

Nasal Microbiology from Patients with Chronic Rhinosinusitis

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The purpose of this study is to establish if the germs that were identified in the bacteriological examination of nasal secretions of patients with CRS without nasal polyposis and patients with CRS with nasal polyposis are different.

Study design: cross-sectional

Keywords: microbiology, chronic rhinosinusitis

The European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS, 2012), states that the prevalence of CRS is increasing annually. Epidemiological studies that explore the prevalence and incidence of CRS with or without nasal polyposis are deficient, especially in European countries - this was affirmed by the most important paper on the subject, EPOS [1].

Most recent data has demonstrated that CRS affects approximately 5–15% of the population in both Europe and the USA [2,3].

Patients with CRS are now divided into those who have nasal polyps (CRSwNP) and those who do not (CRSsNP) [1].

The field has had many breakthroughs in the past few years, but the pathogenic mechanisms and triggering factors are not completely understood. Recently, the bacterial role of CRS pathogenesis has been the subject of intense debate, therefore the microbiology of CRS has been studied extensively [4,5].

Ethics statement. This study has been approved by the ethics committee of the University of Medicine and Pharmacy Craiova. All participants provided written informed consent prior to their participation in the project.

Experimental part

Materials and methods

We analyzed a number of 62 patients that have been diagnosed with CRS with/without nasal polyps that have been admitted in the ENT Clinic of County Emergency Hospital Craiova from January of 2016 to December of 2016.

The study included persons that were aged between 15 and 75, according to an EPOS suggestion [1].

EPOS states that the symptomatology of adults with CRS is [1]:

-The presence of two or more symptoms and at least one is nasal obstruction/congestion or rhinorrhea (anterior or posterior)

-With/without headache or facial pressure

-With/without hypo-/anosmia

-duration of symptoms should be at least 12 weeks, confirmed via telephone or face-to-face.

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At the recommendation of EPOS, we have excluded the next categories of patients that presented associated afflictions: cystic fibrosis diagnosed through the sweat test or DNA analysis, immunodeficiency syndrome (congenital or acquired), mucociliary congenital afflictions (e.g. primary ciliary dyskinesia), fungus-ball or invasive fungal sinusitis, systemic vasculitis, granulomatous diseases, cocaine abuse, cancer [6-14].

Furthermore, because our bacteriological study is based on tissue that comes from the meatus of patients with CRS, we also excluded patients that used systemic or local antibiotics the preceding month.

To appreciate how life-quality of patients with CRS is affected, we used the SNOT-22 test, translated into Romanian [15]. This test scores between 0-110, depending on how much the symptoms of CRS affect the patient.

The clinical examination of the patients was done endoscopically and classically (anterior rhinoscopy) [16]. The endoscopic examination was done under local anesthesia-pulverizations with Lidocaine 10% and by using a 4mm Storz telescope (KARL STORZ GmbH & Co. KG, Tuttlingen, Germany).

Sample Collection

Samples for the microbiological examination were collected after using local anesthetics (Lidocaine 10%) associated with xylometazoline (1 puff for each nasal fossa). Swabs were endoscopically guided (using a 0⁰ endoscope) to the middle meatus region and rotated at least 3 times. All samples were immediately transported to the laboratory.

The germs were isolated by inoculation of the tissue samples on proper culture medium – solid, semisolid, liquid, on plaques or tubes. The inoculated plaques and tubes were then incubated for 24-48h at 37° C in a thermostat, in aerobiosis conditions for proper bacterial growth and multiplication.

The macroscopic aspect of the cultures (using the free-eye, simple lens or stereoscopic lens) guided our next steps. The cultures that were obtained on liquid or solid mediums were identified using morphological, biochemical antigenic and pathogenic criteria.

From an isolated colony, we inoculated a dispersion medium, until the manufacturers turbidity conditions were fulfilled and then verified with a MacFarland nephelometric analyzer.

For the laboratory diagnostic of staphylococcal infections, smear tests were done on Methyl blue and Gram mediums from the pathological products and inoculations on simple liquid and solid culture mediums (simple gelosis) and complex mediums (blood-gelosis, Chapman). The incubation took place in aerobiosis at 37⁰C.

The statistical analysis was done by using the Kruskal-Wallis and chi-square tests.

Results and discussions

Characteristic of the study-lot

The study included 62 patients that were diagnosed with CRS, with/without nasal polyposis, using the mentioned criteria. The age average of the entire lot was 48, the age average of the women was 49 and of the men 46. The conclusion is that the lot was age balanced (Table 1).

Table 1
THE DISTRIBUTION OF PATIENTS WITH CRS BY AGE AND GENDER

	Average age	Standard deviation	Minimal age	Maximum age
Total	48	17.2	20	86
Women	49	17.1	22	86
Men	46	17.4	20	78

We also noticed that the lot was gender balanced (Table 2) – 45% men(M) (28) and 55% women(W) (34).

Table 2
DISTRIBUTION OF PATIENTS WITH CRS BY GENDER

Women	34	55%
Men	28	45%
Total	62	100%

According to the medium of provenance, the patients were 29% from rural mediums (R) (18) and 71% from urban mediums (U) (44). We take note of the high percentage of patients that are from urban mediums, approximately twice as many (Table 3).

Table 3
DISTRIBUTION OF PATIENTS WITH CRS BY
THE MEDIUM OF PROVENANCE

	Rural	18	29%
	Urban	44	71%
	Total	62	100%

	Rural	Urban	Total
Women	10	24	34
Men	8	20	28
Total	18	44	62

By analyzing Table 3 we note that the patients with urban provenance are twice as many that those of rural provenance. The table also demonstrates that both men and women of urban provenance are twice as many than those of rural provenance, which was an expected situation, considering the fact that we established earlier the gender balance of the study lot.

From a clinical point of view, 45(72%) patients have been diagnosed with CRSsNP and 17(28%) with CRSwNP. The age of the patients at the time of diagnosis will be the following: -Table 4

Table 4
DISTRIBUTION OF PATIENTS WITH CRSsNP AND CRSwNP BY AGE
(KRUSKAL-WALLIS TEST, CHI-SQUARE TEST)

	CRSsNP	CRSwNP	Statistical analysis*
Number of patients	45	17	
Age average (years)	46	54	p = 0.0542
Age average W (years)	49	49	p = 0.8815
Age average M (years)	40	58	p = 0.0118
Age average R (years)	47	53	p = 0.6065
Age average U (years)	45	54	p = 0.0929
M/W Raport	18/27	10/7	p = 0.1840

Considering this table, we note the following:

- the age of patients with CRSsNP is not significantly different than that of patients with CRSwPP ($p > 0.05$)
- the age of female patients with CRSsNP is not significantly different than that of female patients with CRSwNP ($p > 0.05$)
- the age of male patients with CRSsPP is significantly different than that of male patients with CRSwNP ($p < 0.05$)
- the age of R patients with CRSsNP is not significantly different than that of R patients with CRSwNP ($p > 0.05$)
- the age of U patients with CRSsNP is not significantly different than that of U patients with CRSwNP ($p > 0.05$)
- The patient lots that have been diagnose with CRPsNP and CRSwNP are omogenous. There are no significant differences. There is no statistical evidence that can indicate that women are more likely to have CRSsNP or CRSwNP, rather than men ($p > 0.05$)

Also:

- there are 4 times as many women with CRSsNP (27) than women with CRSwNP (7)
- there are 2 times more men with CRSsNP (18) than with CRSwNP (10)
- the patients of rural provenance have twice as many CRSsNP (12) than those with CRSwNP (6)
- the patients with urban provenance have 3 times as many CRSsNP (33) than CRSwNP (11)
- altogether, from the 62 patients lot, approximately a quarter have CRSwNP and 3/4 have CRSsNP

We have calculated the differences between the age average of patients with CRSsNP vs. CRSwNP

- of the entire lot, the age average of patients with CRSsNP is 46 years old, and for those with CRSwNP is 54
- for women, the age average of those with CRSsNP is approximately equal with those with CRSwNP (49)
- for men, the age average of those with CRSsNP (40) is a lot smaller than those with CRSwNP (58)

All patients have completed the SNOT-22 test that analyses the life-quality of patients with CRS. The average SNOT-22 score is analyzed in Table 5.

Table 5
CRSsNP AND CRSwNP ANALYSES AND AVERAGE SNOT-22 SCORE

	CRS	CRS+NP	Statistical analyses*
Number of patients	45	17	
SNOT-22 average	58.8	69.8	p = 0.1339

For the statistical analyses we used the Kruskal-Wallis test. The conclusion of Table 5 is that the SNOT-22 average score of patients with CRSsNP is not significantly different than that of patients with CRSwNP ($p > 0.05$).

SNOT-22 average for the entire lot is 61.8; for women the average score is 66.9 and for men 55.7. This shows a significant difference between SNOT-22 average scores of women vs. men - $p = 0.0639$ ($p > 0.05$) according to the Kruskal-Wallis test (Table 6)

Table 6
SNOT-22 AVERAGE SCORE BY GENDER

	SNOT-22 average	Standard deviation	SNOT-22 minimum	SNOT-22 maximum
Entire lot	61.8	22.3	21	106
Women	66.9	21.3	31	106
Men	55.7	22.2	21	95

Bacteriological analyses of nasal secretions of patients with CRS

Of the 62 samples we have studied, 32 (52%) showed no microorganisms growth; in 30 samples (48%) we have found aerobic bacteria. We did not find anaerobic microorganisms (Table 7).

Type of bacteria	Number of patients	CRSsNP	CRSwNP
Citrobacter	2	1	1
E Coli	1	1	0
Klebsiella	3	2	1
Proteus	3	2	1
Staphylococcus haemolyticus	1	1	0
Staphylococcus aureus	19	16	3
Streptococcus alfa	1	1	0
No development	32	21	11
Total	62	45	17

Of the 62 patients, 32 (52%) did not develop any germs and 30 patients (48%) developed one of the seven: White Staphylococcus haemolyticus (1 patients), Alpha Streptococcus (1 patient), E. Coli (1 patient), Citrobacter (2 patients), Proteus (3 patients), Klebsiella (3 patients), Staphylococcus aureus (19 patients). Therefore, out of all the patients that developed one of the seven types of germs (30), those that developed Staphylococcus aureus (19) represent 63%. This means 2/3 out of all the patients that developed a germ (19 out of 30).

Of the patients that have been diagnosed with CRSsNP (45), 21 (47%) have not developed any type of germ; of the 24 patients with CRS that developed a germ, most of them developed Staphylococcus aureus (16 patients = 67%).

Out of the patients diagnosed with CRSwNP (17), 11 patients (65%) did not develop any germs; of the 6 patients with CRSwNP that developed a germ, most of them were Staphylococcus aureus (3 patients=50%).

Out of the 32 patients that have not developed any type of germ, 21 (66%) have been diagnosed with CRSsNP and 11 (34%) with CRSwNP.

Out of the 19 patients that developed Staphylococcus aureus, 16 (84%) have been diagnosed with CRSsNP and 3 (16%) with CRSwNP.

We did not notice differences between the germ that developed in women vs. men and this contradicts data from specialty literature. In a study that was published in 2017 Golan notes that Staphylococcus aureus is most likely present in men [17]. Females are more susceptible to allergic rhinitis during menstruation and pregnancy [18, 19]

Chronic rhinitis has a high prevalence in smoking patients [20, 21, 22, 23]. The sleep-apnea syndrome can be associated with allergic rhinitis, but it doesn't determine the severity of the disease [24, 25]. Another risk factor for rhinitis can be low serum levels of hemoglobin [26, 27].

The role of microorganisms in the pathogenesis of CRS, undisputed for decades, has been vigorously debated in recent years. There is not enough evidence to support the classic bacterial theory, and CRS is no longer considered to be a consequence and result of recurrent acute inflammation episodes. Because of the germ diversity that we have found in the nasal mucosa, there are authors that consider [28] the presence of germs found in the nasal mucosa irrelevant in the physiopathology of CRS.

Many previous investigations addressed CRS microbiology using conventional culture-dependent techniques. Under these conditions, the most common pathogens identified were aerobic bacteria – *S. aureus* and *coagulase-negative Staphylococci* [3,29,30], negative -Gram bacilli [31].

Our study analyses the differences of the bacteriological examination of patients with CRSsNP and CRSwNP. The study protocol allowed us to take samples only from the middle nasal meatus. Our study included CRS patients free of acute exacerbation.

Some authors explain the existence of CRS forms that are resistant to medical therapy caused by intracellular persistence of *S. aureus* in the mucosa of the nasal cavity and paranasal sinuses [32]. However, data on the presence of *S. aureus* in the nasal cavity and paranasal sinuses are quite diverse and largely depend on the sampling method and culturing technique. For example, one study examined the middle nasal meatus microbiology and recovered *S. aureus* in 36% of the cases of CRS patients, and in 39% of those from healthy subjects [33], and 40% developed *Staphylococcus* [34]. Another study identified *S. aureus* in swabs from the inferior turbinate of CRS patients (50%) and of healthy individuals (33%) [35].

In our study we take notice of the frequency of the germs isolated from the middle meatus of CRS patients: *Staphylococcus aureus* is most commonly found, followed by the Gram-negative bacilli (*Klebsiella* and *Proteus*). Mantovani [36] also identifies Gram-negative in CRS patients, especially *Pseudomonas aeruginosa*. We only isolated *Citrobacter* in 1 patient, and this contradicts the speciality literature. because Stern [37] states that *Citrobacter* is the most commonly found Gram-negative germ isolated from the nasal secretions of CRS patients.

Conclusions

In our study, 63% of the probes were positive for *Staphylococcus aureus*. This meant it has the highest frequency in both CRSsNP (67% of the positive probes) and CRSwNP (50% of the positive probes). Gram-negative bacteria have also been isolated, placing them as second in frequency: 8% out of the positive probes in patients with CRSsNP and 32% in patients with CRSwNP.

There were no significant differences in the microbiological features of the middle meatus samples from CRSsNP patients and CRSwNP patients.

More studies are needed to completely elucidate the role of bacteria in the pathogenesis of CRS, in particular by comparing the density and composition of the microbiological community that resides in the nasal cavity and paranasal sinuses under acute exacerbation conditions and remission periods.

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