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Microbiology of chronic hyperplastic sinusitis*

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SUMMARY

Background: *Patients with chronic hyperplastic sinusitis (CHS) form a heterogeneous group with similar symptoms and similar treatment despite of possible different mechanisms behind the disease. In the present study we focused on the microbiological findings in CHS and compared these results to the patient history in order to find out a possible explanation for the aetiology and chronicity of CHS.*

Methods: *In 30 patients the sinus mucus was collected under endoscopic sinus surgery. Samples from 20 healthy volunteers were collected by nasal lavage. Eosinophil staining, bacterial culturing and fungal staining and culturing were done. Histological samples were obtained from all patients.*

Results: *Bacterial cultures were positive in 93% of the patients compared to 70% in controls. Staphylococcus aureus and coagulase-negative Staphylococci were the two most common findings in both groups. A total of seven patients had positive fungal finding. The only fungal genus found was Aspergillus. In the control group no samples were positive for fungi.*

Conclusions: *Microbiological findings do not seem to explain the chronic course of CHS, but fungi may play some part in the pathophysiology of the disease. These results may be more a reflection of a change in the environment in the paranasal sinuses and a change in normal flora than the actual cause of CHS.*

Key words: chronic sinusitis, polyposis, fungi, bacteria, eosinophilia

INTRODUCTION

Chronic sinusitis, defined as sinusitis lasting longer than three months, is a growing problem in the USA and Europe, affecting about 15% of the population (Kaliner et al., 1997). The prevalence of the disease has increased by 50% from 1982 to 1993 in the USA (Collins, 1993) and a similar trend is seen also in the Finnish population (Suonpää and Savolainen, 1998). Approximately 20% of patients with chronic sinusitis have nasal polyposis, a condition termed chronic hyperplastic sinusitis (CHS) (Settipane, 1996). At least 50% of patients with CHS have associated asthma, typically of the non-atopic late-onset type, and 30% to 40% also have aspirin sensitivity (Slavin, 1988; Slavin, 1988; Settipane, 1996). Patients typically have a history of multiple operations.

Acute sinusitis may be perpetuated by factors predisposing to sinus ostial obstruction and infection. Disturbances in mucociliary transport and deficiencies in the immune system, especially abnormalities in production of IgA and IgG, make the patient susceptible to acute sinusitis (Hamilos, 2000). Similar factors may contribute to sinusitis chronicity, but in addition inflammatory mechanisms play a key role. In CHS the sinus mucosa contains eosinophils and IL-5-producing T lymphocytes: a type of inflammation seen also in the asthmatic bronchial mucosa (Schubert, 2001) and termed non-infectious or allergic inflammation. In allergic fungal sinusitis, one type of hyperplastic sinusitis, there is also mucus containing clusters of eosinophils (allergic mucin) (Hamilos, 2000). The exact pathophysiology behind these changes remains unknown.

The role of bacteria is well established in acute sinusitis, in which the most common organisms are *Streptococcus pneumoniae, Hemophilus influenzae* and in children *Moraxella catarrhalis* (Wald, 1998). The microbiology is less clear in

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chronic sinusitis. Some studies have underlined the increased role of *Staphylococcus aureus*, coagulase-negative *Staphylococci* and respiratory anaerobes but these bacteria are also part of the normal nasal flora (Savolainen et al., 1986; Jousimies-Somer et al., 1989). Even more controversial is the meaning of fungal findings in chronic sinusitis. Fungi can cause true invasive infections usually in an immunocompromised host. In non-invasive disease fungi are found within the sinus cavity without penetration of the mucosal barrier. The non-invasive variants of fungal infections include mycetoma or fungus ball and allergic (eosinophilic) fungal sinusitis (AFS). The criteria of AFS are chronic sinusitis, allergic mucin in some of the paranasal sinuses and the presence of fungi within mucin. Some authors include also atopy. Patients usually have nasal polyposis. Ponikau et al. (1999) found fungi in 96% of the patients with chronic sinusitis and 100% of healthy controls. In the same study 93% of patients met the criteria of allergic fungal sinusitis, which is usually considered to be quite rare. Braun et al. (2003) reported similar results using a similar specimen collection and culture technique as Ponikau and colleagues. In both studies the patients and the controls did not differ in terms of positive culture findings and fungal species discovered.

The aim of our study was to chart the medical history and to review the clinical and the microbiological findings as well as to examine the histology of sinus mucosa in patients operated on for chronic hyperplastic sinusitis. We wanted to pay special attention to the bacteria and fungi found and to compare these findings with the normal flora of the nasal cavity in healthy subjects. This type of study, which takes into account both bacteria and fungi in relation to the history of patients entering the study, has seldom been done. Furthermore, we also studied the full clinical spectrum of CHS and not only one manifestation of the disease.

MATERIALS AND METHODS

Patient data

Thirty patients undergoing a paranasal sinus operation due to chronic sinusitis were enrolled. All patients had a history of chronic sinusitis lasting longer than twelve weeks. They also had findings of polyposis and mucus in some of the paranasal sinuses in the preoperative CT-scan. Patient medical history including operations, asthma, allergy, aspirin intolerance, immunodeficiency and their family history of atopy were asked. Immunodeficiency was not tested in laboratory. Twelve patients were men aged 36-68 years (mean 47.3 y) and 18 were women aged 28-66 years (mean 48.4 y). Nineteen (63%) of them had had one or more sinus operation earlier, including polypectomy, antrostomy, endoscopic sinus surgery (ESS) or a Caldwell-Luc-operation. Moreover, two patients had had a (rhino)septoplasty. One male patient had had over 40 polypectomies. Seventeen patients (57%) had asthma and five of them (29%) were aspirin sensitive. These five patients were all operated on at least once previously. Four patients (13%) were immunocompromised having specific immunoglobulin deficiency. All four had had several operations. The patient details are shown in Table 1. Twenty healthy volunteers from the Department of Otorhinolaryngology without any history of nasal or sinus operations served as volunteer controls.

Specimen collection

The nasal lavage method earlier described by Hirvonen et al. (1999) was used for collecting samples for eosinophil staining from the controls and preoperatively from the patients. In the control group the same method was used to collect bacterial and fungal samples. In brief, each nostril was flushed through with 5 ml of phosphate buffered saline (PBS) using a 5 ml sterile syringe and a sterile butterfly cannula of about 2 cm in length. The cut end of the cannula was placed half inch inside the nostril. The patient closed the nostrils by pinching them firmly together and leaned forward. PBS was pushed back and forth twice and finally collected into the syringe. Any residual PBS remaining in the nostril was collected in pan placed underneath the nose and collected into the syringe.

Patients had also an intra-operative specimen collected. Mucus from the paranasal sinus was placed into an empty sample tube for fungal culture or in Transpocult tube for bacterial culture. A specimen for fungal staining was taken with a sterile cotton swab and placed directly onto a microscope slide. The slide was then air-dried. The biopsy for histological analysis was obtained from the same paranasal sinus as the mucus.

The control specimens were collected as follows: 1-2 ml of the lavage fluid was placed into an empty sample tube for fungal examinations. Bacterial culture was collected by dipping a sterile cotton swab soaked in activated charcoal in the fluid. It was placed in modified Stuart transport medium (Transpocult; Orion Diagnostica, Espoo, Finland) for transportation to the bacteriology laboratory.

Specimen handling

The standard clinical methods used in the diagnostic laboratories of Helsinki University Central Hospital to culture and identify both bacteria and fungi were used. The bacterial samples were inoculated onto the following media: chocolate agar for the isolation of aerobes; blood agar with colistin to select for *Streptococcus sp*; fastidious anaerobic agar for all anaerobes; blood agar with neomycin and vancomycin for *Bacteroides sp*; and thioglycollate broth for enrichment culture. Aerobic cultures were incubated at 36°C in an atmosphere containing 5% CO_2. The plates were examined after 24 h and 48 h. The anaerobic cultures were incubated in an atmosphere of 4-10% CO_2, the GasPak, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and examined after 48 h. Both aerobic and anaerobic plates were cultured 5 more days and examined at day 7 in case of negative growth after 48 h.

Table I. The medical history, microbiological findings and histological findings of the patients with chronic hyperplastic sinusitis.

Patient age (y)		Medical history	Bacterial culture	Fungal staining and culture	Histology
Male	36	Asthma IgGIII immunodeficiency	*Staphylococcus aureus* *Haemophilus influenzae*	Yeast cells (not specified) Culture negative	Inflammatio chronica Eosinophilia
Female	49	Allergic rhinitis	*Escherichia coli* *Klebsiella oxytoca* *Staphylococcus aureus*	Fungal hyphae Culture negative	Aspergilloma
Male	36		Staphylococcus aureus	-	Polypus, eosinophilia Fungal hyphae
Female	53	Asthma, ASA sensitivity	*Staphylococcus epidermidis*	-	Sinuitis chronica Eosinophilia
Female	47	Asthma	*Propionibacterium acnes*	-	Eosinophilia
Female	29		*Staphylococcus epidermidis* *Propionibacterium acnes*	-	Inflammatio chronica
Female	53	Asthma, ASA sensitivity	*Escherichia coli*	-	Polypus, eosinophilia
Male	44		-	-	Polypus, eosinophilia
Male	68	Asthma, COPD	*Streptococcus viridans*	-	Polypus, eosinophilia
Female	53	Asthma	*Klebsiella oxytoca*	-	Polypus, eosinophilia
Male	58		*Coagulase negative Staphylococci* *Streptococcus viridans*	Fungal hyphae Culture negative	Polypus, Aspergillus
Female	56		*Klebsiella pneumoniae* *Corynebacterium sp.*	Fungal hyphae *Aspergillus fumigatus*	Polypus, eosinophilia
Female	44	Allergic rhinitis	Normal flora	-	Polypus, eosinophilia
Male	40	Asthma IgGI and IgGIII immunodeficiency	Normal flora	-	Granulationes Eosinophilia
Female	44	Allergic rhinitis	*Staphylococcus aureus*	-	Not taken
Female	56	IgGIII and IgM immunodeficiency	*Staphylococcus aureus*	-	Sinuitis chronica
Female	38	Intrinsic rhinitis, thrombosytosis	*Haemophilus-like bacillus*	-	Sinuitis
Female	28	Asthma	*Staphylococcus epidermidis*	-	Not diagnostic
Female	52	Asthma	Normal flora	-	Sinuitis chronica
Female	66	Asthma, ASA sensitivity	*Staphylococcus aureus*	-	Sinuitis chronica
Female	56	Asthma, ASA sensitivity	*Staphylococcus aureus*	-	Polypus, eosinophilia fungal hyphae
Female	42	Asthma	*Coagulase negative Staphylococci*	-	Sinuitis chronica, fibrosis
Male	36	Asthma IgGI immunodeficiency	*Staphylococcus aureus* *Streptococcus pneumoniae*	-	Inflammatio polypus atypus allergica, eosinophilia
Male	62		*Staphylococcus aureus* *Proteus mirabilis*	-	Polypus, eosinophilia
Female	54	Asthma	-	-	Sinuitis chronica, eosinophilia
Male	48	Asthma	*Propionibacterium acnes* *Coagulase negative Staphylococci*	-	Polypus, eosinophilia
Male	53	Allergic rhinitis	*Klebsiella pneumoniae* *Coagulase negative Staphylococci*	Fungal hyphae *Aspergillus niger*	Polypus, eosinophilia fungal hyphae
Male	45	Asthma	*Staphylococcus aureus*	-	Polypus, eosinophilia
Male	41	Agammaglobulemic anemia	*Stenotrophomonas maltophilia*	-	Sinuitis chronica
Female	51	Asthma, ASA sensitivity	*Stenotrophomonas maltophilia*	-	Polypus, eosinophilia

The fungal specimen was first vortexed to mechanically disperse the mucus. Calcofluor white fluorescence staining was carried out using the processed sample or the sample placed directly onto a microscope slide and the preparation was examined using a fluorescence microscope with 40-fold magnification. The remaining sample was plated out on Sabouraud dextrose agar containing penicillin 6 mg/L and streptomycin 26 mg/L. The cultures were incubated at 28°C and 37°C and examined at 7 and 10 days.

For eosinophil staining the lavage sample was centrifuged 1500 rpm for 10 minutes. Cells were fixed on a microscopy slide air-drying. The slides were stained first in eosin-solution (Merck), rinsed quickly in water followed by rinsing with ethanol and stained again in methylthionin-solution (Merck). The slides were finally rinsed with water and ethanol and air-dried. Sample was considered positive, if any eosinophils were detected under the light microscope. All histological samples were stained with hematoxylin and eosin and with Periodic acid-Schiff staining. If the Periodic acid-Schiff staining was negative for fungi the Gomori methenamine silver staining method was done.

RESULTS

Bacterial findings

Twenty-eight (93%) of bacterial cultures were positive. In ten (33%) cultures two or more bacteria were cultured. At least one species of aerobic bacteria was cultured from 27 patients (90%) and anaerobic bacteria from three patients (10%). The most common aerobic bacteria was *Staphylococcus aureus*, which was isolated from ten patients (33%); followed by coagulase negative *Staphylococci* (23%); the number includes also *Staphylococcus epidermidis*. The only anaerobic species found was *Propionibacterium acnes* in three patients. The findings were similar to those found in patients with asthma. In the control group 14 cultures (70%) were positive. One had two and another had three different species cultured. Coagulase negative *Staphylococcus* species were the most common finding (40%) followed by *Staphylococcus aureus* (25%). No anaerobic bacteria were cultured. Table 2 lists the bacteria found in patients and in controls.

Table 2. Bacterial culture results for the patient group and the control group.

Bacteria	Patients		Controls	
Staphylococcus aureus	10	(33%)	5	(25%)
*Coagulase negative Staphylococci**	7	(23%)	8	(40%)
Propionibacterium acnes	3	(10%)	-	
Streptococcus viridans	2	(7%)	1	(5%)
Stenotrophomonas maltophilia	2	(7%)	-	
Escherichia coli	2	(7%)	1	(5%)
Klebsiella pneumoniae	2	(7%)	1	(5%)
Klebsiella oxytoca	2	(7%)	1	(5%)
Haemophilus influenzae	2	(7%)	-	
Proteus mirabilis	2	(7%)	-	
Streptococcus pneumoniae	1	(3%)	-	
Corynebacterium sp.	1	(3%)	-	
Culture positive	28	(93%)	14	(70%)
Two or more bacteria	10	(33%)	2	(10%)

* includes *Staphylococcus epidermidis*

Fungal findings

A total of seven patients had positive fungal finding. Fungal staining of the mucus was positive in five patients: one had yeast cells and four had fungal hyphae. The cultures were positive in two patients: one *Aspergillus fumigatus* and one *Aspergillus niger*. Both staining and culture were positive in these two cases. There were five specimens positive for fungal hyphae in histological samples stained with Periodic acid-Schiff staining. Only in two of these five specimens were the fungal hyphae visible in samples stained with hematoxylin-eosin staining. Two were morphologically consistent with *Aspergillus*. In both cases the operating surgeon had suspected mycetoma. Only three of these five patients had positive fungal staining and/or culture of the sinus secretion. Gomori staining yielded no additional positive findings. No invasive fungal infection was discovered. In the control group no sample was positive for fungi.

Eosinophilia

Tissue eosinophilia was present in 19 histological specimens. Secretion eosinophilia was also examined from 24 preoperative lavage samples. Eight were positive and four of them had also tissue eosinophilia. All of these eight patients had asthma or allergic rhinitis. Of seven patients with some finding of fungi none had secretion eosinophilia and five had tissue eosinophilia. In the control group all lavage samples were negative for eosinophils.

DISCUSSION

Patients with CHS form a heterogeneous group with similar symptoms and similar treatment despite of the possible different mechanisms behind the disease. In the present study we wanted to focus specifically on the bacterial and fungal findings in chronic hyperplastic sinusitis and compared these results to the patient history in order to find out a possible explanation for the aetiology of CHS and chronicity of the disease.

Patients with CHS have associated asthma in over 50% of the cases and 30-40% has aspirin sensitivity (Slavin, 1988). A similar profile was seen in our study, in which 57% of the patients had asthma and 29% of them were also sensitive to aspirin. The incidence of asthma among these patients was much higher than the general 2-6% incidence of asthma in the Finnish population (Haahtela et al., 1999). Three patients had specific immunoglobulin G subclass deficiency and one patient had both

immunoglobulin G subclass and immunoglobulin M deficiencies. Every patient with immunodeficiency had been operated on earlier. Three of these patients also had asthma but none of them had ASA intolerance. Chee et al. (2001) also found a high incidence of immune dysfunction in patients with refractory sinusitis, i.e. low immunoglobulin G in 17.9%, low immunoglobulin A in 16.7%, and low immunoglobulin M in 5.1%.

CHS and nasal polyposis are known to have a high frequency of recurrence: 63% of the patients in the present study had been operated on earlier and similar numbers have been described in the literature (Hamilos, 2000; Chee et al., 2001). In long-term follow up studies of nasal polyps the ASA intolerant patients had the highest frequency of recurrence and they usually needed re-operation and medication more often than patients with atopic allergy or intrinsic allergy-like disease (Vento et al., 2000). The high recurrence frequency in ASA intolerant patients was also seen in the present study. All the patients with ASA intolerance had been operated on earlier and four of these five patients had had several operations. 75% of asthmatic patients without ASA intolerance had had a previous operation compared to 54% of non-asthmatic patients. The number of patients is too small to make further conclusions but CHS with asthma without ASA intolerance could have poorer prognosis than CHS in non-asthmatic patients.

The bacteria culture results were similar to those of other studies (Hamilos, 2000; Wald, 1998). *Staphylococcus aureus* and coagulase-negative *Staphylococci* were the two most common findings in both the control group and the patient group. Anaerobic bacteria (*Propionibacterium acnes*) were found in the patient group only. Some studies have highlighted the polymicrobial aetiology of chronic sinusitis, particularly aerobic and anaerobic beta-lactamase-producing bacteria (Brook et al., 1994; Bhattacharyya and Kepnes, 1999). Beta-lactamase-producing bacteria were found in the present study, but their pathogenity in chronic sinusitis is unknown. The same bacteria are also the main components of the normal nasal flora (Savolainen et al., 1986). However, it is also hypothesized that the normal nasal flora may be the normal flora solely under healthy conditions (Bhattacharyya and Kepnes, 1999). Aral et al. (2003) found anaerobes in 14.2% of maxillary sinus aspirates taken during functional endoscopic sinus surgery but ethmoid sinus samples and nose swap samples cultured only aerobes in the same patients. Samples taken "deeper" inside the nose could favour anaerobes. On the other hand anaerobic bacteria, especially *Propionibacterium* species are found in up to 100% of nasal cavities of healthy subjects (Jousimies-Somer et al., 1989).

In the present study bacterial cultures taken from asthmatic patients with or without ASA intolerance did not differ from cultures taken from non-asthmatic patients. So, our bacterial findings do not conclusively explain the different prognosis of CHS between these groups. Several bacterial species implicated in chronic sinusitis are potent inducers of IgE pointing to a possible allergic mechanism (Bhattacharyya and Kepnes, 1999). One of the most likely candidates in CHS is an enterotoxin (a superantigen) producing strain of *Staphylococcus aureus* (Schubert, 2001). Specific IgE directed against *S. aureus* enterotoxin has been found in eosinophilic nasal polyps. Patients with enterotoxin producing strains of *Staphylococcus aureus* have more severe symptoms than patients with other types of *S. aureus* (Schubert, 2001). In the present study, eight of ten patients with *Staphylococcus aureus* were operated on at least once earlier, although the prevalence of *Staphylococcus aureus* was about the same as in the control group. Longer courses of the disease and several operations could also have influenced the normal nasal flora, which could favour *Staphylococcus aureus*. In the patient group a greater diversity of bacterial species was found (12 compared to 6), but this has probably no clinical meaning.

Fungi were found in seven patients. Two patients had mycetoma probably caused by *Aspergillus*. The histology and fungal staining of mucus from the sinus cavity of these patients showed fungal hyphae, but both were culture negative. One patient had a mycetoma-like mass in the sinus cavity and the culture was positive for *Aspergillus fumigatus*. These patients were immunocompetent. The 10% prevalence of mycetoma in CHS patients in the present study is high compared to our previous clinical experience. In the remaining four patients with fungal findings one patient had yeast cells seen in direct microscopy and three had fungal hyphae in histological samples stained with PAS. Only one of the patients was culture positive for fungi (*Aspergillus niger*). Two patients (number 1 and number 3 in Table 1) had thick mucus in the sinus cavity, with polyposis and tissue eosinophilia but no secretion eosinophilia. Eosinophil staining was done on the nasal lavage sample, which may not have contained a sufficient concentration of cells and mucus from sinus cavity to detect secretion eosinophilia. The other two patients had polyposis as a main finding.

The first AFS cases described were caused by Aspergillus (Katzenstein et al., 1983), but dematiaceous fungi are more common (Richardson and Warnock, 2003). Until recently AFS was thought to be rare. About 5-10% of patients with chronic sinusitis is estimated to have AFS (Marble, 2001), but it is known to be more common in hot, humid areas (Ferguson et al., 2000). The criteria of AFS vary, so it is difficult to establish the diagnosis. In the present study, two patients (7%) had possibly AFS. The only fungal genus found was *Aspergillus*. The culture was positive in two of seven patients. It is estimated that 20-40% of fungal infections are culture positive, which we also found in this study. Based on these results it can be concluded that fungal staining of mucus as well as histological samples stained with specific fungal staining are needed to detect fungi. Five of seven fungus positive patients (71%) were operated on earlier. That does not differ significantly from the

average 63% of all patients in this study. In our view the presence of fungi does not explain the chronic course of CHS. They may play some part in pathophysiology of CHS. It is also possible that these results are more a reflection of a change in the environment in the paranasal sinuses and a change in normal flora than the actual cause of CHS.

In this study fungi were not found in control subjects. This is in contrast with studies reported by Ponikau et al. (1999) and Braun et al. (2003), who found fungi in almost every nose both in patient-groups and control-groups. Buzina et al. (2003) obtained mucus samples by flushing the noses of chronic rhinosinusitis patients with saline or by ESS and by flushing from healthy controls. The samples showed fungal growth in 91.3%, 84.0% and 91.3% respectively. Some of the most prevalent genera showed a significant seasonal fluctuation with maxima in prevalence in late summer/early autumn. It is hypotised that nasal lavage reflects the fungal spores in the patients environment more than the local environment in the sinuses. However, the lack of fungal positive findings in the control group does not support this view. The control samples in the present study were collected in the middle of the winter. So the Nordic climate may explain this finding.

In conclusion, this and other studies (Gwaltney et al., 1992) re-emphasize that chronic sinusitis represents a repeatedly damaged mucosal lining. Previous results from patients operated on due to chronic sinusitis have showed that the sinus epithelium does not recover fully in six months after surgery (Toskala and Rautiainen, 2003). These studies point towards structural damage and not infection. Future studies are needed to understand the mechanisms of this very common disease.

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