HANTAVIRUS INFECTIONS IN THE UNITED STATES: DIAGNOSIS AND TREATMENT

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In volume three of *Antiviral Chemotherapy: New Directions for Clinical Application and Research*, Dr. Karl Johnson described the spectrum and mechanism of action, pharmacokinetics, and toxicity of ribavirin.[1] Clinical data supporting the use of intravenous ribavirin for several forms of viral hemorrhagic fever, including Lassa fever and hemorrhagic fever with renal syndrome (HFRS), are presented in detail elsewhere and will not be repeated in this chapter.[1-3]

The etiologic agent of Lassa fever is an arenavirus (family *Arenaviridae*, genus *Arenavirus*), Lassa fever virus. HFRS results from infection caused by at least four recognized hantaviruses (family *Bunyaviridae*, genus *Hantavirus*). Both Lassa fever virus and hantaviruses are maintained in nature through chronic infections in rodent reservoir hosts. Although differing markedly in pathophysiology and clinical presentation, severe human cases of Lassa fever or HFRS share the common features of increased capillary permeability, hypovolemic shock and hemorrhagic manifestations.

In May 1993, Dr. Bruce Tempest, an Indian Health Service (IHS) physician in Gallup, New Mexico and Richard Malone and Dr. Patricia McFeeley at the New Mexico Office of Medical Investigator (OMI) noted a cluster of unexplained deaths in previously healthy, young adults from rural region of the Four Corners region of New Mexico and Arizona. After a febrile prodrome, patients rapidly developed shortness of breath and non-cardiogenic pulmonary edema. Those with the most severe form of the disease had lactic acidosis, shock and cardiac arrhythmias and died.[4,5]

A working group, which included representatives from the IHS, OMI, New Mexico State Health Department, University of New Mexico (UNM), and Navajo Division of Health, met and defined the clinical syndrome and pathology and excluded most known causes for the syndrome. In late May, the Centers for Disease Control and Prevention (CDC) was invited to assist in the investigation, and a field team traveled to the Southwest. In early June, Dr. Thomas Ksiazek and other investigators in the Special Pathogens Branch (SPB) at CDC found antibodies in patients' sera in patterns suggesting cross-reactivity (but not identity) with previously known hantaviruses.[6] Other investigators at SBP identified a new hantavirus by genetic studies of autopsy tissue, using reverse transcription-polymerase chain reaction (RT-PCR).[7]

Intravenous ribavirin was made available from June 1993 to September 1994 under an open CDC protocol for treatment of individual patients with suspected hantavirus pulmonary syndrome (HPS). A controlled trial of ribavirin, sponsored by the National Institute of Allergy

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Edited by J. Mills *et al.*, Plenum Press, New York, 1996

and Infectious Diseases (NIAID) - Collaborative Antiviral Study Group (CASG), will begin in 1995. This chapter will describe the etiologic agents, epidemiology, pathology, clinical course and management of hantavirus infection in the United States.

HANTAVIRUSES AND THEIR RODENT RESERVOIRS

Nucleotide sequence analysis of viral genetic material amplified from autopsy tissue by RT-PCR indicated that the agent causing the New Mexico outbreak was a previously unrecognized hantavirus.[7-11] It is more closely related to the previously described New World hantavirus, Prospect Hill virus, and to Puumala virus than to Hantaan virus and Seoul virus (Figure 1). More recently, the agent has been isolated in cell culture.[12,13] The agent has been described in the literature under several names, including Four Corners virus, Muerto Canyon virus, Sin Nombre virus and HPS-associated hantavirus; the formal name will be decided by the International Committee on the Toxomany of Viruses.

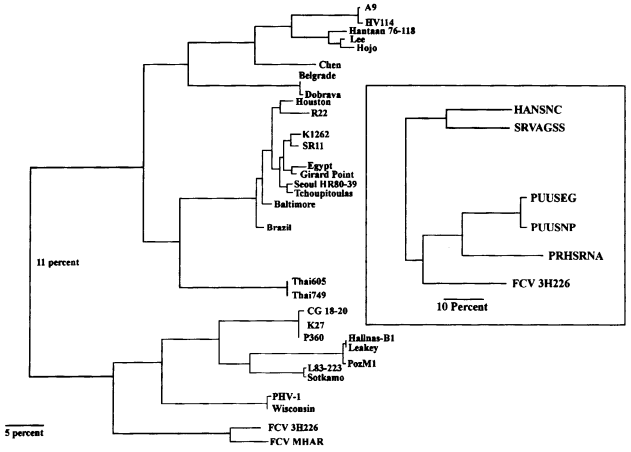

Figure 1. Parsimony tree analysis comparing HARDS-related virus G2 sequences (alleles FCV-3H226 and FCV-MHAR) with other hantavirus sequences. FCV: Four Corners Virus. PHV: Prospect Hill Virus. HV: Haatan Virus. Insert shows a simplified tree analysis for Haatan virus, Seoul virus, Puumala virus, Prospect Hill virus and Four Corners virus. From Reference 9, with permission.

As hantaviruses all have a rodent reservoir, rodents living around the homes of patients were trapped, speciated and studied for hantavirus infection. The predominant rodent species trapped near patients' homes and work areas was the deer mouse, *Peromyscus maniculatus.* Hantavirus antibodies were detected in *P. maniculatus* and related species. Nucleotide sequence analysis of RT-PCR-amplified viral genetic material from patients and from *P. maniculatus* showed that nucleotide sequences were highly related, indicating that the deer mouse is the primary rodent reservoir.[14] More recently, several other hantaviruses have been identified, in part through investigation of hantavirus cases occurring outside the range of the deer mouse.

Mapping of nucleotide sequences from a man who died in Louisiana revealed another related hantavirus, but no rodent reservoir for this virus has been identified[15]. In October 1993, a man in Florida contracted an illness similar to HPS, and hantavirus IgG and IgM antibodies were detected in an acute-phase serum sample. No *Peromyscus sp.* were trapped near his home but a third hantavirus, now tentatively named Black Creek Canal virus, was detected in cotton rats, *Sigmodon hispidus,* trapped at the site.[16] The range of the *P.*

maniculatus and *S. hispidus* and the location of documented human hantavirus infections in the United States and Canada through October 1994 are shown in Figure 2.

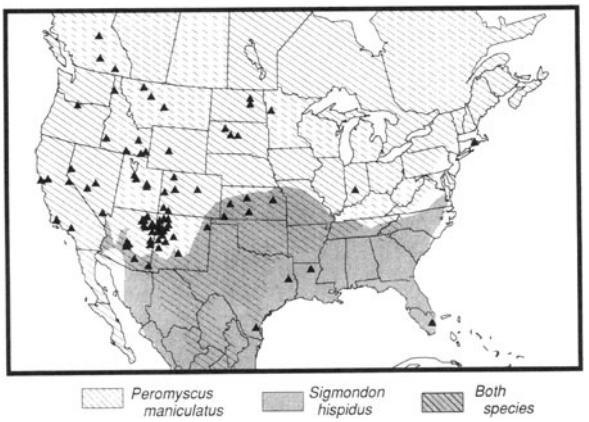

Figure 2. Distribution of human cases with hantavirus pulmonary syndrome (HPS) shown in solid triangles as compared with the distribution of the deer mouse, *Peromyscus maniculatus*, and the cotton rat, *Sigmodon hispidus*. (From Chapman LE & Khabbaz RF. Infect Agents Dis 1994;3:234-244, and Rodent distributions from: Burt WH, Grossenheider RP. A Field Guide to the Mammals. 3rd ed. New York. Houghton Miflin Company. 1980, with permission.)

Hjelle and colleagues have also recently identified yet another novel hantavirus from the harvest mouse, *Reithrodontomys megalotis*.[17] The harvest mouse is distributed throughout most of the western United States, but no human infections with this virus have been identified.

The degree of genetic divergence among the recently identified hantavirses, the fact that the genetic divergence spans the S, M and L genomic sequences rather than a single genomic sequence, the retrospective identification of HPS cases that occurred in the mid and late 1970s and the detection of the HPS-associated hantavirus in deer mice captured in 1983 suggest that these viruses emerged as a result of ancient divergence rather than a recent genetic reassortment.[7-11,15-19] Furthermore, the parallels in the phylogenetic relationships among hantaviruses and the phylogenetic relationships between their rodent hosts suggest that the viruses may have co-evolved with their rodent hosts.[9,11,17]

EPIDEMIOLOGY

Through October 1994, 95 HPS cases have been diagnosed in the United States, including 27 new cases in 1994. Fifty-seven percent of the total and 60% of the cases diagnosed in 1994 occurred in the Four Corners states (Arizona, Colorado, New Mexico and Utah). However, HPS cases have occurred in 16 states, including most states west of the Mississippi River. Of the 54 cases in the Four Corners states, 23 have been diagnosed in New Mexico and 18 have been diagnosed in Arizona. At least one case has been identified monthly since March 1993, and the number of cases reported has increased in the spring and summer months in both 1993 and 1994. The earliest cases reported to date occurred in New Mexico in 1975,[18] and the earliest reported death was in 1978 [19] and in Utah in 1959 (R. Khabbaz, B. Hjelle, personal communication).

Among clinically-identified cases of HPS, the ratio of males and females is approximately 1:1. Of the 95 cases reported between 1992 and October 1994, 54% were non-Hispanic

white, 8% were Hispanic, 36% were Native American and 2% were black. The median age was 35 years (range of 12-69 years). The case-fatality ratio was 53% overall and 41% in 1994.

Most persons with HPS appear to have acquired the infection in rural areas. Risk factors for acquisition have been investigated in a case-control study, using uninfected household members and members of neighboring homes as controls. The most significant risk factor for infection was the presence of increased numbers of small rodents in the household. However, activities likely to increase contact with rodents or aerosolized rodent excreta, such as cleaning food storage areas, agricultural activities and handling rodents, were also associated with HPS.[20]

CLINICAL COURSE

The illness typically begins with a prodrome characterized by fever and myalgia, the latter of which is often prominent.[4,5] Headache, backache, abdominal pain, diarrhea, nausea and vomiting may also be present. Fever and abdominal pain may dominate the clinical presentation; in some cases, this has led to referral to a surgeon and admission to a surgery service. The prodrome, which usually lasts 3-4 days but may last 10-12 days, is followed by the sudden onset of non-cardiogenic pulmonary edema.

The cardiopulmonary stage is heralded clinically by shortness of breath which maybe accompanied by dizziness and cough. All HPS patients have become hypoxic during this stage and have required supplemental oxygen, and about 70% of patients require intubation and assisted ventilation. Mortality has exceeded 50% among those who have required intubation. In patients with moderate to severe disease, monitoring with a flow-directed pulmonary artery catheter typically shows normal pulmonary wedge pressure (PWP), decreased cardiac index (CI), and elevated systemic vascular resistance (SVR) (Table 1).[4,5,21] Within three to four days of the onset of the cardiopulmonary phase, patients who survive usually improve rapidly over a period of a day or less; rarely, the patient may have a prolonged course characteristic of adult respiratory distress syndrome (ARDS).

Table 1. Results of initial hemodynamic and pulmonary studies in five patients with HPS treated with ribavirin.*

Patient No.	Cardiac Index liters/ min/m^2	Systemic Vascular Resistance dyn•sec•cm $^{-5}$	PCWP mm Hg	Peak Inspiratory Pressure cmH^2O	Pulmonary-Artery Pressure† mm Hg	Outcome
1	1.9	1268	8	32	29/16	Survived
2	3.5	812	2	33	11/6	Survived
3	1.6	2701	7	31	38(mean)	Died
4	1.9	1857	5	NA	46(mean)	Died
5	1.8	1598	28	30	33/16	Died

*PCWP denotes pulmonary-capillary wedge pressure, and NA not available.
Pulmonary-artery pressure is expressed as systolic/diastolic pressure.
From Duchin et al, N Engl J Med, 1994;330:949-955, with permission.

Patients with the most severe form of the disease develop shock, followed by profound cardiogenic shock and death despite the ability to maintain adequate oxygenation in intubated patients. Death may occur within hours of the first pulmonary symptoms. Indicators of a

poor prognosis include a lactate of ≥4 mmol/L, marked hemoconcentration, cardiac index of ≤2, and hypotension persisting despite pressors and hydration.

PATHOLOGY

At autopsy, the lungs are rubbery, edematous, and airless and are surrounded by large, protein-rich, pleural effusions.[22] There is intra-alveolar and septal edema, hyaline membranes composed of fibrin, and interstitial lymphoid infiltrates. In contrast to individuals dying from ARDS, the hyaline membranes are without cells or cellular debris and the type I pneumocytes appear intact. Splenomegaly is present in some cases, and immunoblasts are scattered throughout the red pulp and in the periarteriolar white pulp. The lymph nodes are grossly normal, but immunoblasts are present in lymph node paracortex. The liver is of normal size, but mononuclear cells are present in portal triads in about half the cases. The fluid suctioned from endotracheal tubes (and, presumably, the fluid present in alveoli) has a high protein content; typically, serum-to-tracheal-fluid-protein ratios exceed 0:8.[21] Of interest, despite clear evidence of depressed cardiac function, cardiac pathologic features are grossly, histologically and ultrastructurally normal.

DIAGNOSIS

Virologic Diagnosis

Most cases of HPS infection are diagnosed serologically. There is now substantial experience in testing sera obtained from patients in the cardiopulmonary stage of HPS[4-7,23,24] and IgG and IgM antibodies have invariably been present in the first sample tested. There is much less experience with testing of sera from patients during the prodrome, but antibody has also been present in many of those cases as well. However, much more experience will be necessary before any conclusions can be reached regarding the sensitivity of serologic testing during the HPS prodrome.

The serologic tests performed initially at CDC used assays for previously recognized hantaviruses, such as Hantaan, Seoul, Puumala and Prospect Hill, to detect cross-reacting antibodies. Now, both CDC and UNM employ assays using recombinant viral antigens from the newly recognized hantaviruses which were recovered from human or rodent tissue by RT-PCR. An enzyme immunoassay (EIA) developed at CDC is available there and at some state laboratories. The assay measures IgG and IgM antibodies to nucleocapsid antigens.[24,25] An immunoblot assay developed at the UNM is also available,[4,23] which measures IgG and IgM antibodies to the nucleocapsid and gG1 antigens. Antibodies directed against nucleocapsid antigens cross-react extensively among hantaviruses, whereas antibodies to gG1 are restricted to closely related hantaviruses. Arrangements to have the western blot assay performed on an emergency basis can be made by contacting the UNM infectious diseases faculty physician on call at (505) 277-5666 or 843-2111. Testing is also available at CDC for specimens forwarded through the state laboratory systems, and some state laboratories can do the EIA.

Several methods are available for identifying hantavirus in tissues or bodily fluids. RT-PCR can detect hantavirus RNA in peripheral blood mononuclear cells (PBMC) or in autopsy tissue.[26] Immunohistochemical staining with monoclonal antibodies can identify hantavirus antigens in autopsy tissue.[22] The sensitivity and specificity of those have not yet been clearly defined. Virus culture is not used for diagnosis, although technically feasible.[12,13]

Radiologic Findings

Ketai and colleagues have reviewed chest radiographic findings in 16 patients with HPS.[27] Chest radiographs are usually normal during the prodrome and may be normal at the onset of pulmonary symptoms; however, radiographic abnormalities are invariably present shortly after the onset of pulmonary symptoms. Typical early findings include bilateral findings of interstitial edema including Kerley B lines, hilar indistinctness or peribronchial cuffing. Radiographic evidence of bilateral air space disease developed within 48 hours (often within 2-3 hours) in 11 (69%), including all patients who required mechanical ventilation or who eventually died.

Routine Laboratory Tests

Except for thrombocytopenia, laboratory abnormalities are uncommon during the prodrome. Although experience is extremely limited, thrombocytopenia has been present in most samples obtained 24 to 48 hours before the onset of the cardiopulmonary stage of HPS (F. Koster, G. Mertz, unpublished data).

In the cardiopulmonary phase, characteristic hematologic abnormalities include thrombocytopenia, increased white blood cell counts with immature granulocytes and $\geq$10% immunoblasts, and hemoconcentration; hypoalbuminemia, elevation of the serum lactate dehydiogenase (LDH), and lactic acidosis may also be present.[5,22] The immunoblasts are characterized by basophilic cytoplasm (occasionally with coarse granules), prominent nucleoli, and a high nuclear-to-cytoplasmic ratio.[22]

Clinical Diagnosis

Although the cardiopulmonary stage of HPS may be confused with a number of other illnesses, many of the latter can be excluded by history, physical findings, the chest radiograph and routine laboratory tests. When the history and clinical presentation suggest HPS, clinicians at the UNM have found it helpful to examine the peripheral smear and to initiate monitoring with a flow-directed pulmonary artery catheter. To date, all patients at the UNM with the complete triad of thrombocytopenia, increased white blood cell count with immature forms and $\geq$10% immunoblasts among lymphocytes and with pulmonary function abnormalities consisting of a normal pulmonary wedge pressure (PWP), normal or decreased cardiac index (CI), and elevated systemic vascular resistance (SVR), have had HPS [F. Koster, G. Mertz, H. Levy, unpublished].

Recognition of patients who are in the prodromal phase of HPS is very difficult, even for clinicians familiar with this disease. The diagnosis should be considered in persons who present with fever and moderate to severe myalgia, particularly if the person has had rural exposure to rodents or rodent excreta within the past 1-3 weeks. Headache, backache, abdominal pain, nausea, vomiting and diarrhea are present in more than half of patients. A history of any high-risk exposure (i.e. cleaning food storage areas, catching mice, agricultural work or increased numbers of mice in the home) in persons from a rural area should prompt the clinician to consider a diagnosis of hantavirus infection. Whenever practical, a complete blood count, including a platelet count, should be obtained. In the setting described above, the presence of thrombocytopenia should trigger both serologic testing for hantavirus and consideration of referral to a center with critical care capabilities.

MANAGEMENT

Critical Care Management

Principles of critical care management for patients with the cardiopulmonary syndrome are straight forward. Supplemental oxygen should be administered if the patient is hypoxemic, with intubation if necessary. Monitoring of cardiac index, pulmonary wedge pressure and systemic vascular resistance with a flow-directed pulmonary artery catheter should be performed. Volume overload should be carefully avoided to reduce the likelihood of worsening pulmonary edema, and instead, inotropic support and vasopressors should be employed whenever possible.

Antiviral Therapy

Intravenous ribavirin was available for individual patients with suspected HPS from June 1993 until September 1994 through a CDC-sponsored protocol. Therapy without a comparison group was considered justified initially because intravenous ribavirin reduces mortality in hantavirus-associated HFRS and because all previously tested hantaviruses had been sensitive to ribavirin *in vitro*;[1,3] subsequently, similar *in vitro* results were obtained with the hantavirus isolated from *Peromyscus maniculatus* (J. Huggins, S. Ruo personal communication). Non-pregnant adolescents and adults with a presumptive diagnosis of HPS were eligible for enrollment in this open ribavirin protocol. The initial regimen was a loading dose of 30 mg/kg, followed by 15 mg/kg every six hours for four days and 7.5 mg/kg every eight hours for six days; this was later modified to a loading dose of 33 mg/kg, followed by 16 mg/kg every six hours for 15 doses, and then by eight mg/kg every eight hours for nine doses (to conform to the regimen which was effective in patients with HFRS).

Preliminary results of the open trial have been recently presented.[28] Between June 1993 and April 1994, 112 persons suspected of having HPS were enrolled in the open trial. HPS was confirmed in only 21. The mortality was 17 (63%) among 27 persons retrospectively diagnosed before the disease was widely recognized in June 1993. Between June 1993 and April 1994, eight (42%) of 19 ribavirin-treated and two (40%) of five untreated patients within the recognized outbreak area died. Outside the recognized outbreak area, 13 (87%) of 15 patients died, suggesting less-frequent recognition of mild disease outside the recognized outbreak period. The significant regional differences in survival and differences in survival among persons diagnosed before and after recognition of HPS suggested that it would be unlikely that appropriate untreated controls could be identified for comparison with patients treated in the open trial. Similar concerns were raised regarding the interpretation of adverse effects. Ribavirin-associated toxicity developed in many patients. The commonest toxicity was a reversible anemia; however, at least 20 patients required transfusion (L. Chapman, personal communication). The association of other adverse events such as pancreatitis or hyperamylasemia to ribavirin therapy was less clear.

In August 1994, the CDC convened a panel of outside experts to review the experience with the open protocol. The panel concluded that ribavirin was generally well tolerated in patients with HPS but had no clearly positive influence on outcome. The panel recommended that the trial be stopped because definitive conclusions regarding efficacy and safety were impossible from the open trial and recommended that a placebo-controlled trial be initiated.

The NIAID CASG plans to conduct a placebo-controlled trial of ribavirin therapy of HPS which will be initiated at the UNM and other medical centers in the Southwest in 1995. In contrast to the open trial which required progression to the cardiopulmonary phase before therapy could be initiated, the controlled trial will encourage enrollment of patients during the prodrome phase of the illness. Patients with signs and symptoms of cardiopulmonary involvement will also be eligible, but patients at high, immediate risk of death such as those

with a low CI or a lactate of >4 mmol/L, will be offered salvage therapy rather than enrollment in the controlled trial.

Other Investigational Therapies

The role of inflammatory mediators, such as cytokines or nitric oxide, in the pathogenesis of HPS is being investigated through measurement of cytokine and nitric oxide levels in patient samples. If these mediators are found to play a role in the pathogenesis of HPS, protocols to evaluate cytokine inhibitors or nitric oxide synthesis inhibitors may be considered.

Extracorporeal membrane oxygenation (ECMO) has been used at the UNM Health Sciences Center in two patients who fit criteria that were associated with 100% mortality in a retrospective analysis of patients cared for at UNM. One patient treated with ECMO survived. To further investigate the feasibility ECMO, investigators at UNM plan to conduct a limited, open trial of this therapy in patients who have profound cardiogenic shock activity or a CI of ≤ 2 and a lactate of >4 mmol/L; the patient must either have serologic confirmation of infection or meet the hemodynamic and hematologic criteria described earlier.

SUMMARY

Definitive diagnosis of acute hantavirus infection requires demonstration of specific IgG and IgM antibodies and a compatible clinical course; most persons with acute illness will also have viral RNA in PBMC which can be detected after RT-PCR amplification. Characteristic hematologic and hemodynamic findings are helpful in establishing a presumptive diagnosis of HPS in persons with cardiopulmonary symptoms and signs while awaiting the results of serologic testing and PCR. Diagnosis during the prodromal phase is difficult although very limited data suggest that most of these patients also have hantavirus IgG and IgM antibodies.

Patients with suspected hantavirus infection should be treated in a critical care unit, as sudden hemodynamic deterioration can occur at any time. Hemodynamic monitoring is encouraged in patients with cardiopulmonary symptoms and signs, and inotropic support and vasopressors should be used rather than fluids. An uncontrolled study of ribavirin therapy was inconclusive and a placebo-controlled trial is therefore planned. The latter study aims to enroll persons with prodromal findings and early cardiopulmonary disease.

Acknowledgements

We thank Dr. Sarah Allen, Dr. Kathy Foucar, Dr. Diane Goade, Dr. Brian Hjelle, Karen Hyde, R.N., Dr. Steven Jenison, Dr. Gus Hallin, Dr. Loren Ketai, Dr. Frederick Koster, Dr. Howard Levy, Dr. Kurt Nolte, Dr. Steven Simpson, Gaye Walters, R.N., Carolyn Williams, R.N. and Dr. Josephine Williams of the University of New Mexico and Dr. Lori Armstrong, Dr. Ralph Bryan, Dr. Rima Khabbaz, Dr. Ali Khan, Dr. Thomas Ksiazek, Dr. Stewart Nichol, Dr. C.J. Peters, Dr. Pierre Rollin and Dr. Sherif Zaki of the Centers for Disease Control and Prevention for their contributions to the investigations described above; Dr. Ralph Bryan, Dr. Brian Hjelle, Dr. Frederick Koster, Dr. Howard Levy, Dr. Kurt Nolte, and Dr. C.J. Peters for their assistance in reviewing this manuscript, and Ms. Cindy Wootton for her assistance in preparation of the manuscript.

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