

Diversity of T_H cytokine profiles in patients with chronic rhinosinusitis: A multicenter study in Europe, Asia, and Oceania

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Background: To date, no study has evaluated the diversity of T_H cell cytokine patterns of patients with chronic rhinosinusitis (CRS) among centers in different continents using identical methods.

Objective: We sought to assess T_H cytokine profiles in patients with CRS from Europe, Asia, and Australia.

Methods: Patients with chronic rhinosinusitis with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP; n = 435) and control subjects (n = 138) were recruited from centers in Adelaide, Benelux, Berlin, Beijing, Chengdu, and Tochigi. Nasal mucosal concentrations of T_H2, T_H17, and T_H1 cytokines; eosinophilic cationic protein (ECP); myeloperoxidase (MPO); IL-8; and tissue total and *Staphylococcus aureus* enterotoxin (SE)-specific IgE were measured by using identical tools.

Results: Combinations of T_H1/T_H2/T_H17 cytokine profiles in patients with CRSwNP varied considerably between regions. CRSwNP tissues from patients from Benelux, Berlin, Adelaide, and Tochigi were T_H2 biased, whereas those from Beijing

mainly demonstrated T_H2/T_H1/T_H17 mixed patterns, and patients from Chengdu showed an even lower T_H2 expression. Concentrations of IL-8 and tissue total IgE in patients with CRSwNP were significantly higher than those in control subjects in all regions. More than 50% of patients with CRSwNP in Benelux, Berlin, Adelaide, and Tochigi showed a predominantly eosinophilic endotype compared with less than 30% of patients in Beijing and Chengdu. SE-specific IgE was found in significantly greater numbers in patients with CRSwNP from Benelux, Adelaide, and Tochigi and significantly lower numbers in patients from Beijing and Chengdu. Moreover, the T_H1/T_H2/T_H17 cytokine profiles in patients with CRSsNP showed diversity among the 6 regions.

Conclusion: T_H cytokine levels, eosinophilic/neutrophilic patterns, and SE-specific IgE expressions show extreme diversity among patients with CRS from Europe, Asia, and Oceania. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: T_H cytokines, IgE, ECP, chronic rhinosinusitis, phenotype, endotype, Europe, Asia, Australia

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Chronic rhinosinusitis (CRS), a chronic inflammatory disease of the paranasal sinuses, is prevalent in 7% to 27% of adults in European countries, 14% in the United States, and 2.1% to 8% in China.¹⁻⁴ CRS is classified into 2 phenotypes, chronic rhinosinusitis without nasal polyps (CRSsNP) and chronic rhinosinusitis with nasal polyps (CRSwNP), according to US and European clinical guidelines.^{1,5}

Evidence from several studies indicates that there are distinct immunologic mechanisms between patients with CRSsNP and those with CRSwNP and even within a clinical phenotype, suggesting the presence of endotypes.⁶⁻⁸ However, these distinctions between patients with CRSsNP and those with CRSwNP might not be universal and have not been studied on a worldwide scale.⁹⁻¹¹ Differences between white and Chinese patients with CRS (eg, in terms of the frequency of comorbid asthma; the bacterial patterns isolated from the nose, including *Staphylococcus aureus*; and other observations) suggest that there might indeed be differences of CRS endotypes in different regions of the world.¹²⁻¹⁶ Moreover, one recent study has demonstrated that the eosinophilic subtype within CRSwNP has increased significantly during the past 10 years in Thailand, together with an increasing intramucosal presence of *S aureus*, suggesting that the prevalence of a specific endotype can change over time.¹⁷ However, another recent report indicated that second-generation Asian patients with CRSwNP living in the United States maintained their noneosinophilic inflammation in CRSwNP status, probably as a consequence of their genetic background.¹⁸

Abbreviations used

CRS: Chronic rhinosinusitis
 CRSsNP: Chronic rhinosinusitis without nasal polyps
 CRSwNP: Chronic rhinosinusitis with nasal polyps
 ECP: Eosinophilic cationic protein
 MPO: Myeloperoxidase
 NP: Nasal polyp
 SE: *Staphylococcus aureus* enterotoxin

Previous studies have focused on the T_H cell endotypes of CRS in different regions of Europe, Asia, and Australia. Zhang et al¹³ compared the eosinophilic and neutrophilic inflammation patterns and T-cell patterns between European and southern Chinese patients with CRSwNP and showed that there was more T_H2 bias with higher GATA-3 and IL-5 levels, together with more eosinophilic inflammation, in European patients, whereas more neutrophilic inflammation and endotypes with T_H1/T_H17 were found in southern Chinese patients with CRSwNP. Cao et al¹⁵ found that patients with CRSsNP from central China to have a predominantly T_H1 pattern, similar to that demonstrated in European patients with CRSsNP,⁸ whereas patients with eosinophilic CRSwNP had a T_H2 -biased inflammation accompanied by T_H17 reactions. A recent study from Japan has shown that the contemporary cytokine profiles in Tochigi patients with CRSsNP/CRSwNP are similar to those of European patients with CRS.¹¹

In view of these findings, it is tempting to hypothesize that apart from genetic factors, diversity in geographic and climatic conditions might influence the immunologic patterns of disease in subjects living in different regions within a single country or in different countries. Thus in the present study we aimed to evaluate the endotypes of patients with CRS from 2 European regions, Benelux (Belgium, Netherlands, and Luxembourg) and Berlin, Germany; 2 Chinese regions, Beijing in northern China and Chengdu in the Sichuan province of southwestern China; and 1 region each in Japan (Tochigi) and Australia (Adelaide) based on T_H cytokines and related immune factors.

METHODS**Patients**

A total of 573 subjects, including patients with CRSsNP, patients with CRSwNP, and control subjects, were enrolled in this study consecutively over a specific period from Benelux and Berlin (European regions: 197 patients), Adelaide (Australian region: 68 patients), Beijing and Chengdu (Chinese regions: 266 patients), and Tochigi (Japanese region: 42 patients; Fig 1). The general information of subjects from the 6 regions is shown in Table I. Diagnoses of CRS were based on patients' histories, clinical examinations, nasal endoscopies, and computed tomographic scans of the sinuses in accordance with the "European position paper on rhinosinusitis and nasal polyps 2012" guidelines.¹ Patients without any sinonasal disease who presented for septoplasty for anatomic variations were recruited as control subjects from each region. Atopic status was evaluated by detecting IgE antibodies against various common inhalant allergens with the Phadiotop test (Phadia, Uppsala, Sweden) and skin prick tests. Diagnoses of asthma and aspirin sensitivity were made by pulmonologists based on lung function and challenge tests. None of the subjects used oral or nasal corticosteroids 4 weeks before or antibiotics 2 weeks before their surgeries.

The ethics committees of Ghent University Hospital, the Academic Medical Centre of Amsterdam, University Hospital Leuven, Charité Berlin,

Beijing TongRen Hospital, Huaxi Hospital of Sichuan University, the University of Adelaide, and Flinders University of Adelaide and the local ethics committee of the Jichi Medical University Hospital of Tochigi approved the study. All patients provided written informed consent.

Measurement of cytokine and IgE levels in tissue homogenates

All samples were obtained by using the same procedures in the different centers, following collaborations between the centers and the Upper Airways Research Laboratory at Ghent University, as described previously.¹⁰⁻¹² Samples from nasal polyps (NPs) of patients with CRSwNP, ethmoidal mucosa of patients with CRSsNP, and inferior turbinate mucosa of control subjects were processed and analyzed in 2 laboratories, with the laboratory in Ghent University Hospital handling all the samples from Benelux, Berlin, Adelaide, and Tochigi, and the laboratory in Beijing TongRen Hospital handling the samples from Beijing and Chengdu. Briefly, freshly obtained tissue specimens were homogenized, as previously described,¹³ and assayed for IL-5, IFN- γ , IL-17A, IL-8, TGF- β 1, and MPO by using commercially available ELISA kits (Quantikine ELISA, R&D Systems, Minneapolis, Minn; MPO, Oxis International, Portland, Ore). Mucosal tissue total IgE, ECP, and specific IgE levels to *S aureus* enterotoxins (SEs) were measured by using the UniCAP system (Pharmacia, Uppsala, Sweden). Twelve random and blinded NP samples from patients in Belgium were analyzed for IL-5, IL-17, IL-8, and ECP in both laboratories in Belgium and Beijing by using the same brand of ELISA kits (Quantikine ELISA, R&D Systems) to ensure comparability of measurements in the 2 laboratories. Expression of T_H1 , T_H2 , and T_H17 marker cytokines, such as IFN- γ , IL-5, and IL-17, were used to define the inflammatory profiles. When a sample showed protein expression for 2 or 3 of those markers, it was considered double/triple positive.

Comparison of eosinophilic versus noneosinophilic CRSwNP

Eosinophilic and noneosinophilic CRSwNP were defined based on the ECP/MPO ratio, with a ratio of greater than 1 being eosinophilic and a ratio of less than 1 being noneosinophilic/neutrophilic disease.⁸

Statistics

Results were analyzed with SPSS 17.0 software (SPSS, Chicago, Ill). Data were entered as both continuous and categorical variables (less than or greater than the detection limit).

Positivity was defined as the percentage of positive samples expressing a certain marker or expressing a marker above a certain cutoff value. Because all the values for ECP, IL-8, and tissue total IgE were greater than the detection limit, receiver operating characteristic curve analysis was used to obtain cutoff values that could best discriminate between CRSsNP and CRSwNP. Because data were found to be nonparametric, medians and interquartile ranges were reported. The χ^2 or Fisher exact test was used for categorical data, with a 2-tailed Kruskal-Wallis test followed by a Mann-Whitney *U* test used for analysis of ordinal data. Paired-samples tests and Pearson correlation analysis were used to compare the results of the same parameters measured in the 2 laboratories for the same samples. Finally, multiple correspondence analyses (MCA) were performed to present the pattern of relationships among the 3 phenotypes (patients with CRSwNP, patients with CRSsNP, and control subjects), the key $T_H2/T_H17/T_H1$ cytokines (IL-5, IL-17, and IFN- γ), and the world regions. Bonferroni corrections were applied for the analysis of multiple comparators, and *P* values of .05 or less were considered statistically significant.

RESULTS**Patients' characteristics**

Table I shows the demographic and clinical characteristics of the subjects. The proportion of male subjects was higher than that of female subjects in all 3 groups in the 6 regions. Positive

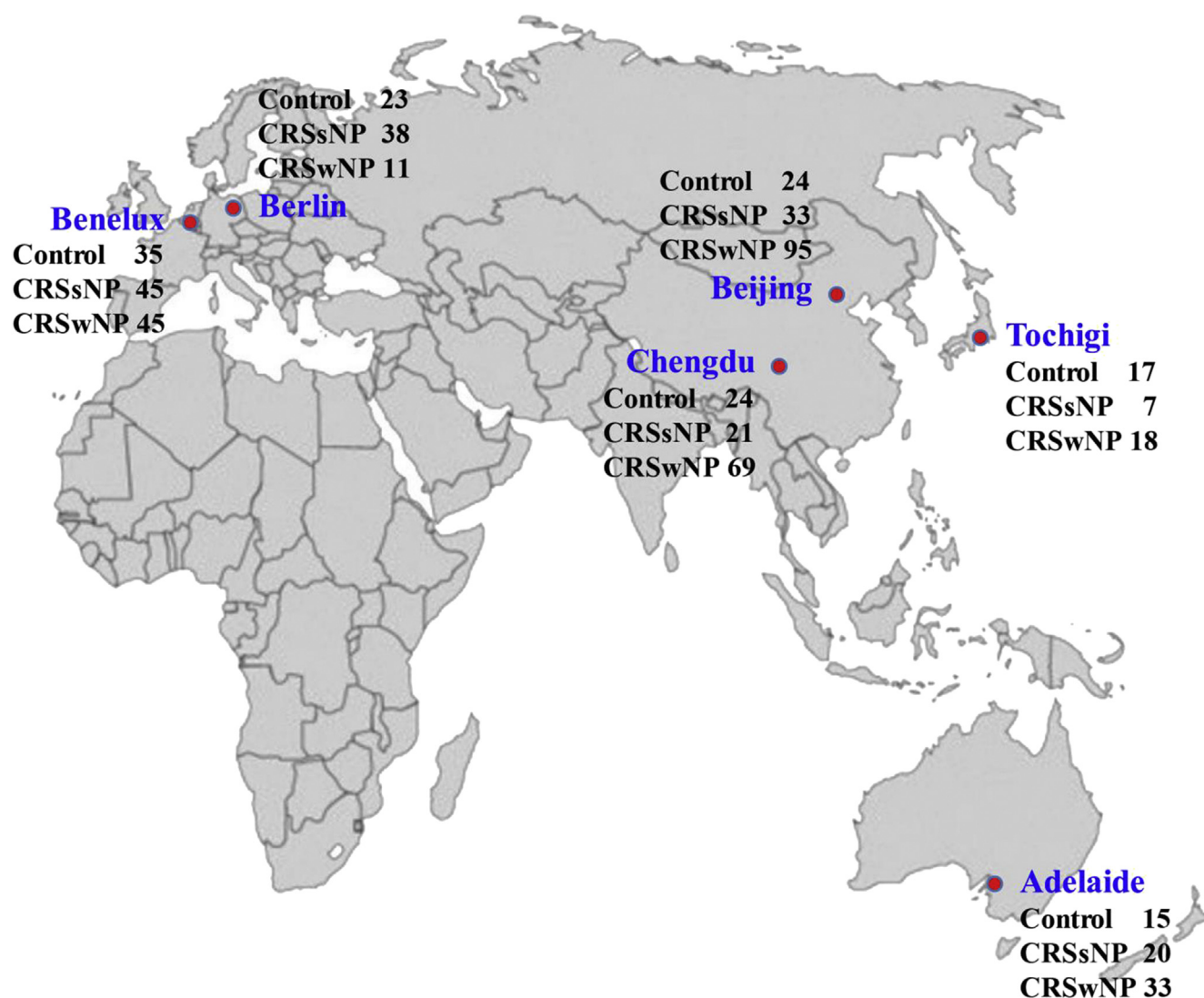


FIG 1. World map with locations of the 6 centers and numbers of patients included in the study.

skin prick test responses were more frequent in patients with CRSwNP than in patients with CRSsNP and control subjects in Beijing only. The prevalence of asthma in patients with CRSwNP in Beijing and Chengdu was lower than that of the other 4 regions.

Comparability of measurements

Assessment of the consistency of measurements between the 2 laboratories measuring all samples, Ghent and Beijing, as determined by using 12 randomly selected samples, which had previously been analyzed in Ghent for IL-5, IL-17, IL-8, and ECP, demonstrated high consistency between the 2 laboratories (Table II).

Multiple correspondence analyses for the interrelationships between phenotypes and $T_H1/T_H2/T_H17$ cytokines

Fig 2 shows the multiple correspondence analyses performed to demonstrate the relationships between CRSwNP, CRSsNP, and

control phenotypes and positive/negative IL-5, IL-17, and IFN- γ expressions and for the CRSwNP phenotype among the 6 regions. The CRSwNP phenotype was located near IL-5 positivity, whereas the CRSsNP phenotype was situated close to IL-17 and IFN- γ positivity (Fig 2, A). In contrast, the control phenotype was situated near IL-5, IL-17, and IFN- γ negativity (Fig 2, A). Assessment of the CRSwNP phenotype by region indicated that in Adelaide, Benelux, Berlin, and Tochigi this phenotype was near IL-5 positivity and IL-17 and IFN- γ negativity. In contrast, the CRSwNP phenotype in Beijing was closer to IL-17 and IFN- γ positivity and near IL-5 positivity (Fig 2, B), whereas in Chengdu this phenotype was near to the negativities of these 3 cytokines (Fig 2, B).

Comparison of IL-5, IL-17, and IFN- γ in patients from different regions

Concentrations of IL-5, IL-17, and IFN- γ in samples of the 3 patient groups from the different regions are shown in Fig 3. Assessment of IL-5 showed that the IL-5 concentrations in patients with CRSwNP were significantly higher than those of

TABLE I. Demographic and clinical characteristics of the subjects in the 6 study regions

	No.	Sex (female/male)	Age (y), median (range)	Allergy, no. (%)	Asthma, no. (%)	Prior surgery, no. (%)
Benelux						
Control subjects	35	13/22	29.0 (17.3-69.3)	8 (22.86)	4 (11.43)	1 (2.86)
Patients with CRSsNP	45	20/25	43.1 (16.8-69.9)	19 (42.22)	6 (13.33)	10 (22.22)
Patients with CRSwNP	45	13/32	46.5 (24.8-71.6)	20 (44.44)	20 (44.44)	22 (48.89)
<i>P</i> value	—	NS	<.001	NS	<.001	<.001
Berlin						
Control subjects	23	9/14	26.9 (18.2-59.9)	10 (43.48)	2 (8.70)	0
Patients with CRSsNP	38	15/23	37.8 (19.4-58.0)	23 (60.53)	4 (10.53)	0
Patients with CRSwNP	11	4/7	42.6 (28.0-53.1)	6 (54.55)	4 (36.36)	0
<i>P</i> value	—	NS	.013	NS	NS	—
Adelaide						
Control subjects	15	8/7	54.0 (46.0-63.0)	—	—	—
Patients with CRSsNP	20	—	59.0 (48.0-68.0)	11 (55.00)	9 (45.00)	—
Patients with CRSwNP	33	—	55.0 (47.0-58.0)	19 (57.58)	14 (42.42)	—
<i>P</i> value	—	—	—	NS	NS	—
Beijing						
Control subjects	24	13/11	39.5 (17.0-68.0)	1 (4.17)	0	0
Patients with CRSsNP	33	28/5	47.0 (13.0-72.0)	8 (24.24)	1 (3.03)	0
Patients with CRSwNP	95	75/20	47.0 (14.0-78.0)	35 (36.84)	13 (13.68)	29 (30.53)
<i>P</i> value	—	.017	NS	.006	.05	—
Chengdu						
Control subjects	24	7/17	31.5 (17.0-60.0)	5 (20.83)	1 (4.17)	—
Patients with CRSsNP	21	9/12	51.0 (16.0-59.0)	8 (38.10)	0	—
Patients with CRSwNP	69	27/42	37.0 (17.0-66.0)	24 (34.78)	5 (7.25)	—
<i>P</i> value	—	NS	NS	NS	NS	—
Tochigi						
Control subjects	17	1/16	60.0 (19.0-80.0)	7 (41.18)	0	—
Patients with CRSsNP	7	4/3	57.0 (29.0-71.0)	2 (28.57)	0	—
Patients with CRSwNP	18	4/14	56.5 (17.0-71.0)	6 (33.33)	6 (33.33)	—
<i>P</i> value	—	.020	NS	NS	.010	—
<i>P</i> value for all	—	<.001	<.001	.006	<.001	—

NS, Not significant.

patients with CRSsNP and control subjects from Benelux, Berlin, Adelaide, Beijing, and Tochigi, with Chengdu as the exception (Fig 3, A, and see Tables E1.1 and E1.2 in this article's Online Repository at www.jacionline.org). Similarly, assessment of IL-17 showed that interleukin concentrations in the CRSwNP groups were significantly higher than those of the CRSsNP and control groups in Adelaide and Beijing, whereas the concentration of IL-17 in the CRSsNP group was much higher than that in the CRSwNP and control groups in Tochigi (Fig 3, B, and see Tables E2.1 and E2.2 in this article's Online Repository at www.jacionline.org). In contrast, concentrations of IFN- γ in the samples from the CRSsNP group were significantly higher than in the CRSwNP and control groups in Beijing but not significantly different among the 3 groups in the other 5 regions (Fig 3, C, and see Tables E3.1 and E3.2 in this article's Online Repository at www.jacionline.org).

Assessment for IL-5, IL-17, and IFN- γ positivity indicated that these were significantly different among the CRSwNP, CRSsNP, and control groups in the 6 regions (data are shown as Fig E1 and Tables E1.3, E1.4, E2.3, E2.4, E3.3 and E3.4 in this article's Online Repository at www.jacionline.org).

Fig 4 shows the T_H1/T_H2/T_H17 cytokine expression in the patients with CRSwNP in the different regions. Between 46% and 82% of the patients with CRSwNP from Benelux, Berlin, Adelaide, and Tochigi were solely IL-5 dominant, with patients from Europe (Benelux and Berlin) showing the highest IL-5 dominance. Assessment of total IL-5 positivity in these 4 regions

showed that this ranged from 58% in Tochigi to 83% in Benelux. Although patients with CRSwNP from China (Beijing and Chengdu) demonstrated much lower single IL-5 dominance, ranging between 16% and 19%, the total IL-5 positivity of 61% in the CRSwNP group from Beijing was similar to that of patients in Benelux, Berlin, Adelaide, and Tochigi and comprised mainly T_H2/T_H1 and T_H2/T_H17 mixed patterns. In contrast, the CRSwNP group from Chengdu showed a lower T_H2 pattern, with 20% of total positivity of IL-5 and 23% of total positivity of comprised of T_H17 and T_H1 patterns.

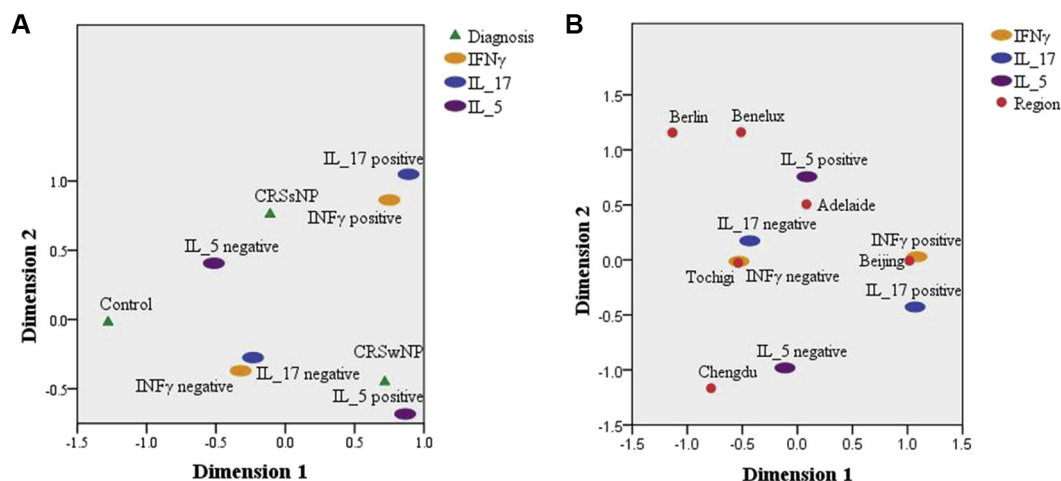
As shown in Fig 5, in patients with CRSsNP, the total IL-5 positivity of patients in Benelux, Berlin, Beijing, and Adelaide ranged from 33% in Benelux to 40% in Adelaide, and single IL-5 positivity in Benelux, Berlin, Adelaide, and Beijing ranged from 3% for Beijing to 32% for Berlin. However, the total IL-5 positivity of the CRSsNP groups from Chengdu and Tochigi were only 5% and 16% (12% of individual IL-5 positivity), respectively. The total positivity of T_H1 and T_H17 cytokines without IL-5 expression among the 6 regions ranged from 13% and 14% in Tochigi and Chengdu to 58% in Beijing.

Marker of eosinophilic (ECP) and neutrophilic (IL-8) inflammation

The control, CRSsNP, and CRSwNP groups all displayed generally high levels of ECP (Fig 3, D, and see Tables E4.1 and E4.2 in this article's Online Repository at www.jacionline.org).

TABLE II. Consistency in analysis of mediators in randomly selected samples (n = 12) from laboratories in Ghent and Beijing

	Ghent, mean ± SEM	Beijing, mean ± SEM	Paired differences, mean ± SEM	t	P value*	r	P value†
IL-5 (pg/mL)	270.30 ± 126.42	291.72 ± 110.63	21.42 ± 31.98	0.670	.517	0.972	<.01
IL-17 (pg/mL)	55.51 ± 26.28	61.00 ± 30.33	5.49 ± 7.97	0.689	.505	0.971	<.01
IL-8 (pg/mL)	11,769.26 ± 3,157.35	12,925.55 ± 3,327.93	1,156.29 ± 816.57	1.416	.184	0.970	<.01
ECP (μg/L)	8,024.68 ± 1,348.69	9,199.93 ± 1,521.15	1,175.25 ± 800.95	1.467	.170	0.851	<.01

*Paired-samples *t* test.†All *P* values of correlations.**FIG 2.** Multiple correspondence analyses plot for the interrelationships between CRSwNP, CRSsNP, and control phenotypes and positive/negative protein expressions for the cytokines IL-5, IL-17, and IFN-γ (all regions **A**); and interrelationships between the 6 regions and IL-5, IL-17 and IFN-γ protein expressions for the CRSwNP phenotype (**B**). Distance between variables (phenotype and cytokine or region and cytokine) indicates the approximate relationship between variables. Thus the nearer they are located together, the stronger the relationship.

However, the concentration of ECP in the CRSwNP groups from Benelux, Adelaide, and Tochigi were higher than those in Chengdu, and the concentrations of ECP in Berlin and Beijing were higher than that of Chengdu, although these did not attain statistical significance. Because there were significant differences in ECP levels between the control and CRSwNP groups among the 6 regions, we defined a cutoff value of greater than 1189.10 μg/L that discriminated patients with NPs from control subjects. In this regard all 6 regions exhibited eosinophilic NPs in more than 60% (data are shown in Fig E1, D, and supplementary Tables E4.3 and E4.4).

IL-8 concentrations were significantly higher in patients with CRSwNP compared with the CRSsNP and control groups in Benelux, Berlin, Beijing, and Chengdu but not in Tochigi (no IL-8 data were available for Adelaide), with concentrations of IL-8 in patients with CRSwNP from Beijing being much higher than those in Benelux, Berlin, and Chengdu (Fig 3, E, and see Tables E5.1 and E5.2 in this article's Online Repository at www.jacionline.org). IL-8 positivity was also found to be significantly higher in the CRSsNP and CRSwNP groups compared with control subjects in all regions, with the highest percentage of IL-8⁺ samples being noted in Beijing (data are shown as Fig E1, E, and Tables E5.3 and E5.4).

Tissue total IgE and specific IgE to SEs

Tissue total IgE levels were generally found to be higher in the CRSwNP groups compared with the CRSsNP and control groups

in the 6 study regions (Fig 3, F). Furthermore, concentrations of tissue total IgE in patients with CRSwNP from Adelaide and Tochigi were comparatively higher than in other regions (Fig 1, F, and see Tables E6.1 and E6.2 in this article's Online Repository at www.jacionline.org), and around 80% of the patients from Adelaide and Tochigi had total IgE levels greater than cutoff values compared with around 50% to 60% of patients from the other regions (data are shown as Fig E1, F, and Tables E6.3 and E6.4).

Fig 6 shows the percentage of samples positive for SE-specific IgE in the NP tissue of patients with CRSwNP in Benelux, Adelaide, Beijing, Chengdu, and Tochigi and illustrates that SE-specific IgE was found in significantly greater numbers of patients from Adelaide and Tochigi and in significantly lower numbers of patients from Beijing and Chengdu compared with patients from other regions. Data for SE-specific IgE were not available for samples from Berlin.

Eosinophilic and noneosinophilic (neutrophilic) dominant endotypes of CRS

We used the ECP/MPO ratio (>1, eosinophilic; <1, neutrophilic) to evaluate the predominantly eosinophilic or neutrophilic patterns of the CRSwNP, CRSsNP, and control groups among the 6 regions (Fig 7 and Tables E7.1 and E7.2 in this article's Online Repository at www.jacionline.org show MPO concentrations). Greater than 50% of patients with CRSwNP in Benelux, Berlin, Adelaide, and Tochigi demonstrated a predominantly

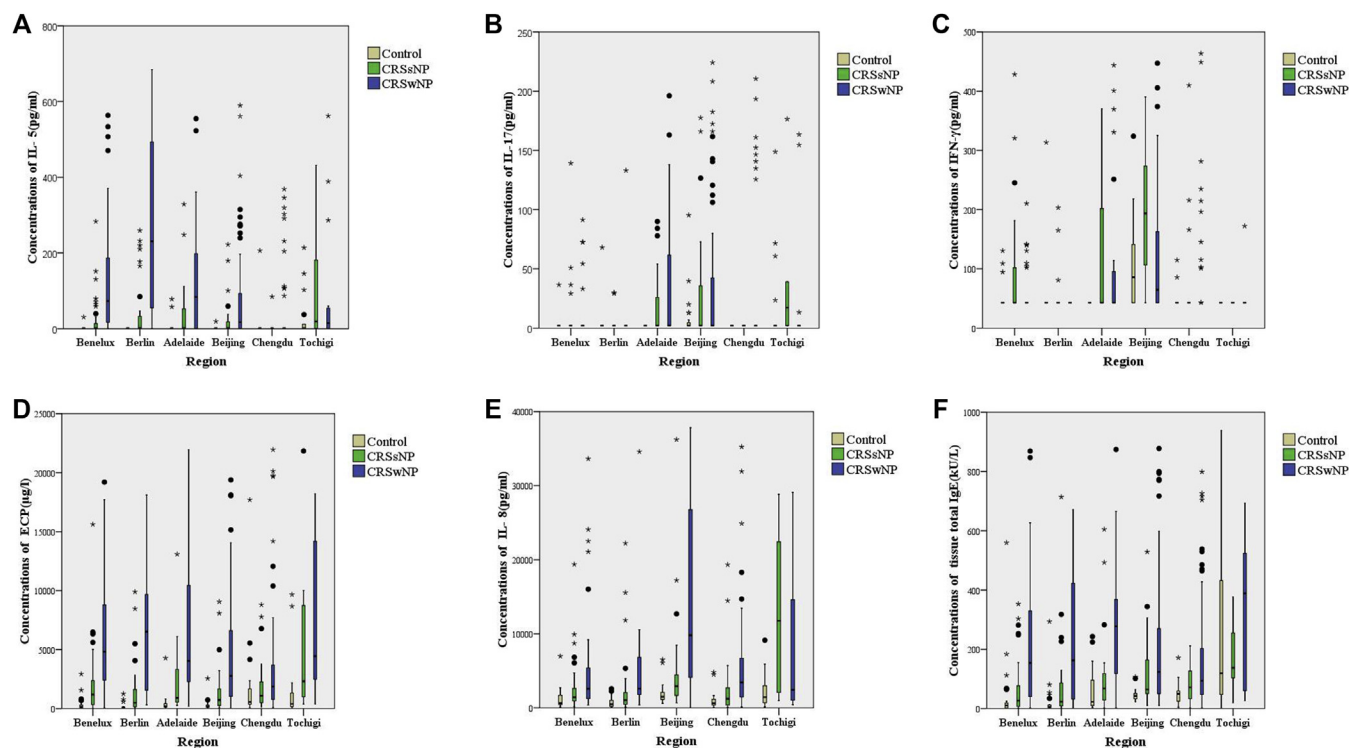


FIG 3. Concentrations of IL-5 (A), IL-17 (B), IFN- γ (C), ECP (D), IL-8 (E), and tissue total IgE (F) in control subjects, patients with CRSsNP, and patients with CRSwNP. Data were not available for IL-8 in samples from patients in Adelaide.

eosinophilic endotype compared with less than 30% patients in Beijing and Chengdu. In contrast, the majority of subjects in the CRSsNP and control groups demonstrated a predominantly neutrophilic endotype in all regions. The eosinophilic endotypes were significantly more frequent in patients with CRSwNP than in patients with CRSsNP in Benelux, Berlin, and Adelaide and also compared with patients with CRSwNP in Beijing, Chengdu, and Tochigi (data are shown as [Tables E8.1 and E8.2](#) in this article's Online Repository at www.jacionline.org).

TGF- β 1

Concentrations of TGF- β 1 were significantly higher in the CRSsNP groups than the CRSwNP groups in Benelux, Berlin, and Chengdu (data are shown as [Tables E9.1 and E9.2](#) in this article's Online Repository at www.jacionline.org). Concentrations of TGF- β 1 in the control and CRSwNP groups from Tochigi were significantly higher than all other regions.

DISCUSSION

In the present study we compared $T_H2/T_H1/T_H17$ cytokine patterns and markers of eosinophilic and neutrophilic inflammation in patients with CRS from 6 regions covering Europe, Asia, and Australia. To our knowledge, this is the first study to evaluate the diversity of immunologic endotypes of CRS among Europe, Asia, and Australia based on T_H cytokines and related markers in a multicenter approach.

In white patients a T_H2 -dominant pattern has generally been considered the main immunologic signature of CRSwNP, whereas CRSsNP displays a T_H1 -dominant pattern.⁸ Studies

comparing European patients with CRSwNP and southern Chinese patients with CRSwNP with regard to inflammation have further shown that European patients present with a T_H2 bias with eosinophilic inflammation, whereas their southern Chinese counterparts often present with a T_H1/T_H17 polarization with neutrophilic inflammation.¹³ Based on the ECP/MPO ratio, we also found a higher percentage of the eosinophil-dominant endotype of CRSwNP in Benelux, Berlin, Adelaide, and Tochigi than in Beijing and Chengdu, thus suggesting the presence of important immunologic differences between European and Chinese patients with CRS. However, these findings raise the possibility that diverse immunologic endotypes can also be prevalent among subjects of similar ethnicity from different national and international regions as a consequence of environmental differences.

In accordance with findings of previous studies,^{8,13} the present study confirmed that CRSwNP is related to IL-5, representing a T_H2 cytokine, whereas CRSsNP relates to T_H1/T_H17 as a general pattern for the 6 regions in the MCA. White patients with CRSwNP showed a relatively stronger T_H2 pattern, whereas Chinese patients with CRSwNP from Beijing showed mixed $T_H2/T_H1/T_H17$ patterns, and patients with CRSwNP from Chengdu showed very low IL-5 positivity. Interestingly, Japanese patients with CRSwNP had higher individual T_H2 patterns than subjects from Beijing but lower patterns than white patients with CRSwNP. In line with the positivity of IL-5, concentrations of IL-5 also showed similar diversity among the 6 regions for CRSwNP. The above results suggest that patients with CRS from Asiatic regions might generally be less susceptible to T_H2 -mediated disease than patients with CRS from European

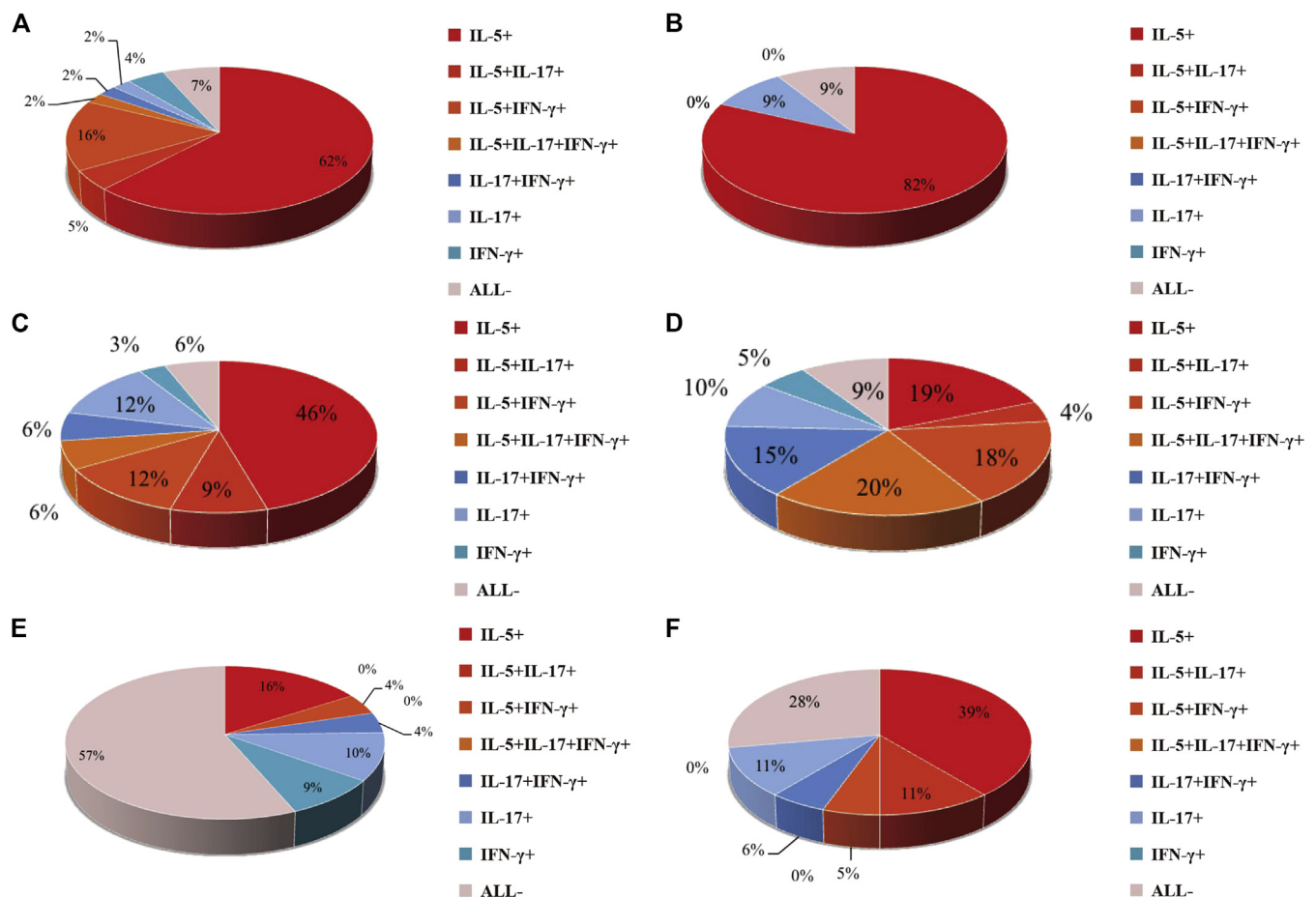


FIG 4. Patterns of $T_H1/T_H2/T_H17$ cytokine expression in samples from patients with CRSwNP: Benelux (A), Berlin (B), Adelaide (C), Beijing (D), Chengdu (E), and Tochigi (F).

regions, possibly because of a combination of both genetic and environmental factors. The distributing patterns of the endotypes based on key cytokines might be crucial for an individualized therapeutic strategy because one previous study with a humanized anti-human IL-5 mAb has demonstrated that this therapy was effective in reducing the size of NPs in only half of the patients with CRSwNP.¹⁹

Although the CRSsNP phenotype has generally been considered T_H1 dominant, with higher levels of IFN- γ in white and Chinese subjects with CRS,^{8,15} the present study showed that only the patients with CRSsNP from Beijing had higher IFN- γ levels compared with patients with CRSwNP and control subjects. Similarly, the patients with CRSsNP from Benelux had higher IFN- γ levels compared with those in control subjects. Moreover, the $T_H1/T_H2/T_H17$ patterns of CRSsNP also showed diversity with 33% to 40% positivity of IL-5 in patients from Benelux, Berlin, Adelaide, and Beijing but only 5% IL-5 positivity in patients from Chengdu. Thus the present study indicates a need for consideration of individualized therapeutic strategies for patients with CRSsNP rather than standardized therapy with steroids and antibiotics.¹

Inflammation and remodeling are 2 aspects of CRS; however, whether these 2 aspects are related or dissociated still needs to be elucidated.²⁰⁻²² In the present study we found an interesting endotype for CRSwNP and CRSsNP. With regard to pangenativity of

$T_H1/T_H2/T_H17$ in all 6 study regions, particularly in Chengdu, 57% of patients with CRSwNP and 81% of patients with CRSsNP demonstrated this endotype, indicating that the vast majority of patients with CRSwNP and those with CRSsNP in Chengdu were independent of $T_H1/T_H2/T_H17$ endotype-mediated reactions. However, in view of a lack of any specific pattern among the different regions for TGF- $\beta 1$, the delegating factor of remodeling, the present study suggests that inflammation based on T_H immunity is unlikely to rationally explain remodeling in patients with CRS.

S aureus can contribute to T_H2 differentiation and amplify the inflammation.²³ Indeed, one recent study has demonstrated that a shift from predominantly neutrophilic to eosinophilic CRSwNP over time, accompanied by an increase in levels of various inflammatory markers, including IgE, was associated with an increase in the intramucosal presence of *S aureus*.¹⁷ Specific IgE against SE has also been shown to act as an independent risk factor for asthma,²⁴ with relatively higher IL-5, ECP, SE-specific IgE, and tissue total IgE levels increasing the risk of comorbid asthma and IFN- γ playing a protective role.¹² The present study demonstrated higher total tissue IgE levels from Adelaide and Tochigi compared with Beijing and Chengdu, as well as higher positivity of SE-specific IgE from Benelux, Adelaide, and Tochigi compared with Beijing and Chengdu. These immunologic patterns of CRSwNP

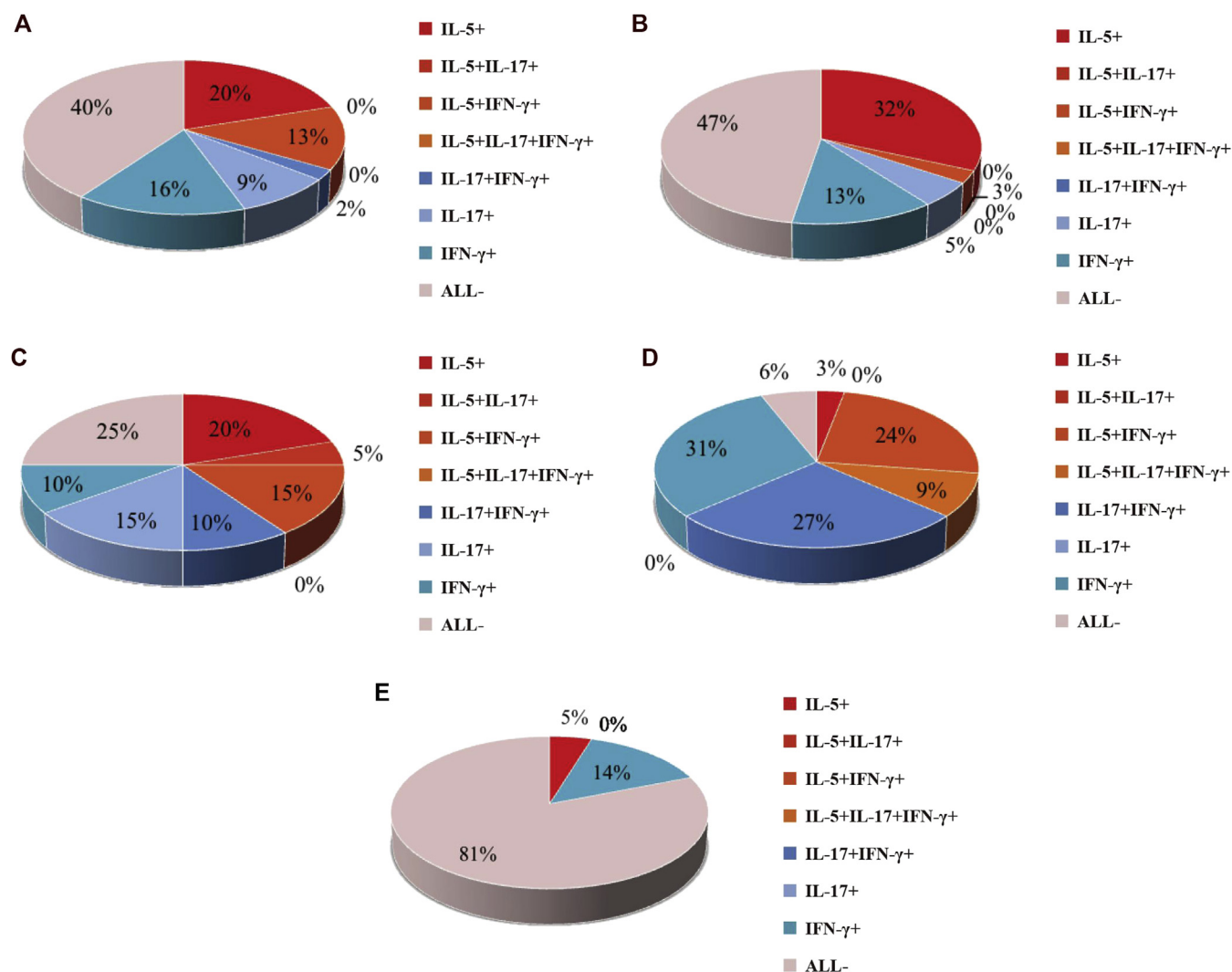


FIG 5. Patterns of $T_H1/T_H2/T_H17$ cytokine expression in samples from patients with CRSwNP (only 7 cases of CRSwNP in Tochigi, data not shown): Benelux (A), Berlin (B), Adelaide (C), Beijing (D), and Chengdu (E).

consisting of low IL-5 levels, low IgE levels, and lack of SE-specific IgE in tissue might explain the lower comorbidity of asthma and atopy in Chinese subjects when compared with white and Japanese subjects. It can be speculated that although the IL-5 positivity in patients from Beijing with CRSwNP was much higher than that in patients from Chengdu, the higher IFN- γ positivity might inhibit the subjects from having asthma, resulting in the approximately similar prevalence of asthma noted in patients with CRSwNP in Beijing and Chengdu. It has also been shown that certain specific patterns of microbiota would be related to a T_H17 polarization or an eosinophilic endotype of CRSwNP.^{25,26} Similarly, air pollution, specifically particulate matter (particulate matter with a diameter $<10 \mu\text{m}$ and smaller), might potentially enhance T_H17 polarization through activation of the aryl hydrocarbon receptor,²⁷ suggesting that the diversity of environmental factors, such as microbiota and air pollution, can result in differences in T_H patterns among the 6 regions to some degree, although this needs to be defined further.

Recently, Mahdavinia et al¹⁸ reported that second-generation Asian American patients with CRSwNP demonstrated lower

levels of tissue eosinophilia than their white American counterparts and suggested that this was mainly due to genetic factors. However, only 11 second-generation Asian Americans with very diverse genetic backgrounds were included, and therefore the authors' interpretation might be questioned. In contrast, the demonstration that tissue eosinophilia in NPs can dramatically increase over a period of 10 to 20 years in the same study cohorts^{17,28} provides support for the involvement of environmental factors, such as air pollution, the microbiome, and also use of antibiotics, in mucosal inflammatory patterns in patients with CRS.

In the present study IL-8 levels and positivity appeared to be upregulated in the CRSwNP groups from all 6 regions, with the highest levels seen in Beijing. Interestingly, the IL-8 level of the control group from Beijing was also much higher than that of the other 5 regions. Because IL-8 synthesis has been shown to be induced by air pollution,²⁹ it is possible that the high levels of IL-8 seen in both patients with CRS and control subjects might be the result of air pollution in Beijing, which has increased significantly in recent years, although further studies are needed to validate this.

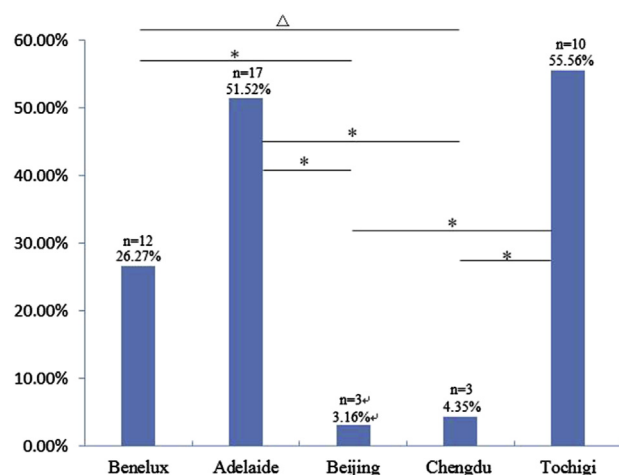


FIG 6. Percentages of positive samples for tissue-specific IgE to SEs in patients with CRSwNP in different regions. $\Delta P = .001$ and $*P < .001$. Data were not available for SE-specific IgE in samples from patients in Berlin.

Based on examination of $T_H1/T_H2/T_H17$ cytokine patterns in patients with CRSwNP and those with CRSsNP, the present study has demonstrated that there appears to be an extreme diversity in terms of the mucosal immunologic endotypes of CRS in Europe, China, Japan, and Australia. However, these findings are somewhat limited by the shortcomings of this study and need to be confirmed in other studies investigating greater numbers of patients from specific environments. In particular, although the samples of this study were randomly selected, this is not a prevalence study and cannot completely represent the populations of these regions. Although analysis of samples in 2 laboratories demonstrated accordance between the 2 laboratories, it would be desirable to further harmonize sampling and measurement techniques. Despite these shortcomings, the present study provides important insights into the diversity of diseases of what was believed to be one umbrella term, CRS, and provides important pointers toward assessing the mechanisms underlying different endotypes of patients with CRS.³⁰ Furthermore, this study highlights the need for future observations on the change in immune patterns over time in patients with airway disease and the consequences thereof in terms of prognosis and management.

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Clinical implications: The cytokine and inflammatory marker profiles of CRS show diversity among European, Asian, and Oceanian patients, suggesting the need for individualized therapeutic strategies.

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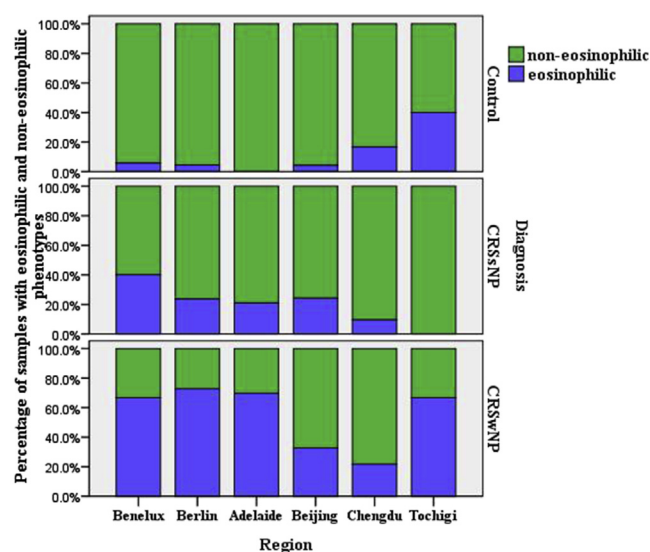


FIG 7. Percentage of the samples with eosinophilic (ECP/MPO ratio >1) and noneosinophilic (ECP/MPO ratio <1) phenotypes.

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