Objective: To study bacteria and viruses in maxillary sinuses of patients with primary hypogammaglobulinemia receiving immunoglobulin therapy.

Design: Prospective cross-sectional study during 6 months.

Setting: Tertiary care university hospital.

Patients: Seventeen patients with primary hypogammaglobulinemia (10 males and 7 females; mean age, 39 years [age range, 11-71 years]). Sixteen patients had common variable immunodeficiency, and 1 patient had X-linked agammaglobulinemia.

Main Outcome Measures: Magnetic resonance imaging and x-ray imaging of paranasal sinuses when patients did not have signs of acute infection and reevaluation 6 months later. Maxillary sinus aspiration and lavage were performed at a follow-up visit. Sinus fluid analysis for bacteria and viruses was performed by culture and by polymerase chain reaction. A questionnaire on symptoms related to sinusitis was administered during the follow-up period.

Results: Among 17 patients, 9 (53%) had radiologically defined sinusitis without subjective symptoms at study enrollment. At reevaluation 6 months later, radiological findings remained unchanged in two thirds of the patients. Among 15 patients, bacteria were found in sinus lavage samples from 13 patients, and viruses were found in samples from 7 patients. Eight patients had 2 pathogens or more on bacterial culture. Rhinovirus was identified from sinus lavage samples in 5 patients (33%), enterovirus in 3 patients (20%), and respiratory syncytial virus in 1 patient (7%). Pathogenic bacteria were found in maxillary sinuses of all patients who tested positive for rhinovirus and enterovirus. No fungi were found. During the follow-up period, 6 patients reported mucopurulent drainage.

Conclusions: Bacteria and viruses were commonly found in maxillary sinuses of patients with primary hypogammaglobulinemia. Yearly evaluation by an ear, nose, and throat surgeon is recommended.

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Patients with primary hypogammaglobulinemia are prone to recurrent bacterial infections of the respiratory tract. They often have experienced recurrent infections for years before diagnosis. Many patients already have pulmonary complications, such as bronchiectasis, at the time of diagnosis. Most patients have a history of chronic sinusitis and successive sinus surgical procedures before recognition of their immunodeficiency.1,2 Long-term or recurrent sinusitis has been documented in up to 60% of patients with humoral immunodeficiency.2 Adequate immunoglobulin therapy with trough serum IgG concentrations exceeding 5 g/L usually prevents bacterial infections, but if tissue damage is already present as a result of long-term infections, the efficacy of immunoglobulin therapy may be poor.2 A predisposing factor to sinusitis in patients with primary hypogammaglobulinemia is the expression of hypogammaglobulinemia in the nasal mucosa. No immunoglobulin-producing plasma cells or secretory IgA or IgM exists in the nasal mucosa.9 Not all patients with hypogammaglobulinemia experience sinusitis, which indicates that other mechanisms such as mucociliary transport, innate immunity, and cell-mediated immunity contribute to mucosal defense.

We previously studied the occurrence of bacteria and viruses in the lower respi-
ratory tract of patients with primary hypogammaglobulinemia and found that the patients harbor bacteria and viruses without any clinical symptoms or signs of acute infection. In this study, we investigated the presence of bacteria, fungi, and viruses in maxillary sinuses of patients with primary hypogammaglobulinemia.

**METHODS**

The study included 17 patients fulfilling the criteria of primary hypogammaglobulinemia1 (16 patients with common variable immunodeficiency and 1 patient with X-linked agammaglobulinemia; 10 males and 7 females; mean age, 39 years [age range, 11-71 years]) at Turku University Hospital, Turku, Finland, at their yearly follow-up visit. Common variable immunodeficiency was characterized by decreased serum immunoglobulin levels (>2 SDs below the age-adjusted mean), defective in vitro antibody formation, and exclusion of other known causes of humoral immune defects. The diagnosis of X-linked agammaglobulinemia was based on male sex, early onset, low serum immunoglobulin concentrations (IgG, <2 g/L), and a lack of circulating B lymphocytes in the peripheral blood. All were receiving regular therapy, 15 with intravenous immunoglobulin therapy was increased in these patients. The 2 patients receiving intramuscular treatment were switched to intravenous treatment. None of the patients were receiving prophylactic antibiotic therapy at the time of the study. All patients were free of acute infections at the time of the first visit, nor did they have any symptoms of sinusitis. A history of recurrent sinusitis before the diagnosis of hypogammaglobulinemia was obtained from 8 of 17 patients. Sinus surgery had been performed in 6 patients, endoscopic antrostomy medialis by fiberoptic surgery in 4 patients, and Caldwell-Luc operation in 3 patients. Eleven patients had bronchiectasis.

Informed consent was obtained from the patients or their parents. The study was approved by the Joint Commission on Ethics of the Turku University Hospital and the University of Turku.

**RADIOGRAPHY AND MAGNETIC RESONANCE IMAGING**

Plain radiography of the paranasal sinuses (occipitomental view) was carried out at the initial visit. It was repeated 6 months later. Magnetic resonance (MR) imaging (T2-weighted sequences) using a 0.23-T MR imaging system (Philips Medical Systems, MR Technologies Finland, Vantaa, Finland) was performed twice in 17 patients, at the first visit and 6 months later. The findings in maxillary sinuses were categorized as normal, mucosal thickening of 5 mm or less, mucosal thickening of more than 5 mm, total opacity, air-fluid level, and a cyst or polyp. Patients with MR imaging showing mucosal thickening of more than 5 mm, total opacity, or air-fluid level were classified as having sinusitis.

**FOLLOW-UP**

The patients completed a questionnaire about symptoms related to sinusitis during the 6-month follow-up period. Possible symptoms included cough, fever, watery rhinitis, purulent rhinitis, nasal obstruction, sputum production, and facial pain, pressure, or fullness.

An ear, nose, and throat surgeon (J.S.) examined the patients at the 6-month follow-up visit. Samples from maxillary sinuses were collected from 15 patients. Both maxillary antra were punctured in 5 patients with sinusitis evident on sinus radiography and in 4 patients consenting to puncture without any radiological changes suggesting sinusitis. A sinus secretion sample was aspirated from the maxillary sinus using a sterile puncture needle. In 6 patients with a history of inferior antrum or middle meatal osteotomy, the specimen was aspirated from the sinus using the antrostomy opening, guided by nasal endoscopy. The aspiration sample was drawn into a 10-mL syringe through a 1.5-mm puncture needle. If the aspirate was negative, 1 to 2 mL of sterile isotonic sodium chloride solution was injected into the antrum and then aspirated again. The syringes were carefully plugged, and samples were delivered within 15 minutes to the laboratories.

**CULTURES**

Conventional methods were used for detection of aerobic and anaerobic pathogens. *Mycoplasma pneumoniae* was cultured using a commercially available kit (Pneumofast; International Mycoplasma, Signes, France).

Viral cultures were carried out according to standard roller tube methods in LLC-Mk2, HeLa Ohio, A549 cells and in human foreskin fibroblasts. The cultures were observed for cytopathogenic effects during 2 weeks. Positive cultures were tested using a time-resolved fluoroenzymoassay for adenovirus, para-influenza virus types 1, 2, and 3 and using respiratory syncytial virus antigens. Rhinoviruses were identified by their acid lability. In addition, a plate culture method with immunoperoxidase staining was used for isolation of cytomegalovirus, herpes simplex virus types 1 and 2, influenza A and B viruses, and fungi. Aspergillus antigen was detected using conventional methods.

**ISOLATION OF RNA AND DNA**

Total nucleic acids from sinus fluid were isolated using proteinase-K (0.5 µg/µL) and sodium dodecyl sulfate digestion (0.2%) at 37°C, followed by phenyl-chloroform extraction and ethanol precipitation. The pellets were dissolved in nuclease-free water, incubated at 56°C for 15 minutes, and stored at −70°C.

**POLYMERASE CHAIN REACTION**

Sinus fluid was analyzed using polymerase chain reaction (PCR) or reverse transcriptase–PCR for the following pathogens: adenovirus, *Chlamydia pneumoniae*, enterovirus and rhinovirus, *mycobacteria*, *M pneumoniae*, and *Ureaplasma urealyticum*. From PCR products, 10 to 20 µL was analyzed in 1.5% to 2% agarose gels containing ethidium bromide (1 µg/mL) and was visualized and photographed under UV illumination. Nucleic acids from the gels were transferred onto nylon membranes (Genescreen Plus; DuPont, Boston, Mass), and the specificity of the signals was confirmed by hybridization with digoxigenin–deoxyuridine triphosphate–labeled (Boehringer Mannheim, Mannheim, Germany) specific probes. The digoxigenin-labeled probes were detected using alkaline phosphatase–conjugated antidigoxigenin Fab fragments and chemiluminescent substrate for alkaline phosphatase (Boehringer Mannheim) according to the manufacturer's instructions.
RESULTS

RADIOLOGICAL FINDINGS

Radiographs of the paranasal sinuses and MR imaging were performed in 17 patients at their usual yearly follow-up visit. None of the patients had symptoms of acute sinusitis at the time of radiological imaging or was receiving prophylactic antibiotic treatment.

Magnetic resonance imaging of the paranasal sinuses showed sinusitis in 9 patients, polyposis in 2 patients, and slight mucosal thickening of the sinus lining in 2 patients. Magnetic resonance imaging was unremarkable in 4 patients.

Radiological findings were concordant with MR imaging results, except for polyposis in 2 patients. Patients with radiological signs of acute sinusitis were not administered antimicrobials because they had no symptoms.

Six months later, radiological studies were repeated in 16 patients. The findings remained unchanged in 10 patients, had improved in 4 patients, and were impaired in 2 patients. Two patients had unremarkable MR imaging and radiological findings at the initial visit and at the 6-month follow-up visit. The radiological changes due to acute sinusitis had cleared in 2 patients. The pathological findings were unchanged in 8 patients: 6 still had changes due to sinusitis, and 2 had polyposis. The acute sinusitis changes had diminished in 2 patients, but mucosal thickening remained. One patient with previously unremarkable imaging results had mucosal thickening on MR imaging, and mucosal thickening in 1 patient had developed to the air-fluid level (Table).

MICROBIOLOGICAL FINDINGS

Bacterial growth was found in samples from 13 patients. Haemophilus influenzae grew in the samples of 4 patients. Eight patients had 2 pathogens or more in their bacterial culture (Table).

Reverse transcriptase–PCR showed rhinovirus in the sinus fluid from 5 (33%) of 15 patients and enterovirus in 3 patients (20%). One patient was positive for respiratory syncytial virus. Two patients were positive for a dual viral infection with rhinovirus and enterovirus. Polymerase chain reaction test results for adenovirus, mycobacterium, M pneumoniae, C pneumoniae, and U urealyticum were all negative. All patients with viral findings also had bacterial growth in their sinus fluid. Fungi were not found (Table).

SYMPTOMS

During the 6-month follow-up, 6 patients had mucopurulent rhinitis, and 5 of them also had cough. Only 1 patient also had alteration in taste and pain in the cheeks. None had fever. None had received antibiotic treatment.

COMMENT

The present study shows that sinusitis is common in patients with primary hypogammaglobulinemia. Despite immunoglobulin therapy, more than half of the patients had radiologically defined sinusitis without remarkable symptoms, and the radiological findings remained unchanged in most patients when reevaluated 6 months later. The interpretation of paranasal sinus MR imaging findings should be considered with care because no healthy control subjects were included in the study. In addition, the interpretation of MR imaging may be difficult after Caldwell-Luc operation. We found total opacity in 3 patients who had undergone Caldwell-Luc operation. These patients had pathogenic bacteria (eg, H influenzae and Moraxella catarrhalis) in maxillary sinus lavage fluid; furthermore, 2 patients had a concomitant virus strongly suggesting infection in the sinus. Consensus definitions of chronic sinusitis include 2 or more of the following symptoms: mucopurulent drainage, nasal obstruction, or facial pain, pressure, or fullness for longer than 12 weeks. By these symptom definitions, only 1 patient fulfilled the criteria for chronic sinusitis.

We found bacteria in the sinuses of most patients when they had no symptoms or signs of acute illness. Haemophilus influenzae and M catarrhalis were the most commonly detected pathogens. Staphylococcus epidermidis, Citrobacter species, Haemophilus alvei, Propionibacterium species, or Proteus species grew in more than half of the patients, but they were usually concomitant with pathogenic bacteria or occasionally concomitant with rhinovirus and enterovirus. Staphylococcus epidermidis may naturally be a contamination, especially if few bacteria are cultured, but its role as a causative agent of infection should be considered in a patient with immunodeficiency.

The occurrence of viruses in the sinuses was an interesting finding. Viruses were found in most patients with no symptoms of sinusitis. Rhinovirus was the most commonly detected virus. Enterovirus was found concomitantly with rhinovirus in 2 patients. Rhinovirus, enterovirus, or both were accompanied by bacteria. The presence of rhinovirus together with bacteria is well established in acute otitis media. In previous studies, rhinovirus, influenza virus, and parainfluenza virus were cultured from the sinus cavities of patients with acute sinusitis. It is well known that viruses pave the way for bacterial infection. Viruses predispose to mucosal inflammation and damage and to invasion of bacteria. Whether rhinovirus has any important role in the development of chronic sinusitis in these patients remains to be established. We did not collect samples from maxillary sinuses at the initial visit. Patients with primary hypogammaglobulinemia are prone to chronic enterovirus infections. However, their susceptibility to chronic rhinovirus infections has not been studied, to our knowledge.

It is not always easy to recognize sinusitis in patients with hypogammaglobulinemia because they often tend to ignore their sinusitis symptoms. In the present study, 3 patients with sinusitis denied having had any symptoms. However, it is important to detect the sinusitis of patients with hypogammaglobulinemia because long-term sinusitis may predispose to chronic pulmonary infection and eventually to pulmonary abnormalities. Most
patients of this study already had bronchiectasis. In a previous study among the same patient group, it was found that bacteria often occurred in the lower respiratory tract in patients without acute illness. Bacteria found in the lower respiratory tract may originate from maxillary sinusitis in chronic sinusitis. Amoxicillin–clavulanate potassium treatment was administered to patients when follow-up imaging showed sinusitis, although antimicrobial therapy is not usually recommended for the treatment of chronic sinusitis without acute infectious exacerbation. It is questionable whether antimicrobial treatment should already have been initiated after the first imaging study.

Patients with primary hypogammaglobulinemia receive regular immunoglobulin therapy. Trough serum IgG concentrations exceeding 5 g/L, preferably within age-adjusted reference values, prevent most breakthrough bacterial infections, and it is assumed that higher serum concentrations also result in higher IgG concentrations in the sinuses. Williams et al found that trough serum IgG concentrations exceeding 4 g/L did not eradicate the symptoms of sinusitis, but the investigators showed penetration of IgG into antral lavage fluid in some patients. In a crossover study, Eijkhout et al compared standard-dose immunoglobulin treatment with high-dose treatment. They found that doubling the standard immuno-

<table>
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<th>Patient No./Sex/Age, y</th>
<th>Sinus Surgery</th>
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<th>Symptoms</th>
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Abbreviations: C-L indicates Caldwell-Luc operation; FESS, functional endonasal sinus surgery.

*Receiving intramuscular therapy.
globulin dosage reduced the number and mean duration of infections but did not decrease the number of positive sputum cultures. In a study by Roifman et al,27 administration of 600 mg/kg of body weight per month resulted in radiological and subjective improvement of chronic sinusitis in 4 of 5 patients, suggesting that higher dosages may be required to eradicate bacteria from the sinuses. In the present study, two thirds of the patients with sinusitis had trough IgG levels exceeding 5 g/L, and one third had levels exceeding 6 g/L, demonstrating that adequate therapy alone was insufficient to clear the infection.

The management of sinusitis in patients with primary hypogammaglobulinemia is controversial. No results of randomized clinical trials are available. Buehring et al31 evaluated the efficacy of azithromycin, N-acetylcysteine, and topical intranasal corticosteroids against chronic sinusitis in patients with humoral immunodeficiency. Despite medication, there was no improvement in sinus inflammation or any significant decrease in the levels of pathogens and inflammatory cytokines. On the other hand, Rusconi et al33 successfully treated patients with hypogammaglobulinemia and chronic sinusitis using amoxicillin-clavulanate and clindamycin hydrochloride for 14 days, followed by isotonic sodium chloride solution nasal washes and intranasal beclomethasone dipropionate for 4 weeks.

CONCLUSIONS

Many patients with primary hypogammaglobulinemia harbor bacteria and viruses in their maxillary sinuses, suggesting that they have chronic maxillary sinus infection. This infection may predispose to lower respiratory tract infections and to bronchiectasis. We suggest a yearly examination by an ear, nose, and throat surgeon. Optimal immunoglobulin therapy is important in these patients, and proper antimicrobial treatment of bacterial sinusitis (although asymptomatic) should be considered.

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Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Kainulainen, Suonpää, Nikoskelainen, Svedström, Vuorinen, Meurman, and Ruuskanen. Acquisition of data: Kainulainen, Suonpää, and Svedström. Analysis and interpretation of data: Kainulainen, Suonpää, Svedström, Vuorinen, Meurman, and Ruuskanen. Drafting of the manuscript: Kainulainen. Critical revision of the manuscript for important intellectual content: Suonpää, Nikoskelainen, Svedström, Vuorinen, Meurman, and Ruuskanen. Administrative, technical, and material support: Kainulainen. Study supervision: Suonpää, Nikoskelainen, Svedström, Vuorinen, Meurman, and Ruuskanen.

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