Body mass index's effect on CRSwNP extends to pathological endotype and recurrence

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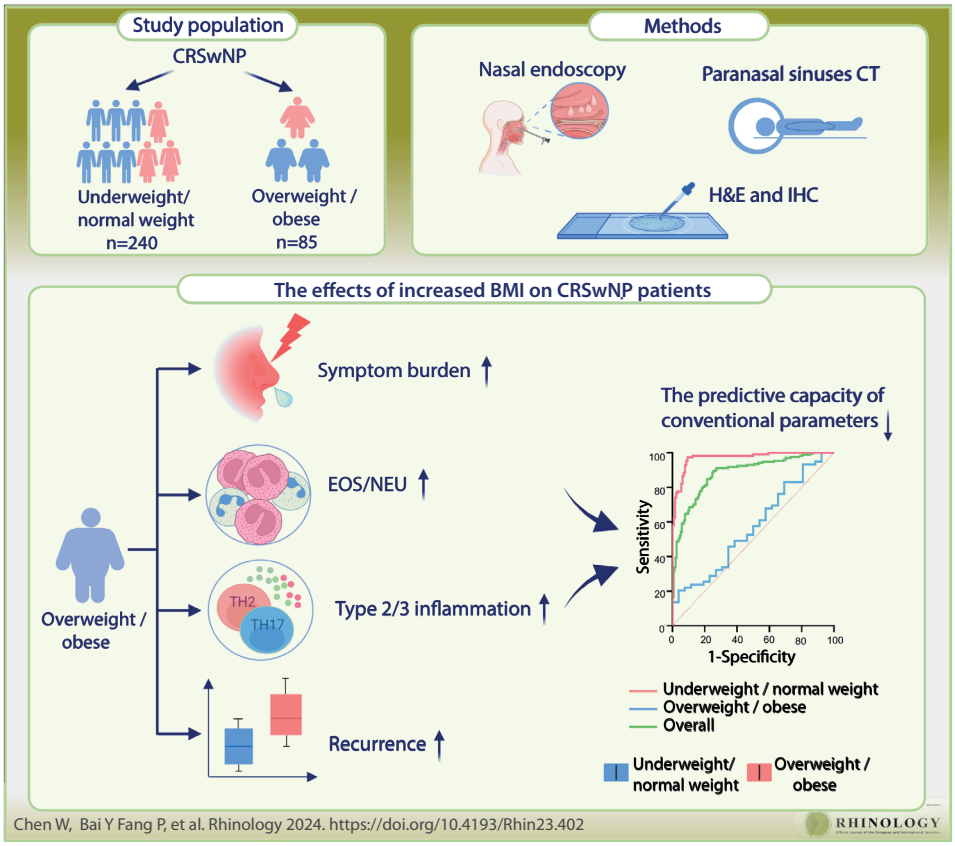
Abstract

Background: Elevated body mass index (BMI) has been recognized as an important contributor to corticosteroid insensitivity in chronic rhinosinusitis with nasal polyps (CRSwNP). We aimed to delineate the effects of elevated BMI on immunological endotype and recurrence in CRSwNP individuals. **Methodology**: A total of 325 patients with CRSwNP undergoing FESS were recruited and stratified by BMI. H&E staining was employed for histological evaluation. Characteristics of inflammatory patterns were identified by immunohistochemical staining. The predictive factors for recurrence were determined and evaluated by multivariable logistic regression analysis and the receiver operating characteristic (ROC) curves across all subjects and by weight group. **Results**: In all patients with CRSwNP, 26.15% subjects were classified as overweight/obese group across BMI categories and exhibited a higher symptom burden. The upregulated eosinophil/neutrophil-dominant cellular endotype and amplified type 2/type 3 coexisting inflammation was present in overweight/obese compared to underweight/normal weight controls. Additionally, a higher recurrent proportion was shown in overweight/obese patients than that in underweight/normal weight cohorts. Multivariable logistic regression analysis identified BMI as an independent predictor for recurrence. The predictive capacity of each conventional parameter (tissue eosinophil and CLCs count, and blood eosinophil percentage) alone or in combination was poor in overweight/obese subjects. **Conclusions**: Overweight/obese CRSwNP stands for a unique phenotype and endotype. Conventional parameters predicting recurrence are compromised in overweight/obese CRSwNP, and there is an urgent need for novel biomarkers that predict recurrence for these patients.

Key words: nasal polyps, body mass index, eosinophils, endotype, recurrence

Graphical abstract



Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a heterogeneous disease with distinct phenotypes and endotypes [1]. The most commonly accepted and well-described endotype is eosinophilic CRSwNP (Eos CRSwNP), characterized by eosinophilia and enhanced type 2 (T2) inflammation associated with elevated IL-5, IL-13, IgE, and Charcot-Leyden crystals (CLCs). It has been appreciated that endotype in Caucasian CRSwNP patients is well characterized by eosinophilia/T2 inflammation, whereas endotype in Asian CRSwNP individuals is dominated by non-eosinophilic inflammation. Recently, several groups have presented compelling evidence that neutrophilic inflammation is present in Western T2 CRSwNP [2-5] and have observed an emerging trend of "eosinophilic shift" inflammatory pattern in Asian patients with CRSwNP [6]. These results suggest a profile of eosinophil-neutrophil coexisting inflammation may be present in CRSwNP under specific conditions.

Obesity is a rapidly increasing global health problem, and affects more than one billion individuals worldwide [7, 8]. Growing clinical evidence has thrown light on the crucial effect of obesity on the pathological endotypes of inflammatory diseases including asthma, atopic dermatitis, eosinophilic esophagitis, and allergic rhinitis [9-13]. In atopic dermatitis, obesity could shift the classical T helper 2 (TH2)-inflammatory pattern to a more severe disease characterized by an augmented TH17-inflammatory type [11, 12, 14]. Emerging epidemiological and clinical evidence has suggested an association between the development of CRSwNP and high body mass index (BMI) [7, 15]. Elevated BMI in CRSwNP patients has also been reported to be associated with increased disease severity [7, 15]. A previous study by Zhang et al. has demonstrated that elevated BMI contributes to glucocorticoid (GC) insensitivity in subjects with Eos CRSwNP [16]. However, whether increased BMI influences the pathological phenotype of CRSwNP has not yet been elucidated.

Despite scientific progress and the development of an increasing number of novel therapeutic regimens including biologics and reboot surgery, CRSwNP continues to exhibit higher recurrent rates. In fact, more than 50% patients with CRSwNP and 98.5% Eos CRSwNP patients were recurrent after undergoing endoscopic sinus surgery (ESS) or pharmacological treatment. Therefore, biomarkers including tissue eosinophils [17, 18], CLCs [18-20], blood eosinophils [21], IL-5 [22, 23], microbiota [24] have been identified as predictors for polyp recurrence. However, there are considerable gaps in our understanding whether elevated BMI influences NP recurrence and predictive capability of parameters.

Given the mounting evidence that elevated BMI plays a crucial role in altering immune response and therapeutic strategies in inflammatory diseases, the goal of this study was to evaluate whether increased BMI would affect immunological endotypes and recurrence in CRSwNP.

Materials and methods

Subjects

A total of 325 patients with CRSwNP who experienced endoscopic sinus surgery (ESS), from January 2021 to June 2022, were recruited and stratified by BMI into underweight/normal weight (≤ 24.9 kg/m^2), overweight (>24.9 kg/m^2), and obese (≥30 kg/m^2) [25]. Due to the limited number of obese CRSwNP patients, obese subjects were combined with overweight individuals in the overweight/obese group [16]. Written informed consent was obtained from all subjects and this study was approved by the Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University (II2023-117-01). The diagnosis of CRSwNP was based on the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012 and EPOS 2020 [26, 27]. Atopy was determined with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) using serological testing or a standard panel of aeroallergens. Allergic rhinitis (AR) and asthma was diagnosed according to the Allergic Rhinitis and its Impact on Asthma (ARIA) 2010 guidelines and Global Initiative for Asthma (GINA) guidelines 2014, respectively [28, 29]. None of the individuals took oral corticosteroids, antibiotics, or biologics within one month before surgery. Patients with allergic fungal rhinitis, cystic fibrosis, immunodeficiency, coagulation disorder, eosinophilic granulomatous polyangiitis or pregnancy were excluded from the study.

Histological evaluation

Nasal polyp (NP) biopsies were collected during the process of ESS procedure. Histological evaluation was determined by hematoxylin and eosin (H&E) staining as previously reported [30]. Eos CRSwNP was defined when eosinophil percentages > 10% of the total infiltrating cells [31]. Eos CRSwNP was also diagnosed by eosinophil counts per high-power field (HPF) > 10/HPF, or 32/HPF, or 55/ HPF, or 70/HPF with specific description [17, 18, 27, 32]. CLCs were identified by the presence of bipyramidal crystal structures [18, 33, 34].

Immunohistochemistry (IHC)

To prepare tissue slides for IHC, NP biopsies were fixed in 4% paraformaldehyde and embedded in paraffin. These 4 μm thick slides were first deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Slides were blocked with 5% BSA and incubated with specific primary antibodies (detailed information for primary antibodies are listed in Table S1) for 12h at 4°C, respectively. The primary antibodies were visualized via a horseradish peroxidase - linked secondary antibody kit according to manufacturer's instructions (Boster, Wuhan, Hubei, China). Isotype controls were included for all samples. Five non-overlapping HPFs with the most count of positive cells were counted, and the mean number of positive cells was calculated.

Classification of NPs based on endotype

Eosinophilhigh (Eoshigh) was defined when eosinophil percentages > 10% of the total infiltrating cells, and neutrophilhigh (Neuhigh) was diagnosed when myeloperoxidase (MPO)$^+$ neutrophils > 10/HPF [31, 35]. Thus, cellular endotype was classified as $Eos^{high}Neu^{high}$, $Eos^{high}Neu^{low}$, $Eos^{low}Neu^{high}$, and $Eos^{low}Neu^{low}$. Cytokine patterns of T1, T2, and T3 were defined by positivity of IFN-γ^+ cells, IL-5^+ cells, and IL-$17A^+$ cells, respectively [36, 37]. Then IFN-γ^+ cells, IL-5^+ cells, and IL-$17A^+$ cells were further classified into groups of high or low based on the median cut-off value of each cytokine. Therefore, the cytokine endotypes were sorted as: IL-5^{high}IL-17^{high}, IL-5^{high}IFNγ^{high}, IL-5^{high}IL-17^{high}IFNγ^{high}, IL-17^{high}IFNγ^{high}, IL-17^{high}, IL-5^{high}, IFNγ^{high}, and All^{low}.

Follow-up and postoperative evaluation

Follow-up was performed 1 week, 1 month, 3 months, 6 months, and 1 year after surgery. All patients undergoing ESS performed nasal irrigation daily and were administrated with both intranasal budesonide spray 128 μg twice a day and oral clarithromycin 250 mg daily for 3 months. Recurrence was defined by the presence of NPs observed under endoscopy, together with at least one symptom (nasal obstruction, rhinorrhea, reduction or loss of smell, headache/facial pain) lasting at least 1 week, despite appropriate medical management (intranasal/systemic corticosteroid and clarithromycin) as well as nasal irrigation as previously reported [20].

Statistical analysis

Statistical analysis was performed with SPSS 23.0 software (SPSS Inc, Chicago, IL, USA). Descriptive statistics were determined. Continuous variables are indicated by medians (25th and 75th percentiles), and categorical variables are indicated with percentages. χ^2 test was used to compare categorical variables. The Kruskal-Wallis H test was used to evaluate significant intergroup variability, and the 2-tailed Mann-Whitney U test was applied for intergroup comparison. A multivariable logistic regression analysis was applied to determine the potential predictors of NP recurrence. Predictive capacity of each biomarker was determined by maximizing the area under the receiver operating characteristic curve (AUC). Regression models were applied across all patients and by weight group. Each regression model was adjusted for gender, age, atopy, and comorbidity of AR and asthma, smoking, revision ESS, as well as general diseases (diabetes, hypertension requiring medication, history of severe COPD, history of stroke, disseminated cancer, phthisis, nephropathy). Each logistic regression model across underweight/normal weight and overweight/obese groups, was also adjusted for BMI. Significance was accepted at a P value of less than 0.05.

Results

Demographics and baseline characteristics

Three hundred and twenty-five patients with CRSwNP were included, and 26.15% subjects were classified as overweight/obese group across BMI categories (Table 1). As expected, BMI was increased in overweight/obese group (26.57 kg/m^2) compared with underweight/normal weight group (21.86 kg/m^2; P < 0.001). The median age for overweight/obese patients with CRSwNP (45 years) was higher compared with that of underweight/normal weight subjects (36 years; P = 0.042). Interestingly, significantly more male patients were found in the overweight/obese CRSwNP group (83.72%) compared with the parallel group (66.25%; P = 0.009). Furthermore, a larger proportion of overweight/obese CRSwNP subjects (65.88%) were comorbid with AR, but not asthma, compared with underweight/normal weight subjects (19.17%; P < 0.001). Presence of atopy was more prevalent in the overweight/obese group than in the underweight/normal weight group since the level of total IgE in serum was elevated in overweight/obese CRSwNP patients. However, atopy frequencies were comparable across BMI groups. The percentage and count of blood eosinophils as well as the percentage of neutrophils were similar across both groups. Although the count of neutrophils in peripheral blood was significantly elevated in the overweight/obese CRSwNP group than that in the parallel group, the difference was small and might not be clinically meaningful. We also found overweight/obese patients with CRSwNP have a higher symptom burden compared with underweight/normal weight controls, as indicated by an increased nasal obstruction score (8.00 vs 5.50, P = 0.080) in overweight/obese subjects, although Lund-Mackay score and Lund-Kennedy score were comparable across BMI groups (Table 1). These results suggest that overweight/obese subjects with CRSwNP are more likely to be male, older, atopic, and to present with more severe clinical symptoms.

Higher BMI augments eosinophil-neutrophil mixed airway inflammation

Since mounting evidence has confirmed that elevated BMI could alter the pathological endotype in patients with asthma and dermatitis [9, 11, 25], it is imperative to delineate the effect of increased BMI on cellular endotypes of overweight/obese CRSwNP patients. Eosinophil counts were significantly elevated in the overweight/obese CRSwNP group (62.40 [25.60, 111.25]) compared with the parallel group (37.80 [7.75, 109.35]) (P = 0.047; Figure 1A) as determined by H&E staining. Furthermore, a larger count and proportion of CLCs, products of hyperactivated eosinophils, were found in overweight/obese CRSwNP subjects (5.00 [0.00, 17.20]; 62.35%) compared with underweight/normal weight subjects (0.00 [0.00, 13.15]; 48.75%), individually (P = 0.033; P = 0.031; Figure 1B and 1C). Then, the presence of elevated eosinophilic inflammation in overweight/obese CRSwNP

Table 1. Demographic data and baseline characteristics of participants by weight group.

Variables	Underweight / normal weight	Overweight / obese	Padj Value
n (%)	240 (73.85%)	85 (26.15%)	-
BMI (kg/m^2)	21.86 [19.90, 23.05]	26.57 [25.68, 28.20]	**<0.001**
Age (years)	36.00 [28.00, 50.00]	45.00 [33.50, 52.00]	**0.042**
Gender, male (%)	159 (66.25%)	72 (83.72%)	**0.009**
Asthma (%)	17 (7.08%)	10 (11.76%)	0.963
Allergic rhinitis (%)	46 (19.17%)	56 (65.88%)	**<0.001**
NERD (%)	0 (0.00%)	0 (0.00%)	1.000
Atopy (%)	144 (60.00%)	61 (71.76%)	0.351
Total IgE (KU/L)	73.00 [31.00, 174.00]	182.00 [55.50, 877.75]	**<0.001**
PB EOS (%)	4.25 [2.20, 7.00]	4.80 [3.20, 7.45]	0.468
PB EOS (*10^9/L)	0.27 [0.13, 0.46]	0.32 [0.21, 0.48]	0.488
PB NEU (%)	53.15 [46.80, 58.80]	54.30 [49.95, 57.30]	0.137
PB NEU (*10^9/L)	3.20 [2.59, 4.12]	3.67 [3.08, 4.36]	**0.004**
VAS scores			
nasal obstruction	5.50 [2.25, 7.00]	8.00 [5.50, 10.00]	0.080
rhinorrhea	3.00 [1.00, 5.75]	5.00 [2.00, 8.50]	0.705
headache	1.00 [0.00, 4.00]	2.00 [0.00, 6.00]	0.405
facial pain	0.00 [0.00, 2.75]	0.00 [0.00, 1.50]	0.096
olfactory disorder	2.50 [0.00, 6.75]	6.00 [2.50, 10.00]	0.265
overall burden	6.00 [4.00, 7.00]	6.00 [4.50, 8.00]	0.718
CT and endoscopic scores			
Lund-Mackay score	16.00 [10.00, 19.00]	14.00 [11.00, 21.50]	0.902
Lund-Kennedy score	9.50 [6.00, 12.00]	8.00 [6.00, 10.50]	0.733

Data are subjected to binary linear regression analysis, and parameters including asthma, gender, and age are adjusted. Date are presented as numbers (percentages) or medians (25th-75th percentiles). Underweight/normal weight, BMI of 24.9 kg/m^2 or less; Overweight/obese, BMI of 25kg/m^2 or greater. BMI, body mass index; PB, peripheral blood; EOS, eosinophil; NEU, neutrophil; VAS, visual analog scale; CT, computed tomography; NERD, nonsteroidal anti-inflammatory drug-exacerbated respiratory disease.

patients was further confirmed by IHC staining, as demonstrated by increased eosinophil cationic protein (ECP)$^+$ eosinophils in the overweight/obese group compared with the parallel control group (P = 0.006; Figure 1D and Figure S1). Additionally, the proportion of Eos CRSwNP, defined by different diagnostic criteria including eosinophil >10% (75.29% VS 63.66%, P = 0.045), or >10/HPF (82.35% VS 70.42%, P = 0.032), or >32/HPF (70.59% VS 52.92%, P = 0.005), or >55/HPF (50.59% VS 41.25%, P = 0.136), or >70/HPF (42.35% VS 36.25%, P = 0.136) was elevated in the overweight/obese than in the underweight/normal weight group (Table S2).

In addition to eosinophils, neutrophil infiltration was also evaluated. An increasing but not significant trend in neutrophil count was observed in the overweight/obese individuals (Figure 1D and Figure S1). Furthermore, the percentage of eosinophilhighneutrophilhigh (Eos^{high}Neu^{high}) subjects was higher in overweight/obese (69.23%) compared to underweight/normal weight (43.76%) subjects with CRSwNP (P = 0.024) (Figure 1E). IHC also identified a significant upregulation of NLRP3$^+$cells, which was associated with GC insensitivity [16], in overweight/obese relative to underweight/normal weight subjects with CRSwNP (P = 0.004) (Figure S2). These results demonstrated that higher and more hyperactivated eosinophilic inflammation as well as Eos^{high}Neu^{high} cellular endotype was present in overweight/obese compared to underweight/normal CRSwNP patients. This suggests that elevated BMI may contribute to the mixed eosinophil-neutrophil inflammation pattern in CRSwNP patients.

Higher BMI amplifies type 2-type 3 coexisting airway inflammation

A mixed inflammatory profile including T helper (TH)1/TH2/TH17 cells has been reported in CRSwNP [31, 37], however, whether elevated BMI alters inflammatory pattern of TH cells in CRSwNP individuals remains underexplored. Thus, the transcription factors (TFs) and inflammatory mediators of TH signatures were analyzed across the groups. The protein level of GATA binding protein 3 (GATA3, TF of TH2; P = 0.002) and IgE (P < 0.001), was robustly increased in overweight/obese compared to underweight/normal weight CRSwNP patients (Figure 2A and Figure

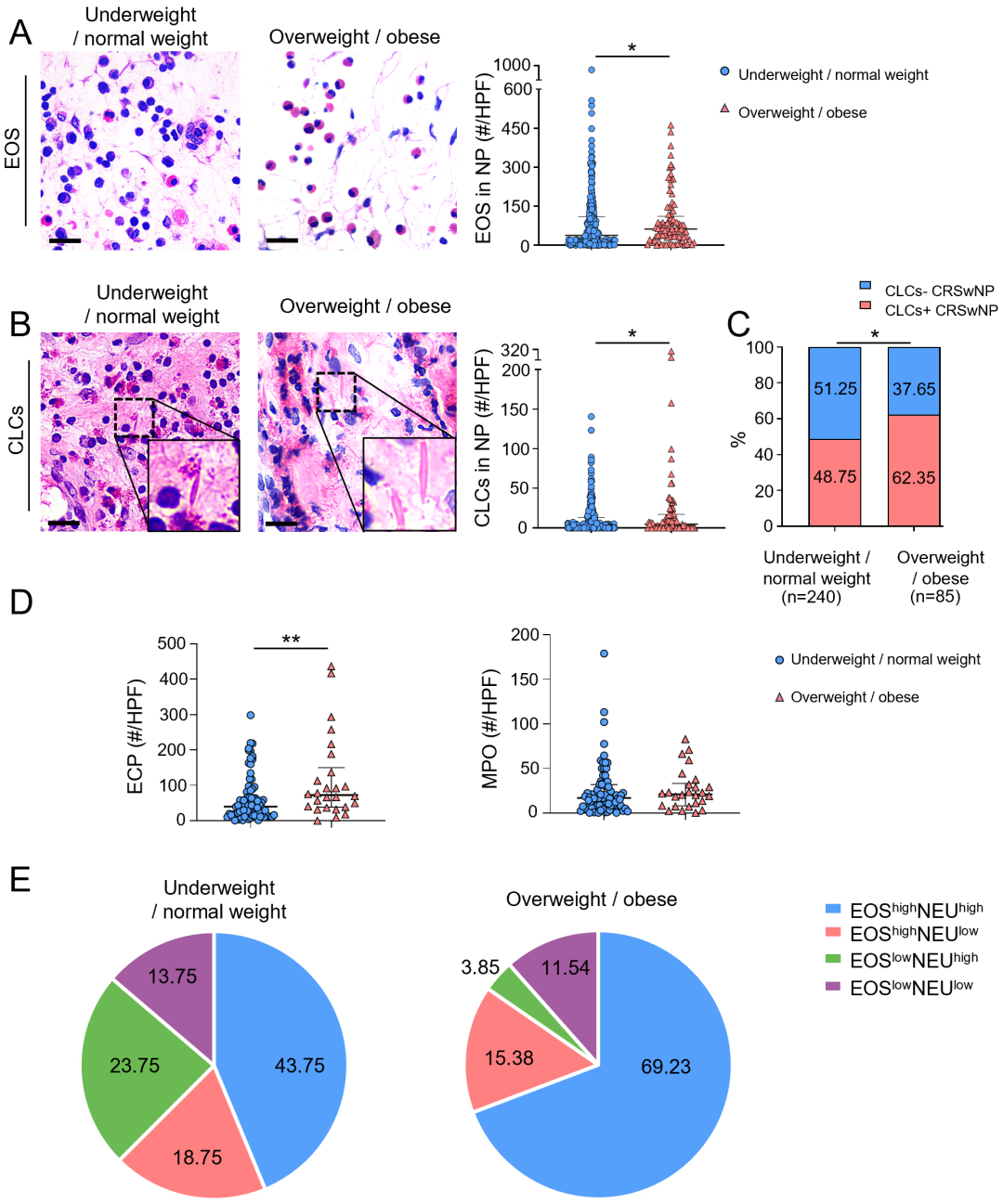


Figure 1. Augmented eosinophil-neutrophil mixed airway inflammation in overweight/obese CRSwNP. (A) Representative photomicrographs stained by H&E and quantitative analysis of eosinophils in CRSwNP individuals by weight group. (B) Representative H&E photomicrographs and quantitative analysis of CLCs in CRSwNP individuals by weight group. (C) The proportion of CLCs+ CRSwNP and CLCs- CRSwNP in CRSwNP subjects by weight group. (D) Quantitative analysis of ECP^{+} cells and MPO^{+} cells in CRSwNP individuals by weight category. (E) Patterns of eosinophils/neutrophils in CRSwNP patients by weight category. Original magnification x400, bar scale=20μm. CRSwNP, chronic rhinosinusitis with nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CLCs, Charcot-Leyden crystals; EOS, eosinophil; HPF, high power field; ECP, eosinophil cationic protein; MPO, myeloperoxidase; (A-C) n=240 Underweight /normal weight and n=85 Overweight / obese; (D&E) n=80 Underweight /normal weight and n=26 Overweight / obese; *: p<0.05, **: p<0.01 compare to Underweight /normal weight, as assessed using unpaired Mann–Whitney test.

S3). Although the count of IL-5^{+}cells showed a tendency of elevation in overweight/obese CRSwNP patients, it did not reach statistical significance (Figure 2A and Figure S3). Interestingly, increased expression levels of RAR-related orphan receptor gamma T (ROR-γt^{+}, TF of TH17; P = 0.005) and IL-17A (P = 0.048) were found in overweight/obese CRSwNP patients compared to underweight/normal individuals (Figure 2A and Figure S3) indicating an amplified TH17 immunity in overweight/obese

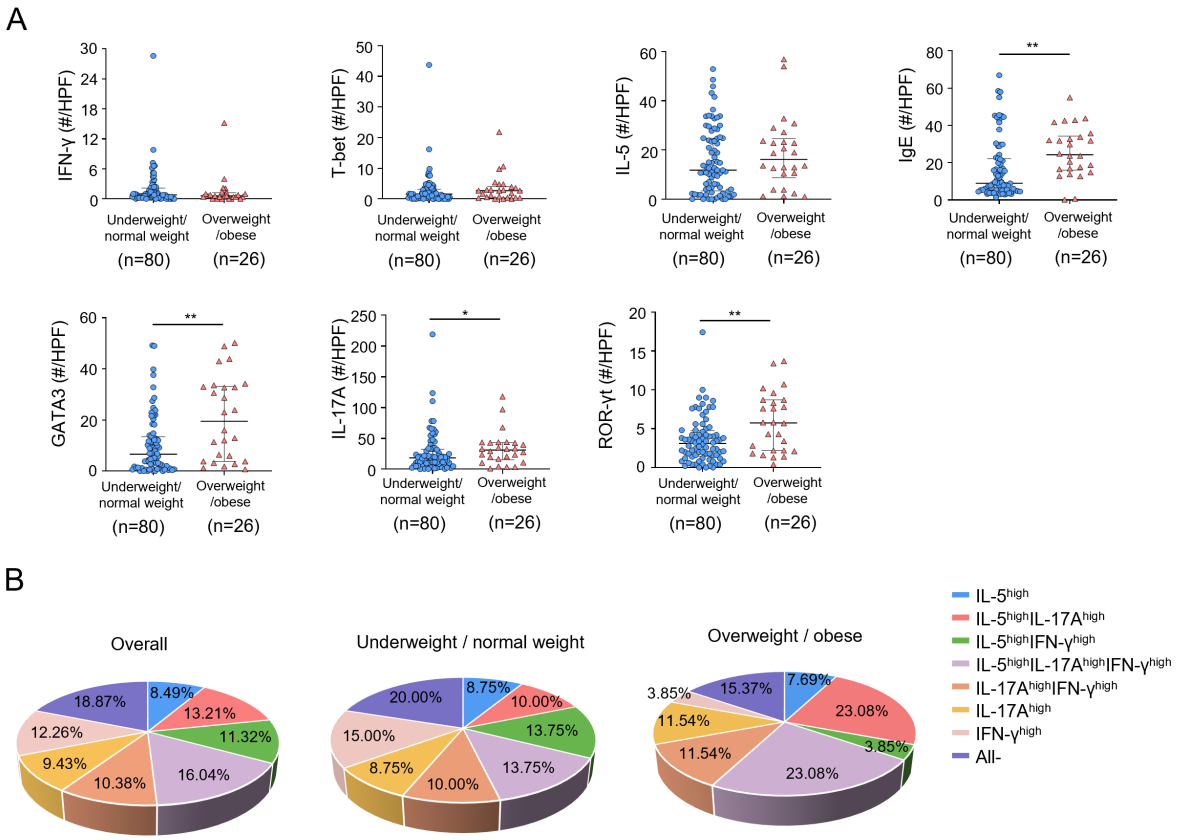


Figure 2. Exacerbated T2/T3 coexisting inflammation in overweight/obese CRSwNP. (A) Quantitative analysis of IFN-γ^+ cells, T-bet$^+$ cells, IL-5$^+$ cells, IgE$^+$ cells, GATA3$^+$ cells, IL-17A$^+$ cells, and ROR-γt^+ cells in CRSwNP patients by weight category. (B) Patterns of T1/T2/T3 cytokine expression in samples from patients with CRSwNP by weight category. CRSwNP, chronic rhinosinusitis with nasal polyps; IFN-γ, interferon gamma; T-bet, T-box transcription factor 21; Ig E, immunoglobulin E; IL, interleukin; GATA3, GATA binding protein 3; ROR- γt, RAR-related orphan receptor gamma T; HPF, high power field. n=80 Underweight /normal weight and n=26 Overweight / obese; *: p<0.05 **: p<0.01 compare to Underweight /normal weight, as assessed using unpaired Mann–Whitney test.

CRSwNP subjects. The TH1 inflammatory pattern, characterized by T-box transcription factor 21 (T-bet)$^+$ cells and interferon (IFN) γ^+ cells, was comparable between the two groups (Figure 2A and Figure S3).

The median cut-off value of each cytokine protein level was applied respectively, to differentiate distinct cytokine patterns (IFNγ^{high}IL-5^{high}IL-17^{high}, IL-5^{high}IL-17^{high}, IL-5^{high}IFNγ^{high}, IL-17^{high}IFNγ^{high}, IL-17^{high}, IL-5^{high}, IFNγ^{high}, and All^{low}). As shown in Figure 2B, the frequency of single IL-5 high inflammation is comparable between the two groups, whereas overweight/obese CRSwNP subjects showed elevated frequencies of IL-5^{high}IL-17^{high} and IL-5^{high}IL-17^{high}IFNγ^{high} inflammatory subsets but reduced frequencies of IFNγ^{high} and IL-5^{high}IFNγ^{high} inflammation subset. In summary, these results suggest that increased BMI possibly could augment TH2/TH17 and TH2/TH17/TH1 dominated mixed inflammatory patterns.

Elevated BMI is involved with higher recurrence in CRSwNP patients

Next, the effect of increased BMI on NP recurrence was analyzed between groups based on BMI. Unexpectedly, we found that the recurrent rate was significantly higher in overweight/obese CRSwNP patients (69.41%) when compared with underweight/normal weight controls (46.67%), indicating elevated BMI might be associated with NP recurrence (Figure 3A and Table S3). Interestingly, the phenomenon of elevated recurrence in overweight/obese CRSwNP is mainly observed in Non-Eos CRSwNP but not in Eos CRSwNP based on different Eos CRSwNP/Non-Eos CRSwNP diagnostic criteria (Figure 3B-F), suggesting elevated BMI exerts differently on recurrence in Eos CRSwNP and Non-Eos CRSwNP. Furthermore, clinical characteristics and inflammatory patterns were compared between recurrent and unrecurrent subjects across all individuals and within distinct weight category (Table S4). Overall, recurrence seems to be more sensitive on male CRSwNP subjects, whereas this trend disappeared when the comparison was done within each weight category. We found that the frequency of AR and asthma comorbidity, positive rate of atopy, count of tissue eosinophils and CLCs,

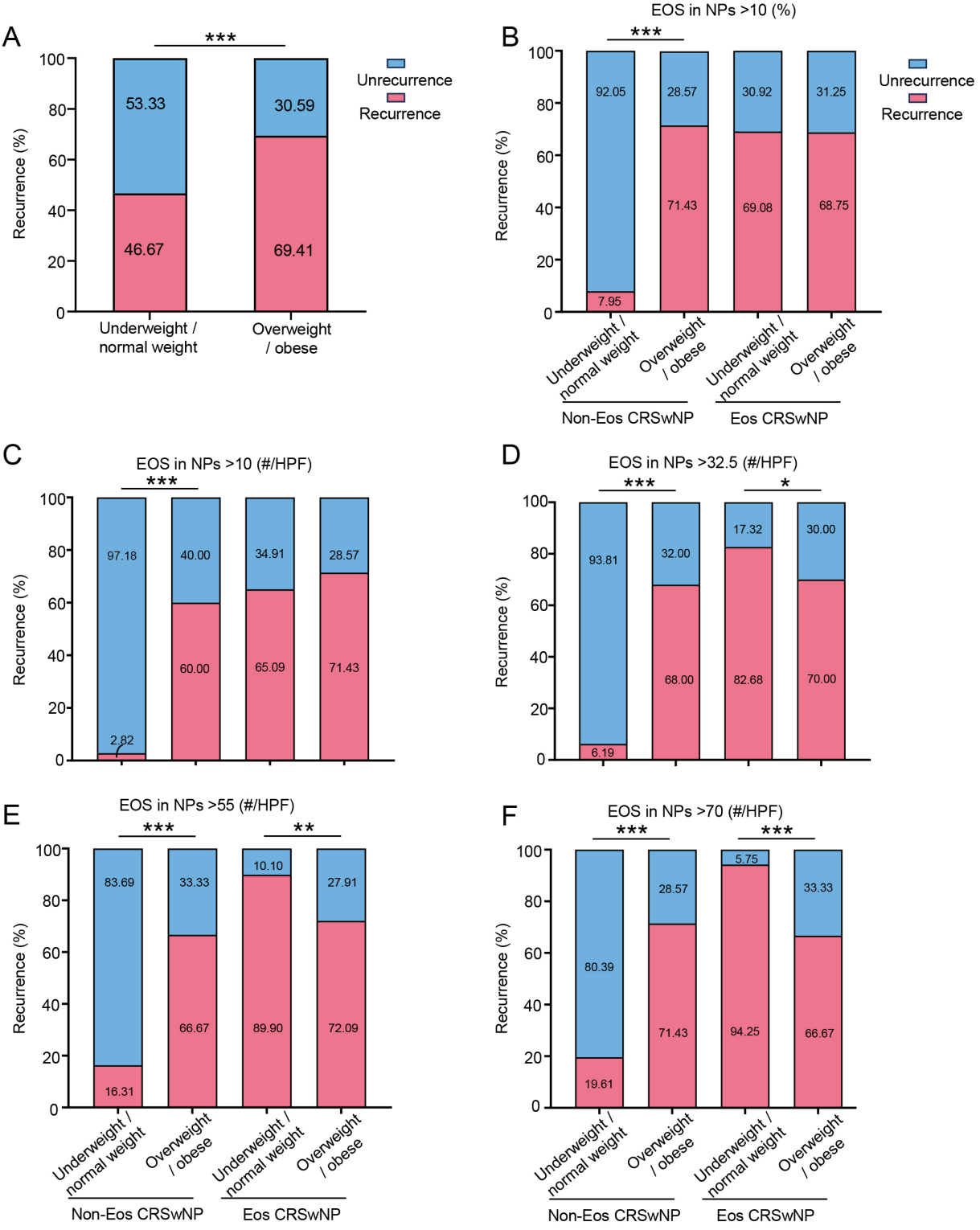


Figure 3. The recurrent rate in patients with CRSwNP by weight category. (A) The recurrence of CRSwNP subjects grouped by weight category. (B-F) The recurrence of individuals with Eos CRSwNP and Non-Eos CRSwNP diagnosed based on EOS in NPs >10% (B), EOS# in NPs >10/HPF (C), EOS# in NPs >32.5/HPF (D), EOS# in NPs >55/HPF (E), EOS# in NPs >70/HPF (F). EOS, eosinophil; NPs, nasal polyps; Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; HPF, high power field. n=240 Underweight /normal weight, n=85 Overweight / obese and n=325 Overall; *: $p<0.05$, *: $p<0.01$, ***: $p<0.001$; as assessed using unpaired χ^2 test.

Table 2. Predicting recurrence of nasal polyps by weight category using conventional predictors.

Variables	OR value	95% CI	P value
Multivariate analysis*			
Overall			
BMI (kg/m^2)	1.087	1.008-1.172	**0.031**
EOS (#/HPF)	1.026	1.019-1.033	**<0.001**
CLCs (#/HPF)	1.179	1.122-1.240	**<0.001**
EOS in PB (%)	1.142	1.057-1.233	**0.001**
Underweight / normal weight			
EOS (#/HPF)	1.057	1.040-1.075	**<0.001**
CLCs (#/HPF)	1.793	1.500-2.144	**<0.001**
EOS in PB (%)	1.385	1.236-1.553	**<0.001**
Overweight / obese			
EOS (#/HPF)	1.002	0.997-1.008	0.395
CLCs (#/HPF)	1.020	0.991-1.049	0.179
EOS in PB (%)	0.951	0.873-1.035	0.242

* Each logistic regression model is adjusted for gender, age, atopy, allergic rhinitis, asthma, smoking, revision ESS, as well as general diseases (diabetes, hypertension requiring medication, history of severe COPD, history of stroke, disseminated cancer, phthisis, nephropathy). Furthermore, each logistic regression model in underweight/normal weight group and overweight/obese group, is also adjusted for BMI. Underweight/normal weight, BMI of 24.9 kg/m^2 or less; Overweight/obese, BMI of 25kg/m^2 or greater. BMI, body mass index; EOS, eosinophil; CLCs, Charcot-Leyden crystals; PB, peripheral blood; HPF, high power field.

percentage of $Eos^{high}Neu^{high}$ in tissue, and blood eosinophil percentage were increased in recurrent group compared with the unrecurrent group in all CRSwNP patients and in underweight/normal group (Table S4). However, these parameters showed no difference when comparing recurrent and unrecurrent within the overweight/obese individuals. These data imply that BMI may serve as a risk factor for recurrence, which prompted us to think whether the predictive capacity of biomarkers for NP recurrence are affected by increased BMI.

Predicting NP recurrence overall and within BMI groups

A multivariable logistic regression model for BMI, eosinophil count (EOS#) and CLCs count (CLCs#) in NPs, and peripheral blood eosinophil percentage (PB EOS%) adjusted for sex, age, AR comorbidity, asthma comorbidity, sIgE, total IgE, smoking, revision ESS, as well as general diseases was used to predict NP recurrence across all subjects and within each weight category (Table 2). Overall, the analysis showed that BMI (odds ratio [OR], 1.087; 95% confidence interval [CI], 1.008-1.172, P = 0.031) was predictive of NP recurrence. As expected, EOS# in NPs (OR, 1.026; 95% CI, 1.019-1.033, P < 0.001), CLCs# in NPs (OR, 1.179; 95% CI, 1.122-1.240, P < 0.001), and PB EOS% (OR, 1.142; 95% CI, 1.057-1.233, P = 0.001) also significantly predicted NP recurrence. When each weight category was modeled separately, EOS# in NPs (OR, 1.057; 95% CI, 1.040-1.075, P < 0.001), CLCs# in NPs (OR, 1.793; 95% CI, 1.500-2.144, P < 0.001), and PB EOS% (OR, 1.385; 95% CI, 1.236-1.553, P < 0.001) were only predictive of NPs recurrence in underweight/normal weight CRSwNP patients but not in overweight/obese subjects. In fact, none of the biomarkers (EOS# in NPs, CLCs# in NPs, or PB EOS%) significantly predicted the recurrence in overweight/obese subjects (Table 2), indicating that elevated BMI may affect efficacy of conventional predictors in overweight/obese CRSwNP subjects.

Elevated BMI compromises predictive capacity of conventional parameters in NPs

The effect of elevated BMI on the capacity of biomarkers to accurately predict underlying NP recurrence is unknown. We used receiver operating characteristic (ROC) curves and the corresponding area under the curves (AUCs) to further evaluate the predictive capacity of conventional parameters across all subjects and within each weight category. Surprisingly, AUC of each biomarker varied based on BMI category. AUC for EOS# in NPs in underweight/normal weight (0.954; 95% CI, 0.931-0.978) and among overall (0.866; 95% CI, 0.826-0.905) are much higher than that in overweigh/obese individuals (0.550; 95% CI, 0.420-0.680) (Figure 4A). Consistent with decreased AUC predictive capacity of EOS# in NPs, tissue CLCs # and PB EOS% also demonstrated compromised AUC in overweight/obese (CLCs, 0.560; 95% CI, 0.435-0.685; PB EOS%, 0.513; 95% CI, 0.375-0.650) compared with underweight/normal weight (CLCs, 0.973; 95% CI, 0.953-0.994; PB EOS% , 0.801; 95% CI, 0.744-0.859) and overall (CLCs, 0.871; 95% CI, 0.830-0.911; PB EOS% , 0.738; 95% CI, 0.638-0.793) subjects, respectively (Figures 4B and 4C). Interestingly, the combination of all 3 biomarkers did not contribute to any improvement in the capacity to predict recurrence in overweight/obese subjects (Figure 4D).

Next, we analyzed the possible causes contributing to the poor

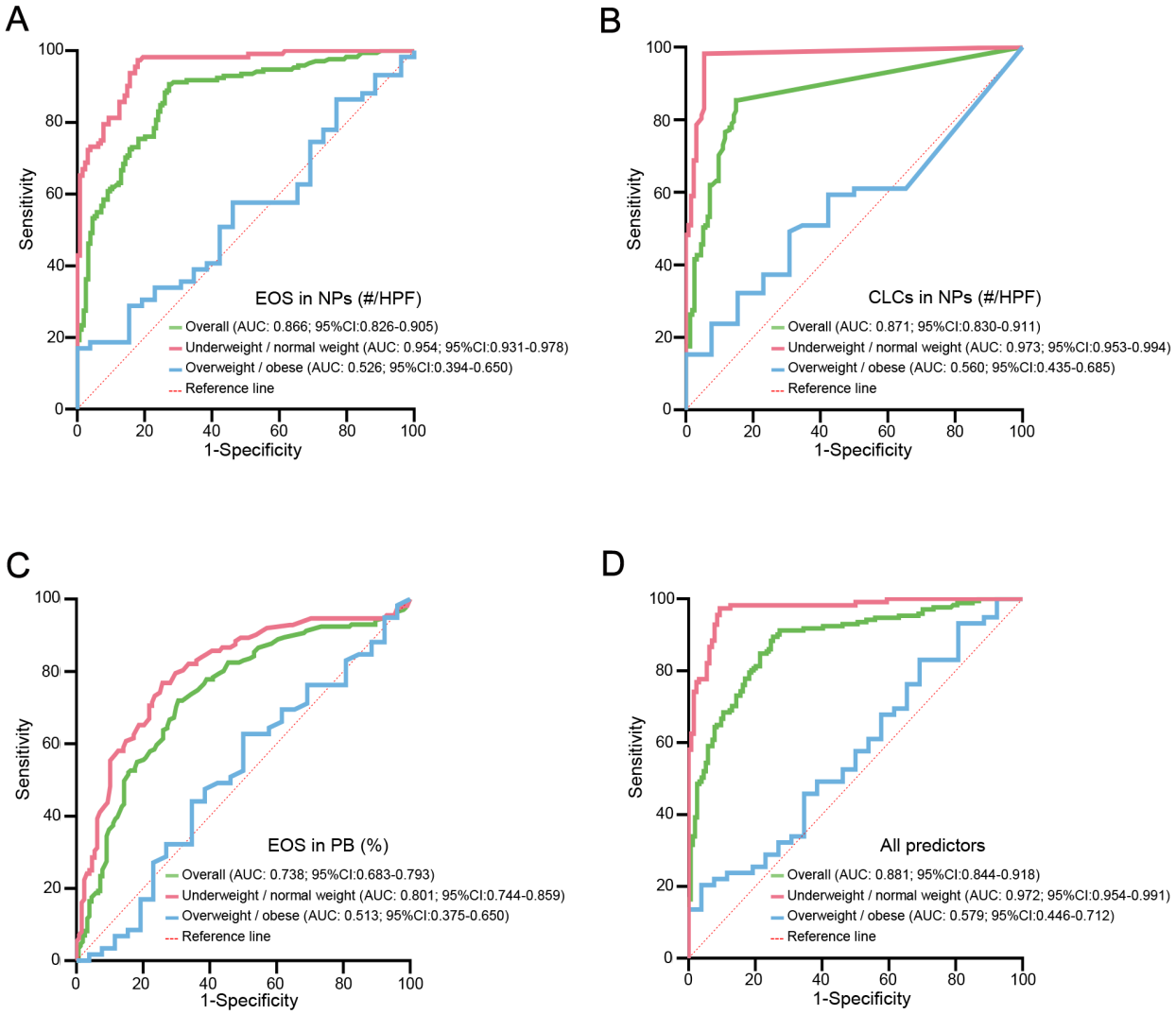


Figure 4. Receiver operating characteristic curves for the predictors of polyp recurrence by weight group. Receiver operating characteristic (ROC) curves for EOS in NPs (A), CLCs in NPs (B), EOS in PB (C), and all predictors (D) for predicting polyp recurrence by weight group adjusted for gender, age, atopy, comorbidity of allergic rhinitis and asthma, and BMI. CLCs, Charcot-Leyden crystals; EOS, eosinophil; NPs, nasal polyps; PB, peripheral blood; HPF, high power field; CI, confidence interval. n=240 Underweight /normal weight, n=85 Overweight / obese and n=325 Overall; as assessed using ROC curves.

predictive capacity of these parameters. The increased count of EOS#, CLCs #, and EOS% in NPs, as well as EOS% in PB was observed in recurrent group compared with unrecurrent group only among underweight/normal weight individuals, but not in overweight/obese subjects (Figure S4). In addition, the elevated recurrent rate in Eos CRSwNP compared with Non-Eos CRSwNP only occurred in underweight/normal weight CRSwNP patients and across all subjects, and the increased trend was absent in overweight/obese subjects (Figure S5). These results indicate that the predictive capacity of conventional biomarkers used for evaluating NP recurrence is compromised in overweight/obese CRSwNP patients, which may attribute to comparable expression levels of these predictive biomarkers in recurrent and unrecurrent CRSwNP subjects with overweight/obesity.

Discussion

Here we have identified that elevated BMI augments eosinophil-neutrophil coexisting inflammation and mixed T2/T3 inflammation and driving a worse clinical burden in overweight/obese CRSwNP individuals. Furthermore, we have found BMI as a predictor for NP recurrence, and demonstrated that conventional biomarkers are poorly predictive of NP recurrence in overweight/obese CRSwNP subjects. Thus, increased BMI could be recognized as an important comorbidity in patients with CRSwNP, and overweight/obese CRSwNP may represent a unique phenotype and endotype [38].

It has been appreciated that elevated BMI could drive eosinophil migration and alter immune response [39-41]. In the current study, we have found eosinophils infiltration and hyperactivation was

increased in overweight/obese CRSwNP subjects, as indicated by elevated eosinophil counts as well as CLCs deposition. These results suggest that elevated BMI appears to drive eosinophilic inflammation, which may result in worse symptom burden. Consistent with our study, increased eosinophil trafficking to the lungs has been revealed in an obese murine asthma model [40]. Furthermore, enhanced submucosal eosinophils has also been reported in asthmatic subjects with elevated BMI [41-43]. Single photon emission computed tomography (SPECT/CT) imaging provided compelling evidence that radiolabeled eosinophils uptake in lung in vivo is enhanced in obese patients with asthma compared to non-obese counterparts [39]. The intertwined relationship between eosinophilic inflammation and BMI has also been evidenced in other diseases including eosinophilic oesophagitis (EoE) and dermatitis [11, 44, 45]. However, the association between eosinophils and elevated BMI remains controversial. Recent studies have proposed discordance between submucosal eosinophilia and peripheral blood and sputum eosinophilia in asthmatic subjects with elevated BMI [41, 46].

We also found an increased trend of neutrophil infiltration and an upregulated EoshighNeuhigh dominated endotype in overweight/obese CRSwNP patients. This phenomenon may contribute to GC resistance, since augmented neutrophilic inflammation has been associated with GC insensitivity in overweight/obese asthmatics [43, 47, 48]. Unfortunately, the GC sensitivity has not been evaluated in our study due to limited clinical information. However, a recent study from Beijing Tongren Hospital demonstrated that elevated BMI results in compromised GC sensitivity in patients with Eos CRSwNP [16]. We also displayed that NLRP3+ cells were increased in overweight/obese CRSwNP patients, suggesting excessive NLRP3 inflammasome activation may play a role in the pathogenesis of neutrophilic inflammation and steroid-resistance [43, 49-51].

In addition to amplified eosinophil-neutrophil cellular endotype, the exacerbated T2/T3 coexisting inflammatory pattern was characterized in overweight/obese individuals with CRSwNP, which suggests that elevated BMI may have a crucial role in reshaping TH immune responses. In agreement with our findings, research on atopic dermatitis has shown that obesity shifts the conventional type 2 TH-predominant disease to a more severe disease with prominent TH17 inflammation, leading to resistance to therapies targeting T2 cytokines (IL-4/IL-13) [11, 12, 14]. The decreased activity of nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) in TH2 cells from overweight/obese mice may contribute to this phenomenon [11]. Also, elevated BMI drives TH17 cell differentiation by inducing acetyl-CoA carboxylase 1, which facilitates appropriate function of RORγt through fatty acid synthesis [52]. Overweight/obesity also promotes TH17-shifting endotype by altering commensal microbiota and damaging the epithelial barrier [53]. Although distinct molecular mechanisms have been elucidated for the augmented T2/T3 coexisting inflammatory pattern, pre-clinical models have shown that overweight/obesity increases the severity of immune-driven diseases, and murine models with obesity often fail to respond to therapies that are effective in lean controls [11]. Benralizumab is directed against the alpha subunit of the IL-5 receptor [54] and the phase III OSTRO Trial (benralizumab-mediated eosinophil depletion for treating CRSwNP, NCT03401229) appears to show decreased efficacy in improving nasal polyp score and nasal blockage score in CRSwNP patients with enhanced BMI [54]. Thus, there is an urgent need for novel therapeutic strategies (including PPAR-γ agonists and inflammasome inhibitors) that target obese/overweight CRSwNP.

Identification of predictors of recurrence in CRSwNP has been an active area of investigation. Mounting clinical evidence has thrown light on the key role of T2 endotype for predicting NP recurrence, and several T2 biomarkers including tissue eosinophils [17, 18], IL-5 [22, 23], peripheral blood eosinophils [21], CLCs [18-20], and serum eosinophil cationic protein [55] have been identified as predictors for polyp recurrence. In the current study, we found that BMI could serve as an independent risk factor for predicting NP recurrence, and overweight/obese subjects have a higher polyp recurrent rate. The increased prevalence of Eos CRSwNP and exacerbated T2-related inflammation may contribute to the elevated recurrence in overweight/obese CRSwNP individuals. However, previous study has demonstrated that BMI levels did not affect NP recurrence in GC-insensitive CRSwNP patients due to the limited population of GC-insensitive recurrent patients [16]. Furthermore, our data revealed conventional biomarkers (including EOS# in NPs, CLCs# in NPs, and PB EOS%) were poorly predictive of NP recurrence in overweight/obese subjects. We noted a decreased trend in AUC of each parameter with increasing BMI, and even the combination of all 3 biomarkers did not show any improvement in the predictive capacity in overweight/obese subjects. This may be attributed to the comparable expression levels of conventional biomarkers in recurrent and unrecurrent groups among overweight/obese patients, where we hypothesize that elevated BMI has distinct effects on Eos CRSwNP versus Non-Eos CRSwNP. Elevated BMI may preferentially affect innated immune responses by promoting eosinophils infiltration and activation, then affect adaptive immune reactions and convert classical T2 inflammatory pattern to T2/T3 mixed profile when eosinophils are hyperactivated to a certain degree. From the perspective of precision medicine, these results imply that overweight/obese individuals could be inaccurately assigned to unrecurrent cohort and possibly be excluded from receiving fine managements and therapies for CRSwNP that could facilitate improved outcomes. Although there is no difference in type/extent of surgeries between the two groups based on BMI (data not shown), overweight/obesity may affect ESS efficacy. A recent article from Cleveland Clinic demonstrated that obese CRSwNP patients had higher postope-

rative SNOT-22 scores (20±17 vs 13±19, P = 0.032) and postoperative extranasal rhinologic domain score (4±3 vs 2±2, P = 0.038) at 3-4 months when compared to non-obese counterparts [56]. This suggests a reduced response to FESS in terms of symptom improvement in obese CRSwNP patients.

This study has several limitations. First, due to the limited number of obese CRSwNP patients, the current study could not differentiate the differential effects of overweight versus obesity on the endotype, phenotype, and recurrence of CRSwNP. Actually, the current endotype and phenotype mainly attributes to overweight. Therefore, any unique effect of obesity alone on CRSwNP should be analyzed in the future. Second, the effect of elevated BMI on efficacy of T2 cytokine-targeted biologics in CRSwNP has not been investigated. Although the phase III Trial of Benralizumab has implied compromised efficacy in improving symptoms in CRSwNP patients with enhanced BMI [54], definitive proof of BMI effect on T2 cytokine-targeted biologics treatment response in CRSwNP has been lacking. Further investigations into the effects of these T2 cytokine-targeted biologics are warranted. Third, validation of results was lacking, particularly from Western countries. Thus, a larger cohort of patients and multicenter study should be conducted to further dissect the effect of BMI on CRSwNP. Fourth, due to limitations of retrospective study, we cannot exclude the impact of socioeconomic status, income, education, and postoperative medications of patients on the association between BMI and recurrence.

Conclusion

CRSwNP is a heterogeneous disease regardless of the presence of overweight/obesity, and elevated BMI has a significant effect on the inflammatory pattern and the capacity of currently available biomarkers to accurately predict NP recurrence. Therefore, it is imperative that the potential confounding effects of overweight/obesity be taken into consideration when interpreting the results of EOS# in NPs, CLCs# in NPs, and PB EOS%. Moreover, these results underscore the need to identify more sensitive biomarkers in overweight/obese patients with CRSwNP, that might permit more precise and tailored therapy.

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Conflicts of interest

All authors declare no conflict of interest.

Authors' contributions

Study idea and design: YZ; Sample screening and collection: WC, YB, PF, JC, XW, YL, XL, JC, ZX, TY, MW, XH and QY; Experiment and data analysis: WC, YB, PF and FS; Manuscript preparation: YZ, WC, YB and PF; Critical review of the manuscript: YZ, RI, QY, and DF; Follow up: YZ, QY and WC; Final approval: all authors.

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SUPPLEMENTARY MATERIAL

Table S1. Primary antibodies used in immunohistochemistry.

Antibody	Application	Host	Concentration	Clone ID	Reference	Source
ECP	IHC	Rabbit	1:400	Polyclonal	PA5-48595	Thermo Scientific (Rockford, IL, USA)
MPO	IHC	mouse	1:400	Polyclonal	AF3667	R&D Systems (Minneapolis, MN, USA)
IFN-γ	IHC	mouse	1:200	Polyclonal	MM700B	Thermo Scientific (Rockford, IL, USA)
T-bet	IHC	mouse	1:100	Polyclonal	13700-1-AP	Proteintech (Wuhan, China)
IL-5	IHC	Rabbit	1:200	Polyclonal	26677-1-AP	Proteintech (Wuhan, China)
IgE	IHC	Rabbit	1:200	Polyclonal	ab75673	Abcam (Cambridge, UK)
GATA3	IHC	Rabbit	1:100	D13C9	5852	Cell Signaling Technology (Danvers, MA, USA)
IL-17A	IHC	Rabbit	1:400	polyclonal	ab79056	Abcam (Cambridge, UK)
ROR-γt	IHC	Rabbit	1:100	Polyclonal	bs-3863R	Bioss (Beijing, China)
NLRP3	IHC	Rabbit	1:100	Polyclonal	19771-1-AP	Proteintech (Wuhan, China)

ECP, eosinophil cationic protein; MPO, myeloperoxidase; MCT, mast cell tryptase; IFN-γ: interferon gamma; T-bet, T-box transcription factor 21; IL, interleukin; IgE, immunoglobulin E; GATA3, GATA binding protein 3; ROR-γt, RAR-related orphan receptor gamma T; NLRP3, NOD-like receptor family pyrin domain containing 3; IHC, immunohistochemistry.

Table S2. Comparison of Eos CRSwNP proportion among participants by weight category.

Variables	Underweight / normal weight	Overweight / obese	P Value
n (%)	240 (73.85%)	85 (26.15%)	-
Eos CRSwNP grouping (>10%)[1] Eos CRSwNP Non-Eos CRSwNP	 152 (63.33%) 88 (36.67%)	 64 (75.29%) 21 (24.71%)	**0.045**
Eos CRSwNP grouping (>10/HPF)[2] Eos CRSwNP Non-Eos CRSwNP	 169 (70.42%) 71 (29.58%)	 70 (82.35%) 15 (17.65%)	**0.032**
Eos CRSwNP grouping (>32.5/HPF)[3] Eos CRSwNP Non-Eos CRSwNP	 127 (52.92%) 113 (47.08%)	 60 (70.59%) 25 (29.41%)	**0.005**
Eos CRSwNP grouping (>55/HPF)[4] Eos CRSwNP Non-Eos CRSwNP	 99 (41.25%) 141 (58.75%)	 43 (50.59%) 42 (49.41%)	0.136
Eos CRSwNP grouping (>70/HPF)[5] Eos CRSwNP Non-Eos CRSwNP	 87 (36.25%) 153 (63.75%)	 36 (42.35%) 49 (57.65%)	0.319

Underweight/normal weight, BMI of 24.9 kg/m^2 or less; Overweight/obese, BMI of 25kg/m^2 or greater. Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; CLCs, Charcot-Leyden crystals; EOS, eosinophil; Non-Eos CRSwNPs, non-eosinophilic chronic rhinosinusitis with nasal polyps; HPF, high power field.

Table S3. Recurrent rate between groups based on BMI under different cutoff-ranges of eosinophils.

Group vs P value	<10/HPF	10-32.5/HPF	32.5-55/HPF	55-70/HPF	>70/HPF
Underweight / normal weight	2 (0.83%)	5 (2.08%)	16 (6.67%)	7 (2.92%)	82 (34.17%)
Overweight/obese	9 (10.59%)	8 (9.41%)	11 (12.94%)	7 (8.24%)	24 (28.24%)
P Value	< 0.001	< 0.001	0.172	0.571	< 0.001

Underweight/normal weight, BMI of 24.9 kg/m^2 or less; Overweight/obese, BMI of 25kg/m^2 or greater. HPF, high power field

Table S4. Characteristics of relapsed CRSwNP patients by weight category.

	Overall (n=325)			Underweight / normal weight (n=240)			Overweight / obese (n=85)		
Variables	Unrecurrence (n=154)	Recurrence (n=171)	P value	Unrecurrence (n=128)	Recurrence (n=112)	P value	Unrecurrence (n=26)	Recurrence (n=59)	P value
Age (years)	36.00 [29.00,52.25]	40.00 [30.00, 50.00]	0.609	35.50 [28.00, 52.00]	38.50 [28.00, 49.00]	0.902	43.50 [32.75, 53.00]	46.00 [34.00, 52.00]	0.909
Gender, male (%)	101 (65.58%)	130 (76.02%)	0.038	80 (62.50%)	79 (70.54%)	0.189	21 (80.77%)	51 (86.44%)	0.503
Asthma (%)	1 (0.65%)	26 (15.20%)	<0.001	0 (0.00%)	17 (15.17%)	< 0.001	1 (3.84%)	9 (15.25%)	0.142
Allergic rhinitis (%)	37 (24.03%)	95 (55.56%)	<0.001	21 (16.41%)	55 (49.11%)	<0.001	16 (61.54%)	40 (67.80%)	0.575
Atopy (%)	75 (48.70%)	130 (76.02%)	<0.001	57 (44.53%)	87 (77.68%)	<0.001	18 (69.23%)	43 (72.88%)	0.730
Total IgE (IU/ml)	68.00 [29.50,217.00]	103.00 [43.00,287.00]	0.122	70.50 [29.25, 193.50]	75.00 [39.00, 168.00]	0.579	23.00 [56.00, 683.50]	189.00 [65.00, 895.50]	0.409
EOS (#/HPF)	10.60 [2.40, 33.98]	93.80 [46.60, 186.20]	<0.001	9.15 [2.20, 21.03]	115.40 [61.50, 206.58]	<0.001	46.15 [14.53, 90.43]	57.60 [30.20, 132.40]	0.703
CLCs (#/HPF)	0.00 [0.00, 0.00]	11.60 [3.70, 26.40]	<0.001	0.00 [0.00, 0.00]	14.30 [5.40, 27.85]	<0.001	3.30 [0.00, 12.30]	7.40 [0.00, 18.00]	0.367
PB EOS (%)	3.05 [1.90, 5.20]	6.30 [3.90, 8.30]	<0.001	2.60 [1.80, 4.48]	6.65 [4.40, 9.33]	<0.001	4.45 [3.18, 7.48]	5.00 [3.30, 7.50]	0.852
$EOS^{high}NEU^{high}$ (%)*	22 (38.60%)	31 (63.27%)	0.011	19 (35.85%)	16 (59.26%)	0.046	3 (75.00%)	15 (68.18%)	0.575

Underweight/normal weight, BMI of 24.9 kg/m^2 or less; Overweight/obese, BMI of 25kg/m^2 or greater. EOS, eosinophil; CLCs, Charcot-Leyden crystals; HPF, high power field; PB, peripheral blood; NEU, neutrophil.

*, of the 325 patients, nasal polyps from 106 patients including 80 underweight/normal weight (53 unrecurrent and 27 recurrent) and 26 overweight/obese (4 unrecurrent and 22 recurrent) were undergone both H&E staining for detecting eosinophils and immunohistochemical staining for myeloperoxidase (MPO, neutrophils).

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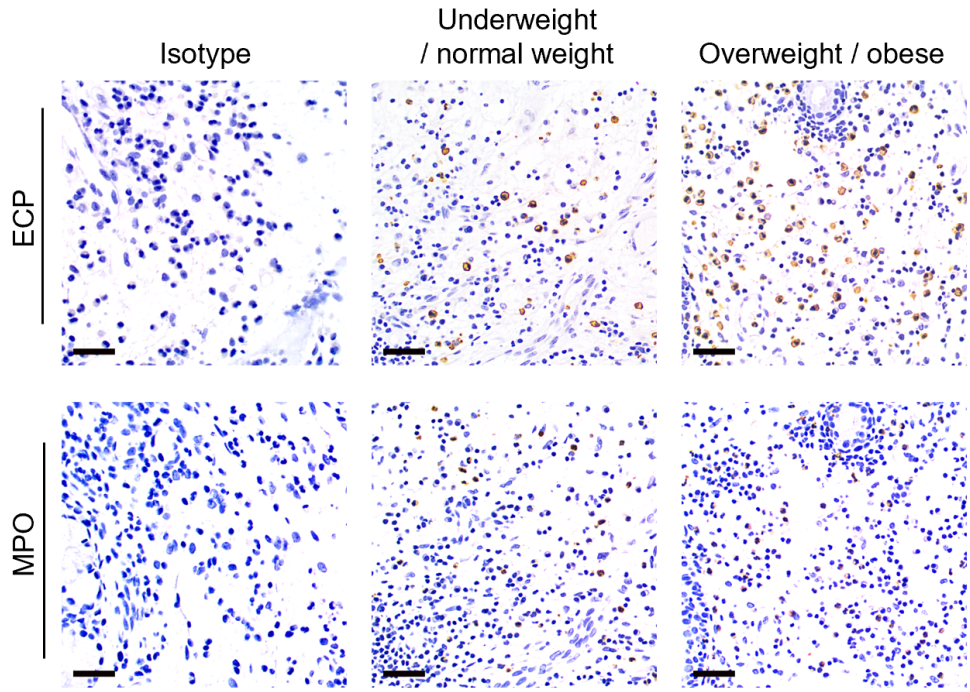

Figure S1. Enhanced eosinophil-neutrophil mixed airway inflammation in overweight/obese subjects with CRSwNP. Representative photomicrographs of ECP^{+} cells and MPO^{+} cells in CRSwNP individuals by weight category (original magnification x400). CRSwNP, chronic rhinosinusitis with nasal polyps; ECP, eosinophil cationic protein; MPO, myeloperoxidase.

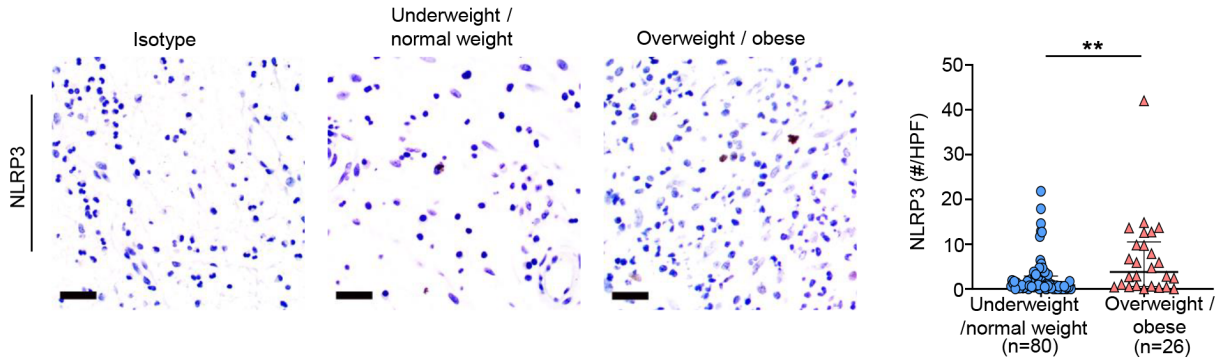

Figure S2. Increased accumulation of NLRP3^{+} cells in overweight/obese subjects with CRSwNP. Representative photomicrographs (original magnification x400, bar scale=50µm) and quantification showing NLRP3^{+} cells in CRSwNP patients with underweight/normal weight (n=80) and overweight/obesity (n=26). Quantification graph show individual patient data with bars representing median±interquartile range Comparison of groups was carried out using Mann-Whitney test. **: p<0.01. NLRP3, NLR family pyrin domain containing 3; HPF, high power field.

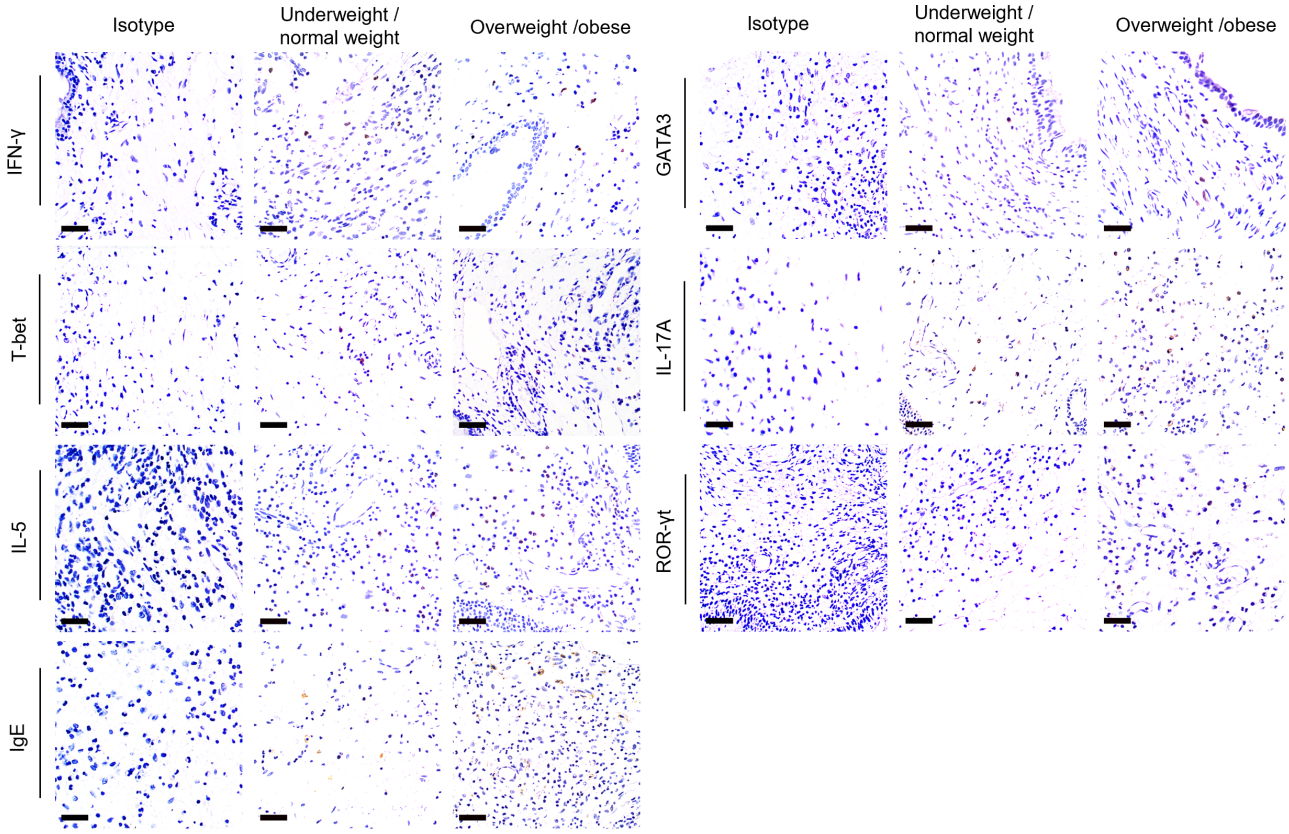

Figure S3. Exacerbated T2/T3 coexisting inflammation in overweight/obese CRSwNP. Representative photomicrographs of IFN-γ^+ cells, T-bet^+ cells, IL-5^+ cells, IgE^+ cells, $GATA3^+$ cells, IL-$17A^+$ cells, and ROR-γt^+ cells in CRSwNP patients by weight category (original magnification x400, bar scale=50μm). CRSwNP, chronic rhinosinusitis with nasal polyps; IFN-γ, interferon gamma; T-bet, T-box transcription factor 21; IgE, immunoglobulin E; IL, interleukin; GATA3, GATA binding protein 3; ROR- γt, RAR-related orphan receptor gamma T.

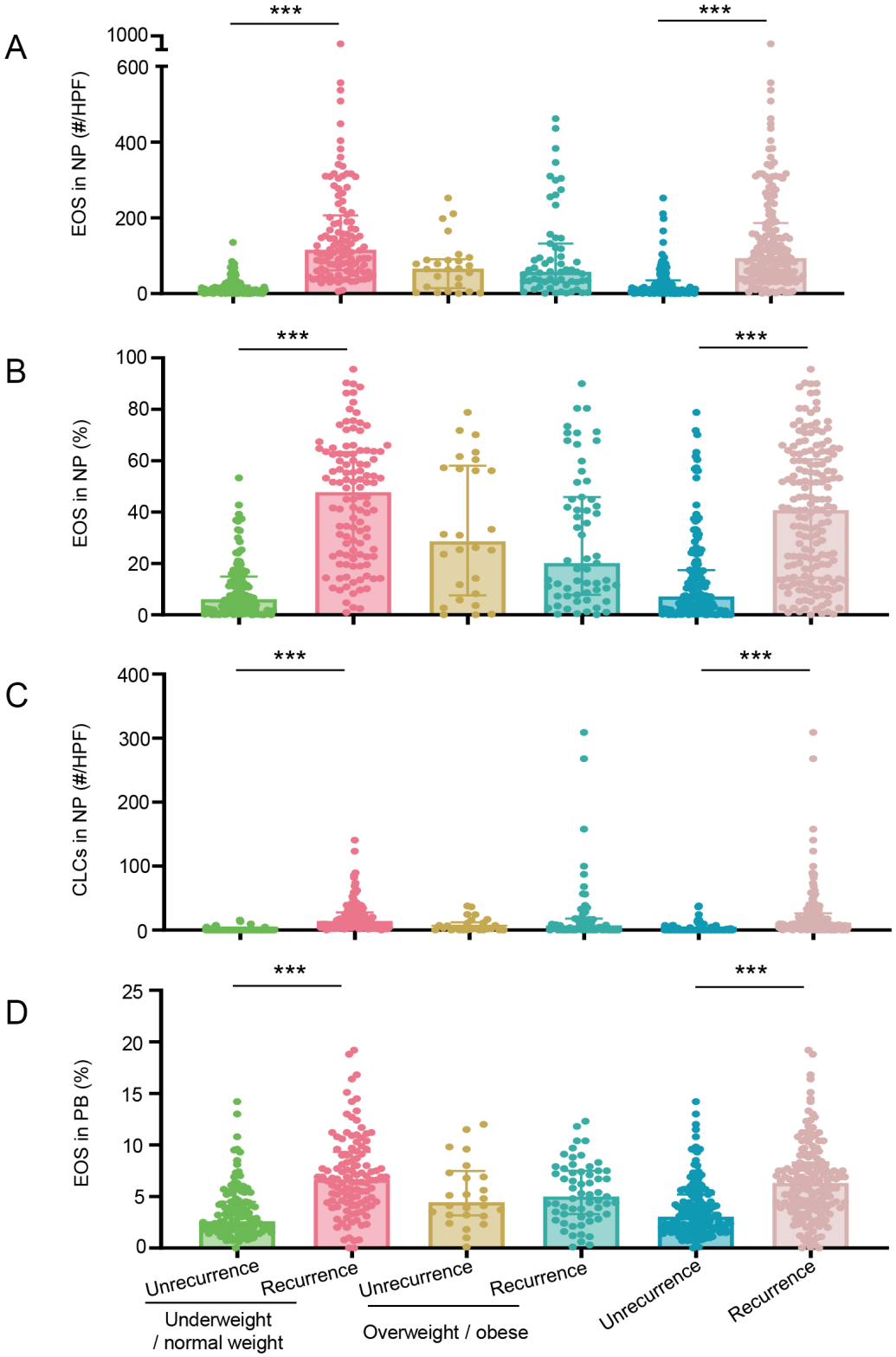

Figure S4. The expression level of biomarkers in CRSwNP patients. The expression level of eosinophil count (A) and percentage (B) in NPs, CLCs count in NPs (C), and eosinophil count in peripheral blood (D) in CRSwNP patients by recurrence group and/or weight group. EOS, eosinophil; CLCs, Charcot-Leyden crystals; CRSwNP, chronic rhinosinusitis with nasal polyps; NPs, nasal polyps. n=240 Underweight /normal weight and n=85 Overweight / obese; n=154 Unrecurrence and n=171 Recurrence; ***: p<0.001, as assessed using unpaired Mann–Whitney test.

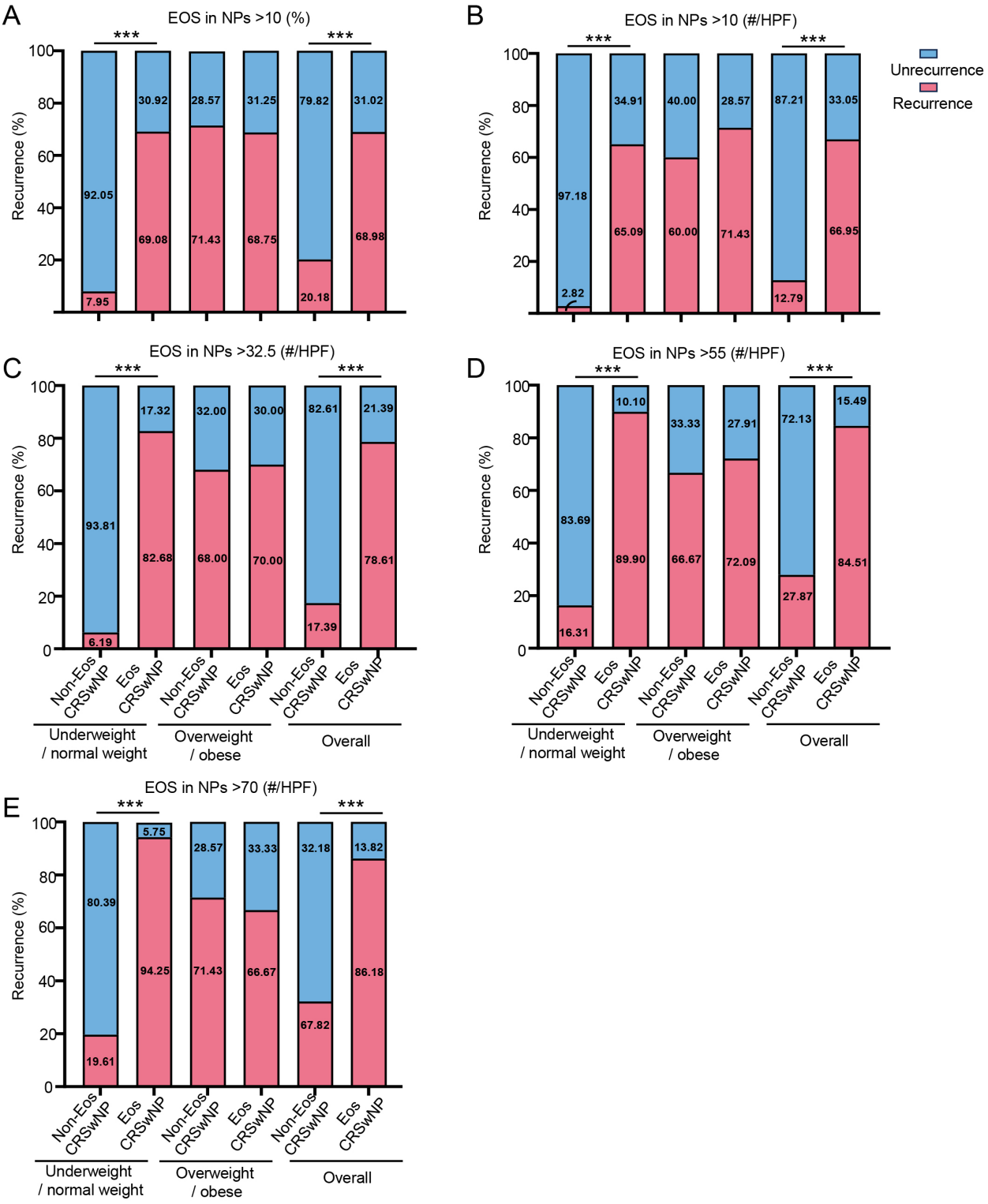

Figure S5. The recurrent rate in patients with Eos CRSwNP and Non-Eos CRSwNP by weight category. The recurrence of individuals with distinct weight category among patients with Eos CRSwNP (diagnosed based on EOS in NPs >10%, EOS# in NPs >10/HPF, EOS# in NPs >32.5/HPF, EOS# in NPs >55/HPF, and EOS# in NPs >70/HPF, respectively) and Non-Eos CRSwNP (diagnosed based on EOS in NPs <10%, EOS# in NPs <10/HPF, EOS# in NPs <32.5/HPF, EOS# in NPs <55/HPF, and EOS# in NPs <70/HPF, respectively). Comparison of groups was carried out using χ^2 test. Patients used in Figure S5 are the same patients as in Figure 3. n=240 underweight/normal weight, n=85 overweight/obese, and n=325 overall. ***: p<0.001. EOS, eosinophil; NPs, nasal polyps; Eos CRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; Non-Eos CRSwNP, non-eosinophilic chronic rhinosinusitis with nasal polyps; HPF, high power field.