The Epidemiology of Parainfluenza Virus Infection in Lung Transplant Recipients

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Human parainfluenza virus (HPIV) is a common cause of seasonal respiratory tract infections. However, little is known about the clinical presentation and impact of HPIV infections in lung transplant recipients. We reviewed HPIV infections at the University of Pittsburgh Medical Center. From January 1990 through May 2000, 32 cases of HPIV infection were identified. HPIV infection was found in 24 lung transplant recipients (75%), all of whom were included in the study group. Diagnosis was established at a median of 2.1 years after transplantation (range, 0.6–5 years). Presenting symptoms included cough (17 patients), shortness of breath (16), and temperature elevation (4). Respiratory failure occurred in 5 patients (21%). The HPIV serotypes were HPIV-1 (7 patients), HPIV-2 (2), and HPIV-3 (15 [63%]). Twenty-two patients underwent transbronchial biopsy, and 18 (82%) showed signs of acute allograft rejection. Seven patients (32%) subsequently were found to have bronchiolitis obliterans.

Lung transplantation has evolved into an accepted and effective treatment for patients with end-stage pulmonary disease. However, the rate of respiratory tract infections among these patients exceeds that among other solid-organ transplant recipients, secondary to altered mucociliary clearance, impact of airway instrumentation, bronchial anastomotic obstruction, and disruption of the lymphatics at the time of surgery [1]. Although bacteria are the most common pathogen isolated, there is evidence that respiratory viruses play an important role in the etiology of infections in lung transplant recipients [2]. One explanation for underestimation of the presence of respiratory viruses in adult lung transplant recipients may be the insensitive methods used for their detection. Most studies have relied on antibody detection that uses CF [3].

Human parainfluenza virus (HPIV) is an enveloped member of the Paramyxoviridae family and is a major cause of respiratory tract infections in children. The spectrum of disease due to HPIV ranges from a mild cold to croup, bronchiolitis, and viral pneumonia. In the adult population, these infections are usually mild and restricted to the upper respiratory tract [4]. HPIV has been reported to cause severe lower respiratory tract disease in patients after bone marrow transplantation [5, 6]. However, data are limited regarding HPIV infections in lung transplant recipients. The purpose of the present study was to investigate the epidemiological and clinical features and the outcome of HPIV infections in lung transplant recipients.

**PATIENTS AND METHODS**

Identification of patients. Patients were selected from a retrospective search of the electronic information database at the University of Pittsburgh Medical Center. Recovery of HPIV from bronchoalveolar lavage...
(BAL) samples (from 23 patients) or transbronchial biopsy samples (from 1 patient) identified the study’s case patients.

BAL was performed on lung transplant patients who had evidence of new lower respiratory tract symptoms, such as shortness of breath, cough, or wheezing, with or without radiological changes. In addition, lung transplant recipients underwent BAL and transbronchial biopsy for surveillance purposes. These procedures were performed quarterly during the first year after transplantation, every 4 months during the second year, and every 6 months during the third year.

Definitions. The “date of diagnosis of HPIV infection” was defined as the date that the specimen yielding HPIV was obtained. “Viral pneumonia” was defined by a new onset or exacerbation of shortness of breath, cough, and fever in association with the presence of a new infiltrate on a chest radiograph and the absence of bacterial pathogen isolation. “Concurrent bacterial pneumonia” was defined by a purulent sputum or BAL specimen (>25 neutrophils per low-power field) plus isolation of a bacterial pathogen together with a respiratory virus. “Respiratory failure” was defined by hypoxemia (Po2 < 60 mm Hg) that required the patient to undergo mechanical ventilation. The diagnosis of acute lung allograft rejection was based on findings from biopsy samples. Acute rejection and bronchiolitis obliterans (OB) were defined in accordance with Lung Rejection Study Group standards [7].

Microbiological analysis. All BAL fluid specimens were obtained by bronchoscopy performed using standard protocols. These samples underwent routine bacterial, fungal, and viral cultures. Silver staining was usually performed to rule out *Pneumocystis carinii* pneumonia. Before 1999, the respiratory viruses were identified by use of conventional viral culture looking for cytopathic effect or hemadsorption. During the last 2 years of the study, specimens used for the detection of respiratory viruses were inoculated into shell vials that contained a mixture of susceptible cell culture lines, including mink lung cells and human adenocarcinoma cells A-549 (Diagnostic Hybrids). The shell vials were incubated and were stained at 24 h and 72 h by use of indirect fluorescent antibody staining technique with the Bartels Viral Respiratory Screening and Identification Kit (Bartels), according to the manufacturer’s protocol. Cells from positive shell vials were scraped and were tested with antibodies for specific respiratory viruses.

Medical records. The hospital’s electronic information database, the Medical Archival Retrieval System, was reviewed to identify all medical records of lung transplant recipients from whom HPIV was isolated. The medical records were reviewed to obtain data on patient age, type of lung transplantation, underlying disease, date that the virus was recovered, presence of respiratory symptoms (cough or shortness of breath) and fever, need for mechanical ventilation, concurrent infections, radiographic findings, pathologic evaluation of the transplanted organ, degree of immunosuppression, antiviral therapy used, and outcome. The primary cause of death was obtained from the discharge summary or the autopsy report.

RESULTS

From January 1990 through May 2000, 32 cases of HPIV infection were diagnosed at the University of Pittsburgh Medical Center. The study group was composed of the 24 lung transplant recipients (5.3% of 454 lung transplant recipients) who were found to have HPIV infection.

Epidemiological findings and clinical features. The patients included 18 recipients of a single-lung transplant and 6 recipients of a double-lung transplant. The median time to diagnosis of HPIV infection was 2.1 years after lung transplantation (range, 0.6–5 years after transplantation). Twenty (83%) of 24 cases occurred at >1 year after transplantation. The patients were 14 women and 10 men. Symptoms, which were reported for 23 patients, lasted for a median of 11 days (range, 1–21 days) before patients were admitted to the hospital. These symptoms included cough (in 17 patients), shortness of breath (16 patients), and temperature elevation (>38°C; 4 patients). Table 1 summarizes the clinical features of the case patients. Chest x-ray films showed infiltrates in 7 patients (29%). Maintenance immunosuppression therapy after lung transplantation consisted of either tacrolimus/prednisone (for 18 patients) or cyclosporine A/prednisone (for 6 patients), in addition to azathioprine or mycophenolate mofetil (for all 24 patients).

Viral isolates were recovered from BAL specimens (from 23 patients) and lung biopsy specimens (from 1 patient). HPIV serotype identification revealed HPIV-1 in 7 patients, HPIV-2 in 2, and HPIV-3 in 15 (63%). The distribution of cases, by serotype, for each month of the year is shown in figure 1.

Table 1. Clinical features of adult lung transplant recipients with human parainfluenza virus (HPIV) infection.

<table>
<thead>
<tr>
<th>Symptom or sign</th>
<th>HPIV-1 (n = 7)</th>
<th>HPIV-2 (n = 2)</th>
<th>HPIV-3 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnea</td>
<td>3 (43)</td>
<td>0 (0)</td>
<td>13 (87)</td>
</tr>
<tr>
<td>Cough</td>
<td>4 (57)</td>
<td>2 (100)</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Temperature &gt;38°C</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>3 (43)</td>
<td>0 (0)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Lower respiratory tract infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral pneumonia</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Concurrent bacterial pneumonia</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Association with rejection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute allograft rejection</td>
<td>4/5 (80)</td>
<td>2 (100)</td>
<td>12 (80)</td>
</tr>
<tr>
<td>Bronchiolitis obliterans</td>
<td>2/5 (40)</td>
<td>1 (50)</td>
<td>4 (27)</td>
</tr>
</tbody>
</table>
Although the majority of the cases (58%) occurred during the summer months, HPIV infections were also observed throughout the year. Viral pneumonia was diagnosed in 4 patients, and secondary bacterial pneumonia occurred in 3 patients. Bacterial pathogens isolated by culture were *Pseudomonas aeruginosa* (2 patients) and *Staphylococcus aureus* (1 patient). Table 1 shows the number of lower respiratory tract infections among the patients, according to 3 HPIV serotypes. Respiratory failure that required patients to undergo mechanical ventilation occurred in 5 of the study patients (21%). None of the study patients received antiviral therapy directed at HPIV. The overall mortality rate was 8% (2 of 24 patients died). These 2 deaths were associated, in time, with recovery of HPIV. The virus may have contributed to death of the patients. One patient died with multisystem failure and herpes simplex pneumonitis. A second patient developed staphylococcal sepsis secondary to pneumonia.

**Lung allograft complications.** Transbronchial biopsy was performed at the time of diagnosis of HPIV infection in 22 patients, and biopsy specimens from 18 of these patients (82%) showed evidence of acute allograft rejection (table 1). Pathologic follow-up surveillance data obtained during the 18 months after diagnosis of HPIV infection revealed that 32% of lung transplant recipients (7 of 22 recipients) developed active OB. The median time from HPIV infection to development of OB was 6 months (range, 1–14 months). The number of OB cases, by HPIV type, is shown in table 1. There were no significant differences in the number of cases of acute allograft rejection or OB among patients with the 3 HPIV serotypes.

**DISCUSSION**

HPIV is a well-known respiratory pathogen in infants and children. The spectrum of disease due to HPIV ranges from a mild cold to croup, bronchiolitis, and viral pneumonia. In adults, these infections are usually mild and are restricted to the upper respiratory tract [4]. However, recent studies of the viral etiology of lower respiratory tract infections have found that HPIV was the cause in 2.5%–11.5% of hospitalized adult patients during the different seasons [8, 9]. Of more importance, these studies indicate that HPIV is an important pathogen in all age groups. Severe HPIV infections have been reported in patients who have undergone bone marrow transplantation [5, 6]. In these patients, HPIV infection has been associated with increased rates of morbidity and mortality. Indeed, 44% of HPIV infections in bone marrow transplant recipients have been associated with pneumonia, and the reported mortality rate has been 37%. However, limited data exist regarding these infections in solid-organ transplant recipients—in particular, lung transplant patients.

In a study by Wendt et al. [10], 10 cases of HPIV infection in lung transplant recipients were described. Infections occurred during all seasons. The serotypes identified were HPIV-3 (in 8 patients) and HPIV-1 (in 2 patients). The predominant clinical signs and symptoms included fever, cough, wheezing, and coryza. One of 10 patients developed acute respiratory failure that required mechanical ventilation, which was associated with concurrent *Pseudomonas* pneumonia. No pathologic findings regarding the lung allograft were given either at the time of or immediately after diagnosis of HPIV infection. However, a significant decrease in spirometry measurements occurred in 5 patients, with decreases in forced expiratory volume in 1 s ranging from 13% to 58% (median decrease, 21%). No direct mortality secondary to HPIV infection occurred in this study. A second report [11] of 10 lung transplant recipients with community-acquired respiratory viral infections described only 2 patients with HPIV infection. Clinical signs and symptoms included dyspnea, cough, and wheezing. No complications were observed in these 2 patients, and both survived the viral infection.

In contrast with findings from the aforementioned reports, our findings indicate that HPIV infections cause significant morbidity and may be a factor contributing to the poor prognosis of lung transplant recipients. Several factors may predispose lung transplant recipients to HPIV infections. In addition to the known factors related to chronic immunosuppression and mechanical factors in the transplanted lung, the most important component appears to be the mucosal immunity in the respiratory tract, a factor also altered in lung transplant recipients [12]. The bronchi-associated lymphoid tissue in the lung plays an important role in the defense against pulmonary infections. It has been shown [12–17] that damage to bronchi-associated lymphoid tissue hampers the ability to clear viruses from the lung. Furthermore, a study of humans after experimental HPIV-1 challenge has indicated that reisolation of virus was inversely correlated with the detection of local neutralizing
antibody in respiratory secretions but not with the detection of antibody in serum samples [13].

Although respiratory viral infections have been described over a wide range of time after transplantation [14], the majority of HPIV infections in lung transplant recipients in our study occurred 1 year after transplantation. Different mechanisms may account for early versus late posttransplant respiratory viral infections in lung transplant patients. Early infections may reflect nosocomial transmission or reactivation of the virus(es), as is the case for adenoviruses and herpesviruses (e.g., cytomegalovirus and Epstein-Barr virus). In contrast, late posttransplant viral infections, such as HPIV infection, are more likely to be community acquired. This may be important, because, given that survival of lung transplant recipients is becoming longer, these viral infections may become recognized as an important factor during the late posttransplant period. In addition, in our cohort of adult lung transplant recipients, HPIV infections were more prevalent during the spring and summer months and were mostly caused by HPIV-3 (63%).

This is in contrast with observations from studies done in the general population that indicated an even distribution of HPIV serotypes all year long [8, 9].

HPIV seems to be capable of activating immunological mechanisms in the transplanted lung, as was evidenced by the high rate of association with acute cellular rejection in our patients. In vitro experiments with respiratory syncytial virus (of the Paramyxovirus genus) have shown that human respiratory epithelial cells and different leukocyte populations elaborate biologically active cytokines, including IL-6, IL-8, and TNF-α, when exposed to this virus [15]. Furthermore, the idea that HPIV infections may contribute to the development of airway damage in lung allograft has been examined in animal models of lung transplantation [16, 17]. After infection with Sendai virus, a murine virus closely related to HPIV-1, lymphocytes started to infiltrate the submucosa of both bronchioles and large airways of the allografted transplanted lung in higher numbers, compared with the nontransplanted lung. Of more importance, these studies have shown that the viral infection was associated with the subsequent development of OB in these animals after lung transplantation. The histological changes were similar in nature to those usually seen in lung transplant recipients [17]. Our findings also point out that HPIV infection in the lung allograft may lead to OB, as was indicated by the significant number of patients who developed OB after HPIV infection (32%). This is a very important finding, because OB, which has an incidence of 41%–63% at 5 years after transplantation, is considered to be the single most important limitation to long-term survival after lung transplantation [18].

Although the etiology of OB after lung transplantation in humans is still unknown, some factors are believed to induce epithelial injury and lead to the development of OB. Among these factors, cytomegalovirus infection, chronic rejection, and biopsy findings of lymphocytic bronchiolitis have been associated with an increased risk of OB [18]. HPIV infection may have a direct cytopathic effect on the epithelium of the airways and may contribute to the development of OB in lung transplant recipients. Thus, treatment strategies against HPIV in this patient population are important. However, at the present time, treatment options for HPIV are limited. Use of aerosolized ribavirin therapy has been reported, in anecdotal cases, after heart and bone marrow transplantation [19, 20], but prospective studies of such therapy in lung transplant recipients have not been conducted. Therefore, in view of the significant morbidity associated with these infections in lung transplant recipients, we recommend that efforts to prevent these infections be focused on strategies directed toward preventing these viruses. Two live attenuated, intranasally administered HPIV-3 vaccines are in development [21]. Results of phase I trials of these vaccines in adults, children, and infants suggest that they are safe and immunogenic [22, 23]. However, there is a need for further studies to determine (1) whether these vaccines can enable patients to mount an immune response and (2) what degree of protection or amelioration of illness is provided by such vaccines.

In conclusion, our findings indicate that HPIV infections are associated with significant morbidity and may have long-term implications in lung transplant recipients. Therefore, development of vaccines and new antiviral agents against HPIV are important for prevention.

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References