

## Antimicrobial peptides in nasal secretion and mucosa with respect to *Staphylococcus aureus* colonization in chronic rhinosinusitis with nasal polyps\*

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### SUMMARY

**Objective:** Nasal carriage of *Staphylococcus aureus* in patients with chronic rhinosinusitis with nasal polyps (NP) is hypothesized to have pathophysiological impact on the disease. Antimicrobial peptides (AMP), especially human beta-defensin-3 (hBD-3) and LL-37, are an important part of the multifactorial defence against microorganisms in barrier organs like the nasal mucosa. The interaction of *S. aureus* colonization and AMP in nasal secretions and mucosa of NP were investigated in this study.

**Patients and Methods:** AMP were quantified in nasal secretions of 13 normal controls (NC) and 12 NP patients, each with and without *S. aureus* colonization, by ELISA. Immunohistochemistry was used to investigate the cellular sources of AMP in the nasal mucosa. To explore the AMP response of primary nasal epithelial cell cultures (NEC) towards *S. aureus* stimulation, a functional assay was established.

**Results:** AMP could be demonstrated in nasal secretions of all groups without differences in hBD-3 concentrations comparing *S. aureus* carriers vs. non-carriers. In NC, higher LL-37 concentrations were observed in *S. aureus* colonized as compared to non-colonized patients. This effect was not detectable in NP patients. Epithelial cells, submucosal glands and cells of the connective tissue could be identified as sources of AMP by immunohistochemistry. An AMP response of NEC towards *S. aureus* stimulation was detected in all groups.

**Conclusion:** In NP patients, LL-37 response towards *S. aureus* colonization is disturbed while the ability of NEC to respond on *S. aureus* challenge is preserved. This deregulation of the nasal barrier could be involved in the multifactorial pathophysiology of NP.

**Key words:** chronic rhinosinusitis, nasal polyps, *Staphylococcus aureus*, antimicrobial peptides, hBD-3, LL-37

### INTRODUCTION

Chronic rhinosinusitis with nasal polyps (NP) is an inflammatory disease of the nose and the paranasal sinuses with polyps as oedematous grape-like structures, infiltrated with inflammatory cells (mainly eosinophils), which have a strong tendency to recur after surgery<sup>(1-3)</sup>. An association of NP, asthma bronchiale and acetylsalicylic acid (ASA) sensitivity can be observed (Samter's triad), but the pathophysiology of NP is not well understood. Multiple

host factors as well as environmental factors are suspected of triggering the inflammatory reaction<sup>(4-7)</sup>. The current therapy of NP bases on surgical intervention and topical intra-nasal as well as systemic immunomodulating therapy<sup>(1)</sup>.

Especially *Staphylococcus (S.) aureus* colonization of the upper airways seems to be one important external trigger. Nasal carriage of *S. aureus* occurs in up to 70% of patients with NP, whereas

*List of abbreviations:* AMP: antimicrobial peptide(s); ASA: acetylsalicylic acid; BSA: bovine serum albumine; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; NC: normal controls; NEC: primary nasal epithelial cell cultures; ELISA: enzyme-linked immunosorbent assay; hBD-3: human beta-defensin-3; IgA: immunoglobulin A; IL: interleukin; LL-37: human cathelicidin; NP: patients with chronic rhinosinusitis with nasal polyps; Psoriasis: S100 A7; RNase-7: ribonuclease 7; RT-PCR: real-time polymerase chain reaction; SD: standard deviation; *S. aureus*: *Staphylococcus aureus*; TBS: Tris-buffered saline; TMB: tetramethylbenzidine; TNF: tumor necrosis factor; WBC: white blood cell count

only 25% of normal controls (NC) are carriers<sup>(8,9)</sup>. Furthermore exotoxins, secreted by *S. aureus*, have the ability to act as superantigens and trigger inflammatory infiltrates characteristic for NP. The organization of secondary lymphoid tissue with polyclonal B-cell activation correlates with the detection of specific IgE antibodies to *S. aureus*-toxins (especially staphylococcal enterotoxin B and toxic shock syndrome toxin 1), located in NP tissue<sup>(3,8,10-13)</sup>. Although *S. aureus* is classified as an extracellular pathogen, it could be detected within nasal epithelial cells and mucous glands of patients with recurrent *S. aureus* rhinosinusitis. This intracellular location affords it to escape from extracellular host defence mechanisms and most commonly used antibiotics, such as  $\beta$ -lactams<sup>(14)</sup>.

The epithelium of the respiratory tract is the first barrier for potential harmful inhaled particles and consists of a mechanical part (epithelial layer, mucociliary clearance) assisted by cellular (neutrophils, macrophages, dendritic cells) and chemical components (secretory IgA, lysozyme, lactoferrin, antimicrobial peptides [AMP])<sup>(15)</sup>. Defects in this barrier can promote antigen passage and processing across the nasal epithelium leading to chronic inflammation<sup>(16)</sup>.

Human beta-defensin-3 (hBD-3) and the only human cathelicidin LL-37 are AMP which exhibit a broad spectrum of antimicrobial activity against *Candida albicans*, enveloped viruses and Gram-negative as well as Gram-positive bacteria like *S. aureus*<sup>(17-21)</sup>. Furthermore they are involved in immunomodulatory processes e.g. chemotactic activity for monocytes and CCR-6 expressing cells like dendritic- and memory T cells, influence on proliferation, release of cytokines, homeostasis, wound healing and balance of proteases and proteases inhibitors. They both are inducible by inflammatory stimuli, such as cytokines (IL-6, TNF- $\alpha$ , IFN- $\gamma$ ) and direct contact with bacteria.<sup>(20,22-28)</sup> HBD-3 and LL-37 are present in barriers like the gastrointestinal tract, skin and airway. Recently, AMP of the major groups (beta-defensins, cathelicidin, psoriasin and RNase 7) could be demonstrated in nasal secretions of healthy volunteers, and epithelial cells, inflammatory cells in subepithelial layers and submucosal glands could be identified as cellular sources by our group<sup>(20,29,30)</sup>.

In this study, we hypothesized a barrier dysfunction of the nasal mucosa caused by aberrances of hBD-3 and LL-37 expression in NP patients with impact on nasal *S. aureus* colonization. Therefore, hBD-3 and LL-37 in nasal secretions of NP patients and NC were quantified by Enzyme Linked Immunosorbent Assay (ELISA). Furthermore, to localize these AMP in corresponding nasal mucosa biopsies, immunohistochemistry was performed. An *ex vivo* stimulation assay was established to determine induction and secretion of these AMP after stimulation of nasal epithelial cells with *S. aureus*.

## PATIENTS AND METHODS

### Patients

Twelve NP patients (4 women, 8 men, mean age 42 years) and 13 NC (4 women, 9 men, mean age 41 years) were included in

this study. In 6 NP patients and 6 NC, endonasal colonization with *S. aureus* was detected. Signs for systemic inflammation were analysed by C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell count (WBC). All NP patients were biopsy proven and in 7 biopsies an eosinophilia was observed. For more details on Asthma and ASA intolerance see patient characteristics Table 1. Data were surveyed between February 2008 and November 2008. Pregnancy and underage as well as haemostatic disorders were exclusion criteria. All participants gave written informed consent and the ethics committee of the University of Kiel, Germany approved the study (AZ A101/07).

### Nasal secretion

Both anterior nostrils were swabbed for 10 seconds with a sterile tip following an internal standard operational procedure. After transferring the tip into a sterile tube, it was incubated for 45 minutes at room temperature under rotation in 500  $\mu$ L of 10 mM sodium phosphate buffer containing 0.1% bovine serum albumin (BSA) and was therefore highly diluted. An aliquot of this solution was preserved at  $-80^{\circ}\text{C}$  until used for ELISA.

### Bacterial culture

To quantify *S. aureus* colonization, 100  $\mu$ l of fresh swab supernatant described above was plated onto Chapman agar. The swab tip was incubated in tryptic soy broth over night at  $37^{\circ}\text{C}$  and the resulting bacterial growth was isolated on sheep blood agar and Chapman agar. Isolates were identified as *S. aureus* by typical appearance of the colonies, haemolysis, expression of clumping factor and protein A, the ability to coagulate citrate plasma, and by acid production from mannitol fermentation.

### Primary nasal epithelial cell cultures (NEC)

Biopsy specimens of the turbinates were taken from a subset of 5 NP patients and 5 NC. NEC were generated using the dispase method (Invitrogen, Karlsruhe, Germany) and cultured in Airway Epithelial Cell growth medium (Promocell, Heidelberg, Germany) under humidified atmosphere of 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ . Homogeneity of NEC and origin of epithelial cells was verified by phase contrast microscopy and exemplary comparison of the cytokeratin profile of biopsies and NEC. The experiments were performed with preconfluent monolayers in 96 well plates (Sarstedt).

### Bacterial stimulation of NEC

A clinical nasal *S. aureus* isolate being described as predominant in Western Europe (T190-2, kindly provided by B.M. Bröker, University of Greifswald, Germany) was used in stimulation experiments<sup>(31)</sup>. After growing the strain overnight at  $37^{\circ}\text{C}$  in tryptic soy (approximately  $5 \times 10^8$  bacteria per ml) the suspension was centrifuged (15 min, 4000 g) and the bacterial supernatant was sterile filtered. Inverted-phase microscopy was used to examine NEC morphology prior to stimulation. To perform the stimulation experiments, the medium was

changed and fresh medium containing bacterial supernatant in a final dilution of 1:5 was added. NEC incubated in fresh culture medium without bacterial supernatant served as negative control. The stimulation was performed for 16 hours. Subsequently, cell culture supernatants were collected and stored at  $-80^{\circ}\text{C}$ . Dose and time dependent stimulation had been tested in preliminary investigations (data not shown). Cell viability after stimulation was checked by trypan blue dye exclusion, and cell morphology was examined microscopically. To prove the absence of bacterial contamination 10  $\mu\text{L}$  of cell culture supernatant was incubated on sheep blood agar at  $37^{\circ}\text{C}$  overnight.

#### ELISA

Nasal secretions and supernatants of stimulated NEC were analysed by sandwich ELISA for hBD-3 and LL-37 in duplicate. For hBD-3 96-well immunoplates (MaxiSorp, Nunc, Roskilde, Denmark) were coated with polyclonal rabbit antibody to hBD-3 (0.6  $\mu\text{g}/\text{ml}$ , Acris, Herford, Germany) overnight. Subsequently, nasal secretions and recombinant hBD-3 (PeptoTech, Hamburg, Germany) as quantification standard were incubated for 2 hours. A biotinylated polyclonal rabbit antibody to hBD-3 (0.25  $\mu\text{g}/\text{ml}$ , Acris, Herford, Germany) was used as detection antibody and incubated for 1 hour. Streptavidin-HRP (R&D Systems, Wiesbaden, Germany; 1:200 in phosphate buffered saline (PBS) + 0.1% BSA) was incubated for 30 minutes. 3,3', 5,5' tetramethylbenzidine (TMB; BD, San Diego, USA) was used as substrate and after 15 minutes the absorbance was measured with a microplate reader (Dynatech MR 5000, Virginia, USA) at 450 nm (reference wavelength 550 nm) according to the manufacturer's instructions. For LL-37, polyclonal rabbit anti-LL-37 (0.5  $\mu\text{g}/\text{ml}$ , Innovagen, Lund, Sweden) was used as primary antibody and biotinylated polyclonal rabbit anti-LL-37 (0.25  $\mu\text{g}/\text{ml}$ , Innovagen, Lund, Sweden) served as detection antibody. As quantification standard human recombinant LL-37 (Innovagen, Lund, Sweden) was used.

#### Immunohistochemistry

Four  $\mu\text{m}$  thick formalin-fixed, paraffin embedded sections of the biopsy specimens were used for immunohistochemistry. The slides were deparaffinised and rehydrated through graded alcohol and then microwave treated in 0.01 M citrate buffer (pH 6.0) for antigen retrieval. After incubation with 0.3% peroxidase/methanol for 10 minutes to block endogenous peroxidase and 3% normal swine serum (Vector, Burlingame, CA, USA) for 15 minutes to avoid non-specific bindings, the slides were incubated with an affinity purified polyclonal rabbit antibody to hBD-3 (5  $\mu\text{g}/\text{ml}$ , Acris, Herford, Germany) for 1 hour at room temperature or with polyclonal rabbit anti-LL-37 (6  $\mu\text{g}/\text{ml}$ , Innovagen, Lund, Sweden). After washing with tris-buffered saline (TBS) they were incubated with biotinylated polyclonal swine anti-rabbit immunoglobulin (4.4  $\mu\text{g}/\text{ml}$ , DAKO, Glostrup, Denmark) for 30 minutes at room temperature. They were rinsed in TBS and incubated with streptavidin-peroxidase reagent 'Vector ABC kit' (Vector, Burlingame, CA,

USA) for 30 minutes. 'Vector NovaRed substrate kit' (Vector, Burlingame, CA, USA) was used to detect the activity of the peroxidase and haematoxylin for counterstaining. Tonsil biopsies served as a positive control as described earlier<sup>(20,32)</sup>. For negative controls the primary antibody was omitted.

#### Statistical analysis

Statistical analysis was performed using SPSS statistical software for windows, 17.0 (SPSS Inc., Chicago, IL, USA). Data were described as mean values,  $\pm$  standard deviation (SD), maximum and minimum.

This exploratory study is based on a limited number of cases in the investigated groups. Therefore the following statistical tests were applied: Although we found no evidence against normal distribution in our data (Kolmogorov-Smirnov-Test), the Mann-Whitney-Test was used to compare concentrations of AMP in nasal secretions between NC and NP (with / without *S. aureus* colonization). Because of small sample sizes ( $n = 5$ ) and very homogenous results we used t-test statistics for the evaluation of stimulation data in LL-37 and hBD-3. As a level of significance we used  $p \leq 0.05$  for all statistical tests.

## RESULTS

#### HBD-3 and LL-37 in nasal secretions

No statistically significant differences in the concentrations of hBD-3 between the groups (NC mean 2.70, SD 5.43, maximum 19.77, minimum 0 and NP mean 0.94, SD 1.89, maximum 5.96, minimum 0 ng/ml;  $p = 0.295$ ) could be detected. As well for LL-37 no difference was detectable (NC mean 17.74, SD 19.04, maximum 61.87, minimum 0 and NP mean 16.37, SD 15.17, maximum 42.79, minimum 0 ng/ml;  $p = 0.852$ ).

No statistical difference in the concentrations of hBD-3 between *S. aureus*-colonized and non-colonized NC (colonized mean 1.04, SD 1.71, maximum 4.04, minimum 0 and non-colonized mean 4.12, SD 7.17, maximum 19.77, minimum 0;  $p = 0.366$ ) and NP patients (colonized mean 1.73, SD 2.50, maximum 5.96, minimum 0 and non-colonized mean 0.15, SD 0.36, maximum 0.88, minimum 0;  $p = 0.310$ ) was found. In contrast, the concentrations of LL-37 were significantly higher in *S. aureus*-colonized than non-colonized NC (colonized mean 31.06, SD 20.36, maximum 61.87, minimum 5.44 and non-colonized mean 6.33, SD 7.11, maximum 20.27, minimum 0;  $p = 0.014$ ). Remarkably, in NP patients no effect of *S. aureus* colonization on the expression of LL-37 could be detected (colonized mean 21.22, SD 16.43, maximum 42.79, minimum 1.87 and non-colonized mean 11.51, SD 13.40, maximum 31.15, minimum 0;  $p = 0.180$ , Figure 1).

#### Immunohistochemistry for hBD-3 and LL-37

All nasal biopsies of NP patients as well as NC showed a positive staining for hBD-3 and LL-37. HBD-3 could be observed in the epithelium and submucosal glands (Figure 2a). Positive staining for LL-37 was found in the epithelial cell layer, submucosal glands and some cells of the connective tissue appearing like granulocytes (Figure 2b).

Table 1. Patient characteristics.

	NC (n = 13)	NP (n = 12)
mean age in years (range)	41 (19 - 68)	42 (18 - 58)
sex	4 female, 9 male	4 female, 8 male
<i>S. aureus</i> colonization (%)	6 (46)	6 (50)
mean CRP in mgdl-1 (SD)	1.3 (0.8)	3.4 (5.1)
mean ESR in mm after the first hour (SD)	7.3 (4.4)	9.3 (3.8)
mean WBC in nl-1 (SD)	6.9 (1.7)	6.1 (1.8)
Asthma* (%)	1 (8)	2 (17)
ASA sensitivity* (%)	2 (15)	1 (8)
Allergies* (%)	3 (23)	5 (42)

NC: normal controls, NP: patients with chronic rhinosinusitis with nasal polyps, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, WBC: white blood cell count, ASA: acetylsalicylic acid, \* anamnestic data.

#### Stimulation of NEC

NEC of NC, stimulated with *S. aureus* culture supernatants, secreted significant higher amounts of hBD-3 (mean 100.73, SD 4.56, maximum 107.38, minimum 95.04) and LL-37 (mean 39.74, SD 22.31, maximum 52.19, minimum 0) than without stimulation (hBD-3 mean 1.34, SD 0.17, maximum 1.63, minimum 1.16 ng/ml;  $p < 0.001$  and LL-37 mean 0.8, SD 0.73, maximum 1.95, minimum 0 ng/ml;  $p = 0.017$ , Figure 3).

The same effect could be observed for NP-NEC with stimulation (hBD-3 mean 78.64, SD 41.58, maximum 103.21, minimum 5.63 ng/ml; LL-37 mean 37.91, SD 21.83, maximum 54.45, minimum 0 ng/ml) and without stimulation (hBD-3 mean 2.28, SD 1.52, maximum 4.91, minimum 1.16 ng/ml;  $p = 0.015$  and LL-37 mean 0.25, SD 0.46, maximum 1.06, minimum 0 ng/ml;  $p = 0.018$ , Figure 3). No statistically significant differences in increase of hBD-3 ( $p = 0.269$ ) and LL-37 ( $p = 0.924$ ) levels between NC and NP patients were detectable.

#### DISCUSSION

Protection of the host against potentially threatening microorganisms is mediated by multiple defence mechanisms. An efficient interaction of all parts of this defence shield is essential<sup>(17)</sup>. Chemical components of the nasal secretion like AMP are one of the first effectors.

By ELISA, hBD-3 could be quantified in nasal secretions in NC as well as in NP patients. For hBD-3 an induction via bacterial challenge, cytokines and calcium is described<sup>(20,33)</sup>, but no such effect of nasal *S. aureus* colonization on hBD-3 concen-

trations in nasal secretions was detectable in this study. This is in line with previous reports describing an absent correlation of nasal *S. aureus* colonization and hBD-3 concentration in nasal secretion in Wegener's Granulomatosis and healthy controls<sup>(34)</sup>. It might be that other factors preponderance in inducing hBD-3 or different *S. aureus* strains elicit variable mRNA expression of hBD-3 in epithelial cells as demonstrated by Quinn et al. for *S. aureus* strains of carriers and non-carriers<sup>(35)</sup>.

In previous reports, LL-37 was detectable in nasal secretions of healthy volunteers by western blot and ELISA in different concentrations<sup>(30,36,37)</sup>. These results could be verified in this study, in which LL-37 was detected by ELISA in nasal secretions of NC and NP patients.

Yeasts and Gram-positive and -negative bacteria induce LL-37<sup>(26,28)</sup>. Interestingly, this effect indeed applied for *S. aureus* carrying NC in this study and already was shown for patients with Wegener's Granulomatosis, where colonized patients showed higher concentrations of LL-37 compared to non-colonized patients<sup>(34)</sup>. Contrary to our expectations, no effect of *S. aureus* colonization was detectable for NP patients. Recently, intracellular survival and replication of *S. aureus* and especially small-colony variants of *S. aureus* is observed for nasal polyp epithelial cells (NEPC) and causes IL-6 secretion. TH-2 (IL-6) cytokine concentration was detected elevated equally in NEPC stimulated with *S. aureus* supernatant and for infected NEPC. Thus *S. aureus* leads to immunomodulatory effects regardless of an intra- or extracellular presence<sup>(38)</sup>. Analogue AMP induction should be detectable even if a disease relevant *S. aureus* infection of epithelial cells might be speculated for NP. A potential reason for the absent LL-37 induction in NP might be a disorder in the complex network of stimulatory and inhibitory effects on LL-37 in the nasal mucosa of NP patients. This might cause higher colonization rates and a perpetuation of inflammatory-triggering factors with effect on tolerance and defence and chronic inflammation as described for other diseases with barrier dysfunction [e.g. inflammatory bowel diseases, atopic dermatitis and cystic fibrosis]<sup>(23,39-41)</sup>.

Antimicrobial activity of AMP *in vitro* is found at concentrations of 1-10 µg/ml<sup>(17,42)</sup>. In this study, the detected concentrations in nasal secretions were lower, but surprisingly higher than in washing fluids of healthy skin<sup>(43)</sup> and comparable to concentrations verified in nasal secretions of healthy volunteers<sup>(30)</sup>. Concentrations on mucosal surfaces are not directly measurable and the concentrations in nasal secretions might underestimate the actual AMP content<sup>(37)</sup>. In this study, the nasal secretion was highly diluted in 500 µl sodium phosphate buffer and therefore the detected concentrations might reach physiological relevant antimicrobial concentrations. In addition, synergistic and additive AMP effects could lead to antimicrobial effects in even lower concentrations<sup>(44)</sup>. Therefore, it is likely that disturbed LL-37 and hBD-3 concentrations in nasal secretions have an important impact on the barrier function of the nasal mucosa.

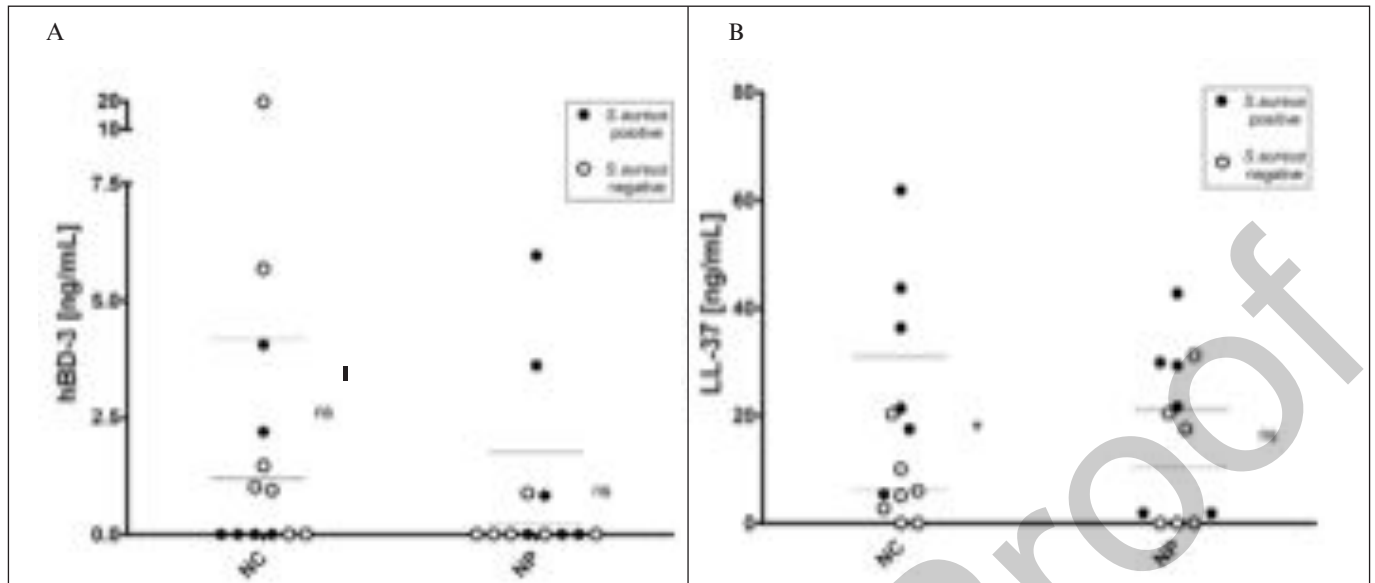


Figure 1. Effect of *S. aureus* colonization (bars indicate mean of colonized [solid line] and mean of non-colonized individuals [dashed line], \*  $p < 0.05$ , ns: not significant).

A: hBD-3 concentrations of nasal secretions from normal controls (NC,  $n = 13$ ,  $p = 0.366$ ) and patients with chronic rhinosinusitis with nasal polyps (NP,  $n = 12$ ,  $p = 0.310$ ).

B: LL-37 concentrations in nasal secretions from NC ( $n = 13$ ,  $p = 0.014$ ) and NP ( $n = 12$ ,  $p = 0.180$ ).

Different cell types, as epithelial cells and granulocytes, were identified to be able to synthesize AMP<sup>(20,21,29)</sup>. HBD-3 could be localized in trachea, tonsil, nasal mucosa and skin in patients with NP and NC<sup>(20,30,45)</sup>. In nasal mucosa, LL-37 could be detected in epithelial cells, submucosal glands and cells of the connective tissue<sup>(25,27,29,30,37)</sup>.

In this study, these results could be verified for NP and NC without detection of any difference between the investigated groups nor colonized / non-colonized participants. Intriguingly AMP could be detected in all samples by immunohistochemistry in contrast to the differentially expression pattern in corresponding nasal secretions which is in line with the literature<sup>(30,34)</sup>.

Epithelial cells are one important part of AMP secretion in nasal mucosa of NC and NP, which is confirmed by the results of the immunohistochemistry and results of the stimulation of primary nasal epithelial cells with *S. aureus* supernatant.

In both groups, *S. aureus* strongly induced AMP in NEC. This effect could also be confirmed in nasal secretions of NC for LL-37 (comparing participants colonized / not-colonized with *S. aureus*) but interestingly not in NP patients as mentioned above. These conflicting results in NEC-stimulation compared to nasal secretions of colonized / not-colonized participants may be the result of modulatory effects in the complex network of defence in nasal mucosa (e.g. auto- / paracrine influence of all cells involved [e.g. granulocytes, macrophages, monocytes, lymphocytes and their local equivalents]) in comparison to the relatively elementary study set up in NEC stimulation (with only additional autocrine stimulation / inhibition of cells). This complex interplay seems to be disturbed in NP concern-

ing LL-37 despite preserved ability to respond to *S. aureus* challenge of the epithelial barrier in NP. AMP response was also not detectable in nasal secretions of *S. aureus* carriers for hBD-3 in NC and NP, although there was a response in NEC stimulation. This also suggests additional mechanisms of inhibition and induction of hBD-3, whereas *S. aureus* remains an important part of the stimulation.

The delicately regulated defence shield of the nasal mucosa therefore seems to be specifically dysregulated and not entirely diminished concerning AMP in NP patients. Careful influence on the involved cell types by stimulation and inhibition [e.g. stimulation of epithelial cells to AMP-secretion by bacterial products or colonization with harmless microorganisms<sup>(46)</sup>] besides locally administered AMP might be new therapeutic options that have to be verified in further studies. Influence on the composition of the nasal commensals by probiotics may be another therapeutic alternative<sup>(47)</sup>.

## CONCLUSION

HBD-3 and LL-37 could be quantified by ELISA in nasal secretions in NP and NC. *S. aureus* colonization had no influence on the concentrations of hBD-3 in nasal secretions. For LL-37 higher concentrations could be observed in *S. aureus* carrying NC but not in NP, which suggests a disturbed immune response in NP patients and might be relevant for new therapeutic strategies. Immunohistochemical staining of nasal biopsies of NP patients and NC demonstrated epithelial cells and submucosal glands as sources of hBD-3. For LL-37 additional cells of the connective tissue could be identified. As epithelial cells of both groups were similarly able to react to *S. aureus* stimulation with higher secretion of hBD-3 and LL-37,

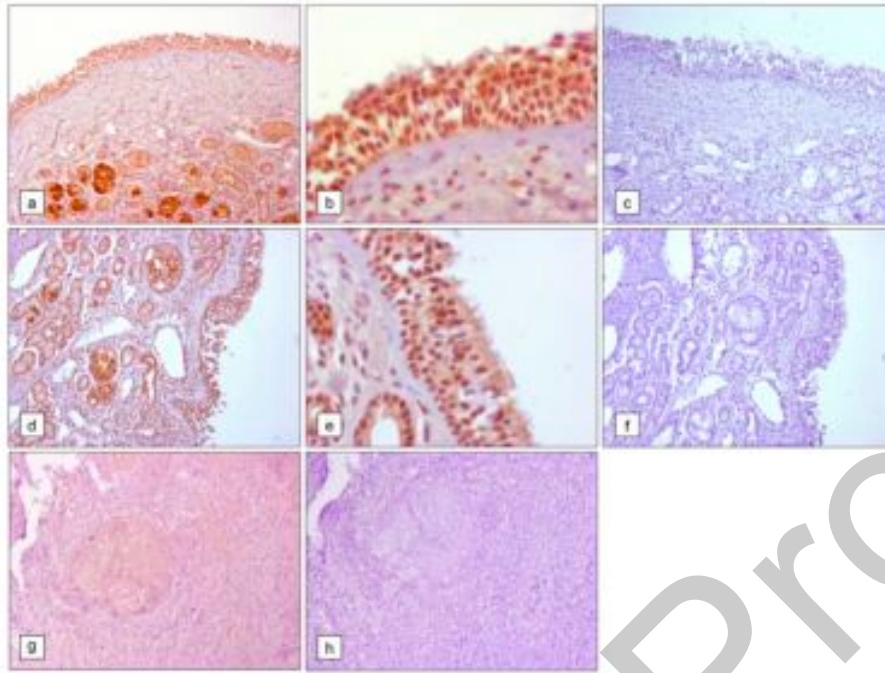


Figure 2a. Immunohistochemistry for hBD-3 in normal controls (NC) and patients with chronic rhinosinusitis with nasal polyps (NP).

- |                            |                            |                        |
|----------------------------|----------------------------|------------------------|
| a: NP with 100x            | b: NP with 400x            | c: NP negative control |
| d: NC with 100x            | e: NC with 400x            | f: NC negative control |
| g: tonsil positive control | h: tonsil negative control |                        |

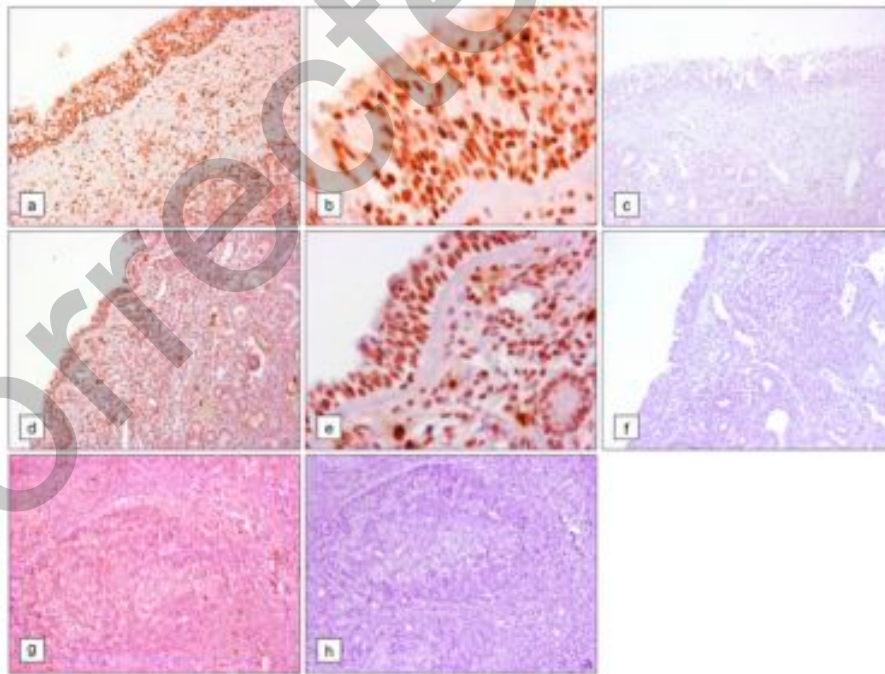


Figure 2b. Immunohistochemistry for LL-37 in normal controls (NC) and patients with chronic rhinosinusitis with nasal polyps (NP).

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|----------------------------|----------------------------|------------------------|
| a: NP with 100x            | b: NP with 400x            | c: NP negative control |
| d: NC with 100x            | e: NC with 400x            | f: NC negative control |
| g: tonsil positive control | h: tonsil negative control |                        |

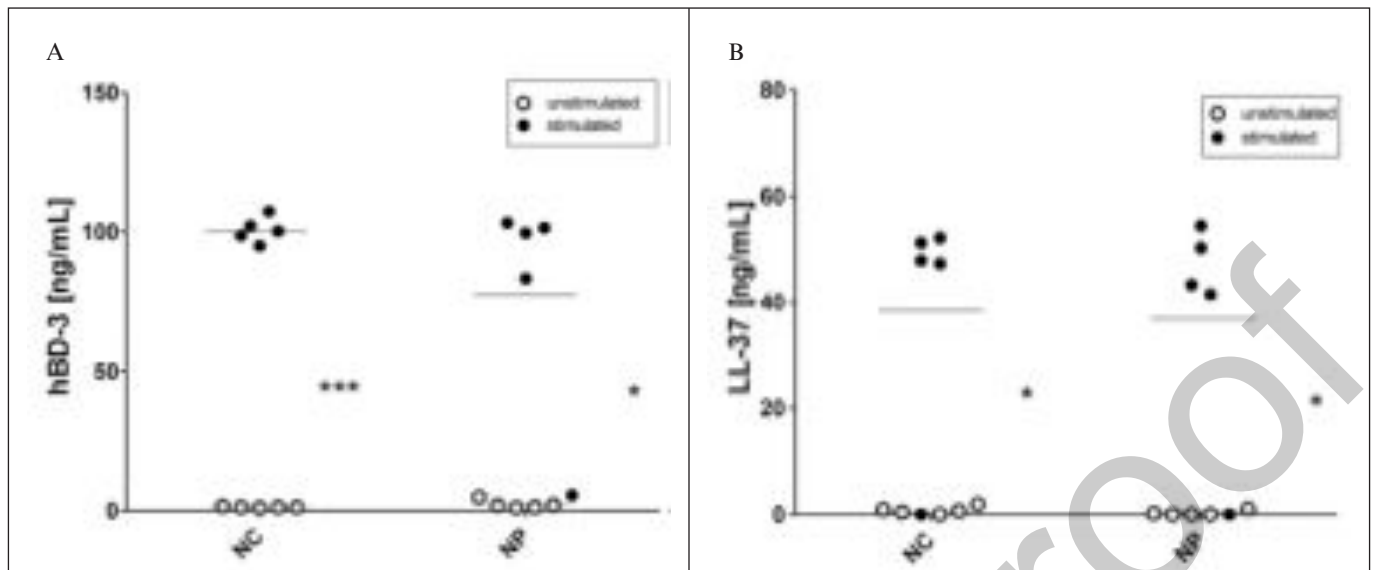


Figure 3. Effect of *S. aureus* culture supernatant stimulation of primary nasal epithelial cell cultures (NEC) of normal controls (NC, n=5) and patients with chronic rhinosinusitis with nasal polyps (NP, n=5, bars indicate mean of stimulated [solid line] and mean of unstimulated individuals [dashed line], \* < 0.05, \*\*\* p < 0.001).

A: hBD-3: human beta-defensin-3, NC p < 0.001, NP p = 0.015.

B: LL-37: human cathelicidin, NC p = 0.017, NP p = 0.018.

a dysbalance in the complex network of stimulatory and inhibitory effects in the nasal mucosa is suggested to be responsible for the detected disturbed LL-37 concentration in nasal secretions of NP patients.

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