Rapid Presumptive Diagnosis of Hantavirus Cardiopulmonary Syndrome by Peripheral Blood Smear Review

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Abstract

Hantavirus cardiopulmonary syndrome (HCPS) is a rare but frequently lethal acute zoonotic viral infection in rural North America. The rapidity of progression from febrile prodrome to cardiogenic shock and noncardiogenic pulmonary edema requiring intensive care creates high diagnostic urgency and a need for a rapid screening tool. In this retrospective cohort study, 2 pathologists scored blinded peripheral blood smears from 52 patients with HCPS and 128 seronegative patients referred for diagnosis of suspected hantavirus infection.

During the prodromal phase, thrombocytopenia was the only consistent abnormality and could be used to indicate hantavirus serologic testing. After the onset of pulmonary edema detected radiographically, the presence of 4 of 5 findings (thrombocytopenia, myelocytosis, hemoconcentration, lack of significant toxic granulation in neutrophils, and more than 10% of lymphocytes with immunoblastic morphologic features) has a sensitivity for HCPS of 96% and a specificity of 99% and missed no patients with HCPS who required intensive care. While each abnormality is commonly seen, the combination of at least 4 of these CBC count data and peripheral blood smear findings can guide early treatment and patient transport decisions until rapid, specific, serologic testing becomes widely available.

In North America hantavirus cardiopulmonary syndrome (HCPS) due to the Sin Nombre virus (SNV) is a rare viral pneumonitis with a high case fatality rate of approximately 45%.1 The highest incidence of this rodent-borne zoonosis is observed in rural areas of the southwestern and northwestern United States and western Canada, and in the eastern United States, as well. HCPS also occurs in multiple regions of South America, where other hantavirus species have been implicated.2 HCPS is characterized by 3 phases, beginning with a nonspecific febrile prodrome lasting 2 to 10 days, followed by the precipitous development of noncardiogenic pulmonary edema and cardiogenic shock, and finally diuresis and convalescence. Since cardiogenic shock is responsible for the majority of deaths, we refer to the syndrome as hantavirus cardiopulmonary syndrome. Before or during the abrupt transition from nonspecific flu-like symptoms to profound shock and pulmonary edema, clinicians in rural regions are faced with several time-sensitive decisions before a serologic diagnosis can be established. Urgent decisions include carefully titrating the dosage of intravenous fluid in the setting of a pulmonary capillary leak syndrome, early use of inotropic agents,3 transport to an intensive care unit before cardiogenic shock supervenes, and enrollment in the National Institutes of Health–sponsored randomized therapeutic trial.4

Definitive diagnosis of SNV infection is made by serologic detection of specific IgM and IgG antibodies to the SNV N antigen in combination with IgG antibodies to the viral G1 glycoprotein. Such antibodies, as well as antibodies capable of neutralizing SNV in vitro, seem to be fully detectable at the earliest stages of illness.5-7 A rapid strip
immunoblot assay is used at the University of New Mexico (UNM), Albuquerque, and results are reported on the same day that the sample is received. Of several hundred tests performed at UNM each year, we are unaware of any false-positive or false-negative interpretations, indicating a sensitivity and specificity of nearly 100%. Despite the rapid turnaround time for the test itself, delays associated with shipping samples impose a minimum 24-hour turnaround time for results.

We sought to identify a method for presumptive diagnosis for HCPS, enabling more cost-effective management decisions early in the disease. An algorithm designed to identify patients likely having HCPS based on symptoms failed to achieve sufficient sensitivity and specificity (M.M., unpublished data). As an alternative approach, we examined, in a blinded manner, the peripheral blood smear of patients with symptoms suggestive of HCPS and compared findings from the CBC count and manual smear inspection between 52 HCPS cases and 128 control subjects representing SNV-seronegative infections clinically mimicking HCPS in the prodromal or cardiopulmonary phase. We identified an aggregate of 5 findings: thrombocytopenia, left shift of the myeloid series, lack of marked toxic granulation of the neutrophils, hemoconcentration, and the presence of immunoblasts. Collectively, this aggregate of 5 findings is sufficiently sensitive and specific to allow a presumptive diagnosis of HCPS at a point in disease evolution characterized by radiographically apparent pulmonary edema.

### Materials and Methods

#### Subjects

Fifty-two (46 at onset of cardiopulmonary phase and 6 only at end of this phase) patients had serologically proven acute SNV infections. Most were treated at the UNM Hospital (n = 38) or the Presbyterian Hospital (n = 3), Albuquerque. Blood smears and serum samples from 11 other patients with HCPS from 5 other states were shipped to UNM for diagnostic evaluation for HCPS. The criteria for “severe” (n = 25, group H2, Table 1) included death occurring as a result of the infection, or, if cardiogenic shock was demonstrated by hypotension unresponsive to fluid resuscitation, an elevated plasma lactate level more than 36.0 mg/dL (4 mmol/L), or myocardial dysfunction documented by a cardiac index less than 2.5 L/min per m². The other 21 cases (group H1) were classified as “mild”; 5 cases required brief mechanical ventilation but had no evidence of shock.

Control subjects (groups C1 through C5) were a nonconsecutive series of 128 patients with febrile illnesses clinically similar to the prodromal or the cardiopulmonary phase of HCPS. The criterion for inclusion in 1 of the 5 control groups was requested serologic testing for SNV at UNM because each patient’s physician was unable to distinguish the illness from possible hantavirus infection on clinical grounds. Sixty-five control subjects (groups C1-C3), all hospitalized at UNM, had infections characterized by...
Fifteen patients (group C1) had acute respiratory distress syndrome; 21 patients (group C2) had blood culture–confirmed bacterial sepsis (gram-negative, 9; gram-positive, 13), and 29 patients (group C3) had lobar or regional pneumonia (10 with positive cultures for gram-positive organisms). Forty-eight control subjects (group C4) were ambulatory patients with febrile illnesses that mimicked the prodromal phase of HCPS and were evaluated in clinics in Gallup, Crownpoint, and Albuquerque, NM. Nineteen of the ambulatory control subjects had culture-confirmed acute influenza A virus infection. In the remaining 29, blood cultures for bacteria and nasopharyngeal cultures for influenza were negative. Fifteen patients (group C5) were diagnosed with a variety of nonrespiratory infections, denoted “other” infections (eg, endocarditis, Rocky Mountain spotted fever, relapsing fever due to *Borrelia* infection, gastroenteritis, hepatitis, encephalitis, infectious mononucleosis, plague, and group A streptococcal infection).

Clinical data were extracted directly from charts in Albuquerque or from data submitted by referring physicians. The study was approved by the institutional review boards of the UNM Health Sciences Center, the Indian Health Service, and the Navajo Nation Department of Health.

### Smear Interpretation

For the study, 220 peripheral blood films and CBC counts from 180 patients were examined by 1 hematopathologist (K.F.), and 100 of these were examined independently by 1 of 3 other hematopathologists (A.S., Y.-Y.C., and R.L.). There were no discrepancies in interpretation among the examiners. All clinical information was blinded to the hematopathologists except for the sex of the patients, which was used in interpreting hemoglobin and hematocrit values. To check consistency of interpretation, 11 slides were submitted in duplicate to 1 hematopathologist (K.F.) for blinded evaluation, and no discrepancies in interpretation were found. Seventy-one slides from the 52 patients with HCPS included 7 slides obtained during the prodromal phase of illness (data not shown), 46 slides from the beginning of the cardiopulmonary phase (Table 1), and 18 slides from convalescence at least 3 days after the onset of pulmonary edema (data not shown).

The CBC count from patients treated at the UNM was performed on the Sysmex SE 9000 analyzer (Toa Medical, Kobe, Japan) and was the source of the hematocrit and platelet count data. The peripheral blood films were made by the Sysmex SP 100 (Toa Medical) automated slide maker and stained with Wright-Giemsa.

Two 100-cell manual differential counts were performed on each slide: a 100-cell differential count of all WBCs and a 100-cell count restricted to cells of the lymphoid system. We assessed the following: (1) left shift, if any, of cells of the granulocytic series; (2) the degree, if any, of toxic changes of neutrophils; (3) the presence of microscopically visible infectious organisms; (4) the presence of nucleated erythroid elements; and (5) the enumeration of the fraction of lymphoid cells with the morphologic features of immunoblasts or plasma cells.

We defined immunoblasts as enlarged lymphoid cells (15-25 µm, or 2 to 3 times the diameter of small lymphocytes) with fair to abundant, moderately to deeply basophilic cytoplasm; a large reticular nucleus with uniform chromatin; and variably prominent nucleoli. Not every cell counted within the immunoblast category looked exactly alike; there was variation in overall size and in the degree of basophilia of the cytoplasm. Owing to their large size, immunoblasts were found in greater numbers at the “feathered” edge and sides of the blood smear and often were best detected on scanning magnification (×10).

Plasma cells were characterized by abundant basophilic cytoplasm, an eccentric round nucleus with clumped chromatin, and a well-defined paranuclear cytoplasmic clearing (Golgi apparatus). A reading of “mild toxic granulation” signifies minimal change in a minority of neutrophils, while “moderate to severe toxic granulation” indicates prominent toxic granulation and Döhle bodies with variable vacuolization in the majority of neutrophils.

From experience examining the peripheral blood films and CBC data of patients with HCPS during the initial outbreak in 1993, 1 hematopathologist (K.F.) found 5 features that were useful in the presumptive diagnosis of florid HCPS:

**Image II** Multiple immunoblasts are evident in the peripheral blood smear from a patient with florid hantavirus cardiopulmonary syndrome. Note the deeply basophilic cytoplasm (Wright-Giemsa, x100).
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Elevated hematocrit value, thrombocytopenia (platelet count, <150 × 10^3/µL [<150 × 10^9/L]), left shift of the myeloid series, lack of severe toxic changes in the myeloid series, and greater than 10% immunoblasts and plasma cells in a 100-cell lymphoid cell count. We tested the hypothesis that a combination of these 5 criteria would be sufficiently sensitive and specific to consistently distinguish HCPS from all other febrile illnesses.

Statistics

The 7 samples of prodromal HCPS were compared with samples from group C4, and separately, the 46 samples from patients with HCPS studied at the onset of pulmonary edema were compared with samples from groups C1 through C3. Group comparisons used 2-sided nonparametric tests, with significance at the \( P < .05 \) level.

Results

Prodromal Phase

Ambulatory patients with prodromal-phase HCPS and ambulatory control subjects with hantavirus-seronegative flu syndrome did not differ by age, sex, or duration and magnitude of fever, nor did they differ by hematocrit value, WBC count, presence of toxic neutrophilia, absolute or reactive lymphocyte count, or monocyte count. Thrombocytopenia (7/7 patients with prodromal HCPS vs 1/48 control subjects with flu syndrome) and left-shifted myeloid series (4/7 patients with prodromal HCPS vs 2/48 control subjects) were present more often in patients with prodromal HCPS than in ambulatory control subjects (\( P < .001 \) and \( P < .05 \), respectively). During the evolution of the prodromal phase, only thrombocytopenia was consistently present, and therefore can be used as a screening criterion for possible hantavirus infection. Before the onset of pulmonary edema visualized on chest radiograph, hemoconcentration...
and large numbers of myelocytes and immunoblasts are not consistently seen.

**Severe HCPS**

Among hospitalized subjects, the group with severe HCPS (H2) had the highest mortality, lowest platelet counts, highest hematocrit values, highest WBC counts, and highest absolute lymphocyte counts compared with all groups of hantavirus-seronegative control subjects and patients with mild HCPS (Table 1). All 25 patients with severe HCPS met 4 of 5 hematologic criteria. Of the patients with severe HCPS, 5 (20%) had moderate toxic granulation of the neutrophils, but none were graded as having severe toxic granulation.

**Mild HCPS**

Of 21 patients with mild HCPS at admission, 19 (90%) met at least 4 criteria. The 2 exceptions lacked left-shifted myeloid series, but these patients did not require supplemental oxygen or hospitalization and would not have been affected clinically by a false-negative diagnosis. More than
half of the group with mild disease had normal hematocrit values, confirming the absence of severe capillary leak. The pentad of abnormal blood findings is transient, and within 3 days after the onset of pulmonary edema, one or more of the abnormalities is lost (data not shown).

Control Groups

As noted in Table 1, each of the individual hematologic pentad features lacks specificity. The presence of immunoblasts was identified in 31% to 43% of each control group. The absence of moderate to severe toxic granulation was noted in 33% to 85% of each control group. The presence of left-shifted myeloid series cells (promyelocytes, myelocytes, and metamyelocytes) was detected in 17% to 60% of each control group. Thus, cases in the control groups met 3 hematologic criteria, including 13% (2/15) of acute respiratory distress syndrome, 18% (4/22) of sepsis, and 20% (3/15) of other infections. However, only 1 control subject, who had fever, dyspnea, thrombocytopenia, and circulating immunoblasts due to bubonic plague, met 4 hematologic criteria (Table 1). In this case, the toxic changes in neutrophils were striking, and the pathologist made the correct presumptive diagnosis (not HCPS).

Sensitivity and Specificity of CBC Count and Peripheral Blood Smear

The calculated sensitivity of 4 or more criteria for all HCPS subjects was 96% (46/48). The calculated sensitivity of detecting HCPS requiring intensive care was 100% (30/30). The calculated specificity of 4 or more criteria to detect only HCPS was 99% (79/80). To calculate positive and negative predictive values, we used an estimated prior probability of 5% prevalence of HCPS among clinically similar thrombocytopenic pulmonary syndromes in the southwestern United States, because that is the percentage of positive serologic assays among specimens submitted for rapid diagnosis to the UNM/Tricore Diagnostic Laboratory, Albuquerque. With an estimated prior probability of 5% among 1,000 tests, the positive predictive value of the 4 of 5 hematologic criteria would be 83% (48/58), and the negative predictive value would be 99.8% (940/942).

Discussion

The definitive diagnosis of acute HCPS depends on the presence of specific IgM antibodies to SNV proteins, detected by several highly specific and sensitive serologic assays. The precipitous onset of shock and pulmonary edema, however, often demand immediate decisions regarding patient transport and intensive care before the onset of shock and well before the serologic tests can be completed. Attempts to use algorithms of clinical signs and symptoms to predict HCPS have not been sufficiently sensitive and specific to guide decisions (M.M., unpublished data). To develop a rapid screening diagnostic algorithm for possible HCPS, we evaluated and defined for the first time a set of hematologic criteria obtained from the CBC count and peripheral blood smear that can be used to evaluate patients in the prodromal and cardiopulmonary phases of SNV infection.

In the first or prodromal phase of HCPS, thrombocytopenia is the first, and usually only, hematologic abnormality and was always present, in our experience, during the cardiopulmonary phase of the disease. In the rural southwestern United States, thrombocytopenia during a viral prodrome–like illness occurs frequently, and, thus, thrombocytopenia alone is not a helpful criterion for prodromal HCPS. Other causes of thrombocytopenia associated with acute febrile illnesses include sepsis, plague, tularemia, borreliosis, Rocky Mountain spotted fever, and parvovirus infection. Furthermore, many drug treatments including antibiotics such as sulfamethoxazole, alcohol toxicity, and a wide variety of other noninfection-related causes in critically ill patients may elicit thrombocytopenia.
Nevertheless, the high frequency of thrombocytopenia in prodromal HCPS demands that febrile patients with a platelet count less than $150 \times 10^3/\mu L$ ($150 \times 10^9/L$) require close follow-up. In our personal experience with more than 70 patients with HCPS, only 2 patients did not have thrombocytopenia at the time of the first health care encounter; these patients had only 1 day of symptoms, and the peripheral blood smears were not available for inclusion in this study. Therefore, we recommend that serial platelet counts should be performed every 12 to 24 hours. In HCPS and other serious infections, the platelet count decreases more than $20 \times 10^3/\mu L$ ($20 \times 10^9/L$) per 12-hour period, while the more prevalent mild viral prodromes are associated with a slower descent in the platelet count (F.K., unpublished data). The presence of a small number of immunoblasts (<10% of lymphoid cells) on the peripheral smear also is seen in most cases of prodromal HCPS. However, circulating immunoblasts typify many transient immune-response processes and are encountered commonly in low numbers on peripheral blood smears.

In the second (cardiopulmonary) phase of HCPS, coincident with the appearance of chest radiographic infiltrates indicative of pulmonary edema, the presence of 4 of 5 hematologic criteria, thrombocytopenia, myelocytosis, lack of severe toxic granulation, immunoblastosis, and hemocytopenia, is sensitive and specific for HCPS. Even in the presence of marked neutrophilia, the neutrophils lacked the striking toxic granulation and Döhle bodies that typify bacterial infections. Several caveats should be noted in interpreting the peripheral smear. Difficulties in the grading of mild toxic changes noted in some HCPS cases make this feature less useful by itself than the other features. Hemocytopenia was present only in the most florid cases. Extreme leukocytosis (leukemoid reaction) often is seen in severe HCPS but never in the prodromal phase. Circulating immunoblasts (subsequently discussed in more detail) decrease or disappear 3 to 5 days after the onset of pulmonary edema, making the peripheral smear not useful during the convalescent stage. Laboratory abnormalities including acidosis, elevated serum lactate dehydrogenase level, elevated serum hepatic transaminase levels, and decreased serum albumin level are present in most cases of HCPS but are common in other infections, particularly sepsis, and fail to provide sufficient predictive value (data not shown).

These hematologic criteria have been observed in HCPS diagnosed in South and Central America (B.H. and F.K., unpublished data) and in hemorrhagic fever with renal syndrome due to Hantaan virus in Asia (K.F., unpublished data). Whether hematologic criteria have presumptive diagnostic value outside North America will require specific evaluation. In regions where leptospirosis, dengue, and other viral hemorrhagic fevers are endemic, the specificity of the hematologic criteria for HCPS would be lower than that in North America.

The hematologic parameters we evaluated included 2 indices available through automated hemogram determination (platelet count and hemoglobin/hematocrit) and 3 that require manual review of the smear (extent of toxic changes and left shift in the neutrophil series and the fraction of lymphocytes that are immunoblasts). The percentage of pathologist-enumerated immunoblasts usually was underestimated by the enumeration of “atypical lymphocytes” with automated cytometry. Morphologic criteria used to define immunoblasts vary. The diagnostician must appreciate that lymphocyte transformation is a dynamic process that impacts the morphologic features of both the nucleus and cytoplasm and that the transformation of cells exists as a continuum from nonactivated to fully activated. In the present study, we placed greater relative weight on the large cell size and deeply basophilic cytoplasm to identify immunoblasts compared with nuclear features, to ensure easy identification that leads to minimal intraobserver and interobserver variability. In this manner, immunoblasts are best detected at low magnification (×10); owing to their large size, these cells often are more numerous along the feathered edge and sides of the smear. A panel of abnormal blood smears showing the spectrum of morphologic features typical of immunoblasts is available. Activated immunoblasts are prominent in lung, liver, lymph nodes, and spleen in fatal cases of HCPS and are thought to participate in the pathogenesis of the capillary leak syndrome.

Requests by clinicians for manual inspection of the peripheral blood smear may be an underused diagnostic tool for blood-borne infections. The presumptive diagnosis of malaria, plague, relapsing fever due to Borrelia organisms, ehrlichiosis, babesiosis, meningococcemia, and disseminated histoplasmosis often can be made by visualization of organisms on the Wright-Giemsa–stained peripheral smear. The infectious mononucleosis syndrome due to Epstein-Barr virus, cytomegalovirus, and HIV is characterized by a striking atypical lymphocytosis. Substantial numbers of circulating immunoblasts typify hantavirus infections, but the unique aggregate of 5 blood features confers specificity for HCPS. Since each of the 5 individual hematologic findings seen in SNV infection are noted in many disorders, the pathologists’ interpretation of the blood smear must search for the aggregate of 5 findings in order to suggest the presumptive diagnosis of hantavirus infection.

The interpretation of our study data has several limitations. The blinded blood smear evaluations in this study were performed by hematopathologists. Although hematology laboratory technologists in our institution accurately interpret HCPS blood smears, we are evaluating whether laboratory
technologists in rural hospitals can recognize the presumptive diagnostic features on blood smear following specific training. Second, although this study includes a substantial percentage of all SNV infections ever described, further experience may identify exceptions, and disease caused by other North American hantaviruses such as Bayou and Black Creek Canal may not mimic that caused by SNV. Finally, HCPS is a rare disease in most regions of the United States. As noted, assuming a 5% prevalence in the Southwest and the sensitivity and specificity statistics of 96% and 99%, the positive predictive value of the 4 of 5 aggregated findings was calculated as 83% (48/58), with a negative predictive value of 99.8% (940/942). In regions with a 0.1% prevalence, for example, the corresponding positive predictive value would be 9.1% and the negative predictive value would be 99.99%. Nevertheless, the early suspicion and recognition of HCPS is critical to the patient’s chances of survival, and use of the blood smear has been useful in the Southwest for accelerating recognition and transport to intensive care.

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