Neuroendocrinology Letters Volume 40 Suppl. 1 2019
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Colonisation of Staphylococcus aureus in patients with Nasal Polyposis

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Submitted: 2019-04-18 Accepted: 2019-07-06 Published online: 2019-09-20

Key words: **Staphylococcus aureus; Nasal polyposis; Colonisation; patients**

Neuroendocrinol Lett 2019; **40**(Suppl.1):37–42 **PMID:** 31785225 NEL400119A10

Abstract

BACKGROUND: Nasal polyps (NPs) are one of the most common inflammatory mass lesions of the nose, affecting up to 0.5–4% of the population. The pathogenesis of NPs has been studied widely, but it is not clearly understood. A possible role of *S. aureus* in nasal polyposis has been suggested by numerous studies. This study aimed to map *S.aureus* colonisation in NP patients in the nose in comparison to healthy controls.

MATERIAL AND METHODS: We identified *Staphylococcus aureus* in nasal mucosal swab, collected from 58 patients with nasal polyposis from the out-patient ENT clinic of the Faculty Hospital in Nitra. We compared them to 50 patients without symptoms of nasal obstruction or NP. Isolated bacterial strains were then further identified.

RESULTS: In nasal mucosa membrane, results were not statistically significant. The selected population consisted of 108 patients, of which 58 (54%) had nasal polyps and 50 (46%) didnt. We collected the following information about patients from both groups: age, gender, smoker, presence of asthma, allergy and presence of *Staphylococcus aureus* by cultivation from nasal mucosa. In addition, for patients with nasal polyposis we have following variables, such as: presence inflammatory diseases, allergy to acylpyrine, cystic fibrosis. Out of 58 patients with nasal polyposis 15% (n=9) were found to have *S.aureus* in nasal mucosa membrane, compared to the healthy controls where 6% (n=3) of patients had *S. aureus*.

CONCLUSION: Our results did not show that *S. aureus* found in nasal mucosa membrane is significantly different in patients with or without NP. However, association of the presence of *S. aureus* in patients with nasal polyposis with asthma, allergy and inflammation has been shown.

INTRODUCTION

Nasal polyps (NPs) are whitish-gray mass-like lesions in the nose. They are outgrowth of nasal mucosa which are smooth, semi translucent, gelatinous and pale mainly situated in the middle meatus, originating from mucous membrane of the ostiomeatal complex, probably beacuse of release of proinflammatory cytokines from epithelial cells as a result of contact between two surfaces of mucosa at this narrow region (Rajguru 2014). NPs are often associated with chronic inflammation of the nose and paranasal, so called rhinosinusitis. According to the European Position Paper on Polyposis and Sinusitis (EPOS), rhinosinusitis can be categorized as acute, subacute, recurrent and chronic rhinosinusitis (CRS). The CRS can be subclassified as CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). CRS with and without nasal polyps differ from each other by several markers related to inflammation and tissue remodeling, the foundation of NPs is a chronic inflammatory and remodeling process of the nasal mucosa (Tantilipikorn *et al.* 2012). NPs usually cause rhinologic symptoms such as nasal blockage, anterior/posterior rhinorhea, and smell disturbance.

Nasal polyposis is a multifactorial condition which is often associated with many diseases and pathogenic disorders included allergy, astma, aspirin sensitivity, infection, cystic fibrosis, allergic fungal sinusitis a role of biofilm formation and the specific impact of superantigens of *Staphylococcus aureus* enterotoxins (SEs) as disease modifiers (Rajguru 2014; Tantilipikorn *et al.* 2012; Cheng et al 2017). Staphylococcal species are the most prevalent bacteria that have been isolated from the nasal mucus of white patients with NP. An increased colonization rate of *S. aureus* has been demonstrated in patients with NP (63.6%) but could not be demonstarted in patients with chronic rhinosinusitis without polyps (27.3%) as compared with control subjects (Pezato *et al.* 2014). Foreman *et al.* (2015) confirmed the hypothesis that biofilms would be present in patients with NP as a nidus from which planktonic *S. aureus* and superantigens are released into the paranasal sinuses.Another study by the North–American Centers for Disease Control and Prevention (CDC) has estimated that at least 65% of all chronic bacterial infections in human involve biofilms. Relevant organisms in otorhinolaryngological diseases, such as *Pseudomonas aeruginosa*, *Streptococcus pneumonie* and *Staphylococcus aureus*, have been shown to form biofilms, (Bezerra 2009). Some studies have suggested that chronic rhinosinusitis with nasal polyposis (CRSwNP) has a relationship with *S. aureus* infection and especially with staphylococcal enterotoxins as a modulator of the disease (Pezato *et al.* 2014; Thunberg 2017). The presence of *S. aureus* biofilms was associated with eosinophilic inflammation, across the spectrum of CRS on the back of T-helper2 skewing of the host's adaptive immune response (elevated ECP and IL-5). This effect could be distinguished from the superantigenic effect resulting in the induction of IgE (Tantilipikorn *et al.* 2012). The aim of this study was to investigate presence of *S. aureus* (SA) isolated from the nose of NP patients in comparison with the samples in healthy controls. The analysis was divided in two parts. In the first part, we examined the dependence of the presence of SA in the group (nasal polyps or no nasal polyps), age, gender, asthma, allergy, smoking conditions). In the second part, we examined the dependence of SA on a larger set of variables in a group of patients with nasal polyposis.

MATERIALS AND METHODS

Clinical bacterial strains

We identified a bacterial strain of *Staphylococcus aureus* with microbiologically proven presence in the clinical material – nasal mucosal swab, collected from 58 patients with nasal polyposis from the out-patient ENT clinic of the Faculty Hospital in Nitra and from 50 patients without symptoms of nasal obstruction without NP that were used as controls. Isolated bacterial strains were further identified at the Institute of Clinical Microbiology of the Faculty Hospital in Nitra using the following methods.

Nasal swab was collected by Stuart medium. In most cases, single swab was used for both nostrils unless the different condition is mentioned. The swab was carefully inserted along the nasal septum into the lower air passage firstly parallel to the nasal edge about 2–3 cm, then by turning it into the horizontal position and then sliding in helical movement into the air passage until resistance was met. The sampling should be massive. The delivered material should be sealed in the original package, stored at room temperature up to 4–6 hours, in exceptional cases at 4–8 ˚C up to 24 hours.

Cultures and identification of S.aureus

Isolated bacteria have been identified down to the species level on the basis of the biochemical properties of the strain using commercial biochemical tests or using special culture media. Species that commonly occur in the upper respiratory tract as a part of a human's physiological flora were identified by genus or species. They are usually not tested for antimicrobial susceptibility. We cultured *Staphylococcus aureus* on a conventional blood agar with 5% sheep erythrocytes, forming convex, round, shiny colonies that are yellow to golden-colored with a zone of marked beta-hemolysis around the colony. We also demonstrated isolates to be *S. aureus* on the basis of biochemical properties using commercially available tests (Staphy test; ERBA Lachema, Brno. Czech Rep.). By further tests for staphylococcus, we examined the cleavage of glycerol, sucrose, trehalose, mannitol, urea, acetoin formation (Voges-Proskauer test – producer or

citation), phosphatase (citation). For epidemiological purposes, phagotyping was performed (citation). Proof of deoxyribonuclease enables to distinguish *S. aureus* from other staphylococci (citation). A plasma coagulase assay was used to distinguish bacteria within the genus *Staphylococcus*. Plasmacoglucose is a protein with enzymatic activity converting soluble fibrinogen to solid fibrin. Formation of plasma coagulase is typical for pathogenic *Staphylococcus* species. We have inoculated the strain in the rabbit plasma. The coagulase-forming strains convert the liquid plasma into solid coagulum. Results were read after 2, 6, 18, 24 hours together with positive and negative control. Controls are necessary for cases where some strains produce staphylokinase (fibrinolysin) that dissolves fibrin, there is a risk of false negativities. Positive control is *S. aureus*, negative control is *S. epidermidis*, or plasma coagulase negative staphylococci (Henry & Isenberg 2004).

The obtained results were analysed by binary logistic regression in accordance with the aims of the study.

RESULTS

The selected population consisted of 108 patients, of which 58 (54%) had nasal polyps and 50 (46%) not. We have the following information about patients from both groups: age, gender (male, female), smoker (yes – no), asthma (yes – no), allergy (yes – no), SA (presence of *Staphylococcus aureus* by cultivation, yes - no).

In addition, for patients with nasal polyposis we know the values of the following variables: inflammatory diseases (yes - frequent inflammatory diseases, no – not the case), acylpyrine (yes – no allergy to acylpyrine), cystic fibrosis (yes - suffers from cystic fibrosis of the lungs, no).

1. Dependence of the presence of SA on selected factors

Patient groups examined have shown to be largely non-homogeneous (Table 1). While in the NP group there are 55% of men, in the non-NP group, there are only 24% men. In the NP patients group there is a significantly higher proportion of asthmatics, allergic patients and smokers – about a third, whereas in the group of patients without NP it is about three times less. Patients with NP are, on average, nearly six years older and their age exhibits greater variability (with NP: mean = 45.48, SD = 16.34, min = 16, max = 78; patients without NP: mean = 39.88; SD = 13.93; min = 18; max = 63).

Tab. 1. Variables in individual patient groups

Variable	Level	NP (N=58)	no NP (N=50)
gender	male	32 (55%)	12 (24%)
	female	26 (44%)	38 (76%)
SA	yes	9 (15%)	3 (6%)
	no	49 (84%)	47 (94%)
asthma	yes	18 (31%)	5 (10%)
	no	40 (68%)	45 (90%)
allergy	yes	20 (34%)	4 (8%)
	no	38 (65%)	46 (92%)
smoker	yes	22 (37%)	6 (12%)
	no	36 (62%)	44 (88%)

As the groups could not be considered homogenous based on several factors (it is questionable whether they are significant), the dependence of the presence of SA on these factors wasl be modelled on the basis of binary logistic regression model. Dependent variable was SA (0 – no, 1 – yes), independent variables: continuous variable age and binary variables group (0 – no NP, 1 – with NP), gender (0 – male, 1 – female), smoking (0 – no, 1 – yes), asthma (0 – no, 1 – yes), allergy (0 – no, 1 – yes). In case of categorical variables, the 0 category is reference.

The results of binary logistic regression showed that the model as a whole was not statistically significant ($\chi^2(6)=7{,}88$; $p=0{,}247$), i. e. the model was not statistically significantly better compared to a model with a constant. Table 2 shows the value of the regression coefficient for each variable, Wald test and odds ratio. The results of Cox and Snell and Nagelkerke R^2 show the model explains 7.0 to 14.0% of the total variability of the dependent variable. The results of Hosmer-

Tab. 2. Results of binary logistic regression

Variable	B	S.E.	Wald	df	Sig.	EXP (B)
Group(1)	0,69	0,82	0,70	1	0,403	1,99
Age	0,03	0,02	1,51	1	0,219	1,03
Gender(1)	0,91	0,86	1,11	1	0,292	2,47
Smoking(1)	0,59	0,81	0,53	1	0,468	1,80
Asthma(1)	0,63	0,73	0,75	1	0,385	1,88
Allergy(1)	0,84	0,70	1,43	1	0,232	2,30
Constant	-4,92	1,28	0,98	1	0,001	0,01

(B – estimate of regression parameter, S.E. –estimated SD of regression parameter, Wald – test statistics value, df – number of degrees of freedom, Sig. – p – value, Exp (B) - odds ratio with respect to reference category).

Tab. 3. Values of variables in the population of patients with nasal polyposis

Variable	Level	With NP (N=58)
acylpyrine	yes no	7 (12%) 51 (87%)
smoker	yes no	22 (37%) 36 (62%)
asthma	yes no	18 (31%) 40 (68%)
Inflammatory disease	yes no	14 (24%) 44 (75%)
allergy	yes no	20 (34%) 38 (65%)
gender	male female	32 (55%) 26 (44%)
Cystic fibrosis	yes no	1 (1%) 57 (98%)
SA	yes no	9 (15%) 49 (84%)

Lemeshow test show the model estimates the data well ($\chi^2(8)=10,83$; $p=0,212$).

The results of Wald test show all variables in the model are statistically insignificant ($p>0,05$). The results also show that the most important predictor for SA is the gender. In women, compared to men, three are a 2.47-times higher odds ($p=0,292$) for the presence of *Staphylococcus aureus*. In patients with allergy, there are 2.30-times higher odds for the presence of SA, in smokers, compared to non-smokers, 1.80-times higher odds for the presence of SA, in asthmatics, compared to non-asthmatics, 188-times higher odds for the presence of SA. An important finding is the patients with nasal polyposis, compared to the patients without NP, the odds are 1.99-higher ($p=0,403$) for the presence of SA. Regarding age, it appears that increasing it by 10 years, the odds for the presence of SA are increased 1.35-times ($p=0,219$).

2. Dependence of the presence of SA on selected factors in the group of patients with nasal polyposis

The procedure is the same as in the above case, so we only show the results. First, however, in Table 3 we present variables in the population of patients with nasal polyposis.

The results of binary logistic regression show that the model as a whole is not statistically significant ($\chi^2(8)=10,46$; $p=0.234$), i. e. the model is not statistically significantly better compared to a model with a constant. Table 4 shows for each variable the value of the regression coefficient, Wald test and odds ratio. The results of Cox and Snell and Nagelkerke R^2 show that the model explains 16.5% to 28.5% of the total variability of the dependent variable. The results of Hosmer-Lemeshow test show that the model estimates the data well ($\chi^2(8)=5,45$; $p=0,708$).

The results of Wald test show that all variables in the model are statistically insignificant ($p>0,05$). Patients with inflammatory diseases – compared to patients without inflammatory diseases – have 2.58-times higher odds for presence of SA, asthmatics vs. non-asthmatics have 1.78-times greater odds ratio for the presence of SA, smokers vs. non-smokers 1.47-times greater odds ratio for the presence of SA. The effect of age and gender is insignificant not only statistically but also practically.

DISCUSSION

Nasal polyposis is a multifactorial disease considered to be a subgroup of chronic rhinosinusitis. The pathogenesis of NP is not yet fully elucidated but, based on multiple studies, it is associated with allergies, infections, asthma, cystic fibrosis, inflammation, acetylsalicylic acid hypersensitivity, various anatomical abnormalities and changes, bacterial superantigens, fungi and bacteria present in biofilm (Rajguru 2014; Cheng et al 2017; Weschta 2003; Okano 2014). In the normal population, the incidence of NPs is about 4%, in asthma patients it is reported at between 7 and 15% (Hedman *et al.* 1999). A prevalence of nasal polyposis of 36-60% is described in patients with an acetylsalicylic acid hypersensitivity

Tab. 4. Results of binary logistic regression

Variable	B	S.E.	Wald	df	Sig.	EXP (B)
Age	-0,018	0,026	,450	1	0,313	0,982
Gender(1)	0,078	1,000	,006	1	0,502	1,081
Smoking(1)	0,384	0,971	,156	1	0,938	1,468
Asthma(1)	0,581	1,029	,319	1	0,693	1,788
Inflammation(1)	0,950	1,127	,710	1	0,572	2,585
Allergy (1)	-18,058	2860,886	,000	1	0,399	1,44E-08
Acylp.allergy(1)	19,514	2860,887	,000	1	0,995	2,99E+08
Constant	-1,584	1,569	1,337	1	0,995	0,205

(Bachert 2006). Several studies associate nasal polyposis with eosinophilic inflammation associated with enterotoxins produced by *Staphylococcus aureus* (SAE) (Bachert 2001). Specific IgE antibodies, which are indicators of the local immune response to classical enterotoxins produced by *S. aureus* (enterotoxins A and B), were found in 50-90% of patients with nasal polyposis (Bachert 2001; Van Zele 2004]. *S. aureus* enterotoxin-specific antibody NP tissue samples exhibit high concentrations of total IgE tissue antibodies and more pronounced eosinophilic inflammation with higher concentrations of eosinophilic cationic protein, IL-5 and eotaxin as compared to *S. aureus* enterotoxin-negative samples (Bachert 2001; Van Zele 2004; Zhang 2005). It has also been shown that 70% of *S. aureus* isolates can produce enterotoxin. These enterotoxins can act as superantigens with strong immunostimulatory properties and with the potential to significantly affect inflammation and lead to polyclonal activation of lymphocytes and secondary immunoglobulin synthesis (Bachert 2009; Bachert 2015). *S. aureus* enterotoxin (SAEs) induced massive inflammatory reaction results from a polyclonal activation of T and B lymphocytes, independent of a specific adaptive immune response enabling to call them superantigens, as first described by Marrack and Kappler in 1990 (Tomassen *et al.* 2011).

Several studies have shown *S. aureus* to be the most common bacterial pathogen in CRSwNP (from 33 to 63.6%), but not in CRSsNP (20–33.3%) or control patients (10–33.3%); leading to the development of the 'staphylococcal superantigen hypothesis,' which proposes that colonizing *S. aureus* superantigenic toxins (SAgs) not just amplify local eosinophilic inflammation but foster polyp formation, too. Moreover, these studies have indicated that the isolation rates for *S. aureus* were greatest in CRSwNP patients with concomitant asthma (Liu *et al.* 2014). Cui *et al.* measured the serum levels of total IgE, specific IgE to SEA, SEB and SEC, and eosinophil cationic protein (ECP) using immuno CAP assays. The positive rate and level of serum specific IgE to SEB, but not to SEA or SEC, were significantly higher in CRSwNP patients compared with the controls (Cui *et al.* 2015).

Another study describes the detection of SA within nasal tissue using the PNA-FISH technique. The presence of SA in the submucosa did not correlate with the amplification of the Th2-related inflammation typically found in CRSwNP patients, but this reaction is dependent on the formation of SAE-IgE within mucosal tissue. This study showed, for the first time, that submucosal SA is a prevalent finding in CRSwNP patients with aspirin exacerbated respiratory disease (AERD) (Corriveau 2009).

Liu *et al.* did not detect *S. aureus* as the most predominant bacterium in the CRSwNP specimens and there were no significant differences between the three patient groups. Results suggest that the bacteriologic profile in the nasal middle meatus is not significantly different in Chinese CRSwNP and CRSsNP patients or control subjects; however, *S. aureus*, *Streptococcus*, *Haemophilus*, *Enterobacter*, and *Corynebacterium* appear to be more frequently associated with CRSwNP patients than CRSsNP patients or control subjects. Furthermore, the isolation rates of *S. aureus* appear to be much lower in Chinese CRSwNP patients compared with their caucasian counterparts (Liu *et al.* 2014). Sachse demonstrated *S. aureus* invasion of the nasal epithelium and submucosa in CRSwNP by PNA-FISH. There was found increased synthesis of the TH-2 cytokine IL-6 but not of IL-13 in cell culture supernatants of infected NPECs. These IL-6 concentrations were as high as induced by extracellular stimulation with staphylococcal supernatants which did not contain any bacterial cells. Thus, regardless of intra- or extracellular presence, *S. aureus* exerted immunomodulatory effects as demonstrated by IL-6 synthesis *in vitro*. As *S. aureus* was detected in CRSwNP by PNA-FISH, its immunomodulatory effects may potentially contribute to the TH-2 cytokine pattern in CRSwNP via IL-6 induction (Sachse 2010).

In our study, we observed the nasal mucosa *S. aureus* settlement of NP patients and compared them with the colonization of healthy individuals without NPs. Our results indicate that nasal mucosa settlement by *S. aureus* from a given set of patients with nasal polyposis showed no significant difference compared to healthy non-NP patients, but association of the presence of SA in patients with nasal polyposis with asthma, allergy and inflammation has been shown.

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