

# Nasal fluid release of eotaxin-3 and eotaxin-2 in persistent sinonasal eosinophilic inflammation

Eugenio De Corso, MD, PhD<sup>1</sup>, Silvia Baroni, MD<sup>2</sup>, Mariapina Battista, MD<sup>1</sup>, Matteo Romanello, MD<sup>1</sup>, Romina Penitente, MT<sup>2</sup>, Walter Di Nardo, MD<sup>1</sup>, Giulio Cesare Passali, MD, PhD<sup>1</sup>, Bruno Sergi, MD, PhD<sup>1</sup>, Anna Rita Fetoni, MD, PhD<sup>1</sup>, Francesco Bussu, MD, PhD<sup>1</sup>, Cecilia Zuppi, MD, PhD<sup>2</sup> and Gaetano Paludetti, MD, PhD<sup>1</sup>

**Background:** The aim of the present study was to measure eotaxin-3 (CCL26) and eotaxin-2 (CCL24) in nasal lavage fluid of patients with different forms of chronic sinonasal eosinophilic inflammation to evaluate their role in the pathophysiology of nasal hypereosinophilia.

**Methods:** The study was an analytic cross-section study, level of evidence 3b. Patients (n = 80) with nasal hypereosinophilia were randomly recruited and grouped in the following categories: persistent allergic rhinitis (AR) (n = 25), nonallergic rhinitis with eosinophilia syndrome (NARES) (n = 30), and chronic rhinosinusitis with polyps (CRSwNP) (n = 25). Non-rhinitic volunteers (n = 20) were recruited as controls. CCL24 and CCL26 concentrations were measured by enzyme-linked immunosorbent assay (ELISA) Quantikine Human Immunoassays (R&D Systems, Minneapolis, MN) in nasal lavage fluids. Differential cell counts were performed by microscopic cytological examination of nasal tissue scraped from the inferior turbinate.

**Results:** Mean CCL26 levels were significantly higher ( $p < 0.05$ ) in AR and in NARES (132.0 pg/mL and 187.63 pg/mL, respectively) than in the control group (13.5 pg/mL); in patients with CRSwNP, CCL26 values were increased compared to controls even though the difference was not statistically significant (58.9 pg/mL vs 16.5 pg/mL). Mean CCL24 levels measured in AR, NARES, and CRSwNP were

significantly increased ( $p < 0.05$ ) compared to controls (96.7 pg/mL, 135.4 pg/mL, and 107.0 pg/mL, respectively, vs 32.2 pg/mL). Moreover, we observed a significant correlation between CCL24 and CCL26 levels, evaluating them intra-individually by Spearman's rank correlation test. Finally, a significant correlation was found between CCL24 and CCL26 levels and the percentage of eosinophilic infiltration of nasal mucosa.

**Conclusion:** Our data suggest that CCL26 and CCL24 are likely involved in the pathogenesis of chronic nasal hypereosinophilia, with a complex cooperation and different involvement of the various members of eotaxin family. Further studies are necessary to better understand the actual physiopathologic mechanism, possible clinical relevance, and therapeutic implications. © 2014 ARS-AAOA, LLC.

## Key Words:

eotaxin-3; eotaxin-2; NARES; nasal polyps; persistent allergic rhinitis

## How to Cite this Article:

De Corso E, Baroni S, Battista M, et al. Nasal fluid release of eotaxin-3 and eotaxin-2 in persistent sinonasal eosinophilic inflammation. *Int Forum Allergy Rhinol.* 2014;4:617–624.

<sup>1</sup>Department Head and Neck Surgery–Institute of Otorhinolaryngology, Catholic University School of Medicine and Surgery, Rome, Italy; <sup>2</sup>Department of Diagnostic and Laboratory Medicine–Institute of Biochemistry and Clinical Biochemistry, Catholic University School of Medicine and Surgery, Rome, Italy

Correspondence to: Eugenio De Corso, MD, PhD, Catholic University School of Medicine and Surgery, Department Head and Neck Surgery, Institute of Otorhinolaryngology, "A. Gemelli" Hospital, Division of Rhinology, Largo A. Gemelli n.1, 00168, Rome, Italy; e-mail: eugenio.decorso@tin.it; eugenio.decorso@rm.unicatt.it

Potential conflict of interest: None provided.

The poster of this article was presented at the 20th European Congress of Clinical Chemistry and Laboratory Medicine (IFCC-EFLM) and the 45th

Chronic sinonasal eosinophilic inflammation is typical of a systemic multi-complex inflammatory disorder characterized by infiltration and accumulation of eosinophils in the nasal mucosa, including allergic airway inflammation, nonallergic rhinitis with eosinophilia syndrome (NARES), and chronic rhinosinusitis with nasal

Congress of the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (EUROMEDLAB meeting, May 19–23, 2013, Milano, Italy) and it was awarded in the abstract/poster competition without financial reward.

Received: 31 October 2013; Revised: 10 April 2014; Accepted: 29 April 2014  
DOI: 10.1002/alr.21348  
View this article online at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).

polyps (CRSwNP). Nevertheless, the mechanisms underlying selective eosinophil infiltration are not fully known. Recently, many authors have emphasized the importance of the microcellular environment, and locally-released chemical mediators in influencing airway inflammation, in particular.<sup>1,2</sup> In fact, understanding the mechanisms governing recruitment and migration of blood-borne eosinophils in the airways can improve knowledge of inflammation leading to mucosal damage in these pathological entities.

Increased production and expression of various chemokines in nasal mucosa has been observed in different chronic sinonasal rhinopathies, and an important role in the guidance of the inflammatory process has been attributed to them.<sup>3–5</sup> The eotaxins are CC-chemokines with the specific function of activation and recruitment (chemotaxis) of leukocytes at sites of inflammation. In particular, the eotaxins play a specific role, first for eosinophils, and second for basophils, mast cells, and T helper 2 (Th-2) lymphocytes. The first discovered was eotaxin 1 (CCL11), in 1993, composed of 73 amino acids. Eotaxin-2 (CCL24) and eotaxin-3 (CCL26) were discovered more recently. The gene for eotaxin-1 has been mapped to chromosome 17q11.2, whereas the genes for CCL24 and CCL26 are on chromosome 7q11.2. Even though there is 37% sequence homology between CCL26 and CCL11, and 34% between CCL26 and CCL24, they seem to have a common feature, namely acting exclusively via a single receptor. Variations in activity may arise from differences in their affinity with the receptor and in their chemotactic effects on eosinophils.<sup>6,7</sup>

The receptor for the eotaxins is CC-chemokine receptor type 3 (CCR-3), which is highly expressed on eosinophils but less so on mast cells, basophils, and Th-2. Immediately after eotaxin binding, CCR-3 is internalized, partially degraded, and finally reexpressed, suggesting that, in addition to stimulating eosinophil migration, the eotaxins may be involved in stopping leukocyte movement by fostering their survival for long periods of time.<sup>8</sup> In the eotaxin family, CCL26 is the most recently discovered, and there are a limited number of reports about its role in eosinophilic chronic inflammation.<sup>9–13</sup> Its production seems to take place in epithelial and endothelial cells, smooth muscle cells, and fibroblasts, following stimulation with interleukin 4 (IL-4), IL-13, and tumor necrosis factor alpha (TNF- $\alpha$ ).

The release of eotaxins is differentially modulated by cytokines. The Th-2 cytokines IL-4 and IL-13 enhance the secretion of all eotaxins, whereas the Th-1 cytokines interferon and TNF- $\alpha$  exclusively promote the release of CCL11 that is secreted by eosinophils, macrophages, lymphocytes, fibroblasts, smooth muscle cells, and endothelial cells. Whereas CCL24 and CCL26 are mainly released by epithelial and endothelial cells. Epithelial cells are a major source of eotaxins, especially CCL26, in particular following Th-2 cytokine stimulation.<sup>4,14</sup> The first objective of the present study was to investigate a possible relationship between eosinophilic inflammation and nasal fluid chemokine

release of CCL24 and CCL26 in an in vivo setting to better understand its role in the pathogenesis of chronic nasal hypereosinophilia in persistent allergic rhinitis (AR), NARES, and CRSwNP. Second, we compared CCL26 levels with CCL24 levels to establish possible cooperation between these 2 chemokines belonging to the same family.

## Patients and methods

### Study design and participants

This was an analytical cross-sectional study with a level of evidence of 3b. It was carried out at the Department of Head and Neck Surgery–Institute of Otorhinolaryngology, “A. Gemelli Hospital,” Catholic University School of Medicine and Surgery, Rome, Italy. Patients were randomly enrolled at our institution in the period between January 2012 and January 2013. During that time, we admitted 80 (37 males, 43 females, mean age 43.7 years) symptomatic patients with chronic nasal eosinophilia lasting for at least 12 weeks and documented by nasal cytology. Exclusion criteria were viral and bacterial acute infective rhinosinusitis, use of systemic or inhaled drugs within the previous 3 months (sodium chromoglycate or nedocromil sodium, antihistamines, cortisone, leukotriene, etc.), immunodeficiency, abnormality of white blood cell counts such as neutropenia, cystic fibrosis, nasal surgery within the previous 6 weeks, pregnancy or lactation, or significant anatomic abnormalities affecting nasal function. Finally, patients with allergic fungal rhinosinusitis were excluded because of its particular clinical features.<sup>15</sup>

All subjects gave written informed consent. Epidemiologic data for the patients is shown in Table 1. All patients underwent allergy testing: total immunoglobulin E (IgE), skin prick testing for common inhalant allergens (at least 18, including house dust, pollen, fungi, dogs, and cats), determination of the levels of specific IgE in serum with radioallergosorbent test (RAST), and nasal provocation test to exclude local allergy in particular in patients who were negative by skin prick testing. All patients underwent nasal cytology, nasal endoscopy, and computed tomography (CT) scan. Diagnosis of asthma was based on clinical history, symptoms, physical examination, and pulmonary function tests according to current international guidelines (National Asthma Education and Prevention Program: Expert panel report III. Guidelines for the diagnosis and management of asthma. MD, Bethesda: National Heart, Lung, and Blood Institute, 2007; 1–61; NIH publication 08–5847; [www.nhlbi.nih.gov](http://www.nhlbi.nih.gov)).

On the same day of the nasal lavage, patients were asked to fill out a questionnaire on the main rhinological symptoms (rhinorrhoea, nasal obstruction, heaviness/facial pain, sneezing, dysosmia, nasal itching, difficulty during sleep, awakenings during sleep, lacrimation). Clinical symptoms were described by patients using a visual analogue scale (VAS) from 0 (minimum) to 10 (maximum) points for each symptom. For each patient, we calculated the “individual

TABLE 1. Demographics of study subjects

|                                          | AR (n = 25)  | NARES (n = 30) | CRSwNP (n=25) | Control (n = 20) |
|------------------------------------------|--------------|----------------|---------------|------------------|
| Age, years, median (IRQ)                 | 31 (18–68)   | 39 (17–70)     | 45 (21–69)    | 40 (21–65)       |
| Sex, male/female, n                      | 12/13        | 13/16          | 12/14         | 10/10            |
| Symptom score, median (IRQ) <sup>a</sup> | 37 (22–43)   | 39 (30–68)     | 30 (22–51)    | 10 (6–15)        |
| Asthma, n/total (%)                      | 7/23 (30.4%) | 7/25 (28%)     | 8/25 (32%)    | None             |
| ASA intolerance, n/total (%)             | 1/25 (4%)    | 5/30 (16.6%)   | 4/25 (15%)    | None             |
| Atopy, n                                 | All          | None           | 9             | None             |

<sup>a</sup>Median value = mean total symptom score divide by the average of the total scores of patients for each group.

AR = persistent allergic rhinitis; ASA = aspirin; Control = healthy subjects; CRSwNP = chronic rhinosinusitis with nasal polyps; IRQ = interquartile range; NARES = nonallergic rhinitis with eosinophilia syndrome.

symptoms score” (sum of scores of each symptom analyzed on the basis of the VAS). For each group, the “mean total score” was calculated (average of the total scores of patients for each group).

Patients were divided according to the type of nasal disorder into the following groups:

- Persistent AR: symptoms of AR and positive allergy tests in vivo and/or in vitro, nasal endoscopy negative for nasal polyposis, and CT scan negative for sinonasal mucosal thickening; group 1 (n = 25). According to Allergic Rhinitis and Its Impact on Asthma (ARIA),<sup>16</sup> we included only persistent allergic patients (more than 4 days per week and for more than 4 weeks), to avoid differences due to seasonal allergen exposure.
- Noninfectious nonallergic rhinitis with eosinophilia syndrome (NARES): typical symptoms of persistent rhinitis (rhinorrhoea, nasal obstruction, sneezing, nasal itching, lacrimation, etc.), no evidence of atopy allergy tests in vivo and in vitro, marked nasal hypereosinophilia highlighted through nasal cytology (>20% of granulocytes or mononuclear cells present excluding epithelial cells); group 2 (n = 30).
- CRSwNP: pathognomonic symptoms of CRS by facial CT or endoscopic signs of nasal polyposis; group 3 (n = 25). Patients were radiologically staged by Lund-Mackay score; the median score value was  $14.2 \pm 2.2$ . CRSwNP was diagnosed according to the European Position Paper on Rhinosinusitis and Nasal Polyps, 2012.<sup>17</sup>
- Healthy subjects without any nasal inflammation, no history of rhinitis or chronic rhinosinusitis, normal endoscopic data, and negative allergy tests served as controls; group 4 (n = 20).

### Nasal cytology

The nasal counts of leukocytes were performed on tissues obtained at the level of the middle third of the inferior turbinate bilaterally by “scraping” the mucosa with a rhinoprobe (Farmark SNC, Milan, Italy). Samples were gently smeared on a glass slide and immediately fixed in 95% ethanol and subjected to May-Grunwald-Giemsa

staining. The eosinophil count was expressed as the percentage of eosinophils observed among all polymorphonuclear and mononuclear cells, excluding epithelial cells, in an average of 10 fields examined at high magnification ( $\times 400$ ). The samples were examined blindly by an experienced cytologist who was unaware about the clinical status of patients.

### Nasal lavage

The nasal wash was obtained with the patient sitting and the head bent forward, instilling 5 mL of isotonic saline (0.9% NaCl) prewarmed to 35°C in each nostril. The liquid was collected in a funnel connected in a 30-mL universal container. The lavage fluid was filtered to remove any nasal mucus and centrifuged immediately at 4000 rpm for 5 minutes (g:1600). It was then divided in aliquots and frozen at  $-80^{\circ}\text{C}$  until assay.

### CCL26 enzyme-linked immunosorbent assay

Eotaxin-3 was measured by enzyme-linked immunosorbent assay (ELISA) (Quantikine Human CCL26/Eotaxin-3 Immunoassay; R&D Systems, Minneapolis, MN), following the manufacturer’s instructions. Samples, analyzed in a single session, were assessed in duplicate and the mean value was calculated. The coefficient of variation (CV) of the assays in duplicate was less than 3%. The sensitivity was 3 pg/mL with a range of 3 to 300 pg/mL.

### CCL24 enzyme-linked immunosorbent assay

CCL24 was assayed by ELISA (Quantikine Human CCL24 Immunoassay; R&D Systems). Samples were analyzed in duplicate during a single session, and the mean value was calculated. The CV of duplicates was less than 3%. The sensitivity was 2.5 pg/mL and the range was 2.5 to 2500 pg/mL.

### Statistical analysis

Statistical analysis was performed using SSPS (IBM Statistical Package for Social Science; version 22.0; Armonk, USA) for Windows. Continuous variables were expressed

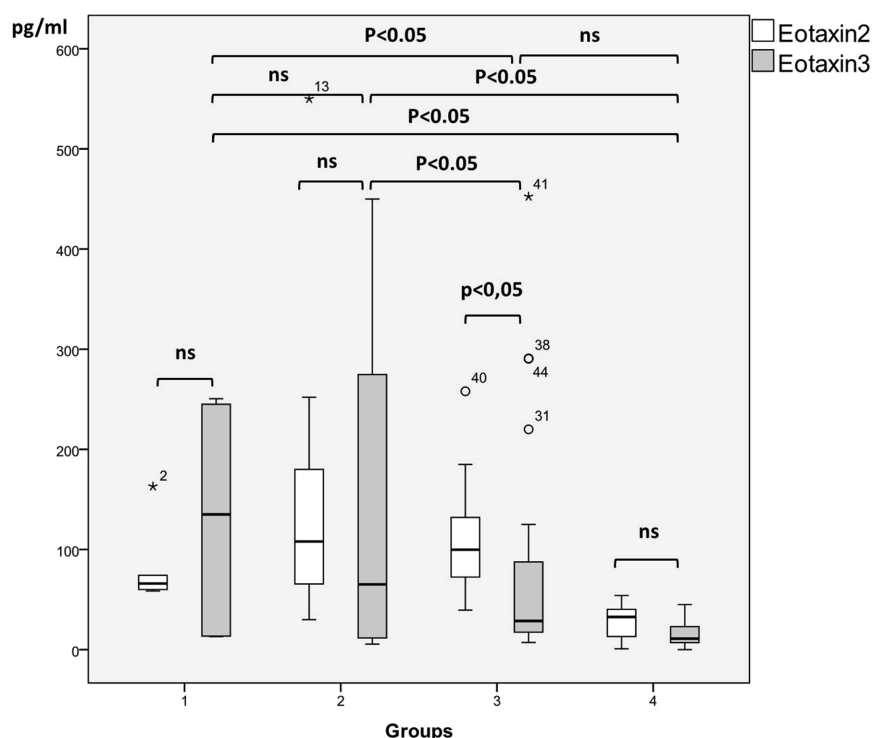


**FIGURE 1.** Levels of eotaxin-2 (CCL24) and eotaxin-3 (CCL26) (pg/mL) in lavages of persistent AR (group 1), NARES (group 2), CRSwNP (group 3), and controls (group 4). The box plots show the median and interquartile range and the error bars show the 5th and 95th percentiles. AR = allergic rhinitis; CRSwNP = NARES = nonallergic rhinitis with eosinophilia syndrome.

as mean  $\pm$  standard deviation (SD). Continual variables were expressed as mean  $\pm$  SD and comparisons between groups were performed using the Mann-Whitney U-test. The strength of the correlation between the 2 parameters was obtained by Spearman's rank correlation test. The results were considered significant for  $p$  values  $< 0.05$ .

## Results

The average concentration of CCL26 in nasal lavage of all patients was significantly higher than in controls ( $148.6 \pm 20.1$  pg/mL vs  $13.5 \pm 5.3$  pg/mL;  $p < 0.05$ ). Statistically significant higher mean levels of CCL26 were observed in lavages of AR and NARES patients ( $132.0 \pm 21.3$  pg/mL and  $187.63$  pg/mL  $\pm 40.3$ , respectively) compared to controls ( $13.5 \pm 5.3$  pg/mL) ( $p < 0.05$ ); in CRSwNP patients, CCL26 values were increased but without a significant difference vs controls ( $58.9 \pm 10.3$  pg/mL vs  $13.5 \pm 5.3$  pg/mL) ( $p > 0.05$ ) (Fig. 1). In order to test if allergy and asthma could be discriminating factors, we measured CCL26 levels in atopic vs nonatopic CRSwNP patients ( $48.21 \pm 10.3$  pg/mL vs  $71.76 \pm 15.3$  pg/mL) and in asthmatic and nonasthmatic patients ( $114.95 \pm 22.3$  pg/mL vs  $132.48 \pm 32.3$  pg/mL). The differences observed were not significant. Finally, no statistically significant correlation was found between CCL26 levels and severity of symptoms score, and in particular with the total symptoms score ( $p > 0.05$ ).

The average concentration of CCL24 in nasal lavage of all patients was significantly higher than in controls ( $134.7 \pm 24.3$  pg/mL vs  $22.77 \pm 8.9$  pg/mL;  $p < 0.05$ ). CCL24 mean levels in AR, NARES, and CRSwNP were significantly increased compared to controls, and  $96.7 \pm 10.3$  pg/mL,  $135.4 \pm 23.4$  pg/mL, and  $107.0 \pm 15.3$  pg/mL, respectively, vs  $22.77 \pm 8.9$  ( $p < 0.05$ ). We did not observe significant difference in CCL24 levels between atopic vs nonatopic ( $103.50 \pm 28.3$  pg/mL vs  $108.5 \pm 25.3$  pg/mL) or between asthmatic and nonasthmatic CRSwNP patients ( $185.9 \pm 40.3$  pg/mL vs  $178.67 \pm 31.3$  pg/mL).

We observed significantly higher levels of CCL24 (107.0 pg/mL), but not CCL26 (58.9 pg/mL), in patients with CRSwNP (group 3). However, CCL24 and CCL26 concentrations in AR (96.7 pg/mL vs 132.0 pg/mL) and NARES (135.4 pg/mL vs 187.63 pg/mL) showed a similar order of magnitude, as shown in Figure 1.

We correlated intraindividual CCL24 and CCL26 levels and observed a significant correlation by Spearman's rank test with a coefficient ( $r_s$ ) of 0.649 ( $p < 0.01$ ), as shown in Figure 2. The same trend was observed between CCL24 and CCL26 for individual patients in AR patients ( $r_s$ : 0.611;  $p < 0.01$ ) and in NARES patients ( $r_s$ : 0.739;  $p < 0.01$ ), but not in CRSwNP ( $r_s$ : 0.412;  $p > 0.01$ ).

Furthermore, we analyzed correlations between CCL24 and CCL26 levels and individual symptom scores. A significant correlation was found between individual symptom scores and nasal fluid CCL24 concentrations (pg/mL) (Fig. 3A), whereas the correlation was not significant with

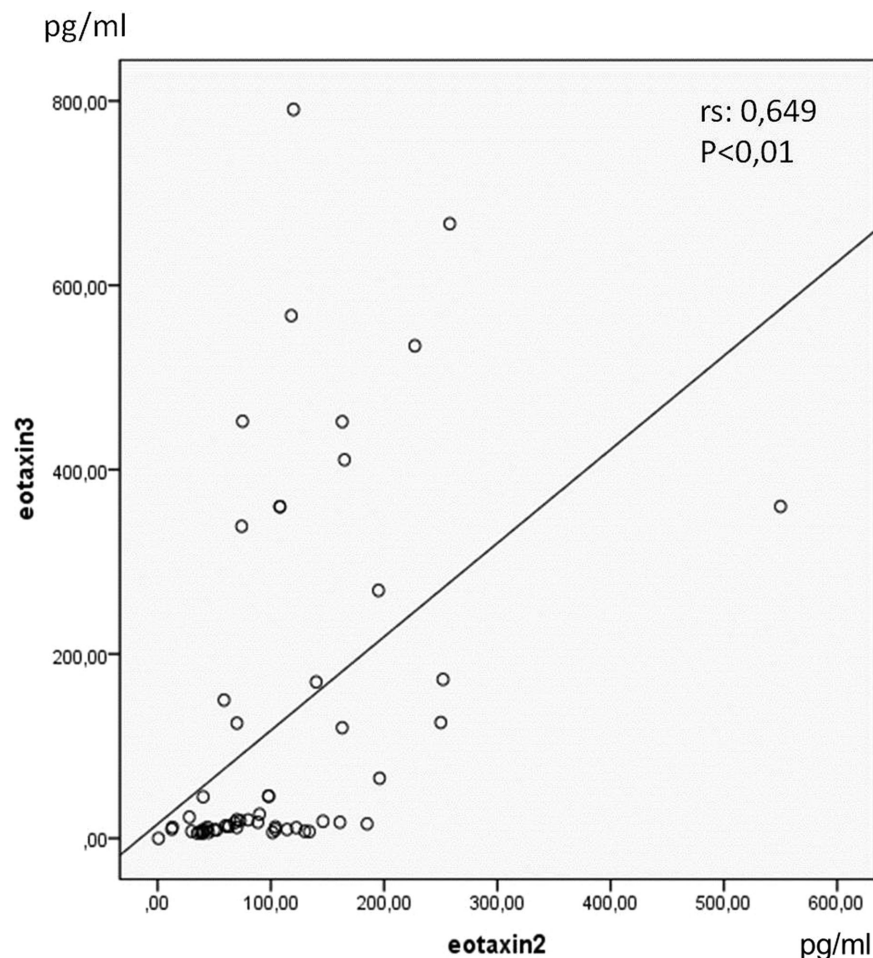


**FIGURE 2.** Scatter plot showing intraindividual correlations between eotaxin-2 (CCL24) and eotaxin-3 (CCL26) levels (pg/mL) in all patients excluding controls. Statistical significance is given as  $p$ .  $r_s$  = correlation coefficient assessed by Spearman's rank correlation test.

CCL26 levels (Fig. 3B). Spearman's rank correlation coefficient was  $r_s = 0.5$  ( $p < 0.05$ ) and  $r_s = 0.3$  ( $p > 0.05$ ), respectively.

Finally, we analyzed correlations between CCL24 and CCL26 levels and percentage of eosinophilic infiltration of nasal mucosa, as shown in Figure 4. A significant ( $p < 0.01$ ) correlation was found in both cases; the Spearman's correlation coefficient ( $r_s$ ) was 0.617 and 0.537, respectively.

## Discussion

Several studies on cytokines and chemokines have rapidly expanded our understanding of inflammatory responses in the pathogenesis of chronic eosinophilic inflammation. Several authors<sup>6,18</sup> have demonstrated that protein expression of CCL11, CCL24 and CCL26 is significantly higher in nasal polyp patients than in controls. The total protein concentrations of samples were measured in homogenized tissue of nasal polyps. There was a direct correlation between the concentrations of all 3 eotaxins and the amount of eosinophilia; the rank order of chemokine protein concentrations was CCL24 > CCL26 > CCL11. The

authors suggested a complex cooperation between all members of the eotaxin family rather than a specific role in the development of eosinophilia. Yao et al.<sup>9</sup> demonstrated that CCL11, CCL24 and CCL26 immunohistochemically stained in nasal mucosa and were present in most infiltrating eosinophils, as well as in other inflammatory cells, epithelial cells, and endothelial cells. Furthermore, the authors illustrated that, compared with non-eosinophilic CRS groups, eosinophilic CRS groups had significant expression of eotaxins in eosinophils. Lam et al.<sup>19</sup> demonstrated that increased CCL26 and other inflammatory mediators is significantly correlated with poorer CT and endoscopic scores in CRS. Finally, Gu et al.<sup>20</sup> showed overexpression of CCL26 mRNA in nasal polyps, demonstrating that CCL26 may lead to eosinophil recruitment and activation in nasal mucosa of patients with nasal polyps.

Hence, all 3 eotaxins have been reported to be increased to different degrees in nasal mucosa of patients with chronic sinonasal eosinophilic inflammation. However, their relative contribution to the pathogenesis and severity of the condition remains poorly understood. In particular, limited data are available for the most-recently-identified CCL26, especially in an in vivo setting. In our series,

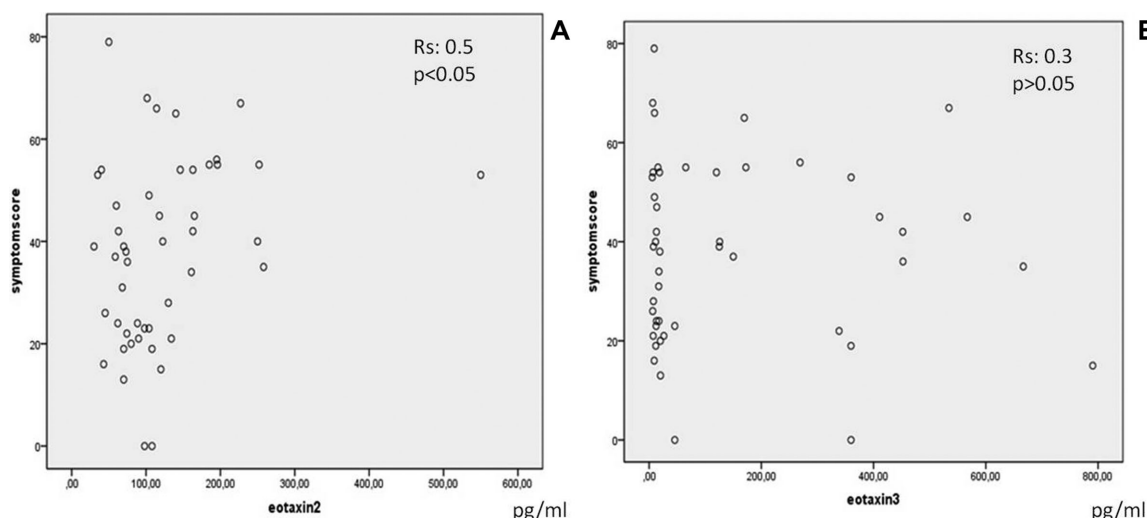


**FIGURE 3.** (A) Correlation between nasal fluid eotaxin-2 (CCL24) concentrations (pg/mL) and individual symptom scores. (B) Correlation between individual symptom scores and eotaxin-3 (CCL26) levels (pg/mL). Individual symptom scores were obtained by adding scores of each symptom for each patient. Statistical significance is given as *p*. *Rs* = correlation coefficient assessed by Spearman's rank correlation test.

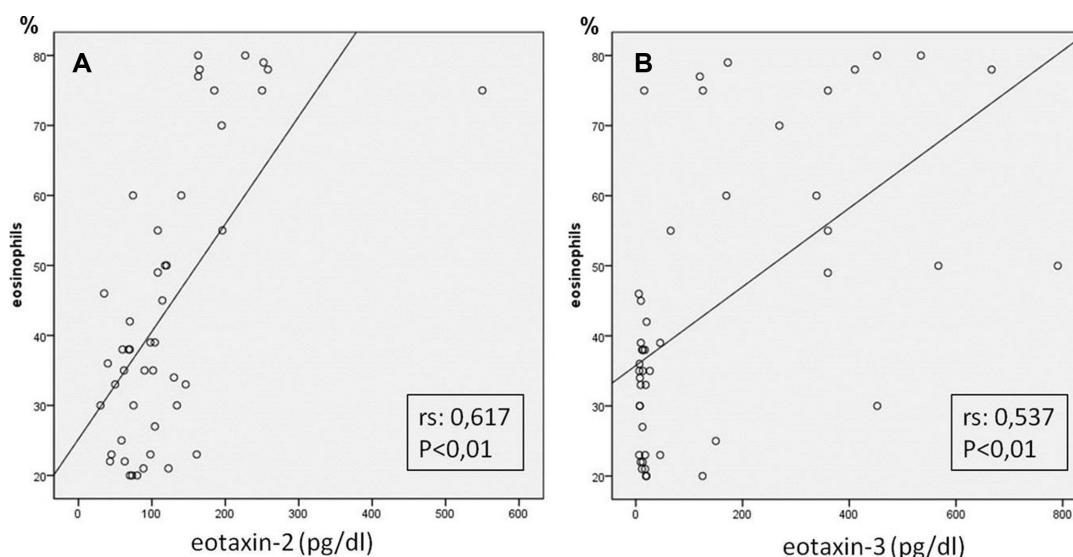


**FIGURE 4.** (A) Correlation between percentages of eosinophilic infiltration of nasal mucosa and eotaxin-2 (CCL24) concentrations (pg/mL). (B) Correlation between eotaxin-3 (CCL26) levels (pg/mL) and percentages of eosinophils. Statistical significance is given as *p*. *rs* = correlation coefficient assessed by Spearman's rank correlation test.

we demonstrated that CCL24 and CCL26 concentrations were increased in the nasal fluid of patients affected by AR and NARES patients compared to controls, confirming their role in the pathophysiology of these diseases. In patients with nasal polyps, CCL24 levels were significantly higher compared to controls, whereas CCL26 levels were increased, but the differences compared to controls was not significant. Furthermore, there were no significant differences in CCL26 levels in atopic vs nonatopic patients and in asthmatic vs nonasthmatic CRSwNP patients. It is difficult to interpret these results, not only because the relative contribution of individual eosinophil-active chemokines in eosinophil transepithelial migration has never been clarified, but also because for CCL26

there have been no such results in an *in vivo* setting until now.

From a pathophysiologic point of view, the lower levels of CCL26 that we observed *in vivo* in nasal polyp patients, compared to the other groups, does not mean that CCL26 is not implicated in the pathogenesis of this disorder. Several possibilities may be taken into account to explain this result. First, Coleman et al.<sup>10</sup> demonstrated that tryptase selectively degrades CCL11 and CCL26, while CCL24 remains stable. Our inability to detect CCL26 in CRSwNP fluid may be related to enzymatic degradation by mast cell tryptase, which is very abundant in nasal polyps. Yuan et al.<sup>21</sup> demonstrated that CCL26 in IL-4 stimulated airway epithelial cells was predominantly to the cell surface

via protein interactions, and that membrane-immobilized CCL26 was the functionally active CCR3 ligand mediating eosinophil transepithelial migration. The CCL26/cell surface association has been previously recognized in endothelial cells,<sup>22</sup> and seems to involve glycosaminoglycans (GAGs). Chemokines have been shown to bind GAG both in vitro and in vivo, and this interaction has important biological functions for in vivo leukocyte trafficking. We cannot exclude that the higher mucosal surface, due to its ballooning, may reduce the measured concentrations of CCL26 in nasal lavage.

The interaction between CCL26 and epithelial cells deserves attention. In fact, recent research<sup>19–23</sup> has implicated these cells as active participants in host immune defense of the upper airway. At the interface between the host and the outside environment, epithelial cells may be induced by both exogenous microbial products and endogenous intracellular signals to express proinflammatory mediators. The mechanisms through which epithelial cells initiate or potentiate inflammation may be important in the pathophysiology of several sinonasal disorders, in particular CRS. Certain epithelial profiles may predict highly dysfunctional epithelial barriers, and prospective evaluation of clinical outcomes from interventions is required. Future endotyping of the epithelium in CRS may be able to provide prognostic information.

Considered together, epithelial cells play an important role in orchestrating mucosal immune responses controlling the recruitment of eosinophils in the mucosa. They are suggested to be the major sources of eotaxins with a site-dependent function. Activated airway epithelial cells are likely implicated by creating a chemotactic gradient of eotaxins that attracts circulating and tissue eosinophils toward the airway lumen. The actual mechanism that initially prompts the continuous accumulation of eosinophils and inflammation mediators, and which ultimately leads to the chronic propagation of the disease, is still unknown.

Local chemokine concentrations are clearly and intimately involved because they induce and activate eosinophils. CCR3, the main eosinophil chemokine receptor, regulates this process; however, the respective contribution and relationships between individual CCR3 ligands in eosinophil transepithelial migration are still less understood.<sup>24,25</sup> Our data suggest that there is significant correlation between levels of the members of the eotaxin family. In fact, measuring intraindividual CCL24 and CCL26 levels, we observed a statistically significant correlation, as shown in Figure 3. The same trend is maintained in patients with AR and in NARES patients, but not in CRSwNP patients. This phenomenon may be related to the lower levels of CCL26 measured in nasal fluid of CRSwNP patients. As previously explained, the low levels observed may be because the test that we used may be not able to detect CCL26 because it is mainly bound to the surface of epithelial cells in these patients.

Many authors have been interested in the special features of eotaxin chemokines because they interact exclusively

with the CCR3 receptor, which is relatively selectively expressed on eosinophils and represents a key eosinophil chemokine receptor.<sup>26</sup> Future studies should investigate if antagonists of CCR3 may attenuate the eosinophilic inflammatory reaction. Blocking activation and migration of eosinophils could provide a new treatment option for inflammatory diseases.<sup>27</sup>

From a clinical point of view we observed a statistically significant correlation between CCL24 and CCL26 levels and the percentage of eosinophilic infiltration of nasal mucosa. Furthermore, we observed a significant correlation between symptom scores and CCL24, whereas correlation with CCL26 levels was not significant. These data are in agreement with our previous report in which we observed that nasal lavage CCL24 levels correlates with severity of symptoms.<sup>28</sup> Furthermore, CCL24 levels were significantly higher in all subgroups compared to controls, while high CCL26 levels were measured only in allergic and eosinophilic nonallergic rhinitis patients. CCL24 measured in nasal lavage seems to be a potentially more stable eosinophilic marker compared to CCL26. Similar differences were described by studying the clinical value of these cytokines in lower airway inflammation in asthmatic patients.<sup>10,24,25</sup> Future studies should confirm our preliminary suggestion.

## Conclusion

Our results indicate that CCL24 and CCL26 are over-secreted in the nasal lavage fluid of patients affected by persistent hypereosinophilia. The data also suggest that there is a significant correlation between levels of these chemokines and percentages of eosinophilic mucosa infiltration, with a mutual and close relationship between them, suggesting possible cooperation, the mechanism of which requires further clarification. Simultaneous expression of all members of the eotaxin family or reaching a certain concentration threshold could possibly be preconditions for stronger eosinophil migration. High levels of CCL24 and CCL26 are undoubtedly involved in a vicious cycle in which both may primarily be produced by airway cells, and later by eosinophils themselves recruiting more eosinophils from the circulation with a self-amplifying process, which contributes to exacerbation of sinonasal inflammation. In agreement with previous reports,<sup>20</sup> we believe that the extent of tissue eosinophilia is not determined by different chemokines fulfilling specific individual tasks, but rather to the complex interplay of multiple chemokines, chemokine receptors, and cells. Nevertheless, within this spectrum of possibilities, investigation of individual mediators is vital for improving the understanding of different steps in the pathogenesis of the disorder, which is indispensable for the formulation of new therapeutic strategies. Future studies should investigate therapeutic strategies that are able to reduce eotaxin levels in the mucosa with the aim of decreasing eosinophil mucosal infiltration and related damage. Ⓔ

## References

- Di Lorenzo G, Drago A, Esposito Pellittieri M, et al. Measurement of inflammatory mediators of mast cells and eosinophils in native nasal lavage fluid in nasal polyposis. *Int Arch Allergy Immunol*. 2001;125:164–175.
- Terada N, Gorai S, Jeong KW, Nomura T, Numata T, Konno A. Mechanisms of eosinophilic inflammation in the mucosa of the nasal cavity and paranasal sinus. *Allergol Int*. 2001;50:273–279.
- Kupczyk M, Kurmanowska Z, Kupryś-Lipińska I, Bocheńska-Marciniak M, Kuna P. Mediators of inflammation in nasal lavage from aspirin intolerant patients after aspirin challenge. *Respir Med*. 2010;104:1404–1409.
- Chao PZ, Chou CM, Chen CH. Plasma RANTES and eotaxin levels are correlated with the severity of chronic rhinosinusitis. *Eur Arch Otorhinolaryngol*. 2012;269:2343–2348.
- Graziano FM, Cook EB, Stahl JL. Cytokines, chemokines, RANTES, and eotaxin. *Allergy Asthma Proc*. 1999;20:141–146.
- Ponath PD, Qin S, Post TW, et al. Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils. *J Exp Med*. 1996;183:2437–2448.
- Weng Y, Siciliano SJ, Waldburger KE, et al. Binding and functional properties of recombinant and endogenous CXCR3 chemokine receptors. *J Biol Chem*. 1998;273:18288–18291.
- Olze H, Förster U, Zuberbier T, Morawietz L, Luger EO. Eosinophilic nasal polyps are a rich source of eotaxin, eotaxin-2 and eotaxin-3. *Rhinology*. 2006;44:145–150.
- Yao T, Kojima Y, Koyanagi A, Yokoi H, Saito T, Kawano K. Eotaxin-1, -2, and -3 immunoreactivity and protein concentration in the nasal polyps of eosinophilic chronic rhinosinusitis patients. *Laryngoscope*. 2009;119:1053–1059.
- Coleman JM, Naik C, Holguin F, et al. Epithelial eotaxin-2 and eotaxin-3 expression: relation to asthma severity, luminal eosinophilia and age at onset. *Thorax*. 2012;67:1061–1066.
- Bartels J, Maune S, Meyer JE, et al. Increased eotaxin-mRNA expression in non-atopic and atopic nasal polyps: comparison to RANTES and MCP-3 expression. *Rhinology*. 1997;35:171–174.
- Yamamoto S, Kobayashi I, Tsuji K, et al. Upregulation of interleukin-4 receptor by interferon-gamma: enhanced interleukin-4-induced eotaxin-3 production in airway epithelium. *Am J Respir Cell Mol Biol*. 2004;31:456–462.
- Provost V, Langlois A, Chouinard F, et al. Leukotriene D4 and interleukin-13 cooperate to increase the release of eotaxin-3 by airway epithelial cells. *PLoS One*. 2012;7:e43544.
- Shin SH, Park JY, Jeon CH, Choi JK, Lee SH. Quantitative analysis of eotaxin and RANTES messenger RNA in nasal polyps: association of tissue and nasal eosinophils. *Laryngoscope*. 2000;110:1353–1357.
- Posteraro B, Scarano E, La Sorda M, et al. Eosinophilic fungal rhinosinusitis due to the unusual pathogen *Curvularia inaequalis*. *Mycoses*. 2010;53:84–88.
- Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group; World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol*. 2001;108:147–334.
- Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl*. 2012;(23):3 p preceding table of contents, 1–298.
- Cavallari FE, Valera FC, Gallego AJ, et al. Expression of RANTES, eotaxin-2, ICAM-1, LFA-1 and CCR-3 in chronic rhinosinusitis patients with nasal polyposis. *Acta Cir Bras*. 2012;27:645–649.
- Lam M, Hull L, McLachlan R, et al. Clinical severity and epithelial endotypes in chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2013;3:121–128.
- Gu Z, Jin M, Cao Z. Role of eotaxin-3 in chronic rhinosinusitis with nasal polyps. *Otolaryngol Head Neck Surg*. 2011;145:324–326.
- Yuan Q, Campanella GS, Colvin RA, et al. Membrane-bound eotaxin-3 mediates eosinophil transepithelial migration in IL-4-stimulated epithelial cells. *Eur J Immunol*. 2006;36:2700–2714.
- Cuvelier SL, Patel KD. Shear-dependent eosinophil transmigration on interleukin 4-stimulated endothelial cells: a role for endothelium associated eotaxin-3. *J Exp Med*. 2001;194:1699–1709.
- Lalaker A, Nkrumah L, Lee WK, Ramanathan M, Lane AP. Chitin stimulates expression of acidic mammalian chitinase and eotaxin-3 by human sinonasal epithelial cells in vitro. *Am J Rhinol Allergy*. 2009;23:8–14.
- Pease JE, Williams TJ. Eotaxin and asthma. *Curr Opin Pharmacol*. 2001;1:248–253.
- Ying S, Robinson DS, Meng Q, et al. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur J Immunol*. 1997;27:3507–3516.
- Forssman U, Ugucioni M, Loetscher P, et al. Eotaxin-2, a novel CC chemokine that is selective for the chemokines receptor CCR-3, and acts like eotaxin on human eosinophils and basophils leukocytes. *J Exp Med*. 1997;185:2171–2176.
- Houmel M, Mazzucchelli L. Chemokine CCR3 ligands-binding peptides derived from a random phage-epitope library. *Immunol Lett*. 2012;149:19–29.
- De Corso E, Baroni S, Romitelli F, et al. Nasal lavage CCL24 levels correlate with eosinophils trafficking and symptoms in chronic sino-nasal eosinophilic inflammation. *Rhinology*. 2011;49:174–179.