Hantavirus Serologies in Patients Hospitalized with Community-Acquired Pneumonia

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In many patients, the etiology of community-acquired pneumonia is not known but may be caused by previously undescribed pathogens in some cases. The recently identified hantavirus Sin Nombre (SN) causes hantavirus pulmonary syndrome. Because sporadic cases have occurred outside the range of its reservoir (the deer mouse Peromyscus maniculatus), an investigation sought to determine whether hantaviruses contributed to cases of community-acquired pneumonia in a large Baltimore hospital. Acute-phase sera from 385 hospitalized patients with pneumonia were examined using an IgG ELISA technique with antigens prepared from several hantaviruses: prototype Hantaan (HTN), Seoul (SEO), Puumala (PUU), Convict Creek (HNI07), and SN. Of 385 sera, 8 (2.1%) showed some reactivity with one or more HTN, SEO, or PUU antigens but none had detectable specific IgM antibodies. No sera were reactive with SN or HNI07 antigens. Thus, hantaviruses are an uncommon cause of community-acquired pneumonia in the Baltimore area.

Despite rigorous investigations, the etiology of community-acquired pneumonia remains unknown in as many as 50% of patients who require hospitalization (reviewed in [1]). Because unknown pathogens may account for some of these cases, continued inquiry into causes of community-acquired pneumonia has been advocated [2]. A previously unrecognized hantavirus was recently identified as the cause of an outbreak of a severe respiratory illness within the southwestern United States [3]. The virus, currently referred to as Sin Nombre (SN), has been linked to the deer mouse (Peromyscus maniculatus) as its principal rodent reservoir [4].

SN is a member of the hantavirus genus, which comprises rodentborne, negative-strand RNA viruses in the Bunyaviridae family. The SN virus causes a rapidly progressive pneumonitis in previously healthy people, which is termed hantavirus pulmonary syndrome (HPS). HPS is characterized by a febrile illness with a brief prodrome that is followed by progressive noncardiogenic edema, leukocytosis, thrombocytopenia, and shock, with high mortality [5]. Although most cases of HPS have been within the known range of the deer mouse [6], others have been reported in Louisiana [7], Florida [8], and Rhode Island (or New York) [9], although some of these cases were due to hantaviruses other than SN [7, 8]. Because Butler and Peters [6] have recommended that the diagnosis of HPS be considered with any compatible clinical illness [6], we examined whether hantaviruses contributed to cases of community-acquired pneumonia in a large urban hospital. Acute-phase sera from 385 consecutive patients hospitalized for community-acquired pneumonia were screened by IgG ELISA for the presence of antibodies reacting to hantavirus antigens prepared from a recombinant protein of SN and other hantaviruses, which may detect a different but related virus.

Materials and Methods

Study design. All adults with pneumonia admitted to the Department of Medicine, Johns Hopkins Hospital, Baltimore, from 14 November 1990 to 13 November 1991 were eligible for study. Patients were hospitalized at the discretion of the admitting physician. The definition of community-acquired pneumonia was adapted from that of Fang et al. [2]: age >17 years and tentative diagnosis of pneumonia within the first 24 h after admission, a new pulmonary infiltrate shown on chest radiographs, and confirmatory
clinical findings of one “major criterion” (cough, sputum production, or fever >37.8°C) or two “minor criteria” (pleuritic chest pain, dyspnea, altered mental status, pulmonary consolidation by physical examination, or white blood cell count >12,000/mm³). We excluded patients hospitalized within 2 weeks before admission and those transferred from another hospital. All enrolled patients satisfied the criteria for community-acquired pneumonia and did not have an alternative diagnostic explanation (e.g., congestive heart failure, pulmonary embolus, or endocarditis). The diagnostic methods and microbiologic results of other evaluations for this cohort of patients are reported elsewhere [10]. For this study, we examined acute-phase sera obtained within the first week of hospitalization that had been stored at -20°C.

Serologic techniques. Stored sera were tested for IgG antibodies by ELISA using hantavirus antigens bound directly to a microtiter plate as previously described [11]. Sera were screened at a 1:100 dilution against prototype Hantaan (HTN) strain 76-118, Seoul (SEO) strain 80-39, Puumala (PUU) strain P360, Convict Creek (California) strain HNI07, and an Escherichia coli recombinant protein from the nucleocapsid region of SN (provided by T. Ksiazek, CDC, Atlanta). Optical density ELISA values were considered negative if <0.2, marginal if 0.2–0.5, moderately reactive if 0.6–1.0, and highly reactive if >1.1. Sera that were IgG-positive for any of these antigens, and a small subset of negative controls, were tested for the presence of hantavirus-specific IgM by the ELISA IgM capture method [12].

Statistical methods. Data were analyzed using the Statistical Analysis System (SAS Institute, Cary, NC). χ² or Fisher’s exact tests were used to compare demographic or serologic characteristics. Any probability values were two-tailed.

Results

Study demographics. A total of 385 patients satisfied criteria for enrollment into the study, and 385 acute-phase sera were available for hantavirus serologic evaluation. Most patients lived in Baltimore (336 [87.3%]) and many were infected with the human immunodeficiency virus (HIV; 180 [46.8%]). The causes of community-acquired pneumonia in this group are detailed elsewhere [10]. Despite extensive diagnostic procedures, the etiology was unknown in 122 (31.7%) patients. Of the 57 patients (14.8%) admitted to the medical intensive care unit (ICU), 48 (12.5%) required mechanical ventilation. For 14 patients (3.6%) admitted to the ICU, the microbiologic diagnosis was not determined.

Hantavirus serologies. Eight (2.1%) serum samples had some reactivity by IgG ELISAs to HTN, SEO, or PUU antigens. As shown in table 1, 3 patients had strongly or moderately reactive sera and 5 patients had marginally reactive sera. These 8 reactive sera and 7 randomly chosen IgG-negative controls were subsequently tested for the presence of IgM antibodies to HTN, SEO, or PUU antigens. None were reactive. No sera reacted to either SN or HN107 antigens by IgG ELISA.

Two patients in the group with strongly or moderately reactive ELISAs were HIV-positive. The cause of the pneumonia was not determined for 4 patients (50%) with positive hantavirus serologies compared with 31.7% (P = .27) for the entire pneumonia patient cohort. None of the 8 patients with reactive hantavirus serologies died.

Discussion

In this study, we were unable to find evidence of hantavirus infection as a cause of community-acquired pneumonia. The 8 sera that reacted with HTN, SEO, or PUU antigens appear to represent prior infection, since they lacked specific IgM antibodies. There was also no evidence of acute or prior infection with SN or Convict Creek viruses. The failure to detect any sera positive for SN or HN107 reinforces the epidemiologic premise that urban residents are at low risk for HPS.

Despite the recent characterization of HPS, it appears that several different hantaviruses may cause similar pulmonary pathology. Of these, SN remains the most common. Most cases have occurred within the mostly rural range of its rodent reservoir P. maniculatus, which covers the lower 48 United States, except for the southeastern and eastern regions. Its range may include western Maryland, but it is not thought to include Baltimore or eastern Maryland [6]. A case of HPS in Louisiana appears to represent a unique hantavirus (Bayou virus), although its rodent host has not been identified [7]. Another new strain of hantavirus (Black Creek Canal virus), isolated from the cotton rat (Sigmodon hispidus), has been implicated as the cause of HPS for a patient in Dade County, Florida [8].

Because different or novel hantavirus strains may react imperfectly with characterized hantavirus antigens, we used a panel of antigens to hantavirus strains that cause pulmonary or renal syndromes. HTN, SEO, and PUU viruses may cause hemorrhagic fever with renal syndrome or the milder disease nephropathia epidemica in Asia and Europe [6]. Although the possibility remains that we failed to detect a new, geographically distinct hantavirus, the inability to detect acute seroconversion by the lack of IgM antibodies to any of the screened antigens suggests that hantaviruses are an uncommon cause of pneumonia in our region. IgG to hantavirus antigens is present within the acute-phase sera of patients with HPS as detected by immunoblot technique [13]. Therefore, lack of sufficient detection of hantavirus IgG is an unlikely explanation for our findings. We cannot rule out that we missed an IgG-negative, IgM-positive case of hantavirus infection, but the IgG screen should detect most infections.

Hantavirus SEO circulates among Norway rats (Rattus norvegicus) in Baltimore, and antibodies to the SEO virus have been documented in humans from Baltimore [14]. Serologic studies suggest that hantavirus antibodies are consistently higher in patients with hypertensive renal disease [15]. This last study established a baseline seroprevalence of 0.25% in patients with no known medical risk factors and rates as high as 6.5% in patients with end-stage renal disease. Although our study found a seroprevalence that is higher than reported for asymptomatic persons (2.1%), this rate is similar to that re-
Table 1. Characteristics and microbiologic findings for patients with positive ELISA hantavirus serologies.

<table>
<thead>
<tr>
<th>ELISA value, patient, age, sex</th>
<th>Pneumonia cause</th>
<th>Specific hantavirus antigen(s)</th>
<th>IgG OD values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly reactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D75,* 28M</td>
<td>Cryptococcus neoformans</td>
<td>HTN, SEO, PUU</td>
<td>2.28, 0.60, 0.29</td>
</tr>
<tr>
<td>Moderately reactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M40,* 32M</td>
<td>Pneumocystis carinii</td>
<td>HTN, SEO</td>
<td>0.80, 0.31</td>
</tr>
<tr>
<td>P32, 72,F</td>
<td>Unknown</td>
<td>HTN, SEO</td>
<td>0.73, 0.39</td>
</tr>
<tr>
<td>Marginally reactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A43, 52,M</td>
<td>Unknown</td>
<td>HTN</td>
<td>0.22</td>
</tr>
<tr>
<td>D63, 45,M</td>
<td>Streptococcus pneumonia</td>
<td>PUU</td>
<td>0.35</td>
</tr>
<tr>
<td>D32, 72,F</td>
<td>Unknown</td>
<td>PUU</td>
<td>0.25</td>
</tr>
<tr>
<td>M38, 23,F</td>
<td>Staphylococcus aureus</td>
<td>PUU</td>
<td>0.28</td>
</tr>
<tr>
<td>P15, 74,M</td>
<td>Unknown</td>
<td>SEO</td>
<td>0.38</td>
</tr>
</tbody>
</table>

NOTE. M, male; F, female; HTN, prototype Hantaan, PUU, Puumula; SEO, Seoul; OD, optical density.
* Human immunodeficiency virus—positive.

Imported for patients with kidney disease. Reactive serologies to PUU antigens may reflect cross-reactivity with other hantaviruses, as PUU is not known to circulate in North America [6].

We believe our study was the first to look at serologic prevalence of hantaviruses among patients hospitalized with community-acquired pneumonia. We found no evidence of acute hantavirus infection; therefore, HPS is an uncommon cause of community-acquired pneumonia within our geographic area. Further studies will be required to determine whether hantavirus pulmonary infection contributes mostly to sporadic disease or if infection is endemic in some communities.

References