleural, perirenal, pericardial, ascites) were found in 13 patients, all in the primary study. Perirenal fluid was demonstrated on the right side in 4, on the left in 1 and bilaterally in 1 case.

Qualitative findings

Altogether 117/250 (47%) of the patients showed qualitative findings in renal US, increased cortical echogenicity (36%) and cortical swelling (28%) being the most common findings (II) (Table 12); 62 (53%) of these patients evinced mild, 22 (19%) moderate and 33 (28%) severe changes.

Table 12. Prevalence of pathologic qualitative renal US findings in 250 NE patients (II)

Renal US findings	**Number of patients**	**Percentage of patients**
Increase in cortical echogenicity (slight/appreciable/marked)	91 (65/17/9)	36 (26/7/4)
Increase in parenchymal swelling (slight/appreciable/marked)	71 (57/8/6)	28 (23/3/2)
Disturbance in corticomedullary border differentiation (slight/appreciable/impossible)	40 (18/16/6)	16 (7/6/2)
Presence of patchy pattern in parenchymal echo-texture (slight/appreciable)	38 (29/9)	15 (12/4)
Perirenal/ascites fluid with other pathological US findings	24	10
Perirenal/ascites fluid without other pathological US findings	4	2
Sum of abnormalities	117	47

A total of 20/23 (87%) of the patients yielded qualitative findings in renal US, assuming that change in these findings between the primary and repeat study is to be regarded as a sign of abnormality (IV). Presence of parenchymal swelling and overall rating of pathology in 87% of patients were the most common findings (Table 13).

21 of the 23 patients (91%) showed qualitative changes in renal US assuming that a comparative study is available (IV). Presence of parenchymal swelling and disturbance in corticomedullary border differentiation in 91% of patients were the most common findings (Table 13).

Table 13. Prevalence of pathologic qualitative renal US findings in 23 NE patients without/with comparison image (IV)

Renal US findings	Number of patients	Percentage of patients
Increase in parenchymal swelling	20/21	87/91
Overall rating of pathology	20/20	87/87
Disturbance in corticomedullary border differentiation	15/21	65/91
Presence of patchy pattern in parenchymal echo-texture	18/15	78/65
Increase in cortical echogenicity	14/18	61/78
Sum of abnormalities	20/21	87/91

In conclusion, quantitative renal US findings were recorded in every patient and qualitative US findings in almost all. The most frequent finding was swelling of the kidney. Quantitative and qualitative renal US findings are not disease-specific in NE. The renal involvement of the acute disease seems to be transient, even though in the repeat study RI was abnormal in two patients and not all the subjective qualitative US findings were evaluated as normal.

Associations between renal ultrasonography findings and clinical course

Quantitative findings

Increased RI and the presence of fluid collections in the primary study as well as a greater change in length and RI between the primary and repeat studies were to some extent associated with clinical fluid volume overload (high maximum daily urine excretion, substantial change in body weight between the anuric and polyuric phases and low hematocrit value), degree of clinical renal insufficiency (high maximum serum creatinine and urea concentration) and leukocytosis (III) (Table 14).

Table 14. Associations of quantitative renal US findings with clinical course in 23 NE patients (III, median values)

	RI in the first study (%)			Fluid collections			Change in length (mm)			Change in RI (%)		
	<71.5	≥71.5	p-value	no fluid	fluid	p-value	<13	≥13	p-value	decreased≤4.5 or increased	decreased>4.5	p-value
Clinical findings												
Change in body weight (g)	1950	3350	0.347	1150	3650	0.045	1450	3600	0.254	3050	3200	0.566
Max daily urine excretion (ml)	3690	4505	0.052	3700	6217	0.039	3690	3905	0.140	3850	3800	0.880
Laboratory findings												
Max blood leukocyte count (10^9/l)	9.2	11.3	0.799	8.6	13.4	0.024	9.2	11.7	0.582	9.2	11.2	0.786
Max CRP (mg/l)	84	76	0.932	94	68	0.219	69	76	0.872	99	61	0.032
Max serum creatinine (μmol/l)	122	325	0.052	111	273	0.052	106	240	0.159	115	332	0.032
Max serum urea (mmol/l)	9.9	26.1	0.024	12.7	26.6	0.052	11.5	26.1	0.109	11.1	26.4	0.017
Max hematocrit	38	36	0.034	37	36	0.171	38	34	0.001	38	36	0.023

Qualitative findings

The severity of findings in renal US was to some extent associated with clinical fluid volume overload, degree of clinical renal insufficiency and leukocytosis (II) (Table 15), (IV) (Table 16).

The severity groups of renal US findings did not correlate positively with the clinical severity of NE (II).

In conclusion, quantitative and qualitative renal US findings were to some extent associated with clinical fluid volume overload, degree of clinical renal insufficiency and leukocytosis. Qualitative US findings are as sensitive as quantitative in the assessment the clinical course and recovery of NE patients´.

Table 15. Associations of qualitative renal US findings with clinical course in 250 NE patients (II, median values)

	Renal US normal/abnormal	p-value
Clinical findings		
Duration of hospital care (days)	8/8	0.737
Change in body weight* (g)	3348/4040	0.049
Min daily urine excretion (ml)	1480/1349	0.273
Max daily urine excretion (ml)	4205/4635	0.031
Max systolic blood pressure (mmHg)	142/141	0.714
Max diastolic blood pressure (mmHg)	87/86	0.674
Laboratory findings		
Max blood leukocyte count (10^9/l)	11.1/12.3	0.061
Max CRP (mg/l)	77/67	0.379
Max serum creatinine (µmol/l)	328/426	0.025
Max serum urea (mmol/l)	19.2/22.3	0.252
Max daily urine protein excretion (g)	2.0/2.6	0.046
Min serum protein (g/l)	60/58	0.346
Min serum albumin (g/l)	30/29	0.267
Min blood thrombocyte count (10^9/l)	96/84	0.115
Min hematocrit	37/36	0.019

*Difference between highest and lowest weight during hospital care

Table 16. The most interesting associations of the change in qualitative renal US findings with clinical course in 23 NE patients (IV, mean values)

	Parenchymal swelling mild/severe	**Cortical echogenicity** mild/severe	**Corticomedullary differentiation** mild/severe	**Presence of patchy pattern** mild/severe	**Overall rating of pathology** mild/severe
Clinical findings					
Change in body weight* (g)	1855/4900**	2909/3933	3317/3582	2831/4240	3382/3500
Min daily urine excretion (ml)	1919/1160**	1846/1226	1626/1410	1484/1573	1897/1180**
Max daily urine excretion (ml)	4441/5119	4849/4745	4983/4589	4499/5180	5255/4373
Max systolic blood pressure (mmHg)	137/145	138/144	137/146	142/140	142/141
Max diastolic blood pressure (mmHg)	84/91	84/90	82/93**	85/91	85/90
Laboratory findings					
Max blood leukocyte count (10^9/l)	8.3/13.6	9.9/12.2	10.9/11.3	8.7/14.1**	10.2/11.9
Max CRP (mg/l)	88/73	92/69	80/80	89/69	94/67
Max serum creatinine μmol/l)	151/347**	178/323	246/261	181/348**	199/303
Max daily urine protein excretion (g)	1.5/2.6	2.1/2.1	2.0/2.3	1.6/2.9	1.8/2.5
Min blood thrombocyte count (10^9/l)	97/75	87/85	78/94	85/87	79/92
Min hematocrit	38/36	38/36	36/37	37/36	37/36
Max hematocrit	41/47**	43/45	44/44	43/45	44/45

*Difference between highest and lowest weight during hospital care

**P-value < 0.05, between mild and severe groups

Renal magnetic resonance imaging findings

The mean parenchymal volume (right and left) was greater in the primary as compared to the repeat study in all 20 patients (range of change 54-172cm^3). The mean renal length was greater in the primary as compared to the repeat in all (range of change 2-13mm) and the mean parenchymal thickness greater in the primary as compared to the repeat study in all patients (range of change 1.0-8.5mm). Edema/fluid collections were found bilaterally in all anatomical areas (perirenal, inside/outside Gerota fascia) in 16 patients, all in the primary study. Right and left kidney did not differ significantly from each other in respect of these MRI findings (V).

In conclusion, quantitative renal MRI findings were recorded in every patient. Renal MRI findings are not disease-specific in NE. The renal involvement of the acute disease was regarded as transient.

Associations between renal magnetic resonance imaging findings and clinical course

Greater change in parenchymal volume, renal length and parenchymal thickness between the primary and repeat study as well as the presence of edema/fluid collections in the primary study showed no significant association ($p>0.01$) with any clinical or laboratory findings. These findings, however, evinced a mild association with clinical fluid volume overload (change in body weight, lowest daily urine excretion), degree of clinical renal insufficiency (highest serum creatinine and urea value), high blood pressure level (highest systolic and diastolic blood pressure level), inflammation (highest blood leukocyte count and CRP value) and thrombocytopenia (lowest blood thrombocyte count) (V) (Table 17).

In conclusion, renal MRI findings were to some extent associated with the clinical course of NE.

Table 17. Associations of renal MRI findings with clinical course in 20 NE patients (V, mean values)

	Change in parenchymal volume (cm^3)		**Change in length (mm)**		**Change in parenchymal thickness (mm)**		**Fluid collections**	
	<80.4 (10)	≥80.4 (10)	<9.5 (9)	≥9.5 (11)	<5.3 (10)	≥5.3 (10)	no fluid (4)	fluid (16)
Clinical findings								
Change in body weight (g)	1580	4060	2600	3000	1830	3810	2250	2963
Min daily urine excretion (ml)	1947	1403	1632	1710	1753	1597	2590	1446
Max systolic blood pressure (mmHg)	140	150	144	146	141	149	143	146
Max diastolic blood pressure (mmHg)	87	91	87	90	87	91	95	88
Laboratory findings								
Max blood leukocyte count (10^9/l)	10.9	13.4	12.3	12.0	11.1	13.3	10.5	12.6
Max CRP (mg/l)	89	136	93	128	107	118	68	123
Max serum creatinine (μmol/l)	257	426	326	353	297	385	356	337
Max serum urea (mmol/l)	15.3 (7)	28.4 (5)	19.6 (4)	21.3 (8)	17.9 (7)	24.7 (5)	12.7 (4)	24.8 (8)
Min blood thrombocyte count (10^9/l)	110	57	63	100	103	64	160	64

Differences between two groups in renal MRI findings were non-significant, Mann-Whitney U test

DISCUSSION

Chest radiograph findings

Pulmonary findings were fairly common in these hospital-treated patients, occurring in about one third (I,II). This is consistent with earlier reports with fewer patients (Lähdevirta 1971, Linderholm et al. 1992, Mustonen et al. 1994a). Mild pulmonary findings predominated, including pleural effusion, atelectasis and interstitial infiltrates often resembling changes in viral pulmonary infection, as in an earlier report (Linderholm et al. 1992). In a study with 10 Dengue hemorrhagic fever patients who underwent both sonography and chest radiography, sonography detected pleural effusion in all 10, whereas radiography detected it in only 3 cases (Thulkar et al. 2000). It has been stated that the minimum pleural fluid volume detectable on standing posteroanterior radiographs is 175ml (Collins et al. 1972). It is possible that smaller amounts can be detected with digital radiography; 51% of NE patients here underwent digital chest radiography (II).

Severe findings including marked alveolar edema or intense infiltrates were seen in 2-4% of the patients (I,II). Only one patient in study I and two in study II showed rapid and massive extension of edematous opacities as in the adult respiratory distress syndrome (ARDS). Ketai and associates (1994) describe 16 HPS patients with typical pulmonary involvement. In chest radiographs interstitial edema was a typical finding in most cases. Two thirds of the patients subsequently developed bilateral air space disease, which was typically bibasilar and perihilar. Pleural effusion was also a common finding. These patients had symptoms of ARDS and half of them died in consequence of the development of pulmonary edema.

In conclusion, the findings were less severe than in HPS and the most obvious similarity was the early accumulation of pleural fluid (Ketai et al. 1994). Chest radiograph findings can be taken not to be disease-specific in NE even though the findings were not compared to the other diseases. The three most characteristic types of lung involvement in NE were determined as a) accumulation of pleural fluid and atelectasis, b) infiltrates and interstitial and peribronchovascular streaks most profusely in the lower parts of the lung resembling the features of viral pulmonary infection with the

exception that there was no hilar adenopathy and c) slight interstitial or typical alveolar edema. The chest radiograph findings proved to be transient.

Associations between chest radiograph findings and clinical course

The occurrence and severity of radiograph findings were associated with fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, leukocytosis and thrombocytopenia (I,II). Patients yielding pathologic radiograph findings had more severe hypoproteinemia than those with normal chest radiograph. Low serum protein concentration was an independent risk factor for pathologic findings in chest radiography (I). However, the direct causes and sequelae of hypoproteinemia in NE are difficult to assess. In general, hypoproteinemia may be caused by the loss of protein into the urine, intestine or perivascular tissue ensuing from endothelial cell damage or increased capillary permeability. It may also reflect suppressed liver synthesis of albumin in an acute-phase response to inflammatory disease (Ballmer et al. 1992). In the present studies, the acute-phase protein CRP did not differ between the groups yielding normal or pathologic chest radiographs (I,II). The amount of peak proteinuria and the lowest serum protein count did not correlate implying additional reasons for hypoproteinemia.

Capillary leakage has been suggested to be important in hantavirus infections and to constitute the putative mechanism of non-cardiogenic pulmonary edema in HPS. In SNV infection the alveolar space protein concentration is increased up to 80% of the serum level due to severely increased capillary permeability (Ketai et al. 1994). However, in subjects with NE the alveolar fluid contained an increased amount of fibronectin while the alveolar protein content was comparable to that in control subjects (Linderholm et al. 1993). Although the theory of alveolar capillary leakage lacks direct evidence, it may play an important role in individual severe cases of NE lung involvement. In addition, the accumulation of pleural fluid in NE could also be taken as a sign of capillary leakage.

In contrast to what is known of SNV-derived HPS, the present data suggest that the degree of ARF was associated with the presence of chest radiograph findings (I,II). Moreover, in the two present studies all patients with pulmonary edema had renal insufficiency, although this was not severe in all subjects. It is known that in ARF fluid overload may result in pulmonary edema mediated by increased hydrostatic pressure and subtle capillary injury secondary to uremia (Kjellstrand et al. 1988). In NE

increased vascular permeability could further increase fluid retention. In the present study the change in body weight between the oliguric and recovery phases of the disease, reflecting the fluid volume overload, was an important predictor of the presence and severity of chest radiograph findings (I).

Most patients with pulmonary edema evinced slight enlargement of the heart (I,II). Venous congestion was also evident in almost all of these patients. It is possible that venous congestion could be the sole or a coexisting cause of findings in some patients with pulmonary infiltrates and pleural effusion. The assessment of venous congestion was difficult, however, as many of the chest films in the acute phase were taken with the patient supine. Clinically heart failure was in no case suspected as the cause of pulmonary abnormalities. It is interesting that one third of NE patients have transiently decreased left ventricular contractility during the acute phase of the disease (Mustonen et al. 1995).

The HPS patients who did not survive might also have had concurrent myocardial disease (Duchin et al. 1994). Despite adequate assisted ventilation and oxygenation HPS patients have manifested progressive hypotension and lethal arrythmias (Duchin et al. 1994, Morrison and Rathbun 1995, Nolte et al. 1995). The myocardium was histologically normal in HPS (Nolte et al. 1995), although hantaviral antigen could be detected in heart tissue (Zaki et al. 1995). However, during pulmonary edema enlargement of the heart was an infrequent finding and the pulmonary capillary wedge pressure was normal in most patients. This excludes heart failure as a major causative factor for alveolar flooding in HPS (Duchin et al. 1994, Ketai et al. 1994).

Local and systemic inflammatory responses have been considered important in the pathogenesis of both NE and HPS. In NE the highest blood leukocyte count and ESR correlate with the severity of renal disease (Mustonen et al. 1994b). The association of inflammation with pulmonary involvement in the present studies could therefore be indirect and mediated by severe renal insufficiency. Clement and associates (1994) described 7 non-lethal NE subjects from Belgium with severe arterial oxygen desaturation, bilateral interstitial infiltrates and pleural effusion without signs of heart failure. They had markedly elevated inflammatory parameters. Consistent with the present results, in a Swedish study patients with pleural effusion and pulmonary infiltrates had higher blood leukocyte and lower blood thrombocyte counts as compared to those with normal chest radiographs, but there was no difference between these groups in the severity of renal involvement (Linderholm et al. 1992). This suggests that inflammation could also be an independent factor in pulmonary involvement in NE. In HPS, the lung histopathological feature is interstitial pneumonitis with a variable mononuclear cell infiltration, edema and focal hyaline membranes (Zaki et al. 1995). In spite of the verified wide presence of hantaviral

antigens in the endothelial cells of the microvasculature (Zaki et al. 1995), in most cases pneumocytes have been found intact and the virus itself was not cytopathic (Nolte et al. 1995, Zaki et al. 1995). Inflammatory cells and cytokines could thus play an important role in transient functional changes in endothelial cells in the pathogenesis of capillary leakage.

The virulence factors of hantaviruses or other mediators responsible for variable clinical outcomes in humans are not known. When patients with viral pneumonia-like findings were studied separately and compared to patients with other kinds of chest radiograph findings, clinical or laboratory findings did not differ (I). Radiological findings suggesting viral pneumonia cannot therefore be used to differentiate a clinically distinct group of NE patients. PUUV is more closely related to SNV than Hantaan virus (Nichol et al. 1993), but NE nonetheless resembles more Hantaan virus-caused HFRS than HPS. In addition to their individual properties encoded into the viral sequence, genetic determinants of the host inflammatory response may also be involved in the severity of findings. Severe forms of NE with shock and renal failure requiring dialysis treatment and the occurrence of pathologic chest radiograph findings have been found to be associated with HLA haplotype B8 and DRB1*0301 (Mustonen et al. 1996). Pathological or altered immune-mediated responses or virus host interactions may thus play an initiating role in the complex mechanisms leading to severe pulmonary involvement. On the other hand, in pediatric patients no significant differences in clinical picture have emerged between patients with and without HLA B8 and DRB1*0301 (Mustonen et al. 2004). A chest radiograph was obtained in 17 cases and the finding was abnormal in only 1 patient, reflecting the benign course of NE in children.

Pathogenesis of pulmonary involvement and radiograph findings in nephropathia epidemica

Comparisons with the clinical and laboratory data suggest that capillary leakage and inflammation, and in contrast to what is known for HPS, also the fluid volume overload associated with renal insufficiency are important factors in the pathogenesis of chest radiograph findings in NE (Figure 3).

Quantitative and qualitative renal ultrasonography findings

Renal US findings were analyzed in three studies (II,III,IV). Apart from blood flow measurement and fluid collections, a single quantitative measurement of kidney size or parenchymal thickness cannot be associated with the severity of the NE disease. There are, however, several qualitative US findings which are used in a clinical setting to analyze the status of the kidney. In order to obtain relevant results, the examination technique in renal US is crucial. In ARF it is important to study by Doppler US both kidneys and at several sites, since for instance in acute tubular necrosis the vascular alterations may be patchy (Brezis et al. 1986, Keogan et al. 1996).

In study IV qualitative US findings were analyzed in two different ways: with and without comparison image. With the two-phase blinded study design it was possible to observe both whether there was qualitative pathology present and whether this pathology could be detected in a clinical situation where there are usually no control images for comparison. Nevertheless in the final analysis change in these US findings between primary and repeat study was regarded as a sign of abnormality. Qualitative US findings were not analyzed at bedside as in that way blinding could not have been implemented; therefore only captured US images were used and the study was retrospective (IV). The agreement on qualitative renal US findings between the three radiologists in both phases (without/with comparison image) was assessed (IV). Consensus on parenchymal swelling was moderate and on other qualitative findings fair or poor.

Quantitative renal US findings were recorded in every patient (III). The mean renal length was greater in the primary as compared to the repeat study in all patients. However, qualitative renal US studies suggest that this change was so small that it could not always be detected in a clinical situation, especially if no comparison image is available. Isotope nephrography has been reported to be more sensitive than US in identifying renal functional impairment in NE patients (Paul et al. 1991). However, only qualitative US findings (renal edema and obliteration of the border between the renal cortex and medulla) were analyzed in that study, the analysis was not blinded and there was no follow-up. In the present studies (II,IV) the radiologists knew that all these patients had NE but were unaware of their clinical data. Altogether 47% yielded qualitative findings in renal US (II). In that study 2 radiologists evaluated renal US findings together. Only 8% of these patients had a comparison study. Of the total, 87% produced qualitative US findings, assuming that change in these findings between the primary and repeat study is to be regarded as a sign of abnormality (IV); 91% yielded qualitative US

findings assuming that a comparison study was available during evaluation (IV). In study IV 3 radiologists evaluated renal US findings independently and mean values were used. These two different analyzing modes explain the percentage differences between these two studies (II,IV). In a study by Paul and colleagues (1991), renal US was pathological in 78% of NE patients. In the present studies the most typical qualitative renal US finding in NE patients were the presence of parenchymal swelling, disturbance in corticomedullary border differentiation and increase in cortical echogenicity (II,IV).

In the previous study with nine patients the isotope method used showed a slightly higher retention value for the right kidney as compared to the left (Paul et al. 1991). US was abnormal in seven right but in only three left kidneys. In the present study perirenal fluid was found on the right side in five patients and on the left in two (III). It is possible that anatomical conditions make it easier to detect fluid on the right side. In some findings in the study IV the pathology of the left kidney was estimated to be more severe than that of the right. Anatomical conditions make the left kidney more difficult to scan than the right, this possibly mimicking pathology and explaining the tendency observed. In study II qualitative US findings were analyzed in both kidneys, but because findings were mostly equal between both kidneys a common statement was made.

In conclusion, quantitative renal US changes occurred in every patient and qualitative US findings were registered in almost every patient assuming that a comparative study was available and change between two studies is a sign of abnormality. The most frequent finding was swelling of the kidneys. Renal US findings may be assumed not to be disease-specific in NE even though the findings were not compared to other diseases.

Associations between renal ultrasonography findings and clinical course

Quantitative and qualitative renal US findings in relation to the typical clinical and laboratory findings in NE are a matter of debate. A number of studies have assessed the relation between RI and laboratory data reflecting kidney function, for example serum creatinine and creatinine clearance (Platt et al. 1989, Kim et al. 1990b). Here the higher RI in the primary study and the greater change in RI at follow-up were associated with fluid volume overload and the degree of clinical renal insufficiency, assuming

that maximum daily urine excretion during the polyuric phase and a substantial change in body weight between the anuric and polyuric phases are measures of fluid volume overload (III). Two previous renal biopsy studies and a study of ARF due to acute tubular necrosis have found only a weakly positive correlation between creatinine level and RI (Platt et al. 1990, Mostbeck et al. 1991, Platt et al. 1991b). Systemic hypotension has been reported to produce an RI greater than 0,9 (Pozniak et al. 1988). In the present study blood pressure was not associated with high RI in the primary study, nor with greater change in RI (III). RI did not differ between right and left kidney, which is in accord with a previous report (Keogan et al. 1996).

The presence of fluid collections in US was associated with clinical fluid volume overload and the degree of clinical renal insufficiency (III). The association between leukocytosis and fluid collections remained obscure (III). Fluid volume overload, which is associated with renal insufficiency and capillary leakage, may also give rise to pathological radiograph findings in NE (I,II). Inflammation as expressed by an increased CRP level did appeared to be related, and thus is not likely to be a significant causative factor in pathologic quantitative or qualitative renal US findings (II,III,IV).

Change in renal length showed some association, but change in renal cortical parenchymal thickness none, with the clinical course of the patients (III). This may be a consequence of the relatively small number of patients studied. Theoretically it is possible that some of the decrease in renal length during follow-up might in part suggest irreparable NE-induced parenchymal damage rather than simply a return to the normal condition. Despite this possibility the renal involvement in the acute disease was regarded as transient. In the present series with MRI no specific findings emerged in the repeat study (V). Nonetheless it can be concluded that the swelling of the kidney in NE does not predict the clinical course as clearly as RI or the presence of fluid collections (III).

Isotope nephroangiography has showen all five NE patients involved to have greatly reduced renal function and increased vascular resistance (Lingårdh et al. 1975). The renal blood flow, which was initially normal or slightly reduced, increased during convalescence, and renal function returned to normal. Also in the present study RI was normal in 21 patients and only slightly abnormal in two in the follow-up (III). Both of these patients were over 50 years. The laboratory parameters reflecting renal function were also normal or close to normal at discharge.

The severity of the qualitative US findings was associated to some extent with fluid volume overload, degree of clinical renal insufficiency, high blood pressure level and leukocytosis (II,IV). The

degree of parenchymal swelling had more statistically significant associations with the clinical course than the other qualitative US findings (IV).

In conclusion, qualitative US findings are as sensitive as quantitative in the assessment of NE patients´ clinical course and recovery, assuming that a comparison study is available. US using only quantitative or qualitative findings is somewhat limited when evaluating NE patients´ clinical situation. The most useful US finding in clinical practice does not require a comparison study. When considering both quantitative and qualitative US findings, if no comparison study is available, the best correlation with the clinical course of NE is obtained by analyzing for the presence of fluid collections and evaluating the RI value by Doppler US.

Renal magnetic resonance imaging findings

The aim here was to evaluate the presence of abnormal findings and their different types in renal MRI in the acute phase of the disease and their progress during recovery. In order to obtain relevant results, the examination technique in renal MRI is crucial. The patients were used as their own control by comparing the primary and repeat MRI findings. MRI findings did not differ significantly between right and left kidney, which is in accord with the US results (II,III,IV). In the present study mean values of the right and left kidney were used (V).

There are no data in the literature regarding accurate computerized renal volume estimation by MRI in NE or in HFRS caused by other viruses. Few earlier works have evaluated the use of MRI in calculating renal volume. In a study where kidney volume was evaluated using MRI, volume was calculated by two different methods, i.e. ellipsoid formula and voxel-count (Bakker et al. 1998). The result was an average of 24% underestimation (range 5-48%) of renal volume calculated with the ellipsoid formula. No significant deviation from the true renal volume was encountered in MRI by the voxel-count method. Repeatability was also greater by the latter method. In the present study the voxel-count method was used and repeatability was excellent (V).

Renal parenchymal volume decreased in all patients during follow-up (V). Renal length decreased in every patient in the US study (III). 3D US was not used to measure parenchymal volume (III). Renal volume is underestimated on an average by 25% with US and the correlation between renal length and renal volume is weak (Bakker et al. 1999). In the present study the correlation between

parenchymal volume and renal length was likewise weak (V). In another study in which renal parenchymal volume was evaluated in children, 3D US proved feasible and comparable to CT and MRI (Fritz et al. 2003).

Normally the relaxation time of the renal medulla is longer than that of the renal cortex and the two tissues can be differentiated from each other on T1-weighted images. Corticomedullary signal differences disappear in many renal diseases (Marotti et al. 1987). In the present study it was sought to measure both cortical and medullar volume, but this proved impossible because in most of the patients in the primary study the corticomedullary difference had disappeared (V). Regardless of the underlying cause of ARF, a serum creatinine value greater than 264 μmol/l results in loss of the corticomedullary difference on T1 fast-spin echo images, while levels above 880 μmol/l result in loss of corticomedullary difference on gadolinium-enhanced gradient echo images (Semelka et al. 1994).

Renal length decreased in all patients during follow-up (V). The mean difference was 9 mm, in the US study 14 mm (III). The clinical severity of NE was fairly similar in these two studies as judged by clinical and laboratory findings. In a study by Bakker and colleagues (1998) it was found that when measuring renal length, repeatability was better with MRI compared to US. The repeatability in the present study was also excellent (V). Renal parenchymal thickness decreased in all patients during follow-up (V). In the US study, the thickness decreased in 19/23 patients (III).

No disease-specific MRI findings emerged even though the findings were not compared to those in other diseases (V). Renal MRI in KHF patients showed low signal intensities along the medulla, especially the outer medulla, on T2-weighted images (Kim et al. 1990a). Intense vascular congestion and hemorrhage in the renal medulla occur in KHF in the acute phase of the disease. While NE is a mild type of HFRS, renal biopsy findings have nonetheless shown hemorrhage in the outer renal medulla or corticomedullary junction in 20-60 % of patients in the acute phase (Collan et al. 1991, Mustonen et al. 1994b). In three cases MRI was performed in the convalescent phase of KHF. The low signal intensity in the medulla encountered in these patients may represent intertubular fibrosis (Kim et al. 1990a).

Intense edema/fluid collections were found bilaterally in 16 patients in the acute phase of NE (V). In the US study, fluid collections were detected in 13/23 patients (III). Mild edema cannot be seen in US, which explains the difference.

In conclusion, measurable renal MRI findings occurred in every NE patient. No disease-specific MRI findings were recorded. Renal involvement in the acute disease was regarded as transient.

Associations between renal magnetic resonance imaging findings and clinical course

The aim here was to establish whether MRI findings were associated with the clinical picture of NE. Change in parenchymal volume showed a mild association with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, inflammation and thrombocytopenia (V). These associations were not significant, this possibly being attributable to the relatively small number of patients studied. Severe quantitative renal US findings evinced a mild association with clinical fluid volume overload and degree of clinical renal insufficiency (III). Change in parenchymal volume was more clearly associated with a severe clinical course than the other MRI findings evaluated. In the US study swelling of the kidney in NE did not predict the clinical course as clearly as RI or the presence of fluid collections (III).

Change in renal length evinced a mild association with high blood pressure level and degree of clinical renal insufficiency (V). In the US study change in renal length was associated with clinical fluid volume overload and degree of clinical renal insufficiency (III).

Change in parenchymal thickness evinced a mild association with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, inflammation and thrombocytopenia (V). In the study with US, change in cortical parenchymal thickness showed no association with the clinical course (III).

The presence of edema/fluid collections in MRI evinced a mild association with clinical fluid volume overload, inflammation and thrombocytopenia (V), while in the US study the presence of fluid collections in US was associated with clinical fluid volume overload, degree of clinical renal insufficiency and leukocytosis (III). The association between leukocytosis and fluid collections remained obscure. Inflammation, as manifested by increased leukocytosis and CRP levels, correlated slightly with pathologic renal MRI findings but was not an independent factor (V).

In conclusion, the severity of the renal MRI findings evinced a mild association with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, inflammation and thrombocytopenia. Change in parenchymal volume was associated with the clinical course more clearly than the other MRI findings.

Pathogenesis of renal involvement and renal ultrasonography and magnetic resonance imaging findings in nephropathia epidemica

Comparison with the clinical and laboratory data suggests that capillary permeability leads to plasma leakage in the kidneys. This causes perivascular edema and congestion, which in term cause medullary ischemia and tubular injury which worsen the degree of clinical renal insufficiency. On the other hand tubular cells are directly damaged via viral infection. This causes peritubular and interstitial inflammation and tubular injury. Thus the intrarenal events in renal failure, both medullary ischemia and inflammation, play a role in the pathogenesis of tubular injury and findings in renal US and MRI (Figure 4).

Radiological recommendations for clinical practice in nephropathia epidemica

Chest radiography, renal US and MRI findings are not disease-specific in NE. Chest radiography is nonetheless a useful measure if the patients have respiratory symptoms; it can rule out severe findings of NE, other inflammatory diseases and heart failure during NE. The correlation of chest radiography findings to the clinical course is good. Renal US and MRI findings are rather limited in assessing the clinical course. MRI is more accurate than US when evaluating quantitative renal findings and MRI is superior to US in evaluating edema/fluid collections in NE. This notwithstanding, US is more appropriate than MRI when evaluating the clinical course of NE patients by reason of its low cost and availability. The best correlation with the clinical course of NE is obtained by analyzing for the presence of fluid collections and evaluating the RI value by Doppler US. The main reason for undertaking US during the acute phase of NE is to rule out other causes of ARF.

SUMMARY AND CONCLUSION

One third of the hospital-treated NE patients evinced disease-related changes in their chest radiographs. Pleural effusion, atelectasis and interstitial infiltrates were the most common radiographic findings. The occurrence and severity of the findings were associated with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, leukocytosis and thrombocytopenia. Capillary leakage and inflammation play a role in the pathogenesis of NE lung involvement, as in HPS. Differently from HPS, however, the fluid volume overload associated with renal insufficiency seemed to contribute markedly to the chest radiograph changes in NE.

Quantitative renal US findings were noted in every patient and qualitative US findings in almost all cases during the acute phase of NE. Swelling of the kidney was the most frequent finding. The severity of the findings in US was associated to some extent with clinical fluid volume overload, degree of clinical renal insufficiency and leukocytosis.

Quantitative renal MRI findings emerged in every patient; renal parenchymal volume, renal length and parenchymal thickness were decreased in all during follow-up. The severity of the findings in MRI evinced a mild association with clinical fluid volume overload, degree of clinical renal insufficiency, high blood pressure level, inflammation and thrombocytopenia.

Capillary leakage and inflammation play a role in the pathogenesis of NE renal involvement. Fluid volume overload associated with renal insufficiency seemed to contribute markedly to the renal US and MRI findings in NE.

Chest and renal involvement in the acute disease were regarded as transient. Chest radiograph, renal US and MRI findings are not disease-specific in NE. Nonetheless the chest radiograph is useful if patients have respiratory symptoms, in that it can rule out severe findings of NE, other inflammatory diseases and heart failure during NE. Qualitative US findings are as sensitive as quantitative in the assessment of NE patients´ clinical course and recovery, assuming that a comparison study is available. US and MRI are somewhat limited in evaluating patients´ clinical situation. MRI is more accurate than US in assessing quantitative renal findings. MRI is superior to US in evaluating edema/fluid collections in NE patients, but US is nevertheless more appropriate than MRI in evaluating clinical course and renal findings in NE patients by reason of its low cost and availability. The main reason for applying US during the acute phase of NE is to rule out other causes of ARF.

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REFERENCES

Ahlm C, Linderholm M, Juto P, Stegmayer B and Settergren B (1994): Prevalence of serum IgG antibodies to Puumala virus (hemorrhagic fever with renal syndrome) in northern Sweden. Epidemiol Infect 113: 129-36.

Ahlm C (1997): Distribution of Puumala virus in Sweden. Academic Thesis, University of Umeå, Sweden 1-68.

Ahlm C, Juto P, Stegmayer B, Settergren B, Wadell G, Tärnvik A and Elgh F (1997): Prevalence of serum antibodies to hantaviruses in northern Sweden as measured by recombinant nucleocapsid proteins. Scand J Infect Dis 29: 349-54.

Ahlm C, Linden C, Linderholm M, Alexeyev OA, Billheden J, Elgh F, Fagerlund M, Zetterlund B and Settergren B (1998): Central nervous system and ophthalmic involvement in nephropathia epidemica (European type of hemorrhagic fever with renal syndrome). J Infect 36: 149-55.

Ala-Houhala I, Mäkelä S, Koivunen E, Mustonen J and Pasternack A (2000): Pronounced monocytosis in a case of nephropathia epidemica. Scand J Infect Dis 32: 419-20.

Ala-Houhala I, Koskinen M, Ahola T, Harmoinen A, Kouri T, Laurila K, Mustonen J and Pasternack A (2002): Increased glomerular permeability in patients with nephropathia epidemica caused by Puumala hantavirus. Nephrol Dial Transplant 17: 246-52.

Alexeyev OA, Settergren B, Billheden J, Ahlm C, Suzdaltsev A and Tsaig M (1993): Laboratory findings in patients with hemorrhagic fever with renal syndrome in western Russia. Infection 21: 412.

Alexeyev OA and Morozov VG (1995): Neurological manifestations of hemorrhagic fever with renal syndrome caused by Puumala virus: review of 811 cases. Clin Infect Dis 20: 255-8.

Alexeyev OA, Elgh F, Zhestkov V, Wadell G and Juto P (1996): Hantaan and Puumala virus antibodies in blood donors in Samara, an HFRS-endemic region in European Russia. Lancet 347: 1483.

Auwaerter PG, Oldach D, Mundy LM, Burton A, Laird Warner M, Vance E, Moore RD and Rossi CA (1996): Hantavirus serologies in patients hospitalized with community-acquired pneumonia. J Infect Dis 173: 237-9.

Bakker J, Olree M, Kaatee R, de Lange EE and Beek FJ (1998): In vitro measurement of kidney size: comparison of ultrasonography and MRI. Ultrasound Med Biol 24: 683-8.

Bakker J, Olree M, Kaatee R, de Lange EE, Moons KG, Beutler JJ and Beek FJ (1999): Renal volume measurements: accuracy and repeatability of US compared with that of MR imaging. Radiology 211: 623-8.

Ballmer PE, Ballmer-Hofer K, Repond F, Hohler H and Studer H (1992): Acute suppression of albumin synthesis in systemic inflammatory disease: An individually graded response of rat hepatocytes. J Histochem Cytochem 40: 201-6.

Bergmann F, Krone B, Bleich S, Prange H and Paulus W (2002): Encephalitis due to a hantavirus infection. J Infect 45: 58-9.

Bernshtein AD, Apekina NS, Mikhailova TV, Myasnikov YA, Khlyap LA, Korotkov YS and Gavrilovskaya IN (1999): Dynamics of Puumala hantavirus infection in naturally infected bank voles (Clethrinomys glareolus). Arch Virol 144: 2415-28.

Bland JM and Altman DG (1986): Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 8: 307-10.

Boroja M, Barrie JR and Raymond GS (2002): Radiographic findings in 20 patients with hantavirus pulmonary syndrome correlated with clinical outcome. A J R 178: 159-63.

Brezis M, Rosen S and Epstein FH (1986): Acute renal failure. In: The Kidney, 3rd edn. Edited by BM Brenner and FC Rector. Saunders, Philadelphia. 735-99.

Brummer-Korvenkontio M, Vaheri A, Hovi T, von Bonsdorff CH, Vuorimies J, Manni T, Penttinen K, Oker-Blom N and Lähdevirta J (1980): Nephropathia epidemica: detection of antigen in bank voles and serological diagnosis of human infection. J Infect Dis 141: 131-4.

Brummer-Korvenkontio M, Henttonen H and Vaheri A (1982): Hemorrhagic fever with renal syndrome in Finland: Ecology and virology of nephropathia epidemica. Scand J Infect Dis Suppl 36: 88-91.

Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P and Vaheri A (1999): Epidemiological study of nephropathia epidemica in Finland 1989-96. Scand J Infect Dis 31: 427-35.

Chapman LE, Ellis BA, Koster FT, Sotir M, Ksiazek TG, Mertz GJ, Rollin PE, Baum KF, Pavia AT, Christenson JC, Rubin PJ, Jolson HM, Behrman RE, Khan AS, Bell LJ, Simpson GL, Hawk J, Holman RC and Peters CJ (2002): Discriminators between hantavirus-infected and –uninfected persons enrolled in a trial of intravenous ribavirin for presumptive hantavirus pulmonary syndrome. Clin Infect Dis 34: 293-304.

Chen JH, Pu YS, Liu SP and Chiu TY (1993): Renal hemodynamics in patients with obstructive uropathy evaluated by duplex Doppler sonography. J Urol 150: 18-21.

Chu YK, Rossi C, LeDuc JW, Lee HW, Schmaljohn CS and Dalrymple JM (1994): Serological relationships among viruses in the Hantavirus genus, family Bynyaviridae. Virology 198: 196-204.

Clement J, Colson P and McKenna P (1994): Hantavirus pulmonary syndrome in New England and Europe. N Engl J Med 331: 545-6.

Clement J, Underwood P, Ward D, Pilaski J and LeDuc J (1996): Hantavirus outbreak during military manoeuvers in Germany. Lancet 347: 336.

Collan Y, Lähdevirta J and Jokinen EJ (1978a): Electron microscopy of nephropathia epidemica. Renal tubular basement membrane. Am J Pathol 92: 167-72.

Collan Y, Lähdevirta J and Jokinen EJ (1978b): Electron microscopy of nephropathia epidemica. Glomerular changes. Virchows Arch A Path Anat and Histol 377: 129-44.

Collan Y, Mihatsch MJ, Lähdevirta J, Jokinen EJ, Romppanen T and Jantunen E (1991): Nephropathia epidemica: mild variant of hemorrhagic fever with renal syndrome. Kidney Int Suppl 35: 62-71.

Collins JD, Burwell D, Furmanski S, Lorber P and Steckel RJ (1972): Minimal detectable pleural effusion. A roentgen pathology model. Radiology 105: 51-3.

Cosgriff TM (1991): Mechanisms of disease in hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. Rev Infect Dis 13: 97-107.

Demas BE, Stafford SA and Hricak H (1988): Kidneys: In Stark DD, Bradley WG, eds. Magnetic Resonance Imaging. St. Louis: Mosby 1221

Dubbins PA (1989): Doppler ultrasound in renal disease. Curr Imaging 1: 32.

Duchin JS, Koster FT, Peters JC, Simpson GL, Tempest B, Zaki SR, Ksiazek TG, Rollin PE, Nichol S, Umland ET, Moolenaar RL, Reef SE, Nolte KB, Gallaher MM, Butler JC and Breiman RF, Hantavirus Study Group (1994): Hantavirus pulmonary syndrome: a clinical description of 17 patients with newly recognized disease. N Eng J Med 330: 949-55.

Elliott RM (1990): Molecular biology of the Bunyaviridae. J Gen Virol 71: 501-22.

Enria DA, Briggiler AM, Pini N and Levis S (2001): Clinical manifestations of New World hantaviruses. Curr Top Microbiol Immunol 256: 117-34.

Esselink RA, Gerding MN, Brouwers PJ, Solleveld H, Jordans JG, Groen J and Osterhaus AD (1994): Guillain-Barre syndrome associated with hantavirus infection. Lancet 343: 180-1.

Forslund T, Saltevo J, Anttinen J, Auvinen S, Brummer-Korvenkontio M, Korhonen A and Poutiainen M (1992): Complications of nephropathia epidemica: three cases. J Intern Med 232: 87-90.

Forslund T, Liisanantti R, Saijonmaa O and Fyhrqvist F (1993): Raised plasma endothelin-1 concentration in patients with nephropathia epidemica. Clin Nephrol 40: 69-73.

Fraser RD, Pare JA, Pare PD, Fraser RS and Genereux GP (eds.) (1989): Diagnosis of diseases of the chest. W.B. Saunders Company.

Fritz GA, Riccabona M, Bohdal G and Quehenberger F (2003): Accuracy of renal volume assessment in children by three-dimensional sonography. Rofo Fortschr Rontgenstr Neuen Bildgeb Verfahr 175: 540-6.

Gill B, Palmer LS, Koenigsberg M and Laor E (1994): Distribution and variability of resistive index values in undilated kidneys in children. Urology 44: 897-901.

Gottlieb RH, Luhmann K and Oates RP (1989): Duplex ultrasound evaluation of normal native kidneys and native kidneys with urinary tract obstruction. J Ultrasound Med 8: 609-11.

Groen J, Bruijn JA, Gerding MN, Jordans HG, Moll Van Charante AW and Osterhaus AD (1996): Hantavirus antigen detection in kidney biopsies from patients with nephropathia epidemica. Clin Nephrol 46: 379-83.

Groeneveld PH, Colson P, Kwappenberg KM and Clement J (1995): Increased production of nitric oxide in patients infected with the European variant of hantavirus. Scand J Infect Dis 27: 453-6.

Groeneveld PH, Kroon FP, Nibbering PH, Bruisten SM, van Swieten P and van Furth R (1996): Increased production of nitric oxide correlates with viral load and activation of mononuclear phagocytes in HIV-infected patients. Scand J Infect Dis 28: 341-5.

Hansson L and Henttonen H (1985): Regional differences in cyclity and reproduction in Clethrionomys species: Are they related? Ann Zool Fennici 22: 277-88.

Hautala T, Sironen T, Vapalahti O, Pääkko E, Särkioja T, Salmela PI, Vaheri A, Plyusnin A and Kauma H (2002): Hypophyseal hemorrhage and panhypopituitarism during Puumala Virus Infection: Magnetic Resonance Imaging and detection of viral antigen in the hypophysis. Clin Infect Dis 35: 96-101.

Hedman K, Vaheri A and Brummer-Korvenkontio M (1991): Rapid diagnosis of hantavirus disease with IgG avidity assay. Lancet 338: 1353-6.

Hjelle B, Jenison S, Torrez-Martinez N, Yamada T, Nolte K, Zumwalt R, MacInnes K and Myers G (1994): A novel hantavirus associated with an outbreak of fatal respiratory disease in the southwestern United States: evolutionary relationship to known hantaviruses. J Virol 68: 592-6.

Hricak H (1982): Renal medical disorders: the role of sonography. In Sanders R C, ed. Ultrasound Annual, 43-80.

Hricak H, Cruz C, Romanski R, Uniewski MH, Levin NW, Madrazo BL, Sandler MA and Eyler WR (1982): Renal parenchymal disease: sonographic-histologic correlation. Radiology 144: 141-7.

Hörling J, Chizhikov V, Lundkvist Å, Jonsson M, Ivanov L, Dekonenko A, Niklasson B, Dzagurova T, Peters CJ, Tkachenko E and Nichol S (1996): Khabarovsk virus: a phylogenetically and serologically distinct hantavirus isolated from Microtus fortis trapped in far-east Russia. J Gen Virol 77: 687-94.

Jokinen EJ, Lähdevirta J and Collan Y (1978): Nephropathia epidemica: immunohistochemical study of pathogenesis. Clin Nephrol 9: 1-5.

Kallio-Kokko H, Leveelahti R, Brummer-Korvenkontio M, Lundkvist A, Vaheri A and Vapalahti O (2001): Human immune response to Puumala virus glycoproteins and nucleocapsid protein expressed in mammalian cells. J Med Virol 65: 605-13.

Kanerva M (1996): Pathogenesis of Puumala virus infection. Academic Thesis, University of Helsinki, Finland 3-102.

Kanerva M, Mustonen J and Vaheri A (1998): Pathogenesis of Puumala and other hantavirus infections. Rev Med Virol 8: 67-86.

Karabatsos N (1985): Puumala virus in: International catalogue of arboviruses. American Society of Tropical Medicine and Hygiene, San Antonio 492-596.

Keogan MT, Kliewer MA, Herzberg BS, De Long DM, Tupler RH and Carroll BA (1996): Renal resistive indexes: variability in Doppler US measurement in a healthy population. Radiology 199: 165-9.

Ketai LH, Williamson MR, Telepak RJ, Levy H, Koster FH, Nolte KB and Allen SE (1994): Hantavirus pulmonary syndrome: radiographic findings in 16 patients. Radiology 191: 665-8.

Kim SH, Kim S, Lee JS, Choi BI, Kim YG, Han JS, Park JH, Han MC and Kim CW (1990a): Hemorrhagic fever with renal syndrome: MR imaging of the kidney. Radiology 175: 823-5.

Kim SH, Kim WH, Choi BI and Kim CW (1990b): Duplex sonography of the native kidney- resistive index vs. serum creatinine. J Ultrasound Med 9: 25.

Kim YS, Lee JS, Ahn C, Han JS, Kim S, Kim SH and Lee HS (1997): Magnetic resonance imaging of the kidney in hemorrhagic fever with renal syndrome: its histopathologic correlation: Nephron 76: 477-80.

Kitsutani PT, Denton RW, Fritz CL, Murray RA, Todd RL, Pape WJ, Wyatt Frampton J, Young JC, Khan AS, Peters CJ and Ksiazek TG (1999): Acute Sin Nombre hantavirus infection without pulmonary syndrome, United States. Emerg Infect Dis 5: 701-5.

Kjellstrand CM, Berkseth RO and Klinkman H (1988): Treatment of acute renal failure. In RW Schrier, CW Gottschalk (eds.). Diseases of the Kidney. Little, Brown and Company. Fourth ed: 1505.

Kontkanen M (1998): Ophthalmic findings in nephropathia epidemica (Puumala virus infection). A clinical, pathophysiological and epidemiological study. Academic Thesis, University of Kuopio, Finland 14-84.

Krause R, Aberle S, Haberl R, Daxbock F and Wenisch C (2003): Puumala virus infection with acute disseminated encephalomyelitis and multiorgan failure. Emerg Infect Dis 9: 603-5.

Kuhlbäck B, Fortelius P and Tallgren LF (1964): Renal histopathology in a case of nephropathia epidemica Myhrman. A study of successive biopsies. Acta Pathol Microbiol Scand 60: 323-4.

Kurane I and Ennis FE (1992): Immunity and immunopathology in Dengue virus infections. Semin Immunol 4: 121-7.

Launes J and Hautanen A (1988): Nephropathia epidemica encephalitis. Acta Neurol Scand 78: 234-5.

Lee HW, Lee PW, Baek LJ, Song CK and Seong IW (1981): Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent Apodemus agrarius. Am J Trop Med Hyg 30: 1106-12.

Lee HW, Baek LJ and Johnson KM (1982): Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. J Infect Dis 146: 638-44.

Lee HW and Dalrymple JM (1989): Manual of hemorrhagic fever with renal syndrome. WHO publications 1-124.

Lee HW, Lee PW, Baek LJ and Chu YK (1990): Geographical distribution of hemorrhagic fever with renal syndrome and hantaviruses. Arch Virol Suppl 1: 5-18.

Lee JS (1991): Clinical features of hemorrhagic fever with renal syndrome in Korea. Kidney Int 40: 88-93.

Le Guenno B and Coudrier D (1995): Epidemiology of Hantavirus infections in France (1977-1995). In: 3rd International Conference on HFRS and Hantaviruses, Helsinki, Finland, June 11.

Lim TH, Lee JS, Choi BI, Kim IO, Suh CH, Han MC and Kim CW (1987): An explanation of renal hemodynamics in acute renal failure based on sequential CT in patients with Korean hemorrhagic fever. J Comput Assist Tomogr 11: 474-9.

Linderholm M, Settergren B, Ahlm C, Burman LA, Traff S, Backlund U and Juto P (1991): A Swedish fatal case of nephropathia epidemica. Scand J Infect Dis 23: 501-2.

Linderholm M, Billström Å, Settergren B and Tärnvik A (1992): Pulmonary involvement in nephropathia epidemica as demonstrated by computed tomography. Infection 20: 263-6.

Linderholm M, Bjermer L, Juto P, Roos G, Sandström T, Settergren B and Tärnvik A (1993): Local host response in the lower respiratory tract in nephropathia epidemica. Scand J Infect Dis 25: 639-46.

Linderholm M, Ahlm C, Settergren B, Waage A and Tärnvik A (1996a): Elevated plasma levels of tumor necrosis factor (TNF)-alpha, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. J Infect Dis 173: 38-43.

Linderholm M, Groeneveld PH and Tärnvik A (1996b): Increased production of nitric oxide in patients with hemorrhagic fever with renal syndrome- relation to arterial hypotension and tumor necrosis factor. Infection 24: 337-40.

Linderholm M (1997): Nephropathia epidemica. Pathogenetic mechanisms with special reference to pulmonary involvement. Academic Thesis, University of Umeå, Sweden 1-64.

Lingårdh G, Lundström B and Nyström K (1975): Renal blood flow in acute epidemic nephritis. (Nephropathia epidemica of Scandinavia). Scand J Urol Nephrol 9: 57-63.

Lundkvist Å, Hörling J and Niklasson B (1993): The humoral response to Puumala virus infection (nephropathia epidemica) investigated by viral protein specific immunoassays. Arch Virol 130: 121-30.

Lähdevirta J (1971): Nephropathia epidemica in Finland: a clinical, histological and epidemiological study. Ann Clin Res 3 Suppl 8: 1-154.

Lähdevirta J and Elo O (1975): Nephropathia epidemican esiintyminen Suomessa vuosina 1966-73. Suomen Lääkärilehti 30: 677-82.

Lähdevirta J and Korpela H (1977): An emerging zoonosis in Scandinavia- nephropathia epidemica. Nord Vet Med 29: 406-12.

Lähdevirta J, Savola J, Brummer-Korvenkontio M, Berndt R, Illikainen R and Vaheri A (1984): Clinical and serological diagnosis of nephropathia epidemica, the mild type of hemorrhagic fever with renal syndrome. J Infect 9: 230-8.

Lähdevirta J (1989): The minor problem of hemostatic impairment in nephropathia epidemica, the mild Scandinavia form hemorrhagic fever with renal syndrome. Rev Infect Dis 11 Suppl 4: 860-3.

Markotic A, LeDuc JW, Hlaca D, Rabatic S, Sarcevic A, Dasic G, Gagro A, Kuzman I, Barac V, Avsic-Zupanec T, Beus I and Dekaris D (1996): Hantaviruses are a likely threat to NATO forces in Bosnia and Herzegovina and Croatia. Nat Med 2: 269-70.

Marotti M, Hricak H, Terrier F, McAninch JW and Thuroff JW (1987): MR in renal disease: importance of cortical-medullary distinction. Magn Reson Med 5: 160-72.

Mittelstaedt CA (1987): Abdominal ultrasound. New York, N Y, Churchill Livingstone 286-8.

Moll van Charante AW, Groen J and Osterhaus AD (1994): Risk of infections transmitted by arthropods and rodents in forestry workers. Eur J Epidemiol 10: 349-51.

Morrison YY and Rathbun RC (1995): Hantavirus pulmonary syndrome: the Four Corners disease. Ann Pharmacother 29: 57-65.

Mostbeck GH, Kain R, Mallek R, Derfler K, Walter R, Havelec L and Tscholakoff D (1991): Duplex Doppler sonography in renal parenchymal disease. Histopathologic correlation. J Ultrasound Med 10: 189-94.

Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli GP, Mayo MA and Summers MD (1995): Family Bunyaviridae Virus taxonomy. Sixth report of the international committee on taxonomy of viruses. Springer-Verlag, Wien 300-13.

Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K and Vaheri A (1994a): Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis 26: 7-13.

Mustonen J, Helin H, Pietilä K, Brummer-Korvenkontio M, Hedman K, Vaheri A and Pasternack A (1994b): Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin Nephrol 41: 121-6.

Mustonen J, Huttunen NP, Brummer-Korvenkontio M and Vaheri A (1994c): Clinical picture of nephropathia epidemica in children. Acta Paediatr 83: 526-9.

Mustonen J, Groundstroem K, Niemelä K and Pasternack A (1995): Cardiac manifestations in nephropathia epidemica (NE). A prospective study of 20 patients. Abstract in 3rd International Conference on HFRS and hantaviruses, Helsinki.

Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A and Vaheri A (1996): Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int 49: 217-21.

Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A and Vaheri A (1998): Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol 47: 277-9.

Mustonen J, Huttunen NP, Partanen J, Baer M, Paakkala A, Vapalahti O and Uhari M (2004): Human leukocyte antigens B8-DRB1*03 in pediatric patients with nephropathia epidemica caused by Puumala hantavirus. Ped Inf Dis J 23: 959-61.

Myhrman G (1934): En njursjukdom med egenartad symptombild. Nord Med Tidskr 7: 793-4.

Myhrman G (1957): Nya rön om nephropathia epidemica. Läkartidningen 54: 3467-71.

Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Koivisto AM, Vaheri A and Pasternack A (2004): Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. Am J Kidney Dis 43: 809-16.

Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S and Peters CJ (1993): Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262: 914-7.

Niklasson B, Nyman L, Linde A, Grandien M and Dalrymple J (1983): An epidemiological survey of nephropathia epidemica in Sweden. Scand J Infect Dis 15: 239-45.

Niklasson B and LeDuc J (1987): Epidemiology of nephropathia epidemica in Sweden. J Infect Dis 155: 269-76.

Niklasson B, LeDuc J, Nyström K and Nyman L (1987): Nephropathia epidemica: incidence of clinical cases and antibody prevalence in an endemic area of Sweden. Epidemiol Infect 99: 559-62.

Niklasson B, Hornfeldt B, Mullaart M, Settergren B, Tkachenko E, Myasnikov YuA, Ryltceva EV, Leschinskaya E, Malkin A and Dzagurova T (1993): An epidemiological study of hemorrhagic fever with renal syndrome in Bashkirtostan (Russia) and Sweden. Am J Trop Med Hyg 48: 670-5.

Niklasson B, Hornfeldt B, Lundkvist Å, Björsten S and LeDuc J (1995): Temporal dynamics of Puumala virus antibody prevalence in voles and of nephropathia epidemica incidence in humans. Am J Trop Med Hyg 53: 134-40.

Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, Merlin TL, McFeeley PJ, Umland ET and Zumwalt RE (1995): Hantavirus pulmonary syndrome in the United States: a pathologic description of a disease caused by a new agent. Hum Pathol 26: 110-20.

Norris CS, Pfeiffer JS, Rittgers SE and Barnes RW (1984): Noninvasive evaluation of renal artery stenosis and renovascular resistance. Experimental and clinical studies. J Vasc Surg 1: 192-201.

Orion diagnostica (1996): Report of contagious disease in Finland. Espoo, Finland. Orion Corporation Ltd.

Park SK, Kim W, Lee CH, Park TS, Lee DY and Kang SK (1996): Changes of T-lymphocyte subsets in patients with hemorrhagic fever with renal syndrome. Nephron 73: 114-6.

Paul R, Kiiliäinen H, Tarssanen L and Vorne M (1991): ($^{99}Tc^{m}$) MAG 3 gamma camera nephrography in epidemic nephritis. Nuc Med Commun 12: 15-25.

Peters CJ and Khan AS (2002): Hantavirus pulmonary syndrome: the new American hemorrhagic fever. Clin Infect Dis 34: 1224-31.

Pilaski J, Ellerich C, Kreutzer T, Lang A, Benik W, Pohl-Koppe A, Bode L, Vanek E, Autenrieth IB, Bigos K and Lee HW (1991): Hemorrhagic fever with renal syndrome in Germany. Lancet 337: 111.

Platt JF, Rubin JM, Ellis JH and Di Pietro MA (1989): Duplex Doppler US of the kidney: differentiation of obstructive from non-obstructive dilatation. Radiology 171: 515-7.

Platt JF, Ellis JH, Rubin JM, Di Pietro MA and Sedman AB (1990): Intrarenal arterial Doppler sonography in patients with nonobstructive renal disease: correlation of resistive index with biopsy findings. A J R 154: 1223-7.

Platt JF, Ellis JH and Rubin JM (1991a): Examination of the native kidneys with duplex Doppler ultrasound. Semin Ultrasound CT MR 12: 308-18.

Platt JF, Rubin JM and Ellis JH (1991b): Acute renal failure: possible role of duplex Doppler US in distinction between acute prerenal failure and acute tubular necrosis. Radiology 179: 419-23.

Plyusnin A, Vapalahti O, Lankinen H, Lehväslaiho H, Apekina N, Myasnikov Y, Kallio-Kokko H, Henttonen H, Lundkvist Å, Brummer-Korvenkontio M and et al. (1994): Tula virus: a newly detected hantavirus carried by European common voles. J Virol 68: 7833-9.

Plyusnin A, Vapalahti O and Vaheri A (1996): Hantaviruses: genome structure, expression and evolution. J Gen Virol 77: 2677-87.

Pozniak MA, Kelcz T, Stratta RJ and Oberley TD (1988): Extraneous factors affecting resistive index. Invest Radiol 23: 899-904.

Pärssinen O, Klementti A, Rossi-Rautiainen E and Forslund T (1993): Ophthalmic manifestations of epidemic nephropathy. Acta Ophthalmol 71: 114-8.

Rebibou JM, Kiesterman JP, Cercueil JP, Tanter Y, Portier H, Rifle G and Chevet D (1997): Papillary necrosis: a late complication of nephropathia epidemica. Clin Nephrol 48: 263-5.

Rodgers PM, Bates JA and Irving HC (1992): Intrarenal Doppler ultrasound studies in normal and acutely obstructed kidneys. Br J Radiol 65: 207-12.

Rollin PE, Bowen MD, Kariwa H, Saluzzo JF, Guerard S, Flechaire A, Coudrier D, Sureau P, Peters CJ and Nichol ST (1995): Short report: isolation and partial characterization of a Puumala virus from a human case of nephropathia epidemica in France. Am J Trop Med Hyg 52: 577-8.

Rosenfield AT and Siegel NJ (1981): Renal parenchymal disease. Histopathologic-sonographic correlation. A J R 137: 793-8.

Saari KM, Alanko H, Järvi J, Vetoniemi-Korhonen SI and Räsänen O (1977): Nephropathia epidemica. The Scandinavian form of hemorrhagic fever with renal syndrome. JAMA 238: 874-7.

Saari KM and Luoto S (1984): Ophthalmological findings in nephropathia epidemica in Lapland. Acta Ophthalmol 62: 235-43.

Schmaljohn AL, Li D, Negley DL, Bressler DS, Turell MJ, Korch GW, Ascher MS and Schmaljohn CS (1995): Isolation and initial characterization of a newly found hantavirus from California. Virology 206: 963-72.

Schmaljohn CS, Hasty SE, Dalrymple JM, LeDuc JW, von Bonsdorf C-H, Brummer-Korvenkontio M, Vaheri A, Tsai TF, Regnery HL, Goldgaber D and Lee PW (1985): Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. Science 227: 1041-4.

Semelka RC, Corrigan K, Ascher SM, Brown JJ and Colindres RE (1994): Renal corticomedullary differentiation: observation in patients with differing serum creatinine levels. Radiology 190: 149-52.

Settergren B, Juto P, Trollfors B, Wadell G and Norrby SR (1988a): Hemorrhagic complications and other clinical findings in nephropathia epidemica in Sweden: a study of 355 serologically verified cases. J Infect Dis 157: 380-2.

Settergren B, Juto P, Wadell G, Trollfors B and Norrby SR (1988b): Incidence and geographical distribution of serologically verified cases of nephropathia epidemica in Sweden. Am J Epidemiol 127: 801-7.

Settergren B, Juto P, Trollfors B, Wadell G and Norrby SR (1989): Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis 11: 921-7.

Settergren B, Trollfors B, Fasth A, Hultberg B and Norrby SR (1990): Glomerular filtration rate and tubular involvement during acute disease and convalescence in patients with nephropathia epidemica. J Infect Dis 161: 716-20.

Settergren B, Ahlm C, Juto P and Niklasson B (1991a): Specific Puumala IgG virus half a century after hemorrhagic fever with renal syndrome. Lancet 338: 66.

Settergren B, Leschinskaya E, Zagidullin I, Fazlyeva R, Khunafina D and Niklasson B (1991b): Hemorrhagic fever with renal syndrome: comparison of clinical course in Sweden and in the Western Soviet Union. Scand J Infect Dis 23: 549-52.

Settergren B, Boman J, Linderholm M, Wistrom J, Hagg E and Arvidsson PA (1992): A case of nephropathia epidemica associated with panhypopituitarism and nephrotic syndrome. Nephron 61: 234-5.

Settergren B, Ahlm C, Alexeyev O, Billheden J and Stegmayr B (1997): Pathogenetic and clinical aspects of the renal involvement in hemorrhagic fever with renal syndrome. Ren Fail 19: 1-14.

Shesberadaran H, Niklasson B and Tkachenko EA (1988): Antigenic relationship between hantaviruses analyzed by immunoprecipitation. J Gen Virol 69: 2645-51.

Simpson SQ (1998): Hantavirus pulmonary syndrome. Heart Lung 27: 51-7.

Smorodintsev AA, Chudakov VG and Churilov AV (1959): Hemorrhagic nephroso-nephritis. Translated from the Russian by Catherine Matthews. Pergamon Press, London 41.

Sommer AI, Traavik T, Mehl R, Berdal BP and Darymple J (1988): Hemorrhagic fever with renal syndrome (nephropathia epidemica) in Norway: seroepidemiology 1981-1985. Scand J Infect Dis 20: 267-74.

Stuhlfauth K (1943): Bericht uber ein neues schlammfieberähnliches Krankenheitsbild bei Deutschen Truppen in Lappland. Dtsch Med Wochenschr 69: 439-43, 474-7.

Swedish institute for infectious disease control. Stockholm, Sweden (1993): Smittskyddinstitutets årsraport 1993 över raporterade sjukdomar. 18.

Swedish institute for infectious disease control. Stockholm, Sweden (1995): 18: 2.

Temonen M, Vapalahti O, Holthöfer H, Brummer-Korvenkontio M, Vaheri A and Lankinen H (1993): Susceptibility of human cells to Puumala virus infection. J Gen Virol 74: 515-8.

Temonen M, Mustonen J, Helin H, Pasternack A, Vaheri A and Holthöfer H (1996): Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunohistochemical study. Clin Immunol Immunopathol 78: 47-55.

Terry JD, Rysavy JA and Frick MP (1992): Intrarenal Doppler: characteristics of ageing kidneys. J Ultrasound Med 11:647-51.

Thulkar S, Sharma S, Srivastava DN, Sharma SK, Berry M and Pandey RM (2000): Sonographic findings in grade III Dengue hemorrhagic fever in adults. J Clin Ultrasound 1: 34-7.

Toivanen AL, Valanne L and Tatlisumak T (2002): Acute disseminated encephalomyelitis following nephropathia epidemica. Acta Neurol Scand 105: 333-6.

Tsai TF (1987): Hemorrhagic fever with renal syndrome: mode of transmission to humans. Lab Anim Sci 37: 428-30.

Valtonen M, Kauppila M, Kotilainen P, Lähdevirta J, Svartbäck CM, Kosunen O, Nurminen J, Sarkkinen H and Brummer-Korvenkontio M (1995): Four fatal cases of nephropathia epidemica. Scand J Infect Dis 27: 515-7.

van Ypersele de Strihou C and Mery JP (1989): Hantavirus-related acute interstitial nephritis in western Europe. Expansion of a world-wide zoonosis. Q J Med 73: 941-50.

van Ypersele de Strihou C (1991): Clinical features of hemorrhagic fever with renal syndrome in Europe. Kidney Int Suppl 35: 80-3.

Vapalahti O, Kallio-Kokko H, Salonen EM, Brummer-Korvenkontio M and Vaheri A (1992): Cloning and sequencing of Puumala virus Sotkamo strain S and M RNA segments: evidence for strain variation in hantaviruses and expression of the nucleocapsid protein. J Gen Virol 73: 829-38.

Vapalahti O, Kallio-Kokko H, Närvänen A, Julkunen I, Lundkvist Å, Plyusnin A, Leväslaiho H, Brummer-Korvenkontio M, Vaheri A and Lankinen H (1995a): Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response. J Med Virol 46: 293-303.

Vapalahti O, Plyusnin A, Vaheri A and Henttonen H (1995b): Hantavirus antibodies in European mammalogists. Lancet 345: 1569.

Vapalahti O (1996): Genetic and antigen properties of Puumala and Tula viruses. Academic Thesis, University of Helsinki, Finland 6-90.

Vapalahti O, Lundkvist Å, Kallio-Kokko H, Paukku K, Julkunen I, Lankinen H and Vaheri A (1996): Antigen properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J Clin Microbiol 34: 119-25.

Weissenbacher MC, Cura E, Segura EL, Hortal M, Baek LJ, Chu YK and Lee HW (1996): Serological evidence of human hantavirus infection in Argentina, Bolivia and Uruguay. Medicina (Buenos Aires) 56: 17-22.

Williams RJ, Bryan RT, Mills JN, Palma RE, Vera I, De Velasquez F, Baez E, Schmidt WE, Figueroa RE, Peters CJ, Zaki SR, Khan AS and Ksiazek TG (1997): An outbreak of hantavirus pulmonary syndrome in western Paraquay. Am J Trop Med Hyg 57: 274-82.

Vitek CR, Breiman RF, Ksiazek TG, Rollin PE, McLaughlin JC, Umland ET, Nolte KB, Loera A, Sewell CM and Peters CJ (1996): Evidence against person-to person transmission of hantavirus to health care workers. Clin Infect Dis 22: 824-6.

Xiao S-Y, LeDuc JW, Chu YK and Schmaljohn CS (1994): Phylogenetic analyses of virus isolates in the genus Hantavirus, family Bunyaviridae. Virology 198: 205-17.

Yanagihara R and Silverman DJ (1990): Experimental infection of human vascular endothelial cells by pathogenic and nonpathogenic hantaviruses. Arch Virol 111: 281-6.

Zaki SF, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foukar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS, Rollin PE, Ksiazek TG, Nichol ST, Mahy BWJ and Peters CJ (1995): Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol 146: 552-79.

Zeier M, Zoller L, Weinreich T, Padberg-Wolf E, Andrassy K and Ritz E (1992): Severe hemorrhagic complications from infection with nephropathia epidemica strain of Hantavirus. Clin Nephrol 38: 190-2.

Zetterholm SG (1934): Acuta nefriter simulerande acuta bukfall. Svenska Läkärtidningen 31: 425-9.

Zhao X and Hay J (1997): The evolution of hantaviruses. Immunol Invest 26: 191-7.

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