

# CD4<sup>+</sup> and CD8<sup>+</sup> T cells infiltration of the polyp tissue in a series of Greek patients with chronic rhinosinusitis with nasal polyps

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## Abstract

Chronic rhinosinusitis (CRS) presents with two basic phenotypes, CRS with (CRSwNP) or without nasal polyps (CRSsNP). The present study's objective was to evaluate the clinical characteristics in a Greek population of CRSwNP patients and their relation to the frequency of CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as the CD4<sup>+</sup>/CD8<sup>+</sup> ratio as prognostic biomarkers. Thirty-three adult CRSwNP patients were recruited at the ENT Department of "G. Papanikolaou" General Hospital of Thessaloniki. Tissue samples from nasal polyps were collected during functional endoscopic sinus surgery. Hadley's nasal endoscopy scores, preoperative Lund-Mackay CT scores, and 22-item Sinonasal Outcome Test, were recorded for each patient. The presence of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes was evaluated in tissue sections by immunohistochemistry. Blood eosinophil and neutrophil counts were also included in the analysis. All data were analyzed with SPSS (version 21.0). Twenty-one males and 12 females were included in the analysis with mean age of 49.5 ± 14.5. LMS ( $P < 0.001$ ,  $r = 0.961$ ) and HES ( $P = 0.001$ ,  $r = 0.54$ ) were both positively correlated with SNOT 22. Hadley's endoscopic score was also positively correlated with Lund-Mackay CT score ( $P < 0.001$ ,  $r = 0.674$ ). Absolute count and percentage of eosinophils were positively correlated with LMS ( $P = 0.003$ ,  $r = 0.513$ ,  $P = 0.002$ ,  $r = 0.527$  respectively) and HES ( $P < 0.001$ ,  $r = 0.622$ ,  $P = 0.004$ ,  $r = 0.497$  respectively). In a subgroup analysis, CRSwNP patients with blood eosinophils >5%, LMS and SNOT 22 were negatively correlated to CD4<sup>+</sup> cells ( $P = 0.029$ ,  $r = -0.654$ ,  $P = 0.043$ ,  $r = -0.618$ , respectively). In CRSwNP patients with CD4<sup>+</sup>/CD8<sup>+</sup> ratio <0.3, CD8<sup>+</sup> T cells were positively correlated with the absolute count and percentage of eosinophils ( $P = 0.042$ ,  $r = 0.684$ ,  $P = 0.036$ ,  $r = 0.699$  respectively). In this study, we recognized the potential importance of nasal CD8<sup>+</sup> T cells in the pathophysiology of CRSwNP patients, also characterized by eosinophil accumulation. Furthermore, the patients' clinical characteristics were also positively correlated with the eosinophilic inflammation and the severity of the disease.

## Keywords

CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, CD4<sup>+</sup>/CD8<sup>+</sup> ratio, chronic rhinosinusitis, eosinophils, nasal polyps

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## Introduction

Chronic rhinosinusitis (CRS) is a simply prevalent inflammatory disease of the nose and the paranasal sinuses and affects 5%–12% of the general population.<sup>1</sup> CRS presents with two basic phenotypes, CR with (CRSwNP) or without nasal polyps (CRSsNP). Patients with CRSwNP often need recurrent surgical procedures and systemic treatment with corticosteroids, and the condition is frequently associated with severe asthma.<sup>2</sup>

CRSwNP is characterized histologically by an intensely edematous stroma with the formation of pseudocysts, subepithelial, and perivascular infiltration by inflammatory cells, and albumin deposition.<sup>3</sup> More specifically, CRSwNP is a heterogeneous inflammatory condition, and the cellular and humoral profile of the underlying inflammation is not yet fully understood.<sup>4</sup>

As CRS's exact etiology is not known, and although several therapeutic approaches are suggested for CRS management, the disease treatment remains challenging.<sup>5</sup> Factors that have been linked to CRS development include chronic immune stimulation due to increased bacterial and fungal colonization and bacterial superantigen responses, resulting in either a predominantly eosinophil or neutrophil-associated inflammation.<sup>6</sup>

Furthermore, CRSwNP is eosinophilic, while CRSsNP is neutrophilic; however, there is no clear-cut division into two types. The great pathophysiological diversity makes the phenotyping classification of CRSwNP very difficult, while endotyping may adequately reflect the pathogenesis for its diagnosis and treatment. An endotype is a subtype of a condition defined by a distinct functional or pathological mechanism.<sup>7</sup> In specific, endotypes of CRS can be (1) non-type Th2, (2) moderate type Th2, and (3) severe type Th2 immune reactions, characterized by cytokines and mediators such as IL4, 5, 13. CRS endotyping can also include a (1) type 2 cytokine-based approach, (2) eosinophil-mediated approach, (3) immunoglobulin E-based approach, and (4) cysteinyl leukotriene-based approach. Subdivisions of CRSwNP may include nonsteroidal anti-inflammatory drug-exacerbated respiratory disease, allergic fungal sinusitis, and eosinophil pauci-granulomatous arteritis by testing.<sup>7</sup>

Indeed, T lymphocytes play a key role in regulating inflammatory processes at mucosal sites whose balance is disturbed in inflamed mucosa.<sup>8</sup> Besides,

there is enormous plasticity within the T cell subsets, whose characterization has accelerated the understanding of inflammatory and humoral immune responses.<sup>9</sup> It has been extensively demonstrated that eosinophilic immune responses are characterized by Th2 cells, whereas Th1 and Th17 cells are involved in neutrophilic immune responses in inflammatory processes.<sup>10</sup> Furthermore, it has been shown that CRSwNP is a Th2 response, whereas CRSsNP is distinguished by elevated Th1 responses.<sup>11</sup>

In specific, CD4<sup>+</sup> T naive cells, partially determined in the pathogenesis of CRS can produce T helper (Th)1, Th2, Th9, Th17, Th22, or T follicular helper (Tfh) effector cell subset (3) and CD4<sup>+</sup> regulatory T (Treg) cells.<sup>12</sup> Similar to CD4<sup>+</sup> Th cells, CD8<sup>+</sup> T cells can differentiate to cytotoxic T cell subsets: Tc1, Tc2, Tc17 cells, and CD8<sup>+</sup> regulatory T (Treg) cells.<sup>13</sup> Previous studies have demonstrated that the CRS signature is Th1, Th2, and Th17 mixture in airway mucosa.<sup>10</sup>

Although CD4<sup>+</sup> T cells' roles have been described in CRS, the roles of CD8<sup>+</sup> T cells are poorly investigated, including the CD4<sup>+</sup>/CD8<sup>+</sup> ratio.<sup>14</sup> The relative role of CD4 versus CD8 cells is also not well described in this disease. Since direct experiments altering this ratio are not possible in humans, we elected to study the ratio and see if alterations in the ratio are associated with altered disease phenotype. Therefore, the present study's objective was to evaluate the frequency of CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as the CD4<sup>+</sup>/CD8<sup>+</sup> ratio as prognostic biomarkers and their relation to the clinical characteristics in a Greek population of CRSwNP patients.

## Methods

### Patients

Thirty-three CRSwNP patients were eligible to be prospectively recruited at the ENT Department of "G. Papanikolaou" General Hospital of Thessaloniki from January 2018 to June of the same year. Tissue samples from nasal polyps were obtained during functional endoscopic sinus surgery procedures. To avoid any immunological overlapping, patients with known coexisting medical problems, such as antrochoanal polyps, allergic asthma, chronic obstructive pulmonary disease, diabetes mellitus, neoplasia, allergic bronchopulmonary aspergillosis, Churg-Strauss syndrome,

**Table 1.** Demographics and clinical characteristics of the patients.

Characteristics	Mean $\pm$ SD	Median (min–max)
Age (years)	49.5 $\pm$ 14.5	49 (17–74)
Male/female	21 (64%)/12 (36%)	—
Polyps unilateral/bilateral	4 (12%)/29 (88%)	—
Lund–Mackay CT score	11.9 $\pm$ 3.5	10 (6–18)
Hadley's endoscopic score	3.3 $\pm$ 0.5	3 (3–4)
SNOT 22 (QoL)	90.2 $\pm$ 12.6	86 (60–107)

cystic fibrosis, fungal rhinosinusitis, immune deficiency, or on treatment with systemic corticosteroids and/or other immunosuppressive therapies were excluded. Women during pregnancy or lactation were also excluded. Informed consent was obtained from each patient, and the ethical committee of the Aristotle University of Thessaloniki (2/2-MAR-2012) approved the study for collecting human tissue samples.

The diagnosis of CRS was based on history, clinical examination, nasal endoscopy, and computed tomography (CT) of the paranasal cavities according to the current European Position Paper on Rhinosinusitis and nasal polyps.<sup>1</sup> In particular, patients fulfilled two or more of the following criteria: blockage/congestion/obstruction, nasal discharge, facial pain/pressure, decrease or loss of smell for at least 4 weeks. Additionally, all patients underwent: CT scan, Hadley's nasal endoscopy scores (HES), preoperative Lund-Mackay CT scores (LMS),<sup>15</sup> preoperative 22-item Sinonasal Outcome Test (SNOT-22).<sup>16</sup>

#### Blood samples and analysis

Blood samples were obtained from each patient at the time of sinus surgery. Whole blood was analyzed for blood eosinophil and neutrophil counts using a laboratory automated analyzer.

#### Immunohistochemistry

The presence of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes was evaluated in tissue sections by immunohistochemistry using the monoclonal Mouse Anti-Human CD4 (1:100, Clone 4B12, Dako, Glostrup, Denmark) and Mouse Anti-Human CD8 (1:200, Clone C8/144B, Dako) antibodies, respectively. Procedures were performed according to kit instructions. They were mounted in Faramount

Mounting Medium (Dako) before analysis by light microscopy after samples' staining. The number of positive cells in tissue sections was counted by a light microscope at a magnification of 400X, using an Olympus CX-40 microscope by two pathologists blindly.<sup>17</sup> Three individual fields with infiltration by inflammatory cells were counted. The percentage of each positive cell (CD4, CD8) in the total inflammatory cells was calculated as an average of the positive cell percentage.

#### Statistical analysis

All data were analyzed with SPSS (version 21.0). The measured data were presented as the mean  $\pm$  standard deviation or median and min-max range. The correlation parameters were obtained using the Pearson correlation coefficient (*r*). A *P* < 0.05 was considered to indicate a statistically significant difference.

## Results

#### Demographics

Thirty-three patients with CRSwNP were enrolled in our study (21 males and 12 females). The demographics of the patients are shown on Table 1. The mean age of the population was 49.5  $\pm$  14.5, and the median (min–max) was 49 (17–71).

#### Clinical characteristics

We further measured the LMS, the HES, and the SNOT-22 (QoL). The results are shown on Table 1. The mean numbers for the clinical tests are 11.9 for LMS, 3.3 for HES, and 90.2 for SNOT-22. LMS (*P* < 0.001, *r* = 0.961) and HES (*P* = 0.001, *r* = 0.54) were both positively correlated with SNOT 22. HES was positively correlated with LMS score (*P* < 0.001, *r* = 0.674).

**Table 2.** Blood and immunohistochemistry results.

	Mean $\pm$ SD	Median (min–max)
Peripheral blood results		
Neutrophils (%)	58.2 $\pm$ 9.3	57.6 (41.8–86.7)
Neutrophils (absolute count, cells/ $\mu$ L)	4400 $\pm$ 1320	4570 (1900–8900)
Eosinophils (%)	3.96 $\pm$ 3.5	2.9 (0.5–16.3)
Eosinophils (absolute count, cells/ $\mu$ L)	300 $\pm$ 260	200 (100–1200)
Immunohistochemistry results		
CD4 <sup>+</sup> T cells %	12.3 $\pm$ 8.4	20 (2–38)
CD8 <sup>+</sup> T cells %	22.6 $\pm$ 12.1	10 (6–40)
CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	0.7 $\pm$ 0.5	0.5 (0.1–2)

### Peripheral blood neutrophil and eosinophil counts

In our population absolute count and percentage of neutrophils were negatively correlated with absolute count ( $P=0.006$ ,  $r=-0.483$ ) and percentage ( $P=0.007$ ,  $r=-0.476$ ) of eosinophils respectively. Absolute count and percentage of neutrophils were negatively correlated with SNOT 22 ( $P=0.006$ ,  $r=-0.481$ ,  $P=0.004$ ,  $r=-0.508$ , respectively). On the contrary, absolute count and percentage of eosinophils were positively correlated with SNOT 22 ( $P=0.012$ ,  $r=0.443$ ,  $P=0.019$ ,  $r=0.418$ , respectively). Furthermore, absolute count and percentage of eosinophils were positively correlated with LMS ( $P=0.003$ ,  $r=0.513$ ,  $P=0.002$ ,  $r=0.527$  respectively) and HES ( $P<0.001$ ,  $r=0.622$ ,  $P=0.004$ ,  $r=0.497$  respectively) whereas absolute count and percentage of neutrophils were negatively correlated only with LMS score ( $P<0.001$ ,  $r=-0.599$ ,  $P=0.001$ ,  $r=-0.554$ , respectively).

### Infiltration of the polyp tissue by CD4<sup>+</sup> and CD8<sup>+</sup> cells

Immunohistochemistry results for the whole population appear on Table 2. No significant correlations were found between CD4<sup>+</sup>, CD8<sup>+</sup> cells, and HES, LMS, and SNOT 22 score.

The majority of our patients had higher % of CD8<sup>+</sup> (22.6%) compared to CD4<sup>+</sup> cells (12.3%). To identify whether patients with CD4<sup>+</sup> predominance in their polyps exhibited a different phenotype we separated our population in those with CD4<sup>+</sup>/CD8<sup>+</sup> ratio  $>1$  and those  $<1$ . There were no differences in any of the clinical characteristics of the patients or the polyp characteristics between the two groups. Subjects with higher CD4<sup>+</sup>/CD8<sup>+</sup>

ratio also had higher % of eosinophils in the blood ( $P<0.05$ , Table 3).

Since this analysis showed a small number of subjects that had high CD4<sup>+</sup>/CD8 ratio, we also decided to analyze the subjects with the lower ratio separately. To do this we put the cutoff point at a ratio of 0.3, since this gave us a similar group size, but in the opposite direction. Again, there was no significant difference in the clinical characteristics between the two groups. These results indicate that the CD4<sup>+</sup>/CD8<sup>+</sup> ratio is probably not predictive of any clinical or prognostic characteristics in our population. Demographic and clinical characteristics of CRSwNP patients with CD4<sup>+</sup>/CD8<sup>+</sup> ratio  $<0.3$  and  $\geq 0.3$  appear on Supplemental Table S1 (added as Supplemental Material). In CRSwNP patients ( $n=9$ ) with CD4<sup>+</sup>/CD8<sup>+</sup> ratio  $<0.3$ , CD8<sup>+</sup> T cells were positively correlated with the absolute count and percentage of eosinophils (Figure 1) ( $P=0.042$ ,  $r=0.684$ ,  $P=0.036$ ,  $r=0.699$  respectively).

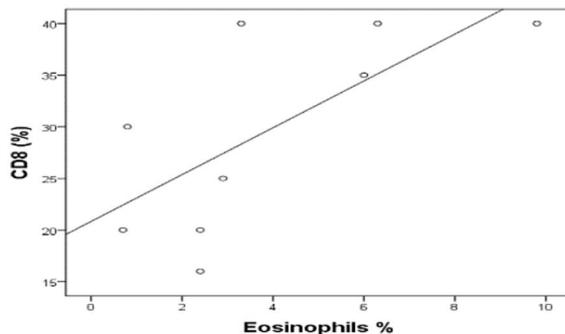
### Subgroup analysis of CRSwNP patients by peripheral blood eosinophil levels

JESREC score uses also the level of eosinophilia in the blood ( $>5\%$ ) as a criterion for eosinophilic chronic rhinosinusitis severity.<sup>18,19</sup> Subgrouping results of our CRSwNP population according to blood eosinophil count of cut off values of 5% appear on Table 4. We separated our patients in these two groups to study whether CD4<sup>+</sup>/CD8<sup>+</sup> ratios correlate with this characterization, but no significant correlation was found. In patients with levels of eosinophils  $>5\%$ , LMS and SNOT 22 were found significantly higher when compared to patients with  $<5\%$ . Besides, these patients were found with significantly lower values of neutrophils when compared to patients with blood eosinophils

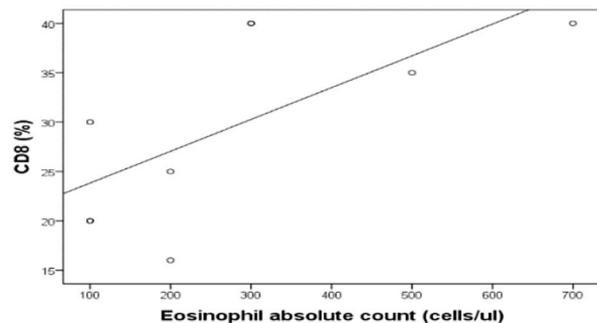
**Table 3.** Demographic and clinical characteristics of CRSwNP patients with CD4<sup>+</sup>/CD8<sup>+</sup> ratio  $\geq 1$  and  $< 1$ .

Characteristics	$\geq 1$ (n=8), mean $\pm$ SD	$< 1$ (n=25), mean $\pm$ SD	P-values
Age (years)	55.6 $\pm$ 10.8	47.6 $\pm$ 15.2	0.204
Male/female	5 (62%)/3 (38%)	16 (64%)/9 (36%)	0.301
Polyps unilateral/bilateral	1 (13%)/7 (87%)	3 (12%)/22 (88%)	0.356
Lund–Mackay CT score	12.5 $\pm$ 3.2	11.7 $\pm$ 3.6	0.522
Hadley's endoscopic score	3.2 $\pm$ 0.5	3.3 $\pm$ 0.5	0.789
SNOT 22 (QoL)	93.5 $\pm$ 11	89.1 $\pm$ 13.2	0.496
Peripheral blood results			
Neutrophils (%)	55.8 $\pm$ 6.7	59 $\pm$ 10	0.492
Neutrophils (absolute count, cells/ $\mu$ L)	4100 $\pm$ 1100	4500 $\pm$ 1400	0.877
Eosinophils (%)	5.3 $\pm$ 2.5	3.5 $\pm$ 3.7	0.048*
Eosinophils (absolute count, cells/ $\mu$ L)	360 $\pm$ 140	290 $\pm$ 300	0.060
Immunohistochemistry results			
CD4 <sup>+</sup> T cells %	18.1 $\pm$ 10.2	10.6 $\pm$ 7	0.005*
CD8 <sup>+</sup> T cells %	13.1 $\pm$ 6.2	25.7 $\pm$ 12.1	0.032*
CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	1.4 $\pm$ 0.4	0.4 $\pm$ 0.2	<0.001*

\*with statistically significant differences between the groups,  $P < 0.05$ .



(a)  $p=0.036$ ,  $r=0.699$



(b)  $p=0.042$ ,  $r=0.684$

**Figure 1.** CRSwNP patients with CD4<sup>+</sup>/CD8<sup>+</sup> ratio  $< 0.3$ : (a) correlation between CD8<sup>+</sup> (%) and percentage of eosinophils ( $P=0.036$ ,  $r=0.699$ ) and (b) correlation between CD8<sup>+</sup> (%) and absolute count of eosinophils ( $P=0.042$ ,  $r=0.684$ ).

**Table 4.** Demographic and clinical characteristics of CRSwNP patients with peripheral blood eosinophil counts  $\geq 5\%$  and  $< 5\%$ .

Characteristics	$\geq 5\%$ (n=12), mean $\pm$ SD	$< 5\%$ (n=21), mean $\pm$ SD	P-values
Age (years)	52 $\pm$ 13.5	48.9 $\pm$ 15.3	0.640
Male/female	7 (58%)/5 (42%)	14 (67%)/7 (33%)	0.567
Polyps unilateral/bilateral	2 (17%)/10 (83%)	2 (10%)/19 (90%)	0.560
Lund–Mackay CT score	14.7 $\pm$ 2.4	10.5 $\pm$ 3.2	0.003*
Hadley's endoscopic score	3.5 $\pm$ 0.5	3.1 $\pm$ 0.4	0.072
SNOT 22 (QoL)	99.5 $\pm$ 7.7	85.9 $\pm$ 12.6	0.002*
Peripheral blood results			
Neutrophils (%)	53.3 $\pm$ 7	60.9 $\pm$ 9.5	0.012*
Neutrophils (absolute count, cells/ $\mu$ L)	3500 $\pm$ 1030	4880 $\pm$ 1250	0.004*
Eosinophils (%)	7.8 $\pm$ 3.1	1.8 $\pm$ 0.9	<0.001*
Eosinophils (absolute count, cells/ $\mu$ L)	520 $\pm$ 260	195 $\pm$ 201	<0.001*
Immunohistochemistry results			
CD4 <sup>+</sup> T cells %	14 $\pm$ 8.6	11.2 $\pm$ 8.6	0.169
CD8 <sup>+</sup> T cells %	21.3 $\pm$ 12	22.1 $\pm$ 12.4	0.919
CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio	1.9 $\pm$ 1.5	2.8 $\pm$ 2.3	0.157

\*With statistically significant differences between the groups,  $P < 0.05$ .

<5%. Furthermore, in CRSwNP patients with eosinophils >5%, LMS and SNOT 22 were significantly negatively correlated to CD4<sup>+</sup> cells ( $P=0.029$ ,  $r=-0.654$ ,  $P=0.043$ ,  $r=-0.618$ , respectively) and HES negatively correlated to CD4<sup>+</sup> cells ( $P=0.07$ ,  $r=-0.564$ ) as well.

## Discussion

CRSwNP is an inflammatory condition that affects the sinonasal mucosa. Lymphocytes are considered to play a significant role in its pathogenesis although its role is not yet fully understood.<sup>20</sup> This is the first study to investigate the frequency and pattern of CD4<sup>+</sup> and CD8<sup>+</sup> T cells accumulation in the polyp tissue of Greek patients with CRSwNP and the relation of CD4<sup>+</sup>/CD8<sup>+</sup> ratio to patient's clinical characteristics.

The CD4<sup>+</sup>/CD8<sup>+</sup> ratio in blood and other biological samples has been used as a prognostic marker in various conditions. It is known that low peripheral blood ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T-lymphocytes (CD4<sup>+</sup>/CD8<sup>+</sup>) is a biomarker of ongoing immune activation in HIV+ patients despite treatment with antiretroviral therapy.<sup>21</sup> Furthermore, a low peripheral blood CD4/CD8 ratio has been associated with pulmonary Emphysema in HIV+ patients.<sup>22</sup> The CD4<sup>+</sup>/CD8<sup>+</sup> ratio in bronchoalveolar lavage fluid (BAL) has been implicated as a diagnostic tool for sarcoidosis in several studies.<sup>23</sup>

In our study, we evaluated the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in polyp tissue. The ratio did not show any correlation with any clinical characteristics. The majority of our patients had higher % of CD8<sup>+</sup> compared to CD4<sup>+</sup> cells. As we did not see any correlation of the ratio with the clinical characteristics in the whole population, we next divided the population in subgroups for further analysis. To identify whether patients with CD4<sup>+</sup> predominance in their polyps exhibited a different phenotype we separated our population in those with CD4/CD8 ratio >1 and those <1. Since this analysis showed a small number of subjects that had high CD4<sup>+</sup>/CD8 ratio, we also decided to analyze the subjects with the lower ratio separately. To do this we put the cutoff point at a ratio of 0.3, since this gave us a similar group size, but in the opposite direction. Again, there was no significant difference in the clinical characteristics between the two groups. These results indicate that the CD4<sup>+</sup>/CD8<sup>+</sup> ratio is probably not predictive of any clinical or

prognostic characteristics in our population. Changes in ratios of CD4<sup>+</sup>/CD8<sup>+</sup> cells are possibly related to inflammatory processes in the respiratory mucosa. One important finding in our study was the positive correlation of CD8<sup>+</sup> T cells with the absolute count and percentage of eosinophils in CRSwNP patients with CD4<sup>+</sup>/CD8<sup>+</sup> ratio <0.3. Indeed, CD4<sup>+</sup> and CD8<sup>+</sup> T cells via the production of IL-4 and IL-5 can stimulate a Th2 phenotype triggering the eosinophilic airway inflammation.<sup>14,24</sup> Besides, studies evaluating CRSwNP patients from Western countries demonstrated that nasal polyps were characterized by eosinophil infiltration, whereas, in Asian populations, polyp tissues are biased toward neutrophilic inflammation.<sup>25</sup>

Moreover, in our study, an observation was that subjects with CD4<sup>+</sup>/CD8<sup>+</sup> ratio >1 showed also higher % of eosinophils in peripheral blood. This may indicate that accumulation of CD4<sup>+</sup> T cells in the polyp tissue correlates with the degree of systemic Th2 inflammation that is probably present in the subjects with the higher numbers of eosinophils in peripheral blood.<sup>14</sup> Whether this subgroup of patients will be the best responders to dupilumab,<sup>26</sup> an anti-IL-4 receptor alpha monoclonal antibody that has been recently approved as a therapeutic for nasal polyps, is an interesting open question.

Additionally, there is evidence for adaptive immune system presence in the tissue (T cells), and the etiology has not clearly been explained. T cells may be the cause of eosinophil attraction, but they may also activate local cells. These cells may be epithelial cells, one of the main sources of eosinophil chemoattractants, such as eotaxins.<sup>27</sup> Furthermore, both CD4<sup>+</sup> and CD8<sup>+</sup> T cells can release IL-13 that is a potent eotaxin inducer in epithelial cells and may be the one attracting eosinophils.<sup>24</sup> Whether one or the other or both produce IL-13 in polyps is an interesting question that needs to be studied. In our study, CD8<sup>+</sup> T cells were found with significantly higher percentages compared to CD4<sup>+</sup> T cells.

We also divided the CRSwNP patients in those with high and low peripheral blood eosinophils as described and we studied these groups in association with the clinical characteristics. We based our subgrouping in the JESREC score which also uses the level of eosinophilia in the blood (>5%) as a criterion for eosinophilic chronic rhinosinusitis severity.<sup>18,19</sup> In the present study, in CRSwNP patients with blood eosinophils >5%, LMS and

SNOT 22 were significantly negatively correlated to CD4<sup>+</sup> cells, possibly reflecting that disease severity is not correlated to CD4<sup>+</sup> cell accumulation in this group. However, a study has already demonstrated that CD4<sup>+</sup> T cells were dominant,<sup>28</sup> whereas most studies have suggested that CD8<sup>+</sup> T cells were the dominant cells in polyp tissues.<sup>29–31</sup> This inconsistency may be due to polyp samples being obtained from varied regions and ethnic groups in different studies.<sup>25</sup> Also, since the ratio does not correlate, maybe the two cell types have overlapping functions in the tissue, and their ratio depends more on other factors, such as the ratio in the blood or the ability of an individual to produce chemotactic factors for one or the other of these cells. Overall, since no other significant differences were found in our analysis, we could say that the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> has not a clear clinical predictive significance; however, our results concerning CD8<sup>+</sup> T cells indicate their potential importance in the pathophysiology of CRSwNP patients as it has been clearly suggested in previous studies.<sup>32</sup> To our knowledge, no other studies have evaluated the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in CRSwNP patients.

We also correlated the different clinical characteristics of the patients to each other. CRS affects the quality of life in some symptom domains<sup>33</sup> and this is evident in the high SNOT-22 score in our study population. SNOT-22 was positively correlated with LMS and HES, as it has already been reported in previous studies.<sup>34</sup> Besides, the LMS increased with increasing polyposis grade, as defined by HES, which has also been demonstrated in other studies.<sup>35</sup> Furthermore, in our study, absolute count and percentage of eosinophils were positively correlated with LMS and HES, possibly reflecting the eosinophilic inflammation and the severity of the disease. In addition, in our subgrouping analysis, CRSwNP patients with peripheral blood eosinophils >5%, were found with statistically significant higher scores of LMS and SNOT 22 when compared to patients with <5%. These results are consistent with previous studies, since eosinophils are inflammatory cells with an important role in the pathogenesis of CRSwNP.<sup>19,36</sup> Besides, levels of peripheral blood eosinophils have been proposed as a factor of disease severity in JESREC study.<sup>19</sup> Moreover, according to recent literature, defining not only the eosinophilic but also the neutrophilic inflammation in CRS patients

could lead to prognosis prediction.<sup>37</sup> Indeed, defining CRS phenotypes is a critical step in determining optimal medical or surgical treatment.

## Conclusions

There are no reports describing the pattern of infiltration of nasal polyps by immune and inflammatory cells in the Greek population. In summary, we recognized the potential importance of nasal CD8<sup>+</sup> T cells in the pathophysiology in a Greek population of CRSwNP patients, characterized by eosinophil accumulation. Moreover, the patients' clinical characteristics were also positively correlated with the eosinophilic inflammation and the severity of the disease. Since our aim was to study whether these biomarkers had a relation to disease severity, no control group was included. In addition, control groups in this case are not easy to obtain as it will be tissue from a different part of the nose and having different pathophysiology. A limitation associated with the present study is the enrollment of 1 group of 33 patients in the 6-month recruitment period. Although our patients were characterized by eosinophilic inflammation, eosinophil tissue counts as well as including more groups of patients would allow further comparisons.

Overall, many subtypes of both CD4<sup>+</sup> and CD8<sup>+</sup> cells have been identified in nasal polyps and have been correlated with the disease's pathogenesis.<sup>20</sup> Additional studies are still required to clarify the underlying mechanisms. Correlation of one of these cell types with specific characteristics of patients' polyps or clinical characteristics may allow us to identify specific roles for these cells. The immune response leading to polyp formation is still not delineated, and future studies need to shed more light.

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## Author contributions

SG, HV, and IK initiated the concept of the project. SG, BI, and PK collected the biological samples. SG analyzed and interpreted the patient data regarding the disease. KD performed the histological and laboratory examination, and SG, KD were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

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The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Ethics approval and consent to participate

The ethical committee of the Aristotle University of Thessaloniki (2/2-MAR-2012) approved the study for collecting human tissue samples. Informed consent to participate in the study has been obtained from the subjects.

### Informed consent

Written informed consent was obtained from all subjects before the study.

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### Supplemental material

Supplemental material for this article is available online.

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