Detection of Rhinovirus in Sinus Brushings of Patients with Acute Community-Acquired Sinusitis by Reverse Transcription-PCR

ANNE PITKÄRANTA,1,2 EURICO ARRUDA,1,3 HENRIK MALMBERG,2 AND FREDERICK G. HAYDEN1*

Departments of Internal Medicine and Pathology, University of Virginia, Charlottesville, Virginia 22908; Department of Otolaryngology, Helsinki University Hospital, FIN-00290 Helsinki, Finland; and Faculty of Medicine, University of Sao Paulo, Ribeirão Preto, SP, Brazil 14049-9003

Received 21 January 1997/Returned for modification 18 March 1997/Accepted 4 April 1997

Of 20 adults with acute community-acquired sinusitis (ACAS), rhinovirus was detected in specimens from 10 (50%) patients, including maxillary aspirates from 8 (40%) patients and nasal swabs from 9 (45%) patients, by reverse transcription-PCR (RT-PCR). Human coronavirus was detected by RT-PCR in nasal swabs from 3 of 20 patients but in no sinus secretions. These findings suggest that rhinovirus is an important cause of ACAS and that viral invasion of the sinus cavity itself may be a common event during the disease.

Most cases of acute community-acquired bacterial sinusitis are believed to be secondary to a preceding viral upper-respiratory-tract infection. This view has been supported by earlier studies in which respiratory viruses were isolated from sinus aspirates of patients with acute community-acquired sinusitis (ACAS) (5, 9). More recently, sinus abnormalities were detected by magnetic resonance imaging in volunteers with experimental rhinovirus colds (17) and by computed tomography in patients with natural rhinovirus colds (7). An important question in understanding the pathogenesis of viral sinusitis is whether direct viral invasion of the sinus cavity or inflammatory events in adjacent areas in the nose, such as the osteeomatal complex, are responsible for the abnormalities in the sinus cavity.

The agents most frequently implicated as causes of viral upper-respiratory-tract infections are human rhinoviruses (HRV) and human coronaviruses (HCV) (6, 15). The advent of reverse transcription-PCR (RT-PCR) has enabled the detection of picornavirus RNA directly in clinical samples with higher levels of sensitivity (2, 8, 11) than viral isolation in cell culture (2, 11). In the present study, the maxillary sinus of adult patients with ACAS was punctured, and specimens obtained with a cytologic brush were tested for HRV RNA and HCV RNA by RT-PCR.

MATERIALS AND METHODS

Patients. Twenty adult patients with diagnoses of ACAS based on clinical findings were studied between May and July 1996 in the Department of Otolaryngology of the University Hospital of Helsinki. All patients had purulent rhinorrhea and nasal obstruction, and most had facial pain. The patients had not been treated with antibiotics, and the duration of their symptoms was less than 2 weeks. Sinus X rays were obtained for 18 of the patients. Ten patients had an air-fluid level in the maxillary sinus, and six of these were culture positive for bacteria (Table 1). Eight patients had mucosal thickening of >8 mm, and two of these had positive bacterial cultures. There were 11 females (ages, 23 to 65 years) and 9 males (ages, 29 to 60 years); the median age was 38 years. Patients for whom frontal sinusitis was suspected were excluded, as were patients with his-
RESULTS

Picornavirus RNA. Picornavirus RNA was detected by RT-PCR in the maxillary sinus brushings from 8 of 20 patients with ACAS (40%) and in the nasal swab samples from 9 of 20 patients (45%) (Table 1). Since the assay used is picornavirus specific (2), we tested the picornavirus-positive samples with an enterovirus-specific probe and found that none of the RT-PCR picornavirus products hybridized with the enterovirus probe. For seven patients (35% of the total), both maxillary sinus and nasal samples were positive for HRV. Overall, HRV was detected in 10 of 20 patients (50%). By culturing of nasal swabs and maxillary brush samples, HRV was detected in both sample types from two patients and in only the maxillary sinus sample from one patient. All HRV culture-positive samples were also RT-PCR positive. The culture-positive cases all presented within 8 days of symptom onset, whereas the majority (five of eight) of those with negative cultures but positive RT-PCR for HRV RNA presented later (Table 1).

HCV RNA. None of the sinus samples were positive for HCV 229E or OC43. Three nasal swab samples were positive for HCV OC43, and two of these patients had positive bacterial cultures (Table 1). All three of the HCV-positive patients were negative for rhinovirus infection by RT-PCR and culture.

Bacterial culture. Bacterial cultures were positive in 10 (50%) of 20 patients. RT-PCR of sinus brushings was positive for HRV RNA in 5 of 10 samples negative for bacteria and in 3 of 10 samples positive for bacteria. Of 10 HRV-infected patients, 5 had positive bacterial cultures of antral washings. In five samples (25% of the cases), both the RT-PCR and bacterial cultures were negative, and in three samples (15% of the cases), both the RT-PCR and bacterial cultures were positive. The two samples from middle-ear effusions were negative by bacterial culture and by RT-PCR for HRV and HCV RNA.

DISCUSSION

In the present study, we found that HRV can be detected by culture in 15% of the brush samples obtained by puncture of the maxillary sinuses in adults with ACAS and can be detected by RT-PCR in 40% of these samples. Our culture results con-
firm earlier studies of ACAS which reported recovery of HRV in maxillary secretions aspirated from 8 and 7% of patients (5, 9). However, the use of RT-PCR enhanced detection of HRV infection, particularly in samples that were obtained during the 2nd week after the onset of illness. These results are consistent with previous studies of persons with acute respiratory illness which found a high prevalence of positive RT-PCR results for picornavirus in association with negative cultures (11). While our findings may have resulted from viral replication in the sinus, they may also have been caused by the presence in the sinus of virions or viral RNA produced by replication elsewhere in the upper-respiratory tract epithelium and introduced during coughing or sneezing, or potentially even by HRV RNA introduced to the sinus at the time of puncture. Direct demonstration of viral replication in situ would provide definitive evidence of productive HRV infection in the sinus mucosa. While not proving direct viral causation for acute sinusitis during the course of HRV infection, the observation that HRV was detected in the sputum in 40% of 20 patients with acute sinusitis and overall in 50% of these patients strongly suggests the importance of HRV infection in predisposing to ACAS.

These samples were also tested for another important cause of upper-respiratory tract infections, HCV (15). Although the coronavirus OC43 was found in three nasal swab samples, none of the sinus samples were positive for HCV 229E or OC43. The 15% coronavirus infection rate is consistent with the findings of prior studies addressing the contributions of coronaviruses to colds (15). Of note, HCVs typically cause infections during the winter months, and our patients were studied during the late spring and early summer. Overall, HCV or HRV infection was documented in 65% of these cases. Other respiratory viruses were not sought in the context of these studies, in part because of the limited sample volume and because of patient sampling after the end of the influenza and respiratory syncitial virus seasons. However, the findings indicate that a preceding respiratory viral infection is associated with the majority of ACAS cases.

These findings support the concept that viruses predispose to bacterial infection of the maxillary sinuses. Although the bacterial causes of ACAS have been extensively studied, few prospective studies have assessed the role of viruses in the etiology of these infections (5, 9). In a study of adults experimentally infected with HRV, changes in the paranasal sinuses were observed by magnetic resonance imaging in 33% of volunteers (17). In natural colds, sinon abnormalities are observed by computed tomography in over 85% of patients (7). These changes resolved spontaneously after several weeks in most patients. These studies indicate that an upper-respiratory viral infection may induce paranasal sinus effusions or mucosal thickening indistinguishable from that observed in acute bacterial sinusitis. We found direct evidence of coinfection by HRV and bacteria in three (15%) of our patients. Although the mechanisms are not defined, earlier studies found that rhinovirus-bacterium coinfection was an indicator of a poor prognosis of acute otitis media during antibiotic treatment (1, 16). While the same could be true with acute sinusitis as well, the limited number of patients we studied does not allow conclusions in this regard. However, the importance of HRV infection in causing sinus disease alone and in conjunction with bacteria raises the possibility that early treatment of HRV colds with specific antirhinovirus agents could have an impact on the risk of development of sinusitis.

ACKNOWLEDGMENTS

This work was in part supported by grants from the Medical Research Council of the Academy of Finland and the Paulo Foundation, Helsinki, Finland.

The authors thank Jack M. Gwaltney, Jr., for his critical review of the manuscript.

REFERENCES