

# 比利時在西元 2007 年到 2018 年之間慢性鼻竇炎之第二型發炎反應的轉變

## Type 2 Inflammatory Shift in Chronic Rhinosinusitis During 2007–2018 in Belgium

Jonstam Karin, MD, PhD ; Delemarre Tim, MSc; Holtappels Gabriele, MLT; Lars-Olaf Cardell, MD, PhD; Westman Marit, MD, PhD;  
Bachert Claus, MD, PhD

新光醫院 蔡易錚醫師

### Commentary

慢性鼻及鼻竇炎在歐洲及美國的盛行率約為 12-16%，每年花費的醫療及勞力損失約有數十億之多。在台灣盛行率則介於 15-20%。慢性鼻及鼻竇炎是一種具有不同炎症類型的異質性疾病，取決於臨床上有息肉的存在 (CRSwNP) 或不存在 (CRSSNP) 以及疾病發生的地理位置。目前在亞洲已經發現了此疾病有向第二型內在型轉變的趨勢。此篇研究的目的即是調查比利時患者是否有第二內在型轉變的趨勢，並根據臨床標記物進一步去找出與內在型的關聯。

此研究為前瞻描述性研究，收集了 2007 年至 2018 年間，在根特大學醫院接受鼻竇手術的 438 例慢性鼻竇炎患者，根據表現型、共病症、組織中的炎症標記物以及兩個不同的手術時間點進行了分層探討。作者對手術組織樣本進行第二型發炎反應標記物分析，並在息肉型鼻竇炎患者的一個子群中探討嗜酸性白血球的角色。研究結果顯示：無論臨床表型如何，在非哮喘、非過敏性的慢性鼻竇炎患者中，與早期手術族群 (2007-2010 手術者) 相比，晚期手術族群 (2016-2018 手術者) 的第二型炎症標誌物有所增加。在息肉型慢性鼻竇炎中，晚期手術族群的 IL-5 陽性患者比例升高。此外，研究也發現：IL-5 陽性的息肉型鼻竇炎和非息肉型鼻竇炎受試者的炎症標誌物和共病症有所不同，此現象在 IL-5 陰性的慢性鼻竇炎組別中則沒有差異，顯示息肉的形成與第二型炎症標誌物的存在或濃度沒有直接關係。根據統計也發現嗜酸性白血球可以與有關共病症的信息一起幫助識別臨床上的第二型息肉型慢性鼻竇炎。

從這篇研究看起來，近八年來，比利時的慢性鼻及鼻竇炎患者也發生了向第二內在型炎症靠攏的轉變趨勢。這種轉變意味著我們預計未來會看到更多嚴重且難以治療的慢性鼻及鼻竇炎病例。在臨床上，我們可以看到許多程度不同的息肉表現型，然而要怎麼藉由臨床表現型來推論疾病的內在型在目前還是一個難解的問題，這篇論文提供了臨床參數 (氣喘/過敏) 和嗜酸性白血球 > 300 個細胞/ $\mu\text{L}$  可用於幫忙識別第二型息肉型慢性鼻竇炎，這個發現對於我們在臨床工作上會有很大的幫助。

關鍵詞：慢性鼻竇炎，鼻息肉，第二型炎症，第二型轉變

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Jonstam Karin, MD, PhD ; Delemarre Tim, MSc; Holtappels Gabriele, MLT; Lars-Olaf Cardell, MD, PhD; Westman Marit, MD, PhD; Bachert Claus, MD, PhD

**Objectives/Hypothesis:** Chronic rhinosinusitis (CRS) is a heterogenic disease with different inflammatory patterns depending on the presence (CRSwNP) or absence (CRSSNP) of polyps and geographical location. A shift toward type 2 endotype has been seen in Asia. We aim to investigate whether there has been type 2 shift in Belgium and to further endotype CRS based on clinical markers.

**Study design:** Prospective descriptive study.

**Methods:** Four hundred and thirty eight patients with CRS undergoing sinus surgery at Ghent University Hospital between 2007 and 2018 were included and stratified based on phenotype, comorbidities, inflammatory markers in tissue, and two different time points of surgery. Tissue samples from surgery were analyzed for type 2 markers. In a subgroup of CRSwNP blood eosinophils (EBC) was available.

**Results:** There was an increase in type 2 inflammatory markers in the latter group versus the earlier, in non-asthmatic, non-allergic CRS patients regardless of phenotype. The proportion of IL-5+ patients was elevated in the latter group in CRSwNP. Inflammatory markers and comorbidities differ between IL-5+ CRSSNP and CRSwNP subjects, no difference was seen in IL-5– CRS. EBC can together with information on comorbidities help identify type 2 CRSwNP in a clinical setting.

**Conclusion:** There is a shift toward type 2 inflammation within the CRS population over recent 8 years also in Belgium. This shift implies that we expect to see more cases of severe and difficult to treat CRS in the future. Polyp formation is not directly linked to the presence or concentrations of type 2 inflammatory markers. Clinical parameters and EBC > 300 cells/μL can be used to identify type 2 CRSwNP.

**Key Words:** Chronic rhinosinusitis, nasal polyps, type 2 inflammation, type 2 shift.

**Level of Evidence:** 3.

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### INTRODUCTION

Chronic rhinosinusitis (CRS) is a frequent disease, with a prevalence of 10%–12% in Europe and USA.<sup>1, 2</sup> CRS is defined as inflammation in the nose and paranasal sinuses for at least 12 subsequent weeks.<sup>3</sup>

From the Department of Clinical Science, Intervention and Technology, Division of Ear, Nose and Throat Diseases (J.K., L.-O.C., B.C.), Karolinska Institutet, Stockholm, Sweden; Department of Ear, Nose and Throat Diseases (J.K., L.-O.C., W.M., B.C.), Karolinska University Hospital, Stockholm, Sweden; Upper Airway Research Laboratory (D.T., H.G., B.C.), Ghent University, Ghent, Belgium; and the Department of Medicine (W.M.), Karolinska Institutet, Stockholm, Sweden.

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Send correspondence to Claus Bachert, MD, PhD, Upper Airway Research Laboratory (URL), Ghent University Hospital, Ghent University, Ghent, Belgium, 9000 Ghent, Belgium. E-mail: claus.bachert@ugent.be

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Clinically, CRS is divided into two different phenotypes based on presence or absence of nasal polyps (CRS with nasal polyps [CRSwNP], CRS without nasal polyps [CRSSNP]). Based on a cluster analysis of inflammatory parameters in nasal tissue,<sup>4</sup> three different endotypes of European CRS have been identified. The first endotype consists of patients with type 1 inflammation, clinically represented mostly by CRSSNP, the second endotype is IL-5 positive with moderately elevated type 2 markers such as immunoglobulin E (IgE) and eosinophil cationic protein (ECP), clinically representing a mix of CRSwNP and CRSSNP. The third endotype is characterized by IL-5 positivity with highly elevated levels of IgE and ECP and the presence of IgE specific to staphylococcal enterotoxins (SE-IgE). This third endotype shows a high prevalence of asthma comorbidity and frequent relapse of disease.<sup>4</sup> In Asia, the inflammatory patterns in CRSwNP are different, with a higher prevalence of type 1 inflammation and lower prevalence of type 2 inflammation<sup>5</sup>; however, a shift toward type 2 inflammation has been seen in parts of Asia.<sup>6–8</sup> It is unknown if there has been an increase in type 2 inflammation or endotype in CRS in Europe. It has been challenging to find easily accessible markers, that can be used to identify the different endotypes of CRS. Serum periostin has been suggested, together with serum IgE and SE-IgE, to be able to identify IL-5 and SE-IgE in tissue,<sup>9</sup> serum periostin is not used in clinical practice.

Having markers for endotyping of disease is crucial to be able to personalize treatment in these patients. The aim of this project was to determine whether the degree of type 2 inflammation in nasal polyps in Belgium/Europe has increased over time, and to investigate the type 2 inflammatory response in tissue in relation to different clinical phenotypes of CRS.

## MATERIALS AND METHODS

### Study Design

Between May 2007 and June 2018, CRSwNP and CRSsNP patients who underwent surgery at Ghent University Hospital, Belgium were asked to participate in a research database. Prior to surgery, clinical characteristics were recorded, and at surgery tissue samples were collected. The total number of patients in the database was 614. All patients were asked to stop intra nasal and systemic corticosteroids prior to surgery, 2 and 4 weeks respectively. In this study, we used patients from the database with complete information on history of asthma, inhalant allergy, and aspirin-exacerbated respiratory disease (AERD) comorbidity as well as results on tissue samples for IgE, IL-5, ECP, and SE-IgE. In a subgroup of CRSwNP patients, blood eosinophilic count (EBC) was also available. Patients with congenital disorders and patients receiving treatment with monoclonal antibodies were not included. AERD was defined as patient-reported respiratory reaction to non-steroidal anti-inflammatory drugs. Asthma was defined as patient reported use of asthma medication. Allergy was defined as patient-reported positive skin prick test or specific IgE. The study was approved by the ethics committee of UZ Ghent Hospital and an informed consent was signed by all participants.

### Sample Collection

During surgery, tissue samples from CRS patients were collected. The tissue was weight and snap frozen in liquid nitrogen and then stored in  $-80^{\circ}\text{C}$  until analysis. Frozen tissue was disrupted and homogenized at 50 Hz for 2 minutes with the

Tissue Lyser LT (Qiagen Benelux, Antwerp, Belgium) and 1 mL 0.9% NaCl in the presence of Complete, an EDTA-free protease inhibitor (Roche Diagnostics Belgium, Vilvoorde, Belgium) was added per 0.1 g of tissue. EBC was analyzed at the university hospital laboratory and information collected from the patient files. Tissue samples were analyzed continuously during the study period using standardized methods, and selected samples have been measured repeatedly to ensure reproducibility.

### Biomarkers

The markers were selected based on their involvement in the inflammatory process in CRS.

Concentrations of ECP, total IgE, and IgE specific to a mixture of *Staphylococcus aureus* enterotoxins (staphylococcal enterotoxins A and C, and toxic shock syndrome toxin-1) were assayed using the UNICAP system (Thermo Fisher Scientific, Phadia, Grootbijaarden, Belgium) according to manufacturer instructions. IL-5 was assayed using the Luminex Performance Assay (Bio-Techne) according to manufacturer instructions and measured with the Bio-Plex 200 system (Biorad, Temse, Belgium). Concentrations in tissue homogenates were expressed as mass versus mass after multiplication with the homogenization dilution factor of 11. IL-5 positivity in tissue was defined as  $\text{IL-5} > 12.98 \text{ pg/g}$  and SE-IgE positivity in tissue was defined as  $\text{SE-IgE} > 3.85_{\text{k}} \text{ UA/g}^4$ . Levels below detection level were set as detection level/2x dilution factor.

### Statistical Method

Graph pad prism 8 for Mac OS X (GraphPad Software, Inc., La Jolla, CA) was used for all non-parametrical statistical analyzes. STATA Statistical Software, version 13.1 (StataCorp, College Station, TX) was used for the prediction model. For tissue markers, the Kruskal-Wallis test with Dunn's multiple comparison test was used when comparing more than two groups, the Mann-Whitney test was used for comparison of two groups. Spearman test was used to assess correlation and the results were interpreted as suggested by Hinkle et al.<sup>10</sup> The difference in proportions between

TABLE I.  
Baseline Statistics.

	CRSwNP	CRSsNP	P Value
n	323	115	
Gender, F/M	112/211	44/71	.498*
Age mean, (min;max)	46.2 (13;81)	41.5 (14;71)	
Asthma, n (%)	132 (40.9)	27 (23.5)	.001*
Allergy, n (%)	134 (41.5)	37 (32.2)	.095*
AERD, n (%)	56 (17.3)	4 (3.5)	<.001*
Tissue markers			
IL-5+, n (%)	271 (83.9)	60 (52.2)	<.001*
SE-IgE+, n (%)	124 (38.4)	16 (13.9)	<.001*
IgE U/g, median (IQR)	434.3 (142.1;871.2)	63.3 (27.3;248.4)	<.001†
ECP ng/g, median (IQR)	14,045.0 (4,455;23,646)	1,587.0 (405.9;7,297.0)	<.001†
IL-5 pg/g, median (IQR)	168.2 (38.6;552.9)	14.4 (1.2;92.3)	<.001†

IL-5+ and SE-IgE + indicates presence of IL-5 and SE-IgE in tissue. Tissue markers IgE, ECP and IL-5 are presented as median and IQR. Significant values are indicated in bold.

\*Fisher's exact test.

†Mann-Whitney test.

AERD = aspirin exacerbated respiratory disease; ECP = eosinophil cationic protein; IgE = immunoglobulin E; IL-5 = interleukin-5; IQR = interquartile range.

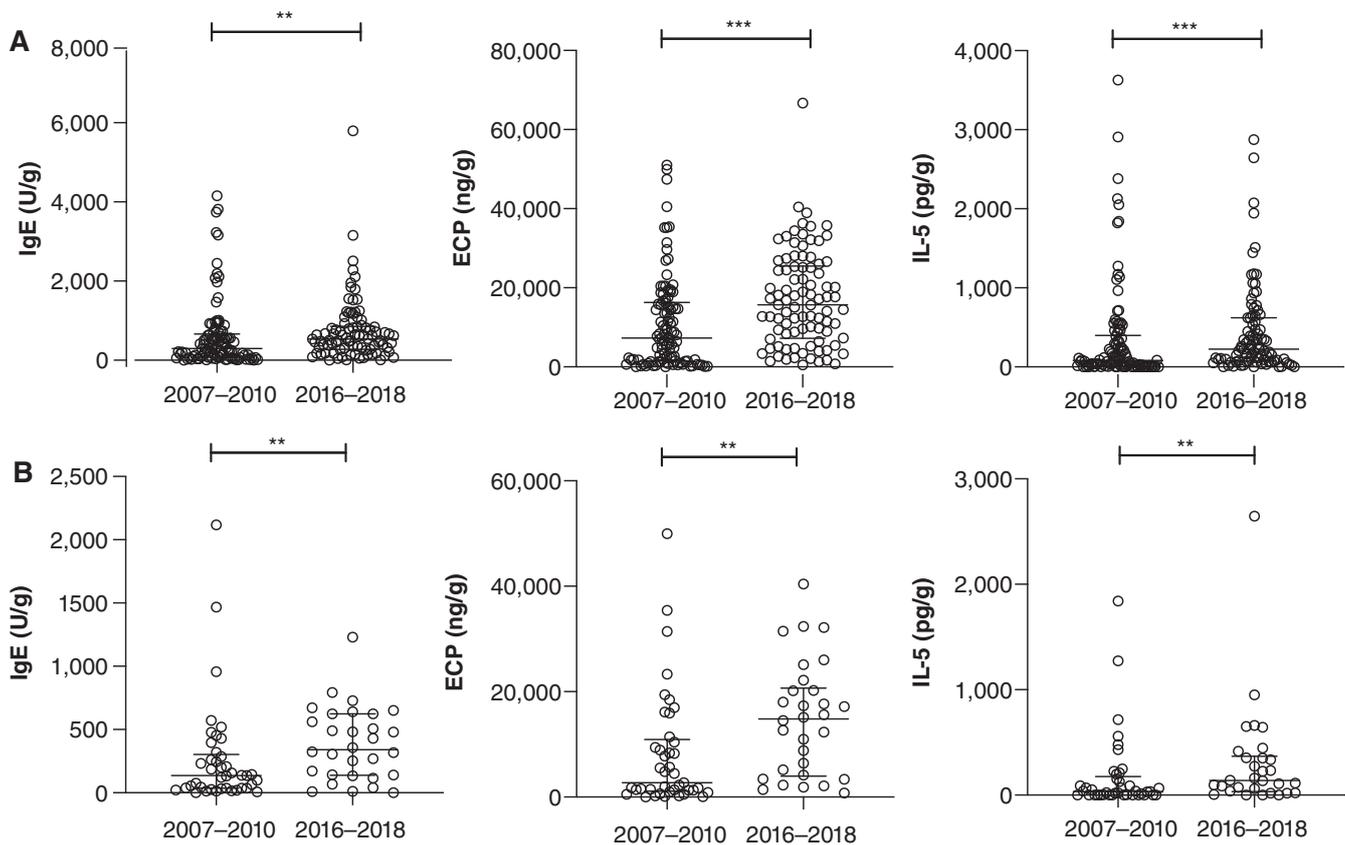


Fig. 1. Levels of IgE, ECP, and IL-5 in CRSwNP patients undergoing surgery in 2007–2010 and 2016–2018 (A) and in non-asthmatic, non-allergic patients (B). Circles represent individual values, whiskers represent median and IQR. \*\*\* $P \leq .001$ , \*\* $P \leq .01$ , \* $P < .05$ . ECP = eosinophil cationic protein; IgE = immunoglobulin E; IL-5 = interleukin-5; IQR = interquartile range.

two groups was tested with  $\chi^2$  test or Fishers' exact when appropriate. A  $P$ -value  $< .05$  was considered significant.

## RESULTS

### Baseline Data

In total 323 patients with CRSwNP and 115 patients with CRSsNP had a full set of required data, as stated in the methodology section, and were included (Table I).

### Type 2 shift in CRSwNP

To investigate whether there has been a change in inflammatory markers in CRSwNP over time, patients who underwent surgery in 2007–2010 ( $n = 102$ ) or 2016–2018 ( $n = 90$ ) were compared. The groups were mutually exclusive. The groups were equal regarding gender, age, allergy, and previous surgery (Supporting Table 1).

Looking at type 2 parameters, the latter group had higher levels of IgE, ECP, and IL-5 in nasal polyp tissue

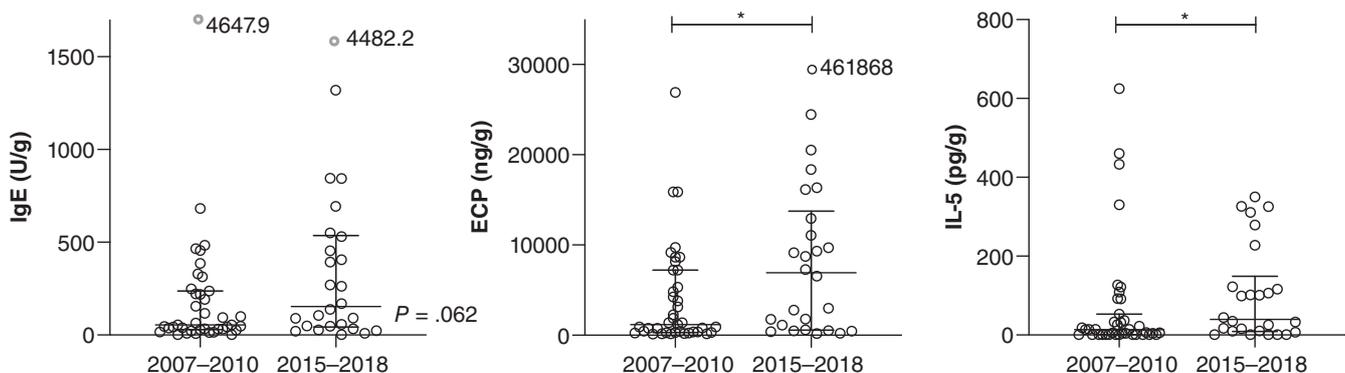


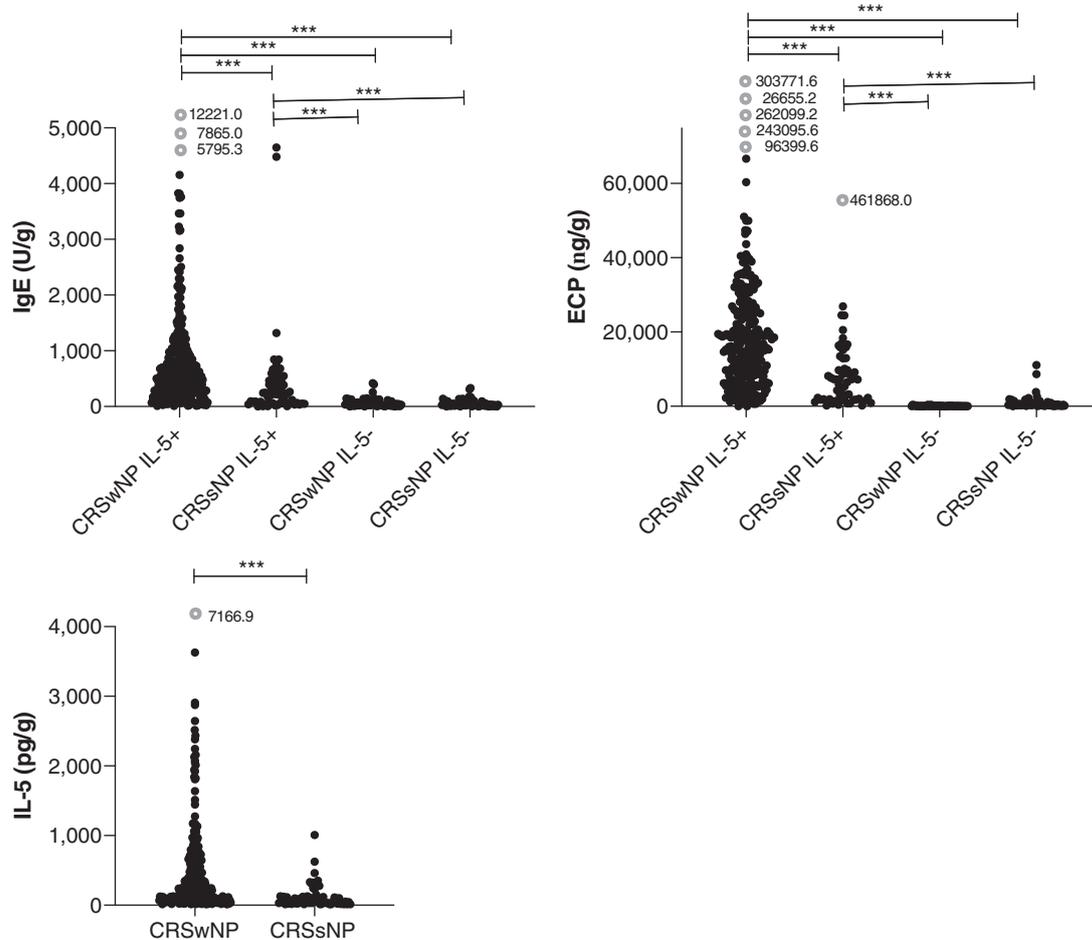
Fig. 2. Levels of IgE, ECP and IL-5 in CRSsNP patients who underwent surgery in 2007–2010 and 2015–2018. Circles represent individual values, whiskers represent median and IQR. Grey circle indicate value above the maximum range of the y-axis. \* $P < .05$ . ECP = eosinophil cationic protein; IgE = immunoglobulin E; IL-5 = interleukin-5; IQR = interquartile range.

than the earlier group (Fig. 1A). Furthermore, a larger proportion of patients in the latter group was IL-5+ in nasal polyp tissue (95.6% vs. 82.4%,  $P = .006$ ) compared to the earlier. There was no difference in EBC between the groups (median [IQR] 2007–2010 293.5 [162.0;574.8] 2016–2018 420.0 [235.0;747.5,  $P = .147$ ]). Further, the two groups were stratified based on asthma and allergy comorbidity. The no allergy/no asthma group had higher levels of IgE, ECP, and IL-5 in the latter group compared to the earlier group (Fig. 1B) and a higher proportion of IL-5+ nasal polyps (from 63.4% to 86.7%,  $P = .033$ ) (For levels of

mediators see Supporting Table 2). In asthmatic and/or allergic patients, no difference was observed between the two groups in any of the parameters analyzed.

### Type 2 Shift in CRSsNP

CRSsNP patients who underwent surgery in 2007–2010 ( $n = 39$ ) and 2015–2018 ( $n = 26$ ) were compared. A shift toward type 2 inflammation was observed, with elevated levels of ECP and IL-5 in the latter group compared to the earlier ( $P = .015$  for ECP and  $P = .041$



IL-5 +	CRSwNP	CRSsNP	p-value	IL-5 -	CRSwNP	CRSsNP	P value
n	272	60		n	51	55	
Asthma n (%)	124 (45.6)	18 (30.0)	0.031*	Asthma n (%)	8 (15.7)	9 (16.3)	>0.999
Allergy n (%)	123 (45.2)	24 (40.0)	0.474	Allergy n (%)	11 (21.6)	12 (21.8)	>0.999
AERD n (%)	54 (19.9)	3 (5.0)	0.004*	AERD n (%)	2 (3.9)	1 (1.8)	0.607
SE-IgE + n (%)	123 (45.2)	16 (26.7)	0.009*	SE-IgE + n (%)	1 (2.0)	0 (0)	0.481

Fig. 3. Levels of IgE, ECP, and in IL-5+ and IL-5-, CRSwNP and CRSsNP and levels of IL-5 in IL-5+ CRSwNP and CRSsNP. Dots are individual values. Circles in grey indicate individual values above the maximal range of the y-axis. \*\*\* $P \leq .001$ , \*\* $P \leq .01$ , \* $P < .05$ . The table shows numbers and proportion of patients with asthma, allergy, AERD, and SE-IgE positivity in tissue in the different groups, significant numbers indicated with \*. AERD = aspirin exacerbated respiratory disease; CRSwNP = chronic rhinosinusitis with nasal polyps; CRSsNP = chronic rhinosinusitis without nasal polyps; ECP = eosinophil cationic protein; IgE = immunoglobulin E; IL-5+ = interleukin-5 positive; IL-5- = IL-5 negative; IQR = interquartile range; SE-IgE = specific IgE to *Staphylococcus aureus* enterotoxins.

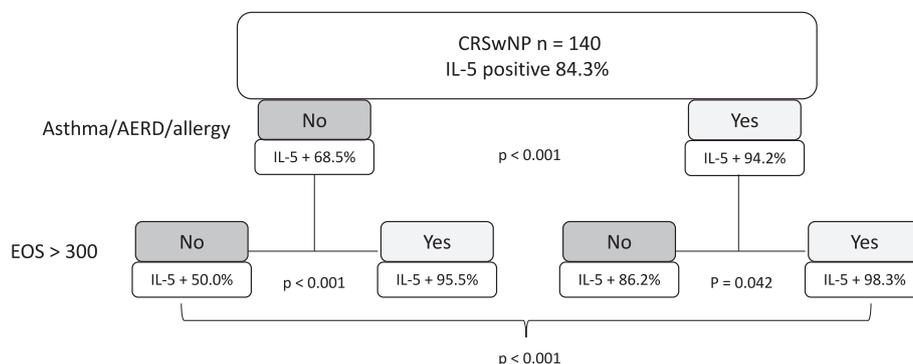


Fig. 4. A prediction model for tissue IL-5 positivity in tissue based on clinical markers, asthma, and/or AERD and/or allergy comorbidity and EBC > 300 cells/ $\mu$ L. AERD = aspirin exacerbated respiratory disease; CRSwNP = chronic rhinosinusitis with nasal polyps; EBC = elevated blood eosinophils; IL-5 = interleukin-5.

for IL-5) (Fig. 2). No difference was seen in the proportion of comorbidities or patients positive for IL-5 or SE-IgE in tissue (Supporting Table 3).

In the non-asthmatic group (n = 31 vs. n = 18), there was an increase in ECP in the latter group compared to the earlier (median (IQR) 1,172.0 (302.5;7,205.0) vs. 7,640.0 (495.0;1,3793.0),  $P = .029$ ). No difference was seen in IgE or IL-5. The proportion of SE-IgE positivity was larger in the latter group (22.2% vs. 3.1%,  $P = .054$ ) although not significant. In the asthmatic group (n = 8 vs. n = 8), no differences were seen.

### IL-5 Positive and IL-5 Negative CRS

In CRS, both CRSwNP and CRSsNP, there are type 2 (IL-5+) and non-type 2 (IL-5-) patients based on concentrations of IL-5 in tissue. IL-5+ CRS had elevated levels of IgE and ECP compared to IL-5- CRS, regardless of the presence of polyps. IL-5+ CRSwNP had elevated levels of IgE, ECP, and IL-5 compared to IL-5+ CRSsNP (Fig. 3). In the IL-5+ groups, there was a difference in asthma and AERD comorbidity and SE-IgE+ in tissue, no such difference was seen between the IL-5- groups.

### Tissue Inflammation in CRSwNP in Relation to Asthma and AERD

The CRSwNP group (n = 323) was stratified into no asthma/AERD, asthma only, asthma, and/or AERD; the groups were mutually exclusive. Levels of IgE, ECP, and IL-5 were elevated in patients with asthma and asthma and/or AERD compared with patients without asthma/AERD. ECP was the only marker elevated in asthma/or AERD compared with asthma only (Supporting Figure 1). All patients with AERD were IL-5 positive in tissue. Patients with asthma and/or AERD had the highest proportion of SE-IgE positivity.

### Blood Eosinophils in CRSwNP

In a subgroup of CRSwNP patients, EBC were available (n = 140). A comparison between the subgroup with EBC results and those without EBC results did not reveal

any differences regarding clinical parameters or levels of type 2 inflammatory markers. Patients with asthma and asthma and/or AERD had elevated blood eosinophils compared to non-asthmatics (Supporting Figure 2). The presence of inhalant allergy did not affect the blood eosinophilic count. There was a rather weak positive correlation between EBC and tissue inflammatory markers; IgE ( $R = 0.415$ ,  $P < .001$ ), SE-IgE ( $R = 0.214$ ,  $P = .011$ ) ECP ( $R = 0.410$ ,  $P < .001$ ), and IL-5 ( $R = 0.489$ ,  $P < .001$ ).

### Identification of Type 2 Tissue Inflammation in Patients with CRSwNP

Out of the 323 CRSwNP patients included, 272 (83.9%) were IL-5+ and 124 (38.4%) were SE-IgE+ in nasal polyp tissue. Similar proportions were seen in the subgroup of 140 patients with results on EBC (IL-5+ 84.3%, SE-IgE+ 40.7%). About 99.2% of SE-IgE+ patients were also IL-5+. A prediction model based on Chi<sup>2</sup>-test (or Fisher's exact test when appropriate) was developed to investigate if any of the clinical markers, asthma, AERD and allergy comorbidity, and EBC > 300 cells/ $\mu$ L can be used to identify patients with or without IL-5+ and SE-IgE+ in nasal polyp tissue. In the total group, the likelihood of IL-5+ was 84.3%. Among patients with asthma or AERD, 94.2% were IL-5+ ( $P < .001$ ). In patients without asthma/AERD, the likelihood was 68.5%, but with an EBC > 300 cells/ $\mu$ L the likelihood increased to 95.2% ( $P < .001$ ). (Fig. 4) 40.7% of the patients were SE-IgE positive. The likelihood of SE-IgE in tissue among patients with asthma/AERD and EBC > 300 was 57.9% compared to 21.9% in patients without comorbidities, and 36.6% in those with additional EBC < 300 cells/ $\mu$ L ( $P = .02$ ).

### DISCUSSION

Here, we show an increase in type 2 inflammation in CRSwNP patients over time in non-asthmatic subjects. Additionally, we have further pinpointed the differences between clinical phenotypes and corresponding type 2 markers in sino-nasal mucosa and, with a prediction

model, identified clinical, easy to access markers, that can help identify type 2 CRSwNP.

CRS is a heterogeneous disease with different underlying inflammation depending on the phenotype and geographical location. Previous projects have reported elevated prevalence of type 1 inflammation in nasal polyp patients in Asia.<sup>5, 11</sup> In Asia, there have been reports on a shift toward more type 2 inflammation in nasal polyp patients over time.<sup>6, 7, 12</sup> Here we show a similar observation in patients undergoing surgery in Ghent, Belgium, comparing CRSwNP patients who had surgery 8–10 years apart. Interestingly, the change was only seen in non-asthmatic/non-allergic patients. Not only were the levels of type 2 inflammatory markers significantly elevated in non-asthmatic/non-allergic patients in the latter group compared to the earlier, but also the proportion of patients with presence of IL-5 in tissue, suggesting an actual change of endotype. This change was not reflected in EBC. There is a possibility that the observed shift toward an increase in type 2 inflammation is due to selection bias. However, there was no difference between the two groups regarding severity of disease (previous surgery, asthma, and AERD comorbidity) suggesting that the patients admitted to surgery have not changed; further, the shift was only seen in non-asthmatic patients, representing the least severe group of CRSwNP patients reducing the risk for our results to be due to selection bias.

We also show a similar but less pronounced shift toward type 2 inflammation in CRSsNP. This change was seen in concentrations of type 2 markers in tissue (ECP and IL-5). Although this does not confirm an actual shift of endotype with significantly increased numbers of type 2 CRSsNP over the years, it demonstrates an increase in type 2 inflammatory burden in these patients. Just as in CRSwNP, the shift is seen in non-asthmatic patients but not in asthmatics. The stratified CRSsNP groups were however quite small why caution must be taken when interpreting these results. Our results are novel findings, and need to be confirmed in larger studies.

It is known that patients with elevated levels of type 2 inflammation are more prone to relapse in their disease.<sup>4</sup> Our findings may suggest that CRSwNP patients now are more likely to develop severe CRSwNP and hence a higher frequency in relapse of nasal polyps might be seen, further illuminating the importance of new treatment methods such as monoclonal antibodies and possibly more extensive surgery. Other atopic diseases, such as asthma and allergy<sup>13</sup> are also increasing. Multiple reasons for this has been proposed, among them increased pollution,<sup>14</sup> changes in the microbiome,<sup>15</sup> and other life style factors. Katotomichelakis et al showed an increase of intramucosal *S. aureus* over the years parallel with a shift to more eosinophils,<sup>6</sup> but could not demonstrate causality. Here, we did not have the possibility to investigate a potential change in microbial colonization as a possible cause for the shift. We did however not see an increase in SE-IgE positivity during the years. Other environmental changes are left to be investigated further.

In the group of CRS patients, IL-5+ CRS, regardless of phenotype, express higher levels of type 2 markers

than IL-5– CRS. Within the IL-5+ group of CRS, CRSwNP patients express higher levels of type 2 markers, have a higher proportion of asthma and AERD comorbidity, as well as SE-IgE positivity compared to CRSsNP. This was not observed in IL-5– CRS. These results indicate that polyp formation, at least in part, is driven by a process separated from the type of inflammation and levels of inflammatory mediators, a notion that is supported by the presence of nasal polyps in non-type 2 disease.<sup>16</sup>

In CRSwNP patients, who underwent surgery in 2007–2018 we saw an increase in type 2 markers in nasal polyp tissue in patients with asthma compared to non-asthmatics, as shown before<sup>4</sup> but AERD did not seem to affect the levels of inflammation, suggesting that asthmatic patients already have a maximal type 2 inflammatory response regardless of AERD comorbidity, ECP was however elevated in asthma/AERD compared to asthma only, coherent with earlier studies.<sup>17</sup> ECP is commonly thought to mirror the numbers of activated eosinophils<sup>18</sup>; however, in AERD patients this elevation of ECP in tissue was not reflected in EBC. Previous studies show that CRSwNP patients with AERD have a higher risk of recurrence<sup>19</sup> and have undergone more sinus surgeries than CRSwNP patients with asthma.<sup>20</sup> Hence, AERD might not influence the inflammatory parameters investigated in our study, but these patients still seem to have a more severe form of disease.

It has been challenging to come up with reliable biomarkers to endotype CRS. Tissue eosinophilia is widely used to diagnose eosinophilic CRSwNP, but there is little or no consensus on cut-off levels of meaningful eosinophilia.<sup>21</sup> Blood eosinophils are suggested as a surrogate to tissue eosinophilia.<sup>22,23</sup> In this study, we had access to EBC in a subgroup of CRSwNP patients. As expected, patients with asthma and with asthma/AERD had elevated levels of EBC. No difference was seen between the groups with asthma only and asthma and/or AERD, again suggesting that asthma alone can cause maximal type 2 inflammation. The increase of EBC in asthmatic patients can probably be explained by the extended volume of inflammatory tissue. The correlation between EBC and type 2 markers in nasal polyp tissue was positive but poor<sup>10</sup> with only IL-5 almost reaching a moderate positive correlation ( $R = 0.489$ ,  $P < .001$ ). A prediction model based on asthma/AERD comorbidity and  $EBC > 300$  cells/ $\mu$ L revealed that  $EBC > 300$  cells/ $\mu$ L can be used to help identify IL-5 and SE-IgE positive and negative patients. A majority of patients with asthma/AERD were IL-5 positive ( $P < .001$ ). Significantly more patients without asthma/AERD but with  $EBC > 300$  cells/ $\mu$ L were IL-5+ in nasal polyp tissue compared to those with  $EBC < 300$  cells/ $\mu$ L (95.5 vs. 50.0%  $P < .001$ ); this can be very useful in a clinical setting identifying IL-5+ patients without asthma/AERD/allergy who still can have a serious form of CRSwNP and require a more extensive form of treatment, surgical or biological. This prediction model was not able to identify the SE-IgE+ endotype in a clinically relevant manner.

These results illustrate the challenge when using blood eosinophils to endotype CRSwNP. It can help to

identify the most severe and the least severe CRSwNP cases, but it only allows limited view on the inflammation inside the polyps. EBC can be altered by various disorders separated from the CRSwNP disease. Consideration on when the EBC test is executed is crucial in order to be able to use it when endotyping CRSwNP. A recent study in asthma showed an inverse relationship between oral GSC dosage and EBC,<sup>24</sup> hence if the patient recently has been on oral GCS, the EBC will most probably be misleading.

## CONCLUSION

We have shown that there has been a type 2 shift, identified by measuring type 2 markers in sinus mucosal tissue, in CRSwNP, seen in non-asthmatic patients over 8–10 years in Belgium, and probably in Western Europe. Our results point toward an actual change of endotype and might mean that we will be seeing more severe cases of CRSwNP in the future, further illuminating the importance of new medical and surgical treatment options for these patients. A shift was also observed in CRSsNP with increased type 2 inflammation levels. Clinical markers can, together with EBC, help identify type 2 CRSwNP, most interestingly patients without asthma/AERD/allergy with IL-5 positivity. EBC alone does, however, not tell what endotype or how severe the inflammation within the polyps are. In the future, it will be necessary to identify better biomarkers in order to accurately endotype CRS, and thereby implement precision medicine in these patients.

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