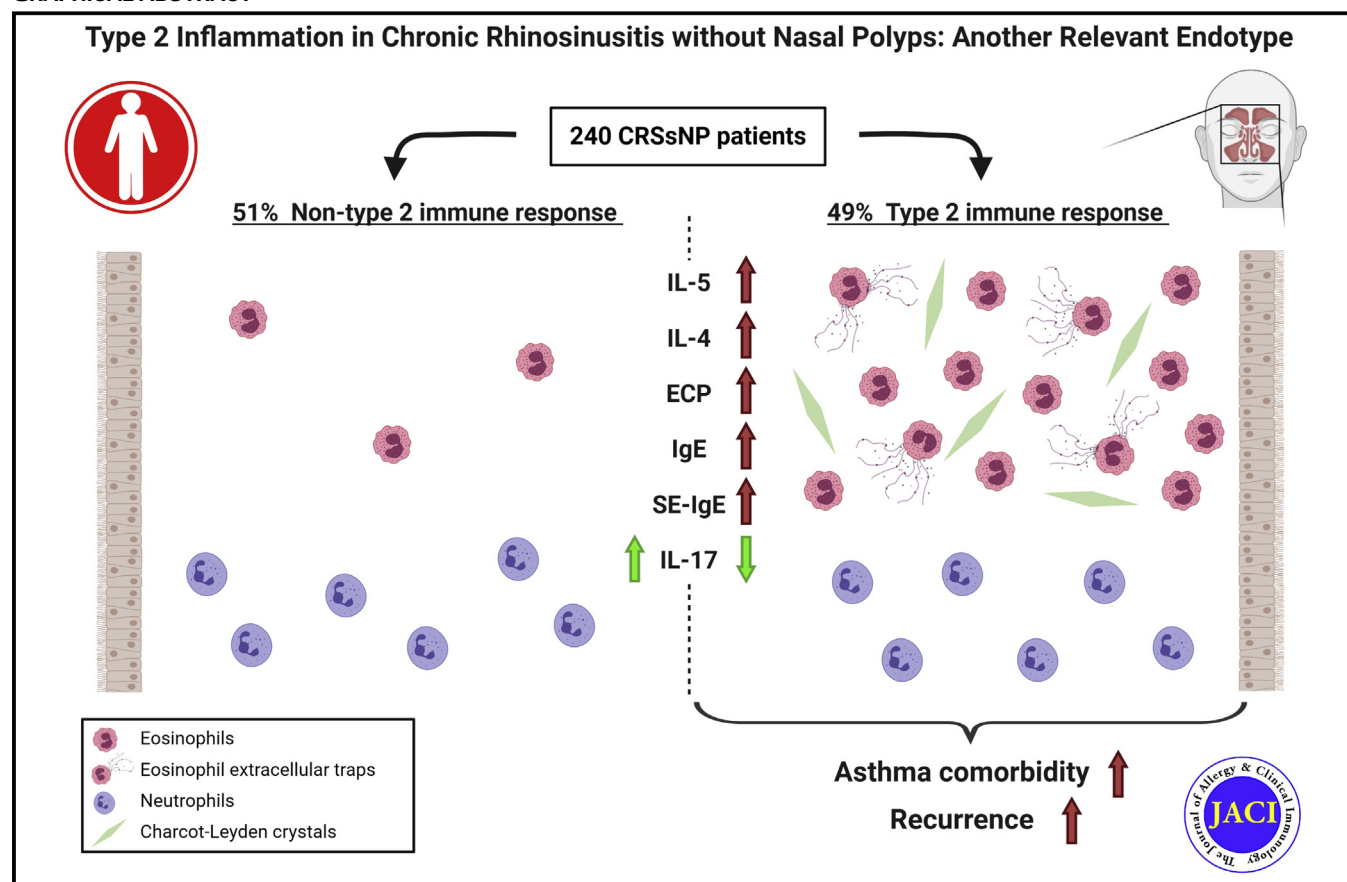


Type 2 inflammation in chronic rhinosinusitis without nasal polyps: Another relevant endotype

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GRAPHICAL ABSTRACT



Background: Chronic rhinosinusitis without nasal polyps (CRSsNP) is mainly considered a type 1 mediated disease. The role and clinical significance of type 2 immune responses in CRSsNP have not been addressed sufficiently; a recent cluster analysis for CRS described the existence of a subgroup of patients with CRSsNP with a type 2 inflammation.

Objective: We aimed to characterize the underlying type 2 immune response and its clinical significance in patients with CRSsNP.

Methods: A total of 240 patients with CRSsNP were endotyped and subdivided on the basis of expression of marker cytokines. Clinical data such as recurrence, comorbid asthma and allergy,

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and numbers of blood eosinophils and neutrophils were collected from all patients. A selection of 15 patients was further characterized for the presence of eosinophils, neutrophils, Charcot-Leyden crystals, and eosinophil extracellular traps in the mucosae.

Results: A type 2 immune response with increased levels of IL-4, IL-5, eosinophil cationic protein, IgE, and *Staphylococcus aureus* enterotoxin-specific IgE was observed in 49% of patients with CRSsNP. Those patients showed increased numbers of blood and tissue eosinophils, and they displayed a considerable eosinophilic inflammation associated with eosinophil extracellular trap cell death and Charcot-Leyden crystals. A significantly increased prevalence of recurrence and asthma was observed in patients with type 2 CRSsNP compared with in patients with non-type 2 CRSsNP. However, only 4 of 117 patients with type 2 CRSsNP developed nasal polyps within 12 years.

Conclusion: This study shows that type 2 immune responses in CRSsNP follow similar patterns but are less pronounced than in chronic rhinosinusitis with nasal polyps. Also CRSsNP with a moderate type 2 immune response showed a considerable eosinophilic inflammation with clinical impact. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: CRSsNP, type 2 inflammation, eosinophils, recurrence, asthma

Phenotypically, chronic rhinosinusitis (CRS) is classified as CRS with (CRSwNP) and CRS without nasal polyps (CRSsNP), representing approximately 20% and 80% of patients with CRS.^{1,2} Although differentiation of CRS based on phenotyping is well established, it has been demonstrated that both phenotypes of CRS can be further differentiated into endotypes with likely importance for the natural course of disease.³ In white patients, endotyping has been used in CRSwNP, which reflects type 2-biased eosinophilic inflammation in about 80% of subjects.^{4,6} On the other hand, CRSsNP is often considered primarily as a type 1 to type 3 inflammation, which is associated with neutrophils and regulated by elevated levels of IL-6, IL-8, IL-17, and TNF- α .^{5,7-9}

Although this dichotomous classification is still used in daily clinics, further endotyping has suggested that CRS is a more complex disease, often with mixed inflammatory patterns.^{4,6,9-11} Remarkably, 30% of European patients and 55% of North American patients with CRSsNP also showed a mild to moderate—and in some cases mixed—type 2 inflammation.^{4,11} Our recent cluster analysis leading to CRS endotyping identified a subgroup of patients with CRS with a moderate type 2 inflammation. Of these patients with CRS, 40% were phenotypically classified as having CRSsNP, but with an asthma prevalence of 33%.⁴ Endotypes may also translate into different symptom patterns, with type 2 inflammation in patients with CRSsNP being associated with loss of smell.¹¹

In CRSwNP, it is well established that disease severity is correlated with the degree of type 2 inflammation.^{6,12,13} Because of the frequent recurrence of disease after surgery and increased asthma comorbidity, research had been focusing on patients with CRSwNP; however, this focus was recently increased with the advent of biologics. In contrast, the observed levels of type 2 cytokines in patients with CRSsNP are rather moderate, and this disease is easier to control in clinical practice.^{2,4} For these

Abbreviations used

CLC:	Charcot-Leyden crystal
CRS:	Chronic rhinosinusitis
CRSsNP:	Chronic rhinosinusitis without nasal polyps
CRSwNP:	Chronic rhinosinusitis with nasal polyps
EETosis:	Eosinophil extracellular trap cell death
FESS:	Functional endoscopic sinus surgery
SE-IgE:	Immunoglobulin E specific for <i>Staphylococcus aureus</i> enterotoxins
ECP:	Eosinophil cationic protein
EET:	Eosinophil extracellular trap
NPV:	Negative predictive value
PPV:	Positive predictive value

reasons, the role and clinical significance of type 2 immune responses in patients with CRSsNP have hitherto been considered less relevant. Therefore, in this study we aimed to define type 2 immune responses and characterize their clinical significance in patients with CRSsNP.

METHODS

Sample collection

Biopsy samples of sinus mucosal tissue samples from white patients with CRSsNP (n = 240) who underwent functional endoscopic sinus surgery (FESS) were collected with approval by the local ethics committee of Ghent University Hospital (B670201525433) and after receiving written informed consent for inclusion. Patients with CRSsNP were qualified for FESS in line with European Position Paper on Sinusitis guidelines.¹ Tissue samples were either snap-frozen or embedded in paraffin. Patients with immunodeficiencies and cystic fibrosis and patients who had used oral or intranasal glucocorticoids within 4 weeks before surgery were excluded from this study. Patients were considered recurrent when they returned with major clinical symptoms such as nasal congestion, facial pain, or smell disturbance. Endoscopy and pharmacologic treatment were reapplied. For patients with persistent recurrence after resuming treatment, revision surgery was discussed, and it was performed on 77% of them. Eight of the 240 patients enrolled into interventional clinical trials after FESS and were excluded from the follow-up study on recurrence. Clinical data and symptom surveys were collected for all patients who enrolled the study (Table 1). Patients were considered to have asthma on the basis of their clinical records or diagnosis by a pneumonologist to whom they were referred when they experienced asthmatic symptoms. Allergy was defined as present when the patient had a positive skin prick test result for at least 1 of the allergens commonly tested in our region. Blood samples for eosinophil and neutrophil analysis were collected before surgery.

Cytokine and protein measurement

Samples were analyzed for common cytokines reflecting previously described inflammatory patterns.^{4,5} Homogenates were prepared from snap-frozen tissues as described earlier.¹⁴ Samples were analyzed for IL-4, IL-5, IL-17, and TNF- α by using Luminex kits commercially available from R&D Systems (Minneapolis, Minn), and IFN- γ using a Quantikine ELISA kit commercially available from R&D Systems. Cytokine levels were measured on a Bio-Plex 200 Array Reader (Bio-Rad, Hercules, Calif). Eosinophilic cationic protein (ECP), IgE, and *Staphylococcus aureus* enterotoxin-specific IgE (SE-IgE [staphylococcal enterotoxin A, staphylococcal enterotoxin C, and toxic shock syndrome toxin-1]) were measured by using the UniCAP method (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden) according to the manufacturer's instructions. Concentrations below the detection limit were considered negative and were given a value of half of the detection limit. Differentiation of patients with

TABLE I. Patient characterization

Indicator	Non-type 2 CRSsNP	Type 2 CRSsNP
Total cases (n)	123	117
Sex (F/M)	58/65	43/74
Age (y), median (range)	43 (18-87)	44 (18-83)
Ethnicity	White	White
Allergy (+/-)	39/84	47/70
Missing cases (n)	—	—
Asthma (+/-)	12/111	34/78
Missing cases (n)	—	5
Tissue concentrations		
IgE (U/g), mean $\pm$ SD	56.88 $\pm$ 67.76	488.47 $\pm$ 1138.56
SE-IgE (UA/g), mean $\pm$ SD	0.33 $\pm$ 1.30	1.77 $\pm$ 3.44
IL-5 (pg/g), mean $\pm$ SD	2.36 $\pm$ 3.57	152.63 $\pm$ 197.60
ECP (μ g/g), mean $\pm$ SD	1.14 $\pm$ 1.82	7.86 $\pm$ 6.67
IL-4 (pg/g), mean $\pm$ SD	15.31 $\pm$ 31.94	38.69 $\pm$ 46.30
IL-17 (pg/g), mean $\pm$ SD	113.46 $\pm$ 173.77	39.56 $\pm$ 82.25
TNF- α (pg/g), mean $\pm$ SD	29.25 $\pm$ 32.80	24.73 $\pm$ 39.50
IFN- γ (pg/g), mean $\pm$ SD	142.39 $\pm$ 268.91	285.79 $\pm$ 518.65

Allergy was defined as present (+) when the patient had a positive skin prick test result for at least 1 of the allergens commonly tested in our region, and negative (–) otherwise.

CRSsNP for type 2 immune response was based on tissue levels of IL-5, as mentioned before (cutoff value 12.98 pg/g).⁴

Staining

Immunohistochemistry. Subsequent to fixation in 4% paraformaldehyde and embedding in paraffin, slides of 4- μ m tissue samples were prepared from a random selection of 15 CRSsNP samples (9 type 2 and 6 non-type 2) that fitted the inclusion criteria. After deparaffinization in xylene, the slides were rehydrated in decreasing concentrations of ethanol. Slides were blocked with 7.5% BSA in PBS and incubated with primary antibodies and isotype control (monoclonal mouse anti-human myelin basic protein, BMK13 [Monosan] and monoclonal mouse anti-human neutrophil elastase, NP57 [Dako]; monoclonal mouse) for 16 hours at 4°C. An alkaline phosphatase–conjugated secondary antibody kit (Dako REAL Detection System, Alkaline Phosphatase/RED, Rabbit/Mouse [Dako, Agilent Technologies, Diegem, Belgium]) was applied following the manufacturer's instructions. The stained slides were counterstained with hematoxylin for 1 minute and mounted with Aquatex mounting medium (Merck). Isotype controls were included for both primary antibodies on all samples. The total numbers of positive cells were counted and expressed as number of cells per mm² tissue.

Immunofluorescence. Paraffin-embedded tissue slides were treated until blocking as mentioned in the preceding section on immunofluorescence. Slides were subsequently incubated with primary antibodies (monoclonal mouse anti-human myelin basic protein, BMK13 [Monosan] and monoclonal rabbit anti-human galectin-10, ab157475 [Abcam]) for 16 hours at 4°C and secondary antibody (fluorescein isothiocyanate–conjugated polyclonal goat anti-mouse IgG, A11017 [ThermoFisher] and fluorescein isothiocyanate–conjugated goat anti-rabbit IgG, respectively, 111034, [ThermoFisher]) for 1 hour at 21°C. Slides were mounted with mounting medium with 4',6-diamino-2-phenylindole (Vector Laboratories, United Kingdom) for nuclear counterstain and analyzed with a confocal laser scanning microscope (Leica Microsystems). For each patient, eosinophil extracellular traps (EETs) and Charcot-Leyden crystals (CLCs) were counted in 10 random fields throughout the tissue. The numbers of EET-involving eosinophils were normalized to the total numbers of eosinophils and expressed as the percentage of EET-generating eosinophils.

Statistical analysis

Statistical analysis was performed by using Prism GraphPad software, version 8 (GraphPad Software, La Jolla, Calif). The Mann-Whitney *U* test was

performed to analyze statistical differences between 2 groups. Correlations were analyzed with a nonparametric Spearman correlation test. A 2-sided chi-square test was performed to evaluate statistical differences between 2 binary variables. Receiver operating characteristic curve analysis was applied to define the best cutoff value and calculate the area under curve, the positive predictive value (PPV), and the negative predictive value (NPV). Where possible, data were presented in dot plots for a better representation of individual variability. Outliers, defined as values more than 3 times the SD above the average value, were excluded from analysis. Levels of significance were expressed as *P* values less than .05, less than .01, less than .001, and less than .0001. *P* values less than or equal to .05 were considered statistically significant.

RESULTS

A considerable fraction of patients with CRSsNP display a type 2 immune response

Patients with CRSsNP (*n* = 240) who underwent FESS were subdivided into a group with an underlying type 2 immune response based on tissue IL-5 levels of 12.98 pg/g (designated as the type 2 CRSsNP group) and a group without an underlying type 2 immune response based on tissue IL-5 levels of 12.98 pg/g (designated as the non-type 2 CRSsNP group) (Fig 1, A). Remarkably, 177 of the patients with CRSsNP (49%) expressed IL-5 in sinus mucosal tissue in concentrations of more than the cutoff value and thus displayed an underlying type 2 response.

Tissue concentrations of total IgE, SE-IgE, ECP (*P* < .0001), and IL-4 (*P* < .01) were also significantly increased in patients with type 2 CRSsNP compared with in patients with non-type 2 CRSsNP (*P* < .0001 [Fig 1, B–E]). In patients with type 2 CRSsNP, significantly positive correlations were observed between tissue levels of IL-5 and ECP (*r* = 0.645 [*P* < .0001]), IL-5 and IgE (*r* = 0.438 [*P* < .0001]), and ECP and IgE (*r* = 0.453 [*P* < .0001]). In contrast, tissue levels of IL-17 were significantly increased in non-type 2 CRSsNP compared with in its counterpart (*P* < .0001) (Fig 1, F), whereas no differences in TNF- α and IFN- γ levels were observed between the 2 groups (Fig 1, G and H).

Blood eosinophils were significantly increased (*P* < .0001) in patients with type 2 CRSsNP compared with in the group with non-type 2 CRSsNP (Fig 1, I). However, receiver operating characteristic curve analysis showed a limited predictive potential for type 2 response in CRSsNP based on the number of blood eosinophils (area under the curve = .7249) (see Fig E1 in this article's Online Repository at www.jacionline.org). The best cutoff value was 211 cells/mL, which gave a PPV of 65% and an NPV of 68%. For the cutoff value of 300 cells/mL, we found a PPV of 59% and an NPV of 75%. No significant difference in blood neutrophils was found between the 2 groups (Fig 1, J).

Type 2 response in CRSsNP results in EETosis and CLC deposition

In CRSsNP, type 2 immune response is characterized by severe tissue eosinophilia, eosinophil extracellular trap cell death (EETosis), and CLC deposition.^{15,16} To investigate whether this is also the case in CRSsNP, we analyzed the extent of eosinophilic inflammation locally in the mucosa in a selection of patients (6 with non-type 2 CRSsNP and 9 with type 2 CRSsNP). The numbers of tissue eosinophils were significantly (*P* < .001) increased in patients with type 2 CRSsNP compared with in patients in the non-type 2 CRSsNP group, in which the numbers

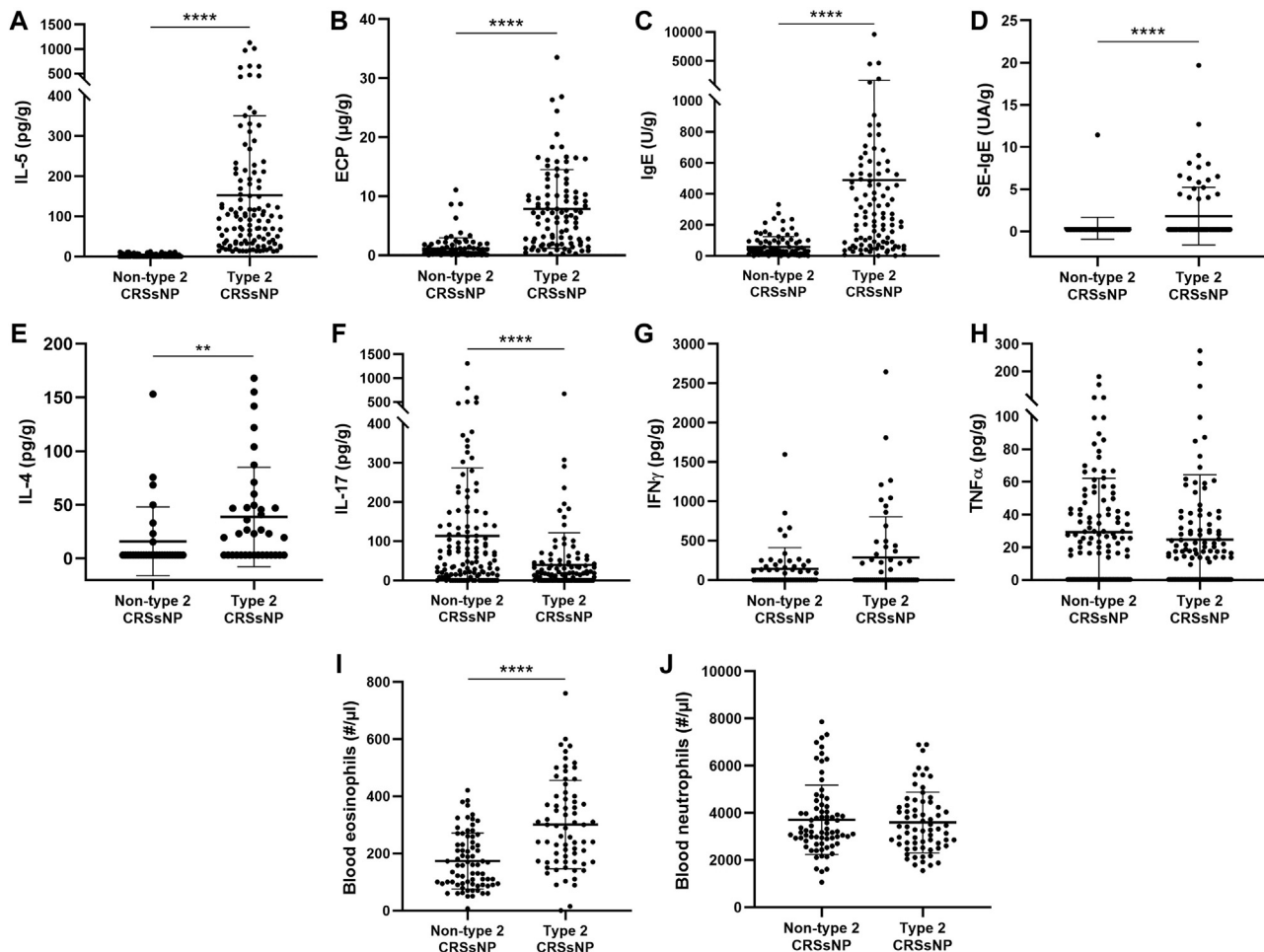


FIG 1. Levels of tissue protein and blood granulocytes in different patient groups. Levels of IL-5, ECP, IgE, and SE-IgE ($P < .0001$) (A-D) and IL-4 (E) ($P < .01$) are significantly elevated in the sinus mucosa of patients with type 2 CRSsNP versus in patients with non-type 2 CRSsNP. F, Levels of IL-17 are significantly ($P < .0001$) elevated in the sinus mucosa of patients with non-type 2 versus in the sinus mucosa of patients with type 2 CRSsNP. G and H, No differences in TNF- α and IFN- γ tissue levels between the 2 patient groups. I and J, Numbers of blood eosinophils ($P < .0001$), but not numbers of blood neutrophils, are significantly elevated in patients with type 2 CRSsNP versus in patients with non-type 2 CRSsNP. Levels of statistical significance are expressed as follows: * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

of neutrophils in the tissue did not differ between the 2 groups (Fig 2, A and B).

EETosis was observed in CRSsNP and was significantly increased in type 2 CRSsNP compared with in non-type 2 CRSsNP ($P < .001$), with an average of 7% of eosinophils undergoing EETosis in patients with type 2 CRSsNP. Moreover, the rates of EETosis in CRSsNP correlated significantly with tissue concentrations of IgE ($r = 0.793$ [$P < .001$]), ECP ($r = 0.782$ [$P < .01$]), IL-5 ($r = 0.755$ [$P < .01$]), and CLCs ($r = 0.705$ [$P < .01$]) (Fig 2, C and see Fig E2 in this article's Online Repository at www.jacionline.org). Remarkably, both Gal10 and CLCs were observed in the patients with CRSsNP (Fig 2, D). In line with the degree of EETosis, CLC deposition was significantly increased in type 2 CRSsNP compared with in non-type 2 CRSsNP ($P < .01$) (Fig 2, E).

Type 2 inflammation in CRSsNP is associated with clinical outcome

All patients were followed up for recurrence for up to 12 years after surgery. In all, 12.9% of all patients with CRSsNP showed

recurrence within 12 years after FESS, with a significantly ($P < .05$) increased recurrence rate for patients with type 2 CRSsNP (19.0%) compared with in patients with non-type 2 CRSsNP (9.2%) (Fig 3). On average, recurrence occurred 6 years after the patients' first FESS and an additional surgical procedure was required for 80.6% of the patients with recurrence. Interestingly, manifest nasal polyp development was observed in only 4 patients with type 2 CRSsNP who were older than 12 years. We collected sinus mucosal biopsy samples from revision surgery performed on 4 patients with recurrent type 2 CRSsNP (3 of whom developed CRSwNP within 8 years after first FESS) and observed increased levels in IL-5 and ECP levels in all 4 samples compared with after the first surgical procedure (see Fig E3 in this article's Online Repository at www.jacionline.org).

The prevalence of comorbid asthma among all patients with CRSsNP was 19.6%, with a significantly increased prevalence of asthma ($P < .0001$) in patients with type 2 CRSsNP (30.2%) compared with in patients with non-type 2 CRSsNP (9.8%). No differences in comorbid allergy were observed between the 2

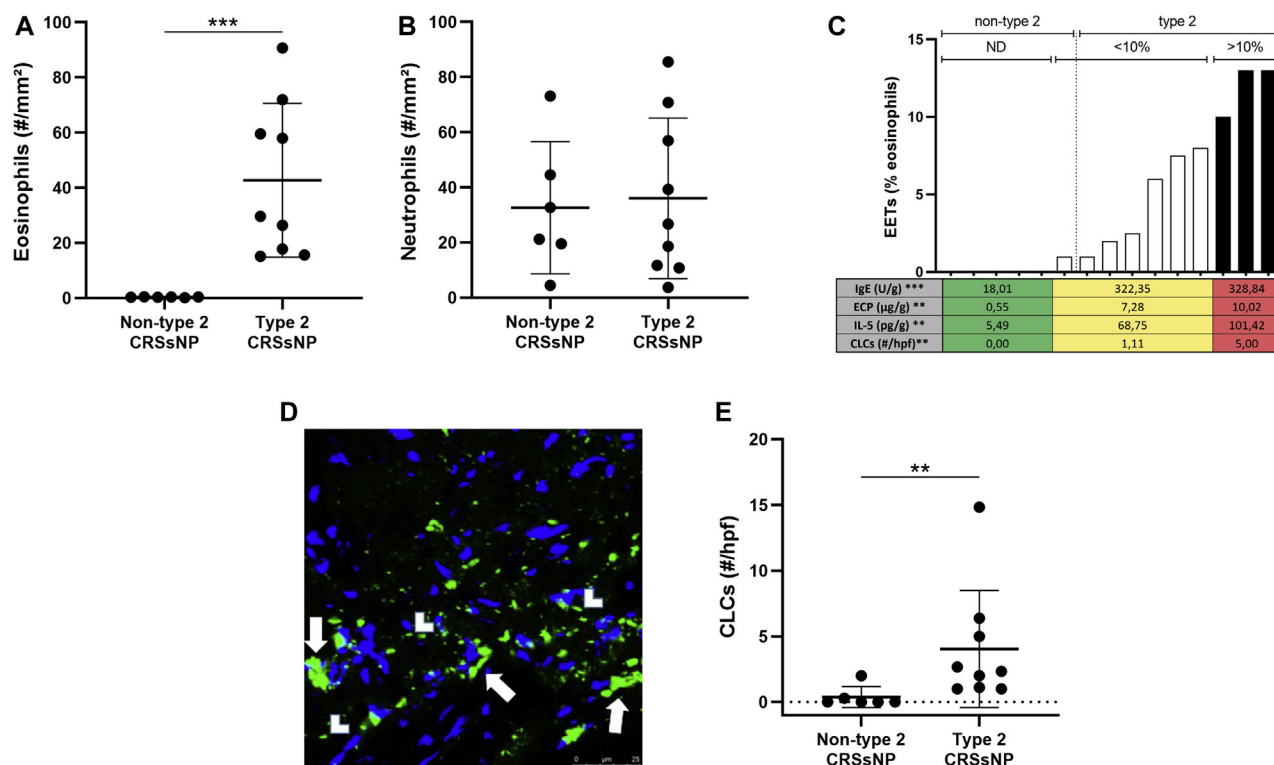


FIG 2. Type 2 CRSsNP associated with eosinophilic inflammation. **A**, Significantly increased numbers of eosinophils in the sinus mucosa of patients with type 2 CRSsNP versus in the sinus mucosa of patients with non-type 2 CRSsNP. **B**, No difference in numbers of neutrophils in the sinus mucosa between the 2 patient groups. **C**, Rates of EETs were significantly ($P < .001$) increased in type 2 CRSsNP and correlated significantly ($P < .01$) positively with tissue levels of IgE, ECP, and IL-5 and the presence of CLCs in the sinus mucosa. **D**, Immunofluorescent staining of galectin-10 (green) and 4',6-diamino-2-phenylindole (DAPI) (blue) in CRSsNP sinus mucosa shows that deposition of galectin-10 (arrows) and CLC formation (arrow heads) were also present in CRSsNP. **E**, Numbers of CLCs were significantly ($P < .01$) upregulated in the tissue of patients with type 2 CRSsNP. Levels of statistical significance are expressed as follows: * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

patient groups (Fig 3). However, among all patients with CRSsNP without allergy, concentrations of IgE were still significantly increased in those patients with a type 2 immune response compared with in those without a type 2 immune response (see Fig E4, A in this article's Online Repository at www.jacionline.org). In addition, we observed that tissue levels of IgE correlated significantly with IL-5 ($r = 0.5946$; $P < .0001$) (Fig E4, B) and ECP ($r = 0.6096$; $P < .0001$) (Fig E4, C) in the patients without allergy. No significant differences were observed in the mean Visual Analog Scale scores for general symptoms, nasal congestion, nasal mucus, facial pain or pressure, reduced smell and/or taste, phlegm in the throat, and cough between non-type 2 and type 2 CRSsNP (see Table E1 in this article's Online Repository at www.jacionline.org).

DISCUSSION

We here confirm that 49% of the patients with CRSsNP display a type 2 immune response. This is in line with earlier publications showing the presence of an underlying type 2 inflammation among heterogeneous inflammatory patterns within the patients with CRSsNP.^{4,6,10,11} The characteristics of type 2 responses in CRSsNP were similar to those of type 2 responses in CRSwNP, as characterized by increased tissue levels of IL-4, IgE, SE-IgE,

and ECP; elevated numbers of blood and tissue eosinophils; and the presence of EETosis associated with CLC deposition. However, the strength of type 2 inflammation is lower in CRSsNP than in CRSwNP (see Table E2 in this article's Online Repository at www.jacionline.org).^{4,5,16-18} Interestingly, the relative fractions of eosinophils that underwent EETosis in type 2 CRSsNP (7.0%) were comparable to those described earlier in severe type 2 CRSwNP (8.8%).¹⁷ However, the absolute numbers of infiltrating eosinophils are extensively (18-fold) lower in type 2 CRSsNP compared with in CRSwNP, which is reflected not only in lower absolute numbers of EETosis, but also in CLC deposition.¹⁷ This confined overall presentation of type 2 cytokines and eosinophil activation in type 2 CRSsNP compared with in CRSwNP might explain why nasal polyp formation does not occur in CRSsNP, despite type 2 presentation. As suggested earlier, excessive levels of eosinophil mediators might cause severe tissue damage associated with vascular leakage, albumin accumulation, and edema formation, inducing nasal polyp formation.^{19,20}

A recent study showed that CLCs can provoke a neutrophilic inflammation in a type 2 inflammatory context such as CRSwNP.²¹ As the extent of both type 2 inflammation and the associated CLC deposition was rather low, a clear link between neutrophilia and CLCs could not be shown in CRSsNP. Likely,

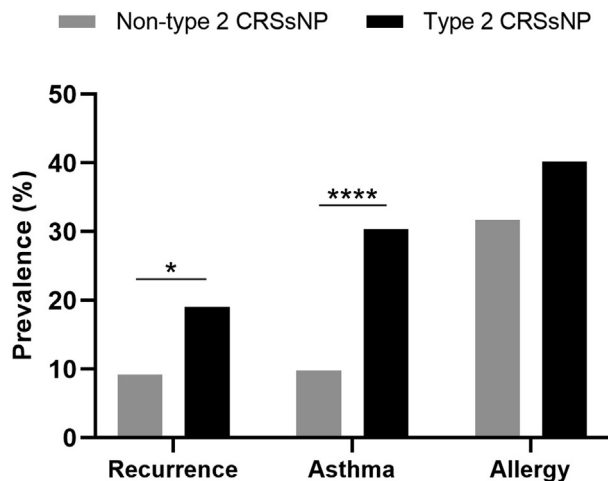


FIG 3. Clinical differences between patients with type 2 CRSsNP and patients with non-type 2 CRSsNP. Patients with type 2 CRSsNP had significantly increased rates of recurrence ($P < .05$) and comorbid asthma ($P < .0001$) compared with the rates in patients with non-type 2 CRSsNP. No differences were observed in prevalence of comorbid allergy between the 2 patient groups. Levels of statistical significance are expressed as follows: * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

the neutrophilic influx is driven by other cytokines that are known to be enhanced in CRSsNP, such as IL-6, IL-8, IL-17, and TNF- α .^{4,5,8,9} Interestingly, here we have demonstrated decreased IL-17 levels in patients with CRSsNP with an underlying type 2 inflammation. This is in line with earlier reports showing that T_H17 response is suppressed in a type 2 environment in CRSwNP, which results in 2 distinctive groups of IL-5^{low}/IL-17^{high} and IL-5^{high}/IL-17^{low} patients with CRSsNP.

Of the patients with CRSsNP, 12.9 % suffered from recurrence within up to 12 years, with an average of 6 years and an increased recurrence rate for patients with CRSsNP who had an underlying type 2 inflammation. The percentage of recurrence in patients with CRSsNP was remarkably lower than that described in patients with CRSwNP, with a recurrence rate of 60% to 70% up to 18 months and 79% up to 12 years after surgery.^{22,23} In another study, no difference in recurrence could be observed within 3 years after surgery on patients with CRSsNP; however, the observation period in this study was shorter than in our current study.²⁴ As shown, patients with CRSsNP with increased mucosal eosinophilia showed less improvement in disease-specific (via the Chronic Sinusitis Survey and Rhinosinusitis Disability Index) and general (via the 36-Item Short Form Health Survey) quality of life than did those patients without eosinophilia.²⁵

Interestingly, our study showed for the first time that a small proportion of patients with type 2 CRSsNP may develop nasal polyps and showed an elevation in type 2 cytokine levels in recurrent biopsy samples. However, the type 2 cytokine levels in the tissue from their first surgery were not necessarily among the highest in the type 2 CRSsNP group. The exact mechanism that induces the shift toward nasal polyp formation therefore remains unclear. Larger studies need to be conducted to confirm this finding and its relevance.

Here we have shown that similar to in patients with CRSwNP, the prevalence of asthma in patients with CRSsNP is associated with an underlying type 2 response. In contrast, another study showed that asthma comorbidity did not affect radiologic disease severity in patients with CRSsNP, whereas this was the case in

CRSwNP.² However, in the latter study, patients with CRSsNP were not further differentiated on the basis of inflammatory patterns. In contrast to our findings for asthma, comorbid allergy was unaffected by the type 2 versus non-type 2 CRSsNP differentiation, whereas local production of IgE was associated with type 2 inflammation in CRSsNP. This suggests a significant role for locally produced polyclonal IgE, rather than allergy, as a driving factor of type 2 immune responses in CRSsNP. Finally, unlike the authors of recent reports, we could not confirm that the presence of a type 2 endotype in CRSsNP was associated with headaches and loss of smell and taste.¹¹

The considerable eosinophilic inflammation and increased recurrence of patients with type 2 CRSsNP raises the question of whether these results should have any impact on disease management in CRSsNP, especially because surgical approaches have been adapted to disease endotypes and treatment with mAbs has become available in patients with severe eosinophilic type 2 CRSwNP.^{3,26-28} However, we have to take into account that type 2 eosinophilic inflammation in CRSsNP is less severe than in CRSwNP and the rates of recurrence and polyp development in type 2 CRSsNP are lower. In addition, no diagnostic markers for type 2 immune response in CRSsNP are available at the moment.

In conclusion, this study shows that type 2 immune responses are not limited to CRSwNP. Also, patients with CRSsNP with a moderate type 2 immune response have a higher rate of recurrence and comorbid asthma. These moderate type 2 immune responses are associated with a significant eosinophilic inflammation in CRSsNP, which may open new perspectives for the implementation of tailored and innovative treatments for patients with severe CRSsNP as well.

Key messages

- In all, 49% of patients with CRSsNP displayed a type 2 immune response associated with eosinophilic inflammation.
- The rates of recurrence and asthma were significantly increased in patients with CRSsNP with an underlying type 2 immune response.
- Development of nasal polyposis was observed in some patients with recurrent CRSsNP.

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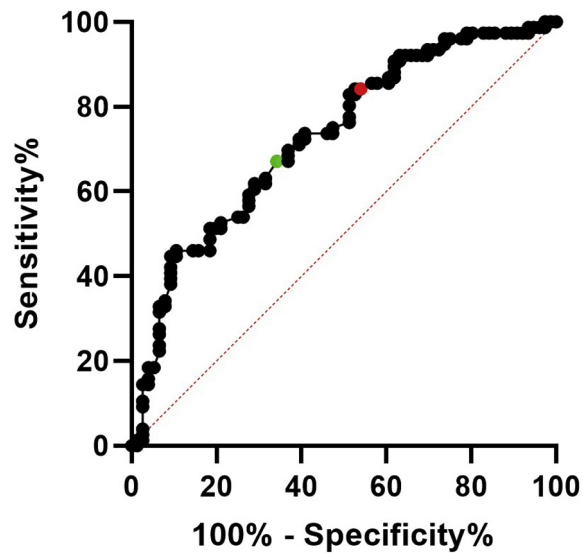


FIG E1. Receiver operating characteristic curve analysis for the presence of type 2 CRSsNP via blood eosinophil counts showed an area under curve of .7249. The best cutoff value (*green*) was 211 cells/mL, with a PPV of 65% and an NPV of 68%. The cutoff value of 300 cells/mL (*red*) showed a PPV of 59% and an NPV of 75%.

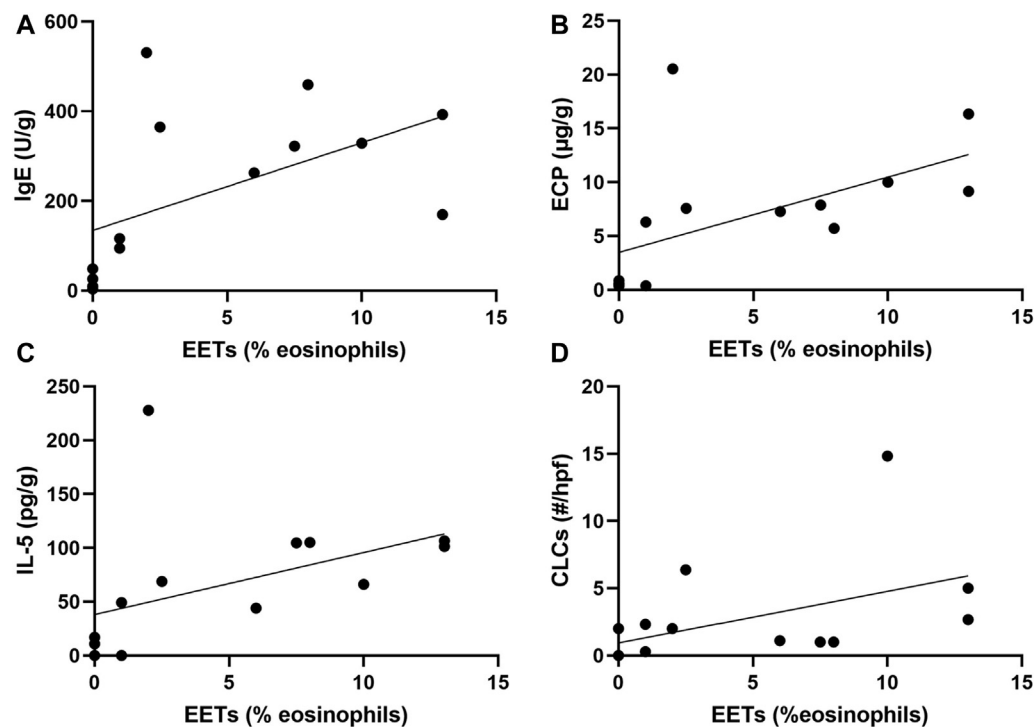


FIG E2. EETosis is associated with other type 2 markers in CRSsNP. Rates of EETs correlated significantly ($P < .01$) positively with tissue levels of IgE ($r = 0.793$) (A), ECP ($r = 0.782$) (B), and IL-5 ($r = 0.755$) (C) and the presence of CLCs ($r = 0.705$) (D) in the sinus mucosa.

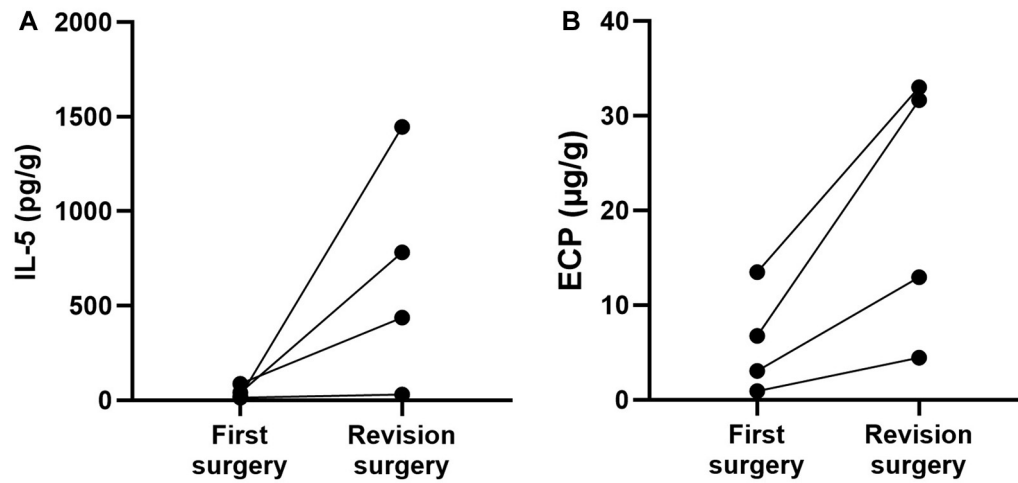


FIG E3. Increased type 2 response in patients with recurrent type 2 CRSsNP. Tissue levels of IL-5 and ECP were compared between tissues collected from first surgery and those of revision surgery. Levels of IL-5 (**A**) and ECP (**B**) tend to be increased in recurrent sinus tissue compared with the levels in the sinus mucosa at earlier FESS. Three of these patients also developed CRSwNP.

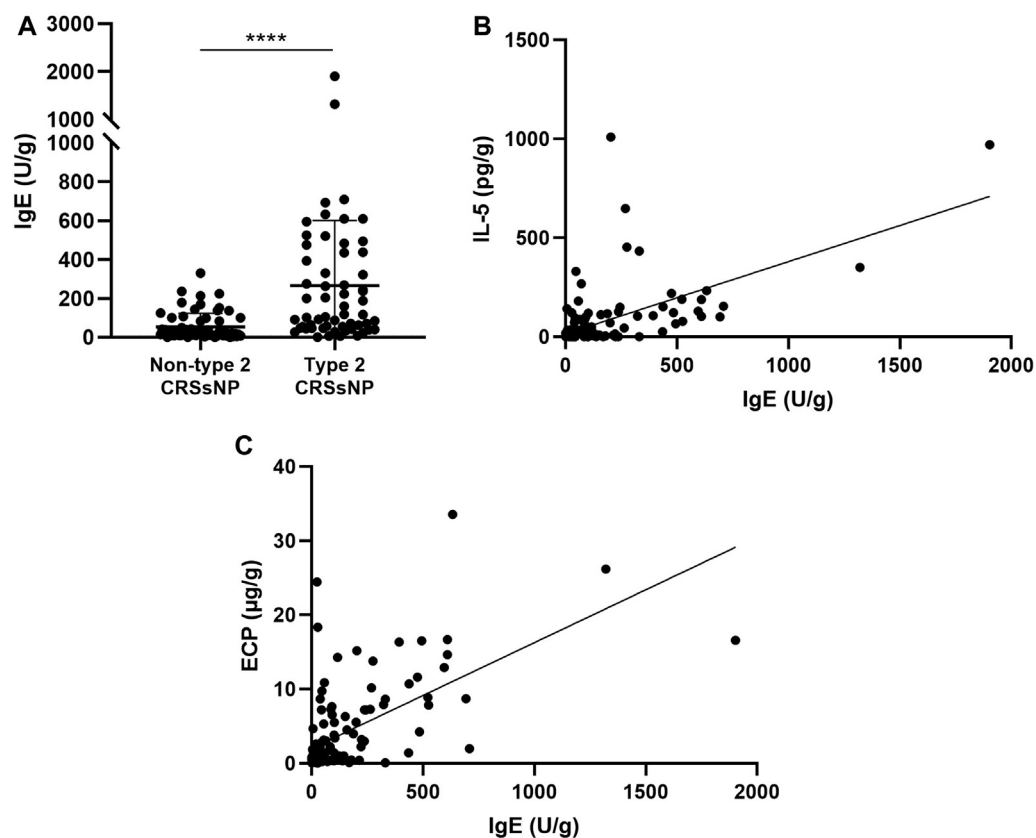


FIG E4. Locally produced IgE associated with type 2 inflammation in patients with CRSsNP without allergy. **A**, Tissue concentrations of IgE are significantly increased in patients with type 2 CRSsNP without allergy versus in those with non-type 2 CRSsNP without allergy ($P < .0001$). **B** and **C**, Among all patients with CRSsNP without allergy, tissue concentrations of IgE correlated significantly ($P < .0001$) with IL-5 ($r = 0.5946$) and EPC ($r = 0.6096$) levels.

TABLE E1. Comparison of symptom scores between patients with non-type 2 versus type 2 CRSsNP

Indicator	Score		<i>P</i> value
	Non-type 2 CRSsNP	Type 2 CRSsNP	
General symptom score	6.88 ± 2.03	6.34 ± 1.88	.106
Nasal congestion	5.90 ± 2.82	5.90 ± 2.52	.750
Nasal mucus	5.16 ± 3.30	5.78 ± 2.68	.401
Phlegm in the throat	6.15 ± 2.89	6.15 ± 2.90	.837
Facial pain/pressure	6.26 ± 3.14	5.44 ± 2.96	.381
Reduced smell/taste	5.23 ± 3.26	5.42 ± 3.11	.715
Cough	3.22 ± 3.03	3.93 ± 3.12	.235

Symptom scores are presented as means ± SDs of the Visual Analog Scale score for each group. *P* value was determined via the Mann-Whitney *U* test.

TABLE E2. Comparison of levels of type 2 mediators in type 2 CRSsNP versus in CRSwNP

Indicator	Type 2 CRSsNP	CRSwNP
Total cases	117	143
Sex (% F/M)	37/63	28/72
Median age, y (range)	44 (18-83)	35 (21-92)
Ethnicity	White	White
Allergy (% positive)	40.2	66.7
Asthma (% positive)	30.4	45.8
Tissue concentrations		
IL-5 (pg/g), mean ($\pm$ SD)	152.63 $\pm$ 197.60	547 $\pm$ 320
ECP (μ g/g), mean ($\pm$ SD)	7.86 $\pm$ 6.67	10.6 $\pm$ 1.33
IgE (U/g), mean ($\pm$ SD)	488.47 $\pm$ 1138.56	989 $\pm$ 502
Eosinophils (/mm ²), mean ($\pm$ SD)	42.75 $\pm$ 27.90	750 $\pm$ 469
EETs (% eosinophils), mean ($\pm$ SD)	7.00 $\pm$ 4.53	8.49
CLCs (/hpf), mean ($\pm$ SD)	4.04 $\pm$ 4.46	10.1

F, Female; M, male.

For the CRSwNP group, we presented the means of historical measurements in CRSwNP, published by our laboratory, with the SDs of these means.^{4,5,16-18}