

Microbial Flora in Chronic Rhinosinusitis with and without Nasalpolyps

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Abstract

Introduction: The most common microbial agents in the etiology of chronic rhinosinusitis are defined in the literature as *Staphylococcus aureus*, *Staphylococcus coagulase-negative* and *Streptococcus* spp. In healthy individuals these same microorganisms are also the most frequent (mainly *Staphylococcus coagulase negative*) as colonizing flora agents. We often encounter a poly microbial colonization of the nose and sinuses. The contribution of the different pathogens for the disease remains uncertain. The aim of this study is to compare the microbial flora found in patients with chronic rhinosinusitis with and without nasal polyps.

Methods: Prospective clinical study. 110 patients with indication for endonasal microsurgery by chronic rhinosinusitis were evaluated. Patients were divided into two groups: with nasal polyps (70) and without nasal polyps (40). During surgery, mucosa of the middle meatus/anterior ethmoid was collected, in the side with the highest score Lund-McKay and sent for bacteriological analysis.

Results: There was a predominance of aerobic Gram-positive bacteria, followed by aerobic Gram-negative and an aerobic bacteria. When evaluated separately the groups with and without nasal polyps, there was a predominance, in both, of Gram-positive bacteria. However, in the group with nasal polyps, there was a higher prevalence of aerobic Gram-negative bacteria and only after an aerobic bacteria while in the group without nasal polyps there was a predominance of an aerobic bacteria in relation to aerobic Gram-negative bacteria.

Conclusion: As recently suggested by several authors, nasal polyps may not be a stage of chronic rhinosinusitis but a distinct disease entity. The existence of distinct microbial flora eventually comes to corroborate this hypothesis.

Keywords: Chronic rhinosinusitis; Polyposis; Microbiology

Introduction

Chronic rhinosinusitis (with or without nasal polyps) is defined as an inflammation of the nose and paranasals in cases that persists beyond 12 weeks, characterized with two or more of the following symptoms: nasal obstruction/congestion, anterior and/or posterior rhinorrhea, localized pain or facial pressure and reduction/loss of smell. In addition to, at least two of these symptoms, there must be polyps/purulent rhinorrhea discharge from the middle meatus/Oedema in endoscopic examination. In the CT scan there must be pathological alteration of the sinuses or at least of the osteo-meatal complex [1,2]. Despite of chronic rhinosinusitis prevalence, its etiology remains a target of great discussion and research.

Chronic rhinosinusitis is the major pathology and nasal polyps are considered a sub-group of this entity. Currently there are many authors who advocate that they consist in two different entities,

based mainly on the difference of inflammatory markers present in patient with and without polyps. This classification change may ultimately lead to different ways of approach and treat the patients [3,4].

The origin of microorganisms in the nasal mucosa may eventually cause sinusitis. It is broadly defined in the literature the type of microorganisms present in chronic rhinosinusitis. There are also some authors that, given the known difference between the rhinosinusitis subgroups (with and without polyps), have compared the microbiological flora between these two groups [5-7].

Objectives

This study aims to evaluate and compare the microbial flora in patients with chronic rhinosinusitis with and without nasal polyps.

Methods

This was a prospective study in the department of otorhinolaryngology at Centro Hospitalar do Porto. The sample size was of 110 patients with chronic rhinosinusitis and with surgical treatment indication. The study took place between January 2010 and March 2014. It was validated by the hospital ethics committee and all patients gave informed consent.

The authors excluded patients under 18 years old, patients with cystic fibrosis, immunodeficiencies, malignant disease, congenital mucociliary disease, fungal disease and vasculitis or granulomatous diseases.

These patients were classified as with and without nasal polyps by endoscopic examination, and the score Lund-McKay was evaluated.

At the beginning of each surgical procedure, a sample of mucosa of the osteomeatal complex region was collected; on the side with the highest score Lund-McKay. All biopsies were collected in sterile container then inoculated into the culture media within 1-4h of collection.

For aerobic culture, biopsies were inoculated in MacConkey agar, Chocolate agar, Sabouraud agar and Cooked meat broth. The media (plates and broth) were incubated at 35°C (Chocolate agar in a 5% carbon dioxide environment). The primary plates and broth were examined daily for at least two days for any microbial growth, the

broth was sub culture at 48h to MacConkey agar and Chocolate agar and examined daily for four more days. Sabouraud was examined daily for seven days for any fungal growth. For an aerobic culture, biopsies were inoculated in Columbia blood agar base with hemin, G.N. an aerobes medium and N.E. an aerobes medium. These media were incubated aerobically at 35°C and evaluated twice a week for any microbial growth, for at least seven days.

Data analysis was performed using IBM-SPSS Statistics version 23. The chi-square test or the Fisher's exact test (when appropriate) was used to determine whether the prevalence of the different types of bacteria was significantly different between the two groups of patients, with polyps (CRSwNP) and without polyps (CRSSNP).

Results

37 different bacteria were identified. In 4 patients with CRSSNP and in 15 patients with CRSwNP, more than one type of bacteria was isolated. Analyzing all patients (n=110) there was a predominance of colonization/ infection by Gram-positive bacteria (65,45%), with predominance of *Staphylococcus* (47,27%). 26,36% (n=29) of the samples showed no cultural growth of microorganisms and were classified as sterile. In 18,82% there was growth of aerobic Gram-positive bacteria. The authors present in the table the Gram-positive and Gram-negative aerobic bacteria and also the total of an aerobic bacteria identification. They identified an aerobic bacteria in 18, 18% of the patients (Table 1 & 2), (Figure 1).

Table 1: Microbiological identification in patients with CRS in 15 patients, more than 1 agent was identified.

Sterile	Total (n=110)	
	29	26,36%
Gram-positive aerobic	72	65,45%
<i>Staphylococcus</i> (total)	52	47,27%
<i>Staphylococcus aureus</i>	20	18,18%
<i>Staphylococcus epidermidis</i>	19	17,27%
<i>Staphylococcus lugdunensis</i>	4	3,63%
<i>Staphylococcus warneri</i>	1	0,90%
<i>Staphylococcus coagulans</i> negativo	6	5,45%
<i>Staphylococcus schleiferi</i>	1	0,90%
<i>Staphylococcus hominis</i>	1	0,90%
<i>Streptococcus</i> (total)	10	9,09%
<i>Streptococcus constellatus</i>	1	0,90%
<i>Streptococcus viridans</i>	1	0,90%
<i>Streptococcus agalactiae</i>	3	2,77%
<i>Streptococcus anginosus</i>	1	0,90%
<i>Streptococcus piogenes</i>	1	0,90%
<i>Streptococcus spp</i>	2	1,81%
<i>Streptococcus pneumoniae</i>	1	0,90%
<i>Corynebacterium pseudodiphthericum</i>	7	6,35%
<i>Corynebacterium pseudotuberculosis</i>	1	0,90%
<i>Corynebacterium urealyticum</i>	1	0,90%
<i>Enterococcus faecalis</i>	1	0,90%



Gram-negative aerobic	13	18,82%
<i>Moraxella catarrhalis</i>	2	1,81%
<i>Escherichiacoli</i>	2	1,81%
<i>Citrobacterfreundii</i>	1	0,90%
<i>Haemophilus influenza</i>	3	2,77%
<i>Proteus mirabilis</i>	2	1,81%
<i>Enterobacter aerogenes</i>	1	0,90%
<i>Klebsiella oxytoca</i>	1	0,90%
<i>Pantoea spp</i>	1	0,90%
Anaerobic (total)	20	18,18%
Gram-positive anaerobic	14	12,73%
<i>Propionibacterium avidum</i>	2	1,81%
<i>Propionibacterium granulosum</i>	1	0,90%
<i>Propionibacterium propionicus</i>	2	1,81%
<i>Propionibacterium acnes</i>	7	6,36%
<i>Propionibacterium spp</i>	1	0,90%
<i>Peptostreptococcus magnus</i>	1	0,90%
Gram-negative anaerobic	6	5,45%
<i>Fingoldia magna</i>	2	1,81%
<i>Veillonella species</i>	2	1,81%
<i>Bacteroides ureolyticus</i>	1	0,90%
<i>Prevotella bivia</i>	1	0,90%

Table 2: Comparative analysis of microbial flora obtained by other authors and present study.

Pathogens	Busaba et al Out of 179(%)	Panduranga Kamathetal out of 100(%)	Brook out of 66(%)	Present study out of 110(%)
<i>Staphylococcus aureus</i>	18,4	46	14	52
<i>Streptococci</i>	1,1	2	14	10
<i>Corynebacterium spp</i>	0	0	NS	9
<i>Pseudomonas</i>	5,0	1	3	0
<i>Enterobacteriaceae</i>	5,6	11	6	7
<i>Actinomycetes</i>	0,0	1	NS	0
<i>Haemophilus influenzae</i>	4,5	0	5	3
<i>Moraxella catarrhalis</i>		0	6	2
<i>Anaerobes</i>	6,1	0		
<i>Peptostreptococcus</i>			56	1
<i>Propionibacterium spp</i>			29	13
<i>Prevotella</i>			47	1
<i>Fingoldia</i>				2
<i>Bacteroides</i>			6	1
<i>Veillonella</i>				2
<i>Fusobacterium</i>			17	

Table 3: Microorganisms frequency in CRSwNP and CRSsNP.

	CRSwNP (n=70)		CRSsNP (n=40)	
Sterile	19	27,14%	10	30%
Gram-positiveaerobic	46	65,71%	26	65,00%
<i>Staphylococcus(total)</i>	31	44,28%	21	52,5%
<i>Staphylococcus aureus</i>	12	17,14%	8	20,00%
<i>Staphylococcus epidermidis</i>	10	14,28%	9	22,50
<i>Staphylococcus lugdunensis</i>	3	4,28%	1	2,50%
<i>Staphylococcus warneri</i>	1	1,43%	0	0%
<i>Staphylococcus coagulansnegativo</i>	3	4,28%	2	5,00
<i>Staphylococcus chleiferi</i>	1	1,43%	0	0%
<i>Staphylococcus hominis</i>	1	1,43%	0	0%
<i>Staphylococcus species</i>	0	0	1	2,50%
Streptococcustotal	9	12,86%	1	2,50%
<i>Streptococcus constellatus</i>	0	0	1	2,50%
<i>Streptococcus viridans</i>	1	1,43%	0	0%
<i>Streptococcus agalactiae</i>	3	4,28%	0	0%
<i>Streptococcus anginosus</i>	1	1,43%	0	0%
<i>Streptococcus piogenes</i>	1	1,43%	0	0%
<i>Streptococcus spp</i>	2	2,86%	0	0%
<i>Streptococcus pneumoniae</i>	1	1,43%	0	0%
<i>Corynebacterium pseudodiphthericum</i>	4	5,71%	3	7,50%
<i>Corynebacterium pseudotuberculosis</i>	1	1,43%	0	0%
<i>Corynebacterium urealyticum</i>	0	0%	1	2,50%
<i>Enterococcus faecalis</i>	1	1,43%	0	0%
Gram-negativeaerobic	10	14,28%	3	7,50%
<i>Moraxella catarrhalis</i>	0	0%	2	5,00%
<i>Escherichia coli</i>	2	2,86%	0	0%
<i>Citrobacter freundii</i>	1	1,43%	0	0%
<i>Haemophilus influenza</i>	3	4,28%	0	0%
<i>Proteus mirabilis</i>	1	1,43%	1	2,50%
<i>Enterobacter aerogenes</i>	1	1,43%	0	0%
<i>Klebsiella oxytoca</i>	1	1,43%	0	0%
<i>Pantoea spp</i>	1	1,43%	0	0%
Anaerobic(total)	14	19,99%	6	15%
Gram-positiveanaerobic	10	14,28%	4	10,00%
<i>Propionibacterium avidum</i>	2	2,86%	0	0%
<i>Propionibacterium granulosum</i>	1	1,43%	0	0%
<i>Propionibacterium propionicum</i>	1	1,43%	1	2,50%
<i>Propionibacterium acnes</i>	5	7,14%	2	5,00%
<i>Propionibacterium spp</i>	0	0%	1	2,50%
Peptostreptococcus magnus	1	1,43%	0	0%
Gram-negativeanaerobic	4	5,71%	2	5,00%
<i>Finegoldia magna</i>	1	1,43%	1	2,50%
<i>Veillonella species</i>	1	1,43%	1	2,50%
<i>Bacteroides ureolyticus</i>	1	1,43%	0	0%
<i>Prevotella bivia</i>	1	1,43%	0	0%

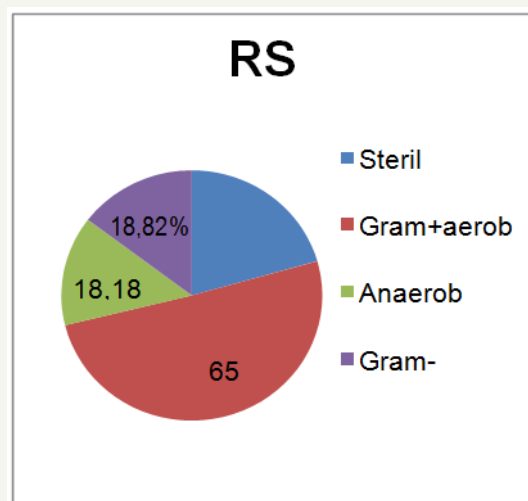


Figure 1: Results in patients with CRS.

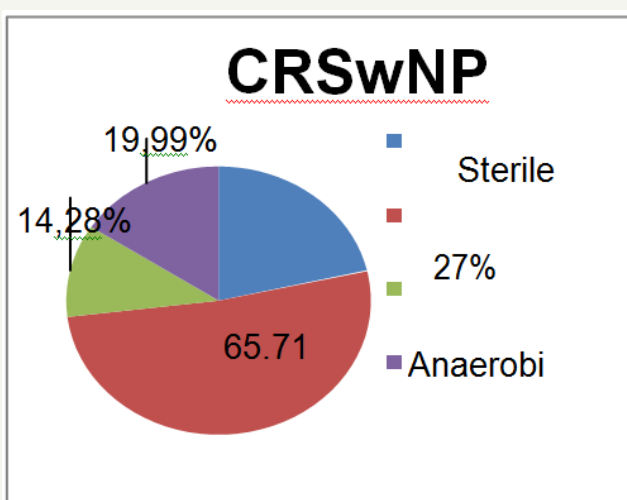


Figure 2: Results in patients with CRSwNP.

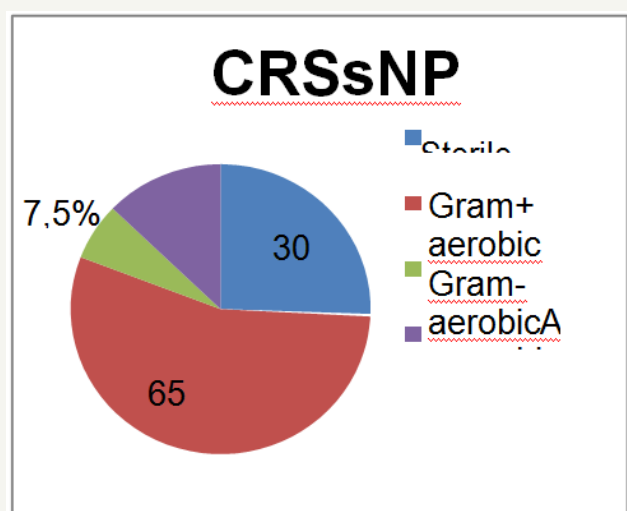


Figure 3: Results in patients with CRSSNP.

Evaluating separately the sub-group with polyps (CRSwNP) and without polyps (CRSSNP), the authors found that the prevalence of aerobic Gram-positive bacteria persists (predominantly

Staphylococcus). However, in both groups the an aerobic stake these cond place in the rank, followed by the Gram-negative aerobic bacteria. The comparison between the two sub-groups show that the aerobic Gram-negative bacteria are more frequent in the CRSwNP group than in the CRSSNP group, although not statistically significant ($p=0.229$, one sided Fisher' sex act test) (Table 3), (Figure 2 & 3).

Discussion

One can find several studies concerning the bacterial identification in patients with chronic rhinosinusitis [5,7-15]. However, most of these did not search for an aerobic bacteria. In our study, we have found more positive identifications of an aerobic bacteria than aerobic Gram-negative bacteria. We isolated 10 different an aerobic bacteria. There so, a study that does not include an aerobic culture is not complete. It is not worthy that we found an aerobic bacteria in both groups. The major difference between the two groups was the prevalence of the aerobic Gram-negative bacteria that was twice higher in the CRSwNP group and, although not statistically significant ($p=0.229$), one sided Fisher' sex act test, it seems that, in a larger population, the significance would be achieved.

Conclusion

Chronic rhinosinusitis, as a single entity, is very heterogeneous and their phenotypic differentiation in chronic rhinosinusite with and without polyposis is consensual. Their distinct peculiarities, despite the phenotype, are related with its inflammatory mediators. The existence of different microbiological flora can be another distinguishing factor. Extrapolation of these differences leads to the need to address these subtypes as distinct clinical entities, which already is suggested by different authors. Perhaps this will conduct to different treatments in the future.

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