

Bacteriological analysis of selected phenotypes of chronic rhinosinusitis with co-existing asthma, allergy and hypersensitivity to non-steroidal anti-inflammatory drugs

Grażyna Stryjewska-Makuch¹, Małgorzata A. Janik², Hanna Klamińska-Cebula³, Bogdan Koleczka¹, Wojciech Ścierański⁴, Grażyna Lisowska⁴

¹Department of Laryngology and Laryngological Oncology, Independent Public Research Hospital No. 7 of the Silesian Medical University in Katowice, Upper Silesian Medical Centre, Katowice, Poland

²Department of Biomedical Computer Systems, Institute of Computer Science, Faculty of Computer Science and Materials Science, University of Silesia in Katowice, Sosnowiec, Poland

³Microbiological Laboratory, Independent Public Research Hospital No. 7, the Silesian Medical University in Katowice, Upper Silesian Medical Centre, Katowice, Poland

⁴Department of Otorhinolaryngology and Oncology in Zabrze, the Silesian Medical University in Katowice, Zabrze, Poland

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Abstract

Introduction: Chronic rhinosinusitis (CRS) is one of the commonest chronic diseases. It is a systemic disease caused by many factors, including bacterial infections. There are two main types of CRS phenotypes: with polyps (CRSwNP) and without polyps (CRSsNP).

Aim: Analysis of sinus mucosal microbiome in patients with CRS depending on the phenotype. Investigating a possible link between the type of bacterial flora and the coexistence of diseases present in the CRS (asthma, allergy or hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) as well as the number of performed operations.

Material and methods: The authors conducted a retrospective study of the bacterial flora of the nasal cavity in patients with CRS operated endoscopically from March 2016 to June 2017. The study consisted of 222 women and 248 men. On the basis of an endoscopic examination, patients were qualified for the phenotype with or without polyps. Based on the medical interview patients were divided into group with asthma, allergy, NSAID hypersensitivity, first and repeated operations. The statistical analysis was made.

Results: There is no statistically significant relationship ($p = 0.8519$) between the CRS phenotypes. In the group with CRSsNP, a statistically significant relationship was found between the observed flora and the coexistence of asthma ($p = 0.0409$), a trend towards significance was also noticed in the case of allergy ($p = 0.0947$). There was no relationship between the flora and NSAID hypersensitivity ($p = 0.7356$). In the group of CRSwNP patients, no statistically significant relationship was found between the observed bacterial flora and the presence of asthma ($p = 0.7393$), hypersensitivity to drugs ($p = 0.1509$) or allergy ($p = 0.7427$). There is no statistical significance between the occurrence of particular flora and the multiplicity of operations in both the CRSwNP ($p = 0.4609$) and CRSsNP phenotypes ($p = 0.2469$).

Conclusions: Gram-positive cocci were equally common in CRSwNP and CRSsNP. In the CRSsNP, there was a correlation between the coexistence of asthma and allergy, and the presence of Gram-positive cocci. There was no statistical significance between the occurrence of particular flora and the multiplicity of operations in both CRS phenotypes.

Key words: chronic rhinosinusitis, bacteriology, asthma, allergy, drug hypersensitivity.

Introduction

Rhinosinusitis with nasal obstruction and/or nasal secretions and at least one of the other symptoms, such

as olfactory disorders or facial pain, lasting over 12 weeks with no complete resolution after maximum medical therapy is qualified as chronic, as detailed in the European Guidelines on Rhinosinusitis and Nasal Polyps 2012

Address for correspondence: Grażyna Stryjewska-Makuch, Department of Laryngology and Laryngological Oncology, Independent Public Research Hospital No. 7, the Silesian Medical University, Upper Silesian Medical Centre, 45/47 Złotowa St, 40-635 Katowice, Poland, phone: +48 32 359 80 00, e-mail: makuch_mg@wp.pl

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(EPOS 2012) [1]. Clinically, on the basis of an endoscopic examination, chronic rhinosinusitis (CRS) is divided into two phenotypes: with polyps (CRSwNP) and without polyps (CRSSNP).

CRS is one of the most widespread chronic diseases in the world, it affects 10.9% of the population in Europe [2] and 12.5% in the United States [3]. It is a systemic disease of unknown cause and complex pathomechanism, which is caused by many systemic and local factors such as allergy, asthma, hypersensitivity to drugs, immune deficiencies, mucociliary dysfunctions, biofilm formation, gastro-oesophageal reflux disease, anatomical variations, osteitis, as well as environmental factors, such as smoking, viral infections, fungal infections and bacterial infections [4–6].

The mechanical barrier, which is mucociliary clearance (MCC) in combination with innate immunity (macrophages and granulocytes), complement system and bactericidal proteins, such as lysozyme, lactoferrin, secretory leukocyte proteinase inhibitor (SLPI), β -defensin, cathelicidin and others, present in the mucus, are the first defence line of the respiratory epithelium upon contact with microorganisms [4, 7, 8]. Toll-like receptors, NOD receptors (nucleotide-binding oligomerization domain), helicases and bitter taste receptors after combining with the pathogen pattern and the stress factor trigger the second, specific line of immunological defence. M cells present in the mucosa of the respiratory tract catch macromolecules and even microorganisms and transport them through the cytoplasm. Next, they are collected by antigen-presenting cells (dendritic cells and macrophages). In the case of extracellular bacteria, the most important role is played by the humoral response and helper lymphocytes Th2 and Th17, and in the case of intracellular bacteria, the cellular response dominates with the participation of macrophages, cytotoxic lymphocytes and NK (natural killer) cells together with Th1 lymphocytes producing interferon- γ , interleukin 2, supporting this type of immunity [9].

Sinus microbiota dysbiosis can lead to an excessive Th2-type inflammatory response with weakening of the innate immunity response by reducing the expression of TLR9 receptor on the respiratory epithelium. In addition, epithelial cells under the influence of the pathogen secrete IL-25, IL-33 and TSLP, which promote a Th2-type response. Th2 lymphocytes produce IL-4, IL-5, IL-10, IL-13 which act as a B-cell growth and proliferation factor [4]. Under the influence of secreted interleukins, and especially IL-5, eosinophilic inflammation develops within the sinus mucosa, usually of considerable severity. A similar mechanism of inflammation has been observed in bronchial asthma, regardless of its phenotype, allergy and hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) [10]. Cope *et al.* [11] has found that the incidence of *Corynebacterium* on the sinus mucosa in patients with CRS is associated with an increased IL-5 gene expression

and a higher risk of developing polyps. Tomassen *et al.* [10] has identified the CRS endotype associated with *S. aureus* and its superantigens, stimulating the local production of IgE antibodies in the development of eosinophilic inflammation. These studies suggest a relationship between the pathomechanism of inflammation and the type of bacterial flora present on the sinus mucosa in chronic inflammation.

Aim

It was decided to compare the bacterial flora in patients with CRS, with and without polyps. Comorbidities such as allergy, asthma and NSAID hypersensitivity, dominated by Th2-biased eosinophilic inflammation, were selected and then it was investigated whether the types of isolated bacteria differ from the flora obtained from patients who did not have these comorbidities. The results from both phenotypes were compared. It was examined whether the type of bacterial flora in patients with CRS depends on the number of performed surgeries.

Material and methods

Study population

The authors conducted a retrospective study of the bacterial flora of the nasal cavity in patients with CRS with and without polyps, operated endoscopically from March 2016 to June 2017 at the Department of Laryngology and Laryngological Oncology in Katowice and at the Clinical Department of Otorhinolaryngology and Laryngological Oncology in Zabrze. The study group consisted of 470 patients, including 222 women and 248 men. The mean age of women was 49.75 ± 13.91 , and that of men – 50.63 ± 14.78 years. All patients were Caucasian.

Inclusion criteria

Patients who had CRS were qualified for surgery in accordance with the EPOS 2012 guidelines.

On the basis of an endoscopic examination, patients were qualified for the phenotype with polyps or without polyps. The size of the polyps was assessed on the Lund-Kennedy score.

Based on the medical interview, patients were divided into a group of first and repeated operations (the latter included patients who underwent endoscopic sinus surgery (ESS) or non-endoscopic surgery like polypectomy, Caldwell-Luc operation).

The extent of inflammatory lesions was assessed on the basis of computed tomography of the sinuses, performed in each patient, on the Lund-MacKay score. After the assessment of the extent of inflammatory lesions, ESS was planned. The surgery was performed under general anaesthesia with hypotension.

Demographic data as well as diseases co-existing with CRS such as asthma, IgE-dependent allergy and NSAID

hypersensitivity were taken into account. The bacterial flora of patients with CRSwNP and CRSsNP was analysed. In each phenotype, two groups of patients were distinguished, namely with allergy, NSAID hypersensitivity and bronchial asthma, and without these comorbidities.

The group of patients with allergy included patients with IgE-dependent allergies to seasonal (early and late blooming trees, grasses, weeds) and perennial airborne allergens (mites and mould spores, cat and dog allergens). Allergies were confirmed by a skin prick test or the presence of specific IgE antibodies in the patient's serum. The examinations were performed on an out-patient basis and correlated with clinical symptoms occurring in the patients. The patients were under constant care of an allergist who applied maximum medical therapy, some patients were also subjected to specific allergen immunotherapy. The patients with clinical symptoms and positive pollen allergy tests were operated outside the pollen season for specific allergens.

The second group included patients with NSAID hypersensitivity. The authors have adopted the term "hypersensitivity to NSAIDs" which is defined by the European Academy of Allergology and Clinical Immunology (EAACI) as objectively reproducible symptoms that are caused by exposure to a specific stimulus in a dose tolerated by other people. Clinical symptoms relate to the mucous membranes of the respiratory and digestive tract as well as the skin, and can be induced not only by aspirin, but also by one or more NSAIDs [12–14].

Patients, qualified to this group by the authors, who reported hypersensitivity to aspirin and other NSAIDs, had dyspnoea attacks, serous fluid discharge/nasal mucosa oedema or acute urticaria after taking the drugs. Aspirin tests were not performed in patients after their admission to the ward. It was the medical history describing the symptoms resulting from taking the drug(s) that played the crucial role.

Patients with bronchial asthma were diagnosed in the outpatient setting according to Global Initiative For Asthma (GINA) [15]. Chronic treatment in outpatient care was recommended by a specialist. The present (the last 4 weeks before the surgical treatment) level of asthma control (good, partial or bad) was important for the authors. In terms of the severity of asthma, assessed on the basis of recommended doses of inhalation drugs and the FEV1 index, patients with mild and moderately severe, well-controlled asthma were qualified for surgical treatment of the sinuses.

Bacteriological examination

Bacteriological and, in justified cases, also histopathological material was collected during ESS. The swabs for bacteriological examination were collected from the middle nasal meatus.

The material was collected with an applicator stick with the AMIES medium. For the growth of aerobic bacteria, the following media were used: the sheep blood

medium (Columbia agar) – Gram-positive bacteria, MacConkey medium (Gram-negative bacteria), Sabouraud medium (fungi growth), chocolate medium in the environment with increased carbon dioxide content (*Neisseria* and *Haemophilus* cultures). Anaerobic bacteria were propagated on Schaedler medium. Each culture was kept at 35–37°C for 24–48 h, except for fungi and bacteria on Schaedler medium, where cultivation was carried out for 7 days. During the cultivation, the material remaining on the applicator stick was stored for 7 days in the propagating medium (cardio-cerebral broth). Identification and drug susceptibility were assessed using the Vitek 2 compact.

The bacteria were divided into two groups: Gram-positive and Gram-negative. The Gram-positive cocci included *Staphylococcus aureus* (*S. aureus*) as well as *Staphylococcus epidermidis* (*S. epidermidis*), haemolyticus, hominis, lugdunensis, *Streptococcus oralis* (mitis), pyogenes, pneumoniae, *Enterococcus faecalis* and *faecium*, all combined in one group. Streptococci differ from staphylococci by the lack of ability to produce catalase, so they are described separately [16].

Gram-negative bacteria included: aerobic gram-negative cocci – the genera *Moraxella* and *Neisseria*, anaerobic Gram-negative bacilli like *Haemophilus influenzae*, as well as non-fermenting *Pseudomonas* and *Acinetobacter* (among others). The *Pseudomonas* species includes *P. aeruginosa*, *fluorescens* and *putida*.

An important group among Gram-negative bacteria were intestinal bacilli – *Enterobacteriaceae*. The following species were included here: *Citrobacter*, *Enterobacter aerogenes* and *cloacae*, *Escherichia*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Serratia* and *Raoultella planticola*.

Exclusion criteria

Patients under 18 years of age, rhinosinusitis exacerbation less than 4 weeks prior to the admission to hospital and the use of antibiotics locally (into the nasal cavity and sinuses) or systemically less than 4 weeks prior to the admission to hospital, partially controlled or uncontrolled bronchial asthma, symptomatic intermittent allergic rhinitis with a documented allergy to grass, tree or weed pollen during the pollen season, immunodeficiency, cystic fibrosis (CF) and those with tumours identified in the histopathological examination of the material collected from the sinuses were excluded from the study. Twelve patients in whom no bacterial growth was observed were excluded from the study, so only 458 patients were included in further analysis. Due to the fact that more than one bacterial strain was grown in some of the patients, 540 results were obtained.

Statistical analysis

The statistical analysis was made using Statistica (Dell Inc. (2016). Dell Statistica (data analysis software system), version 13. software.dell.com). The χ^2 test

was used to assess the relationship between variables. A p -value less than 0.05 was considered statistically significant.

Results

Patients

The study included 458 patients, 214 (46.7%) women and 244 (53.3%) men. Two hundred and thirteen (46.5%) patients had the CRSsNP phenotype. Among them, 35 (16.4%) had IgE-dependent allergy, 9 (4.2%) – NSAID hypersensitivity, 21 (9.9%) – bronchial asthma.

Two hundred and forty-five (53.5%) patients had the CRSwNP phenotype. Among them, 52 (21.2%) had IgE-dependent allergy, 44 (18%) – NSAID hypersensitivity, 83 (33.9%) had bronchial asthma.

One hundred and forty-six patients (31.9% of all) had more than one operation.

Bacteriological results

For statistical purposes, the bacteria were divided into the groups of Gram-positive cocci, where group 1 was *S. aureus*, group 2 – coagulase-negative staphylococci and streptococci. Group 3 was Gram-negative cocci and gram-negative bacilli and group 4 – Gram-negative intestinal bacilli.

A detailed description is given in Table 1.

In the group of CRSwNP patients, no statistically significant relationship was found between the observed bacterial flora and the presence of asthma ($p = 0.7393$), hypersensitivity to drugs ($p = 0.1509$) or allergy ($p = 0.7427$). The percentages of individual groups of bacteria were comparable regardless of the existence or absence of a comorbidity. In the group with CRSsNP, a statistically significant relationship was found between the observed flora and the coexistence of asthma ($p = 0.0409$). In the CRSsNP group with asthma, the group of coagulase-negative staphylococci and streptococci was the largest (33.33%). *S. aureus* and Gram-negative intestinal bacilli constituted the same percentage (29.17% in both groups). However, when asthma did not coexist with CRSsNP, Gram-negative intestinal bacilli were most common (41.36%), coagulase-negative staphylococci and streptococci constituted 33.18%, and *S. aureus* – 24.55%. A trend towards significance was also

noticed in the case of allergy ($p = 0.0947$). *S. aureus* was most common (40%) in the group of allergy patients, and intestinal bacilli (41.2%) in the group without allergies. There was no relationship between the flora and NSAID hypersensitivity ($p = 0.7356$).

There is no statistically significant relationship ($p = 0.8519$) between the phenotype and the observed bacterial flora in the reoperated patients. In patients operated on for the first time, this relationship is also statistically insignificant ($p = 0.4754$). Among these patients, *S. aureus* occurred in 24.7% of cases, *S. epidermidis et al.* in 34.1%, intestinal bacilli in 39.3%, and the remaining strains in 1.9%. Generally, there is no statistical significance between the occurrence of a particular flora and the multiplicity of operations in both the CRSwNP ($p = 0.4609$) and CRSsNP phenotype ($p = 0.2469$).

Discussion

The vast majority of Gram-positive cocci, especially *S. aureus* and coagulase-negative *Staphylococcus*, in both CRS phenotypes (Table 2), prompts consideration of the influence of these microorganisms on the inflammation process in the sinuses. It is difficult to agree with the suggestions presented in earlier publications [17–19] that this is only the effect of contamination. *S. aureus* and *epidermidis*, considered to be commensals on the surface of healthy skin, may cause serious opportunistic infections in other areas. The vast majority of studies describe staphylococcal infections within the skin, soft tissues and gastrointestinal tract [20–22]. However, more and more attention is paid to the importance of *S. aureus* in inflammatory processes within the sinus mucosa, revealing its colonization in at least 60% of patients with CRSwNP and 33% of patients with CRSsNP [23].

In phylogenetic terms, staphylococci are a very homogeneous group, strongly separated from other types. The division into coagulase-positive and negative staphylococci is rooted in the diagnosis. We now know that among the clinical strains of *S. aureus* considered coagulase-positive, there are also strains that do not show this feature [16]. The pathogenesis of *S. aureus* depends on many factors. These include pyrogenic exotoxins together with toxic shock syndrome toxin 1 (TSST1), staphy-

Table 1. Mucosal microbiome in chronic rhinosinusitis

| Type of bacteria | Number | % |
|--|--------|------|
| Group 1 (100% <i>S. aureus</i>) | 130 | 24.1 |
| Group 2 (67.2% <i>S. epidermidis</i>) | 177 | 32.8 |
| Group 3 (55.6% <i>Haemophilus influenzae</i>) | 18 | 3.3 |
| Group 4 (Gram-negative intestinal bacilli) | 215 | 39.8 |
| Total | 540 | 100 |

Table 2. Bacterial flora in patients with CRSwNP and CRSsNP

| Type of bacteria | Phenotype | | % |
|------------------|------------|------------|-------------|
| | CRSwNP | CRSsNP | |
| Group 1 | 57 (23.3%) | 54 (25.4%) | 130 (24.1%) |
| Group 2 | 79 (32.2%) | 72 (33.8%) | 177 (32.8%) |
| Group 3 | 10 (4.1%) | 3 (1.4%) | 18 (3.3%) |
| Group 4 | 99 (40.4%) | 84 (39.4%) | 215 (39.8%) |
| Total | 245 (100%) | 213 (100%) | 540 (100%) |

lococcal enterotoxins, aggresins, invasins, fibronectins or leucocidin, which destroys multinucleated leukocytes and macrophages. Haemolysins, hyaluronidases and lipases facilitate the spread of bacteria, whereas coagulase, clumping factor (CF) and protein A prevent opsonisation and phagocytosis [24]. Stimulation of the polypoid tissue with endotoxin B led to an increase in the cytokines of the Th2 pathway and suppression of T-regulatory lymphocytes, which sustains inflammation. Higher IgE, IgA and IgG levels were found in nasal polyp homogenates in patients with CRSwNP and *S. aureus* infection compared to patients with CRSsNP and the control group [23].

Staphylococcal superantigens (SAEs) are closely related to CRSwNP, especially in the group of patients with allergy, asthma, NSAID hypersensitivity and atopic dermatitis. In 100% of patients with aspirin exacerbated respiratory disease (AERD), specific IgE anti SAE [25] was found in polyp homogenates. In the case of the neutrophilic (non-eosinophilic) endotype of CRSwNP in Chinese population, the concentrations of staphylococcal superantigens and specific IgE are significantly lower than in the case of the eosinophilic endotype of rhinosinusitis, and mainly play the infectious role [26, 27].

Busaba *et al.* [17] just like the authors, did not find significant differences between the phenotypes when examining the bacterial flora of patients with CRSwNP and CRSsNP. The most frequently obtained coagulase-negative staphylococci (51%) were considered to be impurity. In a review study, Hamilos [28] reports that coagulase-negative staphylococci constituted up to 75% and the positive ones – about 50% in CRS in bacteriological examinations. It is suggested that the lack of tolerance towards colonizing bacteria might favour the pro-inflammatory action of commensal bacteria [4]. Biofilm production, mucociliary clearance dysfunction, and further epithelial damage are conducive to eosinophil infiltrations that counteract uncontrolled bacterial invasion. However, long-term stimulation with bacterial superantigens promotes the development of chronic Th2 and Th17 inflammation. Eosinophils fulfil a cytotoxic function in both innate and acquired immune responses. They can participate in antimicrobial defence releasing the so-called extracellular eosinophilic traps (EETs) capable of binding and killing bacteria both inside and outside human diseased mucosal tissue. Patients with CRSwNP show massive reactivity toward *S. aureus* exposure with extensive EET formation. *S. epidermidis* did not induce a significant, rapid increase in EET. However, an increase in TLSP production was observed approximately 2 h after stimulation, which suggests a delayed effect of *S. epidermidis* on the production of EETs [29]. Such observations may be evidence of the influence of coagulase-negative staphylococci on the development of inflammation in the sinus mucosa. The frequent presence of *S. epidermidis* in the authors' study, in the light of the cited publications, suggests its important role in CRS. *S. epidermidis* differs from

S. aureus by the lack of coagulase enzyme. *S. epidermidis* is an important commensal of the skin and mucous membranes, but under special circumstances it may cause chronic opportunistic infections associated with biofilm formation or acute infections like sepsis [30]. *S. epidermidis* owes its special pathogenicity to adhesive abilities dependent on the production of extracellular mucus (extracellular slime substance – ESS) and receptors for many extracellular matrix (ECM) proteins allowing for biofilm formation. Infections associated with the presence of biofilm are extremely resistant to antibiotic treatment due to reduced metabolism and impaired penetration through the extracellular matrix. In addition, *S. epidermidis* biofilms induce attenuation in phagocytic function and weaken the production of anti-inflammatory cytokines as compared to their planktonic form.

In the presented studies, apart from Gram-positive cocci, intestinal bacilli were another important group. A retrospective study of the sinus bacterial flora from the years 1975–2010 carried out by Thanasumpun [31] revealed similar results. Stern *et al.* [32] compared the bacterial flora in both CRS phenotypes stating that Gram-negative bacteria were most common in CRSwNP (in this case, *Citrobacter* spp. 17%). Similarly, the predominance of Gram-negative bacteria in chronic ethmoidal sinusitis is reported by Brook [33]. Tabet *et al.* [34] found that patients with CRSwNP colonized with Gram-negative bacteria (except *Pseudomonas aeruginosa*) showed a similar inflammation profile (Th2) as patients with Gram-positive bacteria, suggesting a mechanism via Toll-like receptor 4 (TLR4)-mediated interleukin 33 (IL-33) production.

Cleland *et al.* [35] just like the authors, did not find any differences between CRS phenotypes, stating at the same time that *S. aureus* was most frequent in reoperated patients. In the authors' study, in reoperated patients, *S. aureus* occurred in 22.7% of cases, *S. epidermidis* with other staphylococci and streptococci constituted 30.1%.

Recent studies show that in atopic dermatitis, *S. epidermidis* is the second, after *S. aureus*, important bacterium colonizing damaged skin in severe cases, which excludes the previously suggested antagonistic relationship between these microorganisms [30].

Polymicrobial biofilm containing *S. aureus* is closely related to persistent rhinosinusitis after endoscopic surgery and is a marker of severe inflammation and worse results obtained after ESS [28, 36, 37].

The results of the bacteriological examination obtained by the authors, revealing the vast majority of Gram-positive cocci, suggest that the bacterial agent might be one of the important elements of CRS inflammation, and the distinguished phenotypes are just one disease at various stages of remodelling. The assessment of the effect of bacterial antigens on inflammation in the mucous membrane of the sinuses and nasal cavity requires further investigation.

Conclusions

Gram-positive cocci were equally common in CRSwNP and CRSsNP and together accounted for more than half of the bacterial flora encountered. There was no statistically significant relationship between the bacterial flora of sinuses and the coexistence of asthma, allergy or NSAID hypersensitivity in the phenotype with polyps. In CRSsNP, this relationship was noted in patients with asthma, and the trend towards significance was noted in the case of allergies. There is no relationship between the flora and NSAID hypersensitivity. Patients after multiple operations showed sinus microbiota similar to that of patients who were operated for the first time.

Conflict of interest

The authors declare no conflict of interest.

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