

## ORIGINAL RESEARCH

# Evaluation of substance P and bradykinin levels in nasal secretions of patients with nasal polyposis with and without sensitivity to non-steroidal anti-inflammatory drugs

Aleksandar Perić MD, PhD<sup>1</sup>  | Svjetlana Matković MD, PhD<sup>1,2</sup> |  
Aleksandra Barać MD, PhD<sup>3</sup> | Tijana Vukadinović MD<sup>1,4</sup> |  
Ljiljana Čvorović MD, PhD<sup>5</sup>  | Danilo Vojvodić MD, PhD<sup>6</sup>

<sup>1</sup>Department of Otorhinolaryngology, Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia

<sup>2</sup>ENT Department, Lovisenberg Diakonale Hospital, Oslo, Norway

<sup>3</sup>Clinical Center of Serbia, Clinic for Infectious and Tropical Diseases, School of Medicine, University in Belgrade, Belgrade, Serbia

<sup>4</sup>Department of Otorhinolaryngology, Clinical Center of Montenegro, Podgorica, Montenegro

<sup>5</sup>Clinical Center of Serbia, Clinic for Otorhinolaryngology and Maxillofacial Surgery, School of Medicine, University in Belgrade, Belgrade, Serbia

<sup>6</sup>Institute for Medical Research, Division of Clinical and Experimental Immunology, Military Medical Academy Faculty of Medicine, University of Defence, Belgrade, Serbia

## Correspondence

Aleksandar Perić, Department of Otorhinolaryngology, Faculty of Medicine of the Military Medical Academy, University of Defence, Crnotravska 17, 11040 Belgrade, Serbia.

Email: [aleksandarperic1971@gmail.com](mailto:aleksandarperic1971@gmail.com)

## Abstract

**Objective:** The role of neurogenic inflammation in pathogenesis of chronic rhinitis is well known. However, very little is known about its importance in pathogenesis of nasal polyposis (NP), especially in form of NP which appears as a part of aspirin-exacerbated respiratory disease (AERD). The aim of this study was to examine the concentrations of neuropeptides substance P (SP) and bradykinin (BK) in nasal secretions of patients with NP.

**Methods:** Fourteen patients with NP as a part of AERD with mild persistent asthma, 14 patients with NP without aspirin sensitivity, and 14 control subjects without nasal inflammation (C) entered this cross-sectional study. Clinical parameters (symptoms, endoscopic, and radiological findings) were assessed. The concentrations of SP and BK were measured in the nasal secretion samples using commercial human enzyme immunoassay kits.

**Results:** The concentration of SP in nasal secretions was significantly higher in NP patients without aspirin sensitivity and AERD patients compared to controls ( $p = .022$ ;  $p < .0001$ , respectively), but higher in AERD than in non-AERD patients ( $p = .018$ ). The level of BK in nasal fluid was higher in non-AERD and AERD NP patients than in controls ( $p < .0001$ ;  $p < .0001$ , respectively), but also higher in AERD than in non-AERD patients ( $p < .0001$ ). We found high positive correlations between BK in nasal fluid and Lund-Mackay computed tomography (CT) score in both non-AERD and AERD groups of NP patients.

**Conclusion:** Our results suggest more intense release of SP and BK from the nasal mucosa in patients with AERD than in patients with NP who do not have aspirin sensitivity. The strong correlation between concentration of BK in nasal secretions and CT score suggests that BK in nasal fluid could be used as a marker for disease severity as measured by the Lund-Mackay score.

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## KEYWORDS

aspirin, bradykinin, hypersensitivity, nasal polyps, neurogenic inflammation, sinusitis, substance P

## 1 | INTRODUCTION

Chronic rhinosinusitis with nasal polyps (CRSwNP), also known as nasal polyposis (NP), is a chronic inflammation of the nasal mucous membrane characterized by epithelial hyperplasia, strong epithelial, and subepithelial infiltration by inflammatory cells, subepithelial edema, and increased production of nasal secretions.<sup>1,2</sup> In more than 90% of cases, NP in the European and North American populations is manifested by dominant eosinophilic infiltration.<sup>1,2</sup> Eosinophils and to a lesser extent mast cells release a large amount of inflammatory mediators, especially cytokines and chemokines, which leads to the attraction of new inflammatory cells to the site of chronic inflammation, and their activation. On the other hand, different enzymes (eosinophil cationic protein [ECP], major basic protein, tryptase, etc.) are released from eosinophils and mast cells, which locally damage the mucosal tissue and create preconditions for tissue remodeling and formation of inflammatory polyps.<sup>1,2</sup>

Neurogenic inflammation is inflammation arising from the local release by afferent neurons of neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A (NKA), bradykinin (BK), and endothelin-3 (ET-3).<sup>3</sup> In such neurons, release of these neuropeptides is thought to be triggered by the activation of ion channels that are the principal detectors of noxious environmental stimuli. Neurogenic inflammation appears to play an important role in the pathogenesis of numerous diseases, including migraine, psoriasis, asthma, eczema, rosacea, multiple chemical sensitivity, and chronic inflammatory diseases of the nasal mucosa.<sup>3</sup> Although the role of neurogenic inflammation in allergic and non-allergic non-infectious rhinitis is relatively well documented, very little is known about neurogenic inflammation in NP.<sup>3,4</sup> Data in the literature range from the suggestion that NP are primarily caused by neurogenic inflammation to the claim that due to the poor sensory innervation of NPs, neurogenic inflammation is completely absent.<sup>5,6</sup> However, recent studies related to releasing of neuropeptides in nasal secretions, especially SP and BK, and monitoring their immunoexpression in NP tissue, clearly suggest that neurogenic inflammation plays a significant role in the pathogenesis of NP.<sup>7-9</sup>

CRS as a part of the aspirin-exacerbated respiratory disease (AERD) is a special clinical phenotype within a wide range of manifestations of this disease.<sup>10</sup> The association of NP with non-atopic asthma and hypersensitivity to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) (so-called Samter's triad) is explained to caused by altered arachidonic acid metabolism.<sup>10</sup> In the genetically predisposed patients, after taking these analgesics, the lipoxygenase metabolic pathway of arachidonic acid is initiated, and the cyclooxygenase metabolic pathway of this acid is blocked. As a result, leukotriene C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> accumulate in the mucosa of the upper and lower

airway, which triggers a strong inflammatory response.<sup>10,11</sup> This form of CRS has a significantly more severe clinical course compared to other patients with NP. Nasal obstruction, olfactory dysfunction, and hyperproduction of nasal secretions are far more pronounced and the patients are much more prone to recurrence of NPs after endoscopic surgical treatment.<sup>11</sup> In particularly severe cases of disease, it is possible to involve the mucous membrane of the middle ear with signs and symptoms of chronic otitis media with effusion, so-called eosinophilic otitis media. Such a special form with the involvement of the entire airway, including the middle ear, has received the colloquial name "Samter's tetrad."<sup>12,13</sup> Due to all the above, this entity requires a special approach in medical and surgical treatment, including the use of biologic agents.<sup>1,2,10,11</sup>

Previous studies have shown that the immunoexpression of leukotriene receptors in NP tissue is higher in patients with AERD compared to NP patients tolerant to NSAIDs.<sup>14</sup> Also, the concentration of leukotriene E<sub>4</sub> in the urine is elevated in AERD patients and correlates well with objective clinical parameters that indicate the degree of clinical manifestation of disease, as is the Lund-Mackay CT score.<sup>15</sup> On the other hand, there is no correlation between urinary leukotriene E<sub>4</sub> and subjective parameters, such as the severity of NP symptoms.<sup>15</sup>

The role of neuropeptides in the pathogenesis of NP is poorly understood and, by literature review, we did not find studies related to the measurement of neuropeptide levels in nasal secretions in patients with AERD. Unlike cytokines and chemokines in nasal secretion samples, which have been found to be able to correlate with clinical disease parameters,<sup>1,13</sup> it is unclear whether this also applies to neuropeptides. In this study, we wanted to compare the production of SP and BK as parameters of neurogenic inflammation in NP patients with AERD in relation to patients with NP who do not have aspirin sensitivity and in subjects without inflammation of the nasal mucosa. We also wanted to examine whether concentrations of SP and BK in nasal secretions correlate with subjective and objective clinical parameters of NP.

## 2 | METHODS

### 2.1 | Ethical consideration

This was a cross-sectional study, conducted according to the Helsinki Declaration. Investigation was approved by the local Ethics Committee and realized between January 2019 and December 2021 at the Department of Otorhinolaryngology and Institute for Clinical and Experimental Immunology of the Military Medical Academy, Belgrade, Serbia. A written informed consent was obtained from all participants. The STROBE reporting method was used to present the results of the study.

## 2.2 | Participants

The “Rhinosinusitis Task Force” and “EPOS 2020” guidelines were used for diagnosis of CRSwNP.<sup>2,16,17</sup> The criteria for inclusion were: (a) symptoms of CRS; (b) bilateral nasal polyps on endoscopy; and (c) presence of soft tissue shadows in both ethmoidal labyrinths on sinus CT scans.

The inclusion criteria for AERD patients were: (a) the presence of bilateral NPs; (b) confirmation of sinusitis on preoperative CT scans; (c) history of aspirin hypersensitivity reaction presented with symptoms of upper and/or lower airways; and (d) confirmation of mild persistent asthma according to the guideline published in the Global Initiative on Asthma<sup>18</sup> (GINA). Due to the potential impact of systemic and inhaled corticosteroid therapy on concentrations of mediators in nasal secretions, only AERD patients with mild persistent asthma were included in the study.

The exclusion criteria were: moderate-to-severe asthma, <18 or >65 years, choanal polyps, systemic diseases manifesting in the sinonasal region, cigarette smoking, previous nasal/sinus surgery. Therapies with topical, oral, or systemic corticosteroids, antihistamines, and antibiotics within the 4 weeks prior to the start of investigation were also criteria for exclusion.

The control group consisted of subjects selected for nasal septum surgery, with no anamnesis, symptoms, and local findings suggesting for nasal inflammation.

## 2.3 | Clinical evaluation

All NP patients and controls assessed and scored their symptoms (nasal obstruction, sneezing, itching, hyposmia, and rhinorrhea) from 0 to 3: 0—no symptom; 1—mild; 2—moderate; 3—severe, resulting in a maximum nasal symptom score of 15, as described in a previous study.<sup>19</sup>

Bilateral nasal rigid endoscopy (0° and 30° Karl Storz endoscope) was done bilaterally in all NP patients and control subjects in a sitting position. In NP patients, the endoscopic scores were calculated according to the estimated size of NPs, presented by Lildholdt et al.<sup>20</sup>: 0—no polyps; 1—mild disease (polyps do not extend to the upper edge of the inferior turbinate—IT); 2—moderate disease (polyps extend to the space between the upper and lower edge of the IT); 3—severe disease (polyps extend to the lower edge of the IT), with a 6 as a maximal endoscopic score.

The Lund–Mackay score was used for the assessment of findings from CT scans.<sup>21</sup> The bilateral opacifications on the CT scans were estimated as 0 (no change), 1 (partial), and 2 (total opacification) for each of the paranasal sinuses. The opacifications of the ostiomeatal complex were graded as 0 (not occluded) or 2 (occluded). The maximum Lund–Mackay score is 24.

## 2.4 | Sampling of nasal secretions and neuropeptide measurement

Nasal fluid samples were obtained from study participants, 28 NP patients and 14 subjects without nasal inflammation. We used an

absorption method with cotton swabs (10 mm long and 4 mm wide) (Torlak). The sticks stood for 5 minutes in the anterior parts of the nasal middle meati, as previously stated.<sup>22</sup> Each piece of cotton wool was then soaked with nasal secretions and then immersed in a 2 ml Eppendorf tube containing 1 ml of transfer medium (penicillin G 340 IU/ml with gentamicin 50 µg/ml and fungizone 500 µg/ml, all dissolved in phosphate-buffered saline). Diffusion of neuropeptides into the transfer medium was allowed for 30 minutes, and then the samples were kept for a maximum of 2 h at a temperature of 4°C. The samples were then centrifuged at 1000g for 10 minutes to separate and precipitate cell elements from the supernatants. The supernatants were frozen at –70°C, for no longer than 2 months, until neuropeptides were detected. The levels of SP and BK were measured in all of the 42 samples using commercial RayBio human enzyme immunoassay kits (RayBiotech). The neuropeptide levels were expressed in nanograms per milliliters (ng/ml). The detection sensitivities and standard ranges (lower and upper limits) were: 0.1 ng/ml (0.1–1000 ng/ml) for SP; 1.4 ng/ml (0.1–1000 ng/ml) for BK. The intra-assay coefficient of variation for both mediators did not exceed 10%.

## 2.5 | Strength of the study and sample size calculation

Subjects were divided into three groups depending on clinical characteristics. The strength of the study should have been at least 80% (0.8), and the probability of error of the first type ( $\alpha$ ) .05. Based on the data from the literature (Schäper et al.<sup>23</sup>), a much higher level of SP could be expected in patients with persistent allergic rhinitis compared to controls (135.3 (32.0) ng/ml vs. 29.6 (6.5) ng/ml;  $p = .003$ ). Also, based on data from the same paper,<sup>23</sup> it could be assumed that the values of standard deviations (SD) will not be high. Therefore, a moderate effect size (0.34) was chosen to calculate the group size. Approximately 42 participants (14 in each group) were required to demonstrate statistical significance at the  $p < .05$  level between groups, with a study strength of 80%. The calculation was performed by the analysis of variance test (ANOVA, fixed effects, omnibus, one-way) and with the use of the available commercial software (GPower 3.1.).

## 2.6 | Statistical analysis

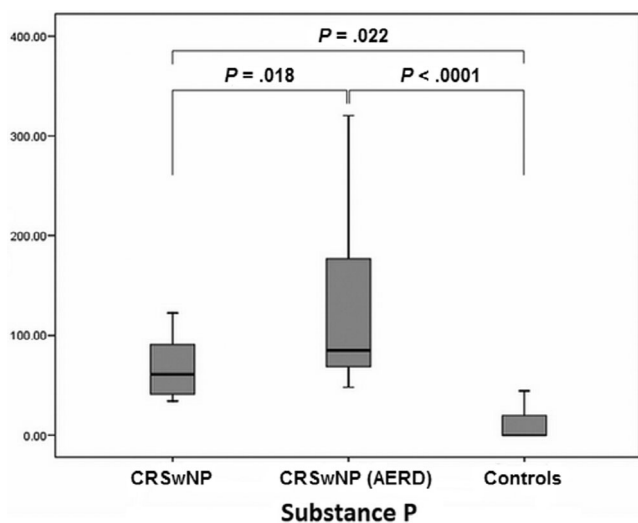
Data are expressed as mean (SD). To calculate the differences between three groups of subjects in the study, we used a one-way ANOVA. Dunn–Bonferroni correction was used to assess the statistical significance of the differences. A  $p$  level of .05 and less was taken as the limit of statistical significance. Correlations between nasal secretion neuropeptide concentrations and clinical parameters were assessed using the Pearson correlation test. For statistical data processing was used the SPSS program, version 17.0 (SPSS Inc.).

**TABLE 1** Demographic, clinical, and biochemical parameters

Participants	CRSwNP	CRSwNP (AERD)	Controls
Number of patients	14	14	14
Age <sup>a</sup> /years	40.6 (10.3)	41.4 (11.2)	40.6 (12.3)
Men/Women	8/6	8/6	9/5
Nasal symptom score <sup>a</sup>	10.6 (2.5)	12.8 (3.0)	3.9 (1.4)
Nasal endoscopic score <sup>a</sup>	4.6 (1.2)	5.0 (1.1)	/
Lund–Mackay CT score <sup>a</sup>	15.5 (6.7)	20.1 (5.5)	/
Substance P (ng/ml) <sup>a</sup>	68.4 (29.3)	127.5 (88.9)	8.8 (10.2)
Bradykinin (ng/ml) <sup>a</sup>	47.6 (8.8)	153.8 (11.9)	15.5 (18.2)

Abbreviations: AERD, aspirin-exacerbated respiratory disease; CRSwNP, chronic rhinosinusitis with nasal polyps.

<sup>a</sup>Expressed as mean (SD).

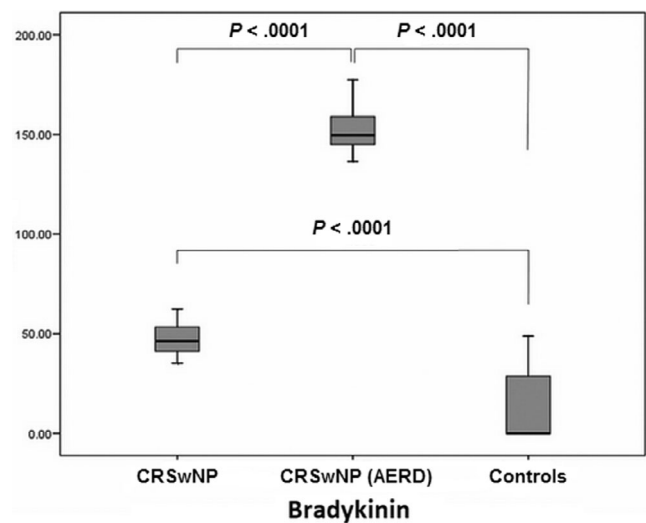


**FIGURE 1** Concentrations of substance P in nasal secretions in patients with CRSwNP, CRSwNP (AERD) patients, and in controls. AERD, aspirin-exacerbated respiratory disease; CRSwNP, chronic rhinosinusitis with nasal polyps

### 3 | RESULTS

Twenty-eight ( $n = 28$ ) NP patients, 14 with and 14 without AERD, and 14 control subjects were included. Data related to demographic, clinical and biochemical parameters are presented in Table 1. We found no statistical difference between CRSwNP without aspirin sensitivity and AERD patients in the nasal symptom and endoscopic score, but the Lund–Mackay score was higher in AERD compared to other NP patients ( $p = .032$ ). Nasal symptom score was higher in NP patients without aspirin sensitivity and AERD patients than in controls ( $p = .001$ ;  $p < .0001$ , respectively).

The mean concentration of SP in nasal secretions was significantly higher in patients with CRSwNP without aspirin sensitivity and AERD patients compared to controls ( $p = .022$ ;  $p < .0001$ , respectively) and the level of SP was higher in AERD than in other CRSwNP patients ( $p = .018$ ) (Figure 1). The level of BK was higher in non-AERD NP patients and AERD patients than in controls ( $p < .0001$ ;  $p < .0001$ ,



**FIGURE 2** Concentrations of bradykinin in nasal secretions in patients with CRSwNP, CRSwNP (AERD) patients, and in controls. AERD, aspirin-exacerbated respiratory disease; CRSwNP, chronic rhinosinusitis with nasal polyps

respectively) and concentrations of BK was higher in AERD than in other NP patients ( $p < .0001$ ) (Figure 2). High positive correlation between BK level in nasal secretions and Lund–Mackay CT score was found in both non-AERD and AERD patients (Table 2).

### 4 | DISCUSSION

Analysis of the composition of nasal secretions can provide us with a lot of valuable information about the inflammatory status of the nasal mucosa, the progression of inflammation, and the respond to applied therapy. Like other mediators of inflammation, neuropeptides, including SP and BK, can be detected in nasal secretions at appropriate concentrations using sophisticated measurement methods. How do neuropeptides reach nasal secretions? SP is a neuropeptide which is released from the sensory nerve endings.<sup>24</sup> SP-containing fibers of the trigeminal nerve have been found in the nasal mucosa, near the

**TABLE 2** Correlations between neuropeptide levels in nasal secretions and clinical parameters in patients with nasal polyps

CRSwNP	NSS	ES	LMS
Substance P	$R = -.142$ $p = .628$	$R = -.129$ $p = .661$	$R = -.087$ $p = .768$
Bradykinin	$R = .145$ $p = .571$	$R = .136$ $p = .618$	$R = .785^{**}$ $p < .000$
AERD			
Substance P	$R = .110$ $p = .708$	$R = .105$ $p = .728$	$R = .005$ $p = .986$
Bradykinin	$R = .121$ $p = .635$	$R = .132$ $p = .594$	$R = .897^{**}$ $p < .000$

Note: \*\*Correlation is significant at the .01 level (two-tailed).

Abbreviations: AERD, aspirin-exacerbated respiratory disease; CRSwNP, chronic rhinosinusitis with nasal polyps; ES, endoscopic score; LMS, Lund–Mackay CT score; NSS, nasal symptom score.

blood vessels of the lamina propria, and within the epithelium. These fibers are unmyelinated afferent C fibers.<sup>24</sup> After stimulation of sensory receptors in the nasal mucosa, the antidromic impulse spreads until the final branching of the fibers in the epithelium and glands, resulting in the release of SP directly in the nasal secretions.<sup>24</sup>

This study may provide insight on neuropeptides as the link between migraine and sinusitis. In migraine, stimulation of the trigeminal nerve receptors also causes neurogenic inflammation via release of neuropeptides, especially SP and NKA.<sup>25</sup> According to the so-called “rhinogenic hypothesis” of migraine origin, nasal mucosa and paranasal sinuses nitric oxide (NO) could be the primary molecule that initiates migraine.<sup>25</sup> Production of paranasal sinus NO is mainly induced by hypoxia due to several factors and the diffusion of NO depends on the vulnerable surface area in the nasal cavity. Two main peripheral trigeminal nerve activating mechanisms may induce pain in migraine. First, the nerve endings of the nasal mucosa are directly stimulated by diffused NO and indirectly stimulated by vasoactive substances released by antidromic activation of the sensory nerve endings, especially by SP and NKA. Second, the perivascular nerve endings of nasal mucosal and meningeal blood vessels are directly stimulated by diffused NO and neuropeptides, which also induces the headache and a sense of pressure in the sinonasal region.<sup>25</sup>

BK is a peptide with an important role in the stimulation and enhancement of inflammatory response in the nasal mucosa.<sup>7,26</sup> In patients with allergic rhinitis, mast cells, basophils, neutrophils, and eosinophils in the nasal mucosa produce and release cytokines and chemokines, and neuropeptides as of BK and SP.<sup>26</sup> Previous investigations indicate a significant increase of BK concentration in the nasal secretions of allergic rhinitis patients in the pollen season.<sup>27</sup>

The results of our study show higher concentrations of SP and BK in the nasal secretions of patients with NP, compared to subjects without nasal inflammation and these data are consistent with the results of a study by Compton et al.<sup>28</sup> Among patients with NP, those with sensitivity to aspirin have higher concentrations of these neuropeptides than those who are not sensitive to NSAIDs. These data suggest that patients with AERD have higher levels of neurogenic

inflammation in the nasal mucosa/paranasal sinuses compared to patients who are not sensitive to aspirin. Neurogenic mechanisms have very important role in the regulation of nasal function. The rich innervation of the nasal mucosa is functionally highly connected with the multitude of cells that participate in the immune response.<sup>24,26,27</sup>

This connection is especially pronounced in patients with chronic inflammation of the nasal mucosa, as is the case with allergic rhinitis.<sup>24,26,27</sup> SP exhibits a number of effects. It stimulates vasodilation and extravasation of plasma in the lamina propria. It stimulates the release of inflammatory mediators from respiratory epithelium.<sup>5–7,24</sup> It strongly stimulates the release of histamine and tryptase from mast cells.<sup>5–7,24</sup> It attracts eosinophils, monocytes, and neutrophils and stimulates macrophages, mast cells, and eosinophils to produce cytokines and products of arachidonic acid metabolism.<sup>3,24</sup> On the other hand, inflammatory mediators secreted in the mucosal layer of the NP patients by inflammatory cells can stimulate and modulate the activity of sensory neurons.<sup>3,24</sup> ECP and tryptase, released from eosinophils and mast cells expose the basement membrane of the nasal mucosa during epithelial damage. In that way, the ends of the sensory fibers become more stimulated to the production of SP, which is released in the nasal secretions of NP patients in larger amounts. Patients with AERD have a higher degree of eosinophilic inflammation in the nasal mucosa and more intensive lipoxygenase metabolism of arachidonic acid compared to other patients with NP.<sup>10,11</sup> This may explain the higher production of SP in patients with AERD compared to other NP patients.

BK is a potent inflammatory mediator. The activity of BK is mediated by the BK B1 and B2 receptors, both expressed in the epithelium, fibroblasts, submucosal glands, vascular endothelium, and smooth muscle cells of the nasal mucosa.<sup>8,9,29</sup> By binding to these receptors, BK stimulates vasodilatation and increases vascular permeability, activation of mast cells, eosinophils, fibroblasts, and macrophages.<sup>8,9,24,29</sup> BK also induces proliferation of both epithelial cells and fibroblasts in NP tissue, which is necessary for the development and growth of NPs.<sup>9</sup> BK, also, increases the eosinophil and neutrophil chemokine expression in the nasal mucosa, stimulating the accumulation of inflammatory cells in the site of chronic inflammation.<sup>30</sup> Finally, BK plays important roles in the pathogenesis of allergic rhinitis and asthma and may activate and increase the arachidonic acid metabolic pathway, all of them strongly associated with the pathogenesis of NP, especially those forms that arise within the AERD.<sup>8,24</sup> In this way, it can be explained why the concentration of BK in nasal secretions is higher in patients with AERD who have a more severe clinical course compared to other NP patients. The fact that the concentration of BK in nasal secretions in both groups of patients with NP strongly correlates with the Lund–Mackay CT score, the only objective clinical indicator of the extent of sinus disease, suggests that in addition to SP, BK also plays a significant role in pathogenesis of NP. Thus, the concentration of BK in nasal secretions could serve as a reliable biomarker for assessing the radiological findings suggesting the extent of paranasal sinus disease.

Our study has limitations. The study sample is relatively small, mostly due to the study's strict exclusion criteria and the fact that the



number of patients with AERD and mild asthma is relatively small. For that reason, all these findings should be further validated in future larger cohorts. Due to financial constraints, our results are limited to measuring the concentrations of biochemical parameters, and not to assessing the immunoexpression of neuropeptides in nasal mucosal tissue and tissue of NPs. So far, we have not dealt with the use of measuring the concentration of neuropeptides in nasal secretions in assessing the effects of NP therapy, but this is one of the topics that should be given special attention in the future.

## 5 | CONCLUSION

In this study, the authors tried to notice the importance of local neuropeptide production in the pathogenesis of NP. Our study is the first one that suggests the more intense production of SP and BK in the mucous membrane of NP patients with AERD when compared to patients with NP who do not have aspirin sensitivity. The concentration of BK in nasal secretions was found to be strongly correlated with the degree of radiologically evaluated NP extent, suggesting that BK in nasal fluid could be used as a marker for disease severity as measured by the Lund–Mackay score.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ORCID

Aleksandar Perić  <https://orcid.org/0000-0002-8453-7272>

Ljiljana Čvorović  <https://orcid.org/0000-0003-3783-1708>

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