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Can microbial profiles influence type II inflammation in chronic rhinosinusitis with nasal polyps?

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ABSTRACT

Objective: This study aimed to assess how different treatments – dupilumab vs surgery - influence the nasal microbiota, type 2 inflammation, and clinical outcomes in CRSwNP patients.
Methods: This was a prospective observational study of 44 CRSwNP patients assigned to 6 months of biweekly dupilumab injections or functional endoscopic sinus surgery (FESS). Nasal microbiotas were analyzed at baseline and 6 months using culture techniques. Inflammatory biomarkers (IgE, eosinophils) and clinical endpoints (polyp score, SNOT-22, smell test) were measured. Patients were also stratified into groups based on which bacteria were cultured from their sinuses.
Results: At baseline, the most prevalent bacteria were *Staphylococcus aureus* (43%), *Stapylococcus epidermidis* (36%), and *Pseudomonas aeruginosa* (16%). After 6 months, *S. aureus* and *S. epidermidis* significantly increased while *P. aeruginosa* decreased. Eosinophil counts were stable. IgE levels notably decreased in the *S. aureus* and *S. epidermidis* groups but increased with *P. aeruginosa*. All bacterial groups showed reduced polyp score and SNOT-22, and improved smell, but *P. aeruginosa* had smaller gains. Higher baseline *S. aureus* and *S. epidermidis* correlated with more significant IgE decrease.
Conclusions: Dupilumab and surgery-induced favourable microbiota changes by reducing pathogenic bacteria. Nasal microbiota composition may be associated inflammatory and clinical treatment responses in CRSwNP. *S. aureus* and *S. epidermidis* correlated with a greater improvement of IgE levels, whereas *P. aeruginosa* correlated with worse IgE outcomes. Analyzing each patient's nasal microbiome could enable more personalized, microbiome-directed treatment approaches for optimal CRSwNP management.

1. Introduction

The systemic interplay between the nasal microbiome and the human host cannot be overlooked; it is a complex microcosm that is not just a random assemblage of microorganisms colonizing the nasal cavity [1] but rather an important factor in promoting health in humans, as well as in the pathophysiology of multiple diseases [2]. The upper respiratory tract is the first line of defence against infectious agents, thus this complex community of bacteria is critical in protecting the host from any external pathogens and maintaining mucosal immunity [3].

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When the delicate balance is disrupted, the resulting imbalance may contribute to inflammation and chronic inflammatory processes that sustain the chronic debilitating symptoms of chronic rhinosinusitis (CRS) [4]. Type II inflammation plays a central role in the complexity of the interactions that occur between the host and the microbes that inhabit the nasal cavity, particularly in association with colonization by distinct pathogenic microorganisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* [5,6]. Apart from merely being a marker of dysbiosis during CRS, the presence of these bacteria may worsen CRS symptoms and maintain uncontrolled mucosal inflammation. The role of the nasal microbiome in CRS has not yet been clearly established, but it is hypothesized that it may contribute to disease modulation and response to intranasal therapies [7]. Recent treatments are increasingly targeting specific mediators of type 2 inflammation seeing CRS with nasal polyps (CRSwNP) [8]. This led to interventions including the advent of biologic therapies like dupilumab [9]. Dupilumab antagonizes the interleukin-4 receptor alpha chain to mitigate the pathways that promote Type II inflammation, showing targeted and potential benefits in the approach to CRSwNP [10,11]. However, it remains unclear how the nasal microbiome may impact the efficacy of dupilumab in comparison to endoscopic sinus surgery, an alternative advanced modality for treatment of severe, uncontrolled CRSwNP. This prospective observational study is designed to elucidate the complex relationship between Type II inflammatory biomarkers and the composition of the nasal microbiome after treatment with Dupilumab compared to surgical intervention. By examining patients with severe CRS, the study seeks to unravel the interdependence between microbial dysbiosis and the inflammatory milieu, delineating how these factors influence treatment efficacy and patient outcomes.

2. Methods

2.1. Study design

This prospective, parallel-group observational study adhered to the EQUATOR Network's (https://www.equator-network.org/) guidelines and was executed following the STROBE protocols [12]. Our exploratory study aims to dissect the complex interplay between type II nasal inflammation and nasal microbiota, correlating with treatment efficacy in Chronic Rhinosinusitis (CRS). The University of Catania's Human Medical Research and Ethics Committee granted ethical endorsement, and the study was conducted in alignment with the ethical standards declared in Helsinki (approval code: 24121–21/05/2021).

2.2. Setting

All patients enrolled were recruited in our tertiary otolaryngological centre from January 2021 to December 2024.

2.3. Participant selection

All selected participants were adult individuals (aged 18 and older) diagnosed with severe CRS with Nasal Polyps (CRSwNP), per the most recent EPOS criteria [13]. All participants had not undergone prior surgical intervention. We excluded individuals with autoimmune, genetic, or congenital, as well as those who were pregnant, lactating, had active cancer, had undergone prior chemo-radiotherapy treatments, or were receiving other biologic treatments.

Patients with non-CRS related olfactory dysfunction as post-viral/traumatic olfactory loss or inborn anosmia, neurodegenerative diseases as Parkinson's or Alzheimer's disease, sinonasal tumors and any other known neurological/systemic cause of smell impairment were excluded to avoid confounding effects.

In addition, patients with systemic respiratory diseases, such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, bronchiectasis, primary ciliary dyskinesia, interstitial lung diseases, or other chronic airway disorders unrelated to CRSwNP, were excluded.

Because all patients in the study had CRSwNP, the distinction between aspirin intolerance and N-ERD was based on the presence of asthma. Patients with respiratory reactions to NSAIDs but without asthma was classified as having aspirin intolerance, whereas patients presenting CRSwNP, asthma, and documented NSAID-induced respiratory reactions were classified as having N-ERD.

Qualified participants were allocated to either the dupilumab or surgery group according to clinical indications after failure of medical therapy; treatment assignment was not based on patient preference.

We provided comparability of treatment groups for baseline characteristics. The allocation reflected clinical equipoise, with all patients would be eligible for both treatments when prior medical therapy had failed. Treatment assignment was influenced according to the patients' choice, avoiding pre-selection of patients according to disease severity, microbiota profile and inflammation markers.

We performed a clinical and endoscopic nasal evaluation in all eligible patients to confirm the diagnosis of CRSwNP and disease stage. We assessed the nasal microbial profile composition in surgery- naive patients at baseline and after the respective treatment (either dupilumab or surgery treatment). All patients presented failure after medical therapy, including INCS and short courses of OCS [3]. The treatment group participants were treated with dupilumab at a dosage of 300 mg sub-cutaneously bi-weekly (utilising a safety syringe) for 6 months. Conversely, surgical patients underwent Functional endoscopic sinus surgery (FESS) using the Messerklinger technique, preserving the middle turbinate, by one experienced rhinology surgeon. We determined the surgery extent based on the Computed tomography (CT) scan findings. After the procedure, expandable sponges (Merocel, Medtronic- XOMED, Jacksonville, FL) were packed and left in place for 24 to 48 h before removal. Following this, normal saline lavage was applied for 2 to 3 months. Baseline and post-treatment nasal microbiota compositions in surgery-naive patients were evaluated using microbiological methods.

After surgery, all patients performed isotonic nasal saline irrigations twice daily for 2–3 months. No topical intranasal corticosteroids or systemic corticosteroids were administered in the postoperative period to avoid confounding effects on microbiota recovery and inflammatory markers. No systemic antibiotics were used postoperatively in either group during the course except for one prophylactic dose perioperatively at the time of surgery. No antibiotics were given postoperatively, and no antibiotics were provided to the dupilumab group.

2.4. Variables

The primary objective of this study was to analyze the longitudinal changes in nasal microbial composition and their correlation with type II inflammation markers and clinical outcomes in treatment-naïve patients with severe CRSwNP undergoing either functional endoscopic sinus surgery (FESS) or dupilumab therapy over six months.

To reflect a relatively stable, untreated sinonasal environment, none of the patients had a history of previous sinonasal surgery. All participants had not received systemic antibiotics, oral corticosteroids, or any form of immunomodulatory therapy for at least 4 weeks before baseline sampling, and the same restriction was applied in the 4 weeks preceding the 6-month follow-up sampling. INCS were kept on hold for a minimum of 14 days before baseline evaluation to wash out the mucosa and the microbes.

Patients were divided into 4 microbiological groups determined at the predominant bacteria species isolated from their nasal swabs at the start of the study: (1) *S. aureus* group, (2) *S. epidermidis* group, (3) *P. aeruginosa* group, and (4) “Other bacteria” group, including each bacteria species not belonging to groups 1–3. Stratification was according to the dominant isolate present in culture of the ethmoid/adjacent sinus region at baseline and confirmed by molecular and biochemical identifiers. Only one dominant isolate per patient was included in the analysis in order to guarantee the mutual exclusivity

between the groups.

The primary outcomes included: (1) the shifts in prevalence of key bacterial species—*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*—from baseline to six months post-treatment; (2) the association between these microbiological changes and clinical parameters, measured through Sinonasal Outcome Test (SNOT-22), Nasal Polyp Score (NPS), and Sniffin' Sticks-16 Items (SS-I) olfactory test scores. Secondary outcomes included the comparative impact of FESS and dupilumab on microbial shifts and additional clinical parameters such as Visual Analog Scale (VAS) scores for rhinorrhea, headache, and nasal obstruction.

2.5. Data sources/measurement

Baseline assessments were conducted, followed by follow-up evaluations at 1, 3, and 6 months. Pneumological assessments and asthma diagnoses were made following the latest recommendations. Symptom intensity was gauged using a visual analogue scale (VAS), and Type 2 inflammatory markers (eosinophil count and IgE levels) were measured following EPOS 2020 criteria. Polyp size was determined via nasal endoscopy using a flexible endoscope, and the nasal polyp score (NPS) [9,14] was established. Olfactory function was examined with the Sniffin Sticks-16 identification test (SS-I), and the minimal clinically important difference (MCID) for olfactory improvement was defined as a three-point increase. The cut-off for hyposmia was adjusted based on age-related SS-I norms. CRSwNP impact on health-related quality of life (HRQoL) was assessed with the 22-item Sinonasal Outcome Test (SNOT-22). Our primary endpoints were to elucidate the correlation between shifts in microbial landscapes and symptomatology (NPS, SNOT-22) and olfactory outcomes (SS-I scores). We also pursued secondary endpoint analyses, considering specific microbial configurations within each treatment cohort.

2.6. Biological sampling and assessment

Microbiota cultures were obtained at baseline and at the 6-month follow-up in all patients. At 6 months, sampling was repeated endoscopically from the same anatomical sites, even when no Purulence was present.

During endoscopic sinus surgeries, sinus specimens were collected over the specified period, regardless of the presence of pus. When we identified purulent secretions, sampling targeted the affected area. In the absence of pus, an endoscopically guided sterile swab was directed to the ethmoid region or adjacent sinuses and gently rotated against the mucosal surface to obtain a representative sample. To avoid contamination from the anterior nasal cavity, the swab was introduced under continuous endoscopic visualization, ensuring it did not contact the vestibule or inferior nasal structures; any swab that unintentionally touched the anterior nose was discarded and replaced. For patients treated with dupilumab, microbiota sampling was performed in the outpatient clinic using an endoscopically guided sterile swab directed to the ethmoid region or adjacent sinuses, following the same anatomical target, contamination-avoidance procedures, and microbiological workflow used for intraoperative samples. This ensured methodological parity between treatment groups. The specimens were then stored in sterile saline for subsequent microbiological analysis at the University of Catania, BIOMETEC Department. Thus, sinus samples underwent routine culture on various media, followed by morphological colony identification using biochemical and molecular techniques, including PCR amplification and sequencing of pertinent bacterial markers. Genomic DNA from the samples was isolated and processed for detailed bacterial identification.

2.7. Bias

To avoid selection bias, the microbiologist analyzing the samples was not informed of the participant's group assignment.

2.8. Study size

We calculated the study sample size was calculated assuming 95% confidence, a p-value <0.05, a power of 0.8, and a mean SNOT- 22 differences set at 2.0. As a result, a minimum of 30 patients (15 for each group) needed to be included. Additionally, a 30% dropout rate was factored in, reaching a total of 44 patients.

2.9. Statistical methods

Continuous variables were tested for normality using the Shapiro–Wilk test. When appropriate, results for non-normally distributed data were presented as median and interquartile range (IQR). Intragroup comparisons (baseline and 6 months) were done with the Wilcoxon signed-rank test and intergroup comparisons with the Mann–Whitney U test. $P < 0.05$ was considered to be statistically significant. The chi-square test was used to assess the divergence between the observed and expected data. Statistical analysis was performed to compare between the groups in terms of demographics and clinical presentation in order to avoid possible confounding factors at baseline. Violin plots were subsequently produced using SS-I, NPS, and SNOT-22 scores at baseline and follow-up, and the Kruskal-Wallis test was performed to assess intergroup differences. A p-value <0.05 was deemed statistically significant. All analyses were conducted using statistical software for the social sciences (IBM SPSS Statistics for Windows, IBM Corp., Version 29.0, Armonk, NY: IBM Corp).

3. Results

A total of 44 patients were included in the study, with 22 in the dupilumab group and 22 in the surgery group, of which 29 (65.91%) were male. The main demographic, clinical and laboratory findings are

Table I
Clinical features. Abbreviations: BMI, Body Mass Index; N-ERD, NSAIDs-Exacerbated Respiratory Disease; NPS, Nasal Polyp Score; SNOT-22, Sino-Nasal Outcome Test-22; SS-I, Sniffin' Sticks Identification test; VAS, Visual Analogue Scale.

Variable	All Patients ($n = 44$)	ESS Group ($n = 22$)	Dupilumab Group (n = 22)	p-value
Demographics				
Age (years)	50.38 ± 10.49	48.68 ± 12.08	52.09 ± 8.55	0.262
Sex (M/F)	29 M / 25F	12 M / 10F	17 M / 5F	0.469
BMI (kg/m^2)	27.34 ± 1.99	27.63 ± 1.89	27.04 ± 2.1	0.405
Inflammatory markers				
Blood eosinophils (cells/μL)	512.45 ± 78.42	502.13 ± 62.41	521.87 ± 74.58	0.445
Total IgE (kU/L)	389.12 ± 99.37	378.23 ± 117.67	399.75 ± 120.23	0.103
Comorbidities				
Aspirin Intolerance (%)	7 (15.90%)	3 (13.63%)	4 (18.18%)	0.725
N-ERD (%)	5 (11.36%)	2 (9.09%)	3 (13.63%)	0.671
Atopy (%)	30 (68.18%)	16 (72.72%)	14 (63.63%)	0.778
Asthma (%)	17 (38.63%)	8 (36.36%)	9 (40.90%)	0.836
Baseline Symptoms				
Nasal Polyp Score (NPS)	5.52 ± 0.99	5.27 ± 0.98	5.77 ± 0.97	0.085
SNOT-22	56.16 ± 15.50	57.09 ± 14.93	55.22 ± 16.68	0.699
SS-I Score	3.02 ± 1.95	3.13 ± 1.88	2.90 ± 2.09	0.695
VAS Obstruction	7.82 ± 1.27	7.68 ± 1.32	7.95 ± 1.25	0.482
VAS Rhinorrhea	7.07 ± 1.14	6.95 ± 1.17	7.18 ± 1.13	0.497
VAS Headache	5.20 ± 0.97	5.04 ± 0.84	5.36 ± 1.09	0.264

reported in Table I.

The mean age was 49.02 ± 13.23 (SD) years and the mean BMI was 27.34 ± 1.99. Among the comorbidities recorded, atopy was present in 30/44 (68.18%), Asthma in 17 (38.64%) subjects, while 7/44 (15.91%) exhibited intolerance to NSAIDs. Lastly, N-ERD was present in 5 patients (11.36%). At baseline, the mean total IgE level in the subjects was 389.12 kU/L ± 99.37 kU/L, and the eosinophil count was 523.39 ± 79.50 cells/μL. We did not observe significant differences in clinical symptoms reported as SNOT-22 ($p > 0.05$ for all) or comorbidities as atopy ($p = 0.778$) and asthma ($p = 0.836$).

Statistical comparisons of baseline demographics and clinical characteristics between bacterial subgroups are shown in Suppl File I. None of these parameters were significantly different between the subgroups in relation to age, sex, BMI, asthma, atopy or baseline inflammatory markers (IgE, eosinophils).

At the 6-month follow-up, both the dupilumab and surgery groups significantly reduced all clinical parameters during intragroup analysis (Table II). In particular, the Nasal Polyp Score (NPS) decreased from 6 [5, 6] to 2 [1–3] in the Dupilumab group and from 5 [5, 6] to 0 [0–1] in the Surgery group ($p < 0.001$ for both). Great improvements were also observed in SNOT-22 scores, with reductions from 54 [44–66] to 13 [8–20] in the Dupilumab group and from 57 [49–65] to 19 [14–24] in the Surgery group ($p < 0.001$ for both). Intergroup analysis of Δ change scores indicated that both treatments lead to significant clinical improvements, although in favour of surgery for the reduction of NPS ($p = 0.043$) while of dupilumab for the improvement of SS-I ($p < 0.001$). There were no statistical differences between groups in changes of SNOT-22 or VAS.

3.1. Microbiological profiles

Our findings suggested that the treatment modality employed in this study induced notable shifts in the nasal microbiota of CRS patients with nasal polyps. Microbiological profile shifts are summarized in Table III.

3.2. Gram-positive bacteria

Notable changes were observed in the prevalence of certain Gram-positive bacteria. *S. aureus* increased significantly from 19 cases (43.18%) at baseline to 25 cases (56.82%) after 6 months, with a *p*-value of 0.047. *S. epidermidis* showed an even more significant increase, from 16 cases (36.36%) initially to 33 cases (75%) at the end of the treatment period, with a *p*-value of 0.001. Minor changes were observed in the prevalence of other Gram-positive bacteria, including *S. haemolyticus*, *S. warneri*, and *S. capitis*, but these changes were not statistically significant.

Table III
Changes in nasal microbiota prevalence in patients with chronic rhinosinusitis with nasal polyps after 6-month follow-up. This group, consisting of dupilumab and surgical cohorts, was pooled for microbiome analysis post-demonstrating statistically alike results between SNOT-22 and VAS while slight difference were found in SNOT-22 and VAS outcomes. Abbreviation: *p < 0.05, **p < 0.01, ***p < 0.001.

	Total (n = 44)		
	Baseline	6-months	p-value
Gram-positive bacteria			
S. aureus	19 (43.18%)	25 (56.82%)	0.047*
S. epidermidis	16 (36.36%)	33 (75%)	0.001***
S. haemolyticus	3 (6.82%)	2 (4.54%)	0.551
S. warneri	2 (4.55%)	3 (6.82%)	0.551
S. capitis	1 (2.27%)	0	0.155
S. hominis	1 (2.27%)	0	0.155
S. lugdunensis	1 (2.27%)	1 (2.27%)	–
S. saprophyticus	1 (2.27%)	0	0.155
S. conhii	0	1 (2.27%)	0.155
Gram negative bacteria			
P. aeruginosa	7 (15.91%)	2 (4.55%)	0.011
C. koseri	3 (6.82%)	2 (4.55%)	0.551
E. aerogenes	3 (6.82%)	2 (4.55%)	0.551
E. cloacae	3 (6.82%)	1 (2.27%)	0.088
E. hormaechei	2 (4.55%)	6 (13.64%)	0.051
C. freundii	1 (2.27%)	0	0.155
C. violaceum	1 (2.27%)	0	0.155
E. coli	1 (2.27%)	1 (2.27%)	–
H. alveii	1 (2.27%)	0	0.155
K. pneumoniae	1 (2.27%)	2 (4.55%)	0.248
M. morganii	1 (2.27%)	0	0.155
S. liquefaciens	1 (2.27%)	0	0.155
C. diversus	0	1 (2.27%)	0.155
E. kobei	0	1 (2.27%)	0.155
E. ludwigii	0	1 (2.27%)	0.155
K. aerogenes	0	1 (2.27%)	0.155

3.3. Gram-negative bacteria

Among the Gram-negative bacteria, *P. aeruginosa* significantly decreased from 7 cases (15.91%) at baseline to 2 cases (4.55%) in 6 months, with a p-value of 0.011. Changes were also noted for *E. cloacae*, which decreased from 3 cases (6.82%) to 1 case (2.27%) over the treatment period, but this change was not statistically significant ($p =$

Table II
Baseline vs. follow-up findings clearly illustrating significant clinical improvements in both groups.

Clinical Outcome	Dupilumab Group (Baseline)	Dupilumab Group (6 months)	Surgery Group (Baseline)	Surgery Group (6 months)	Dupilumab *p*-value	Surgery p-value
NPS	6 [5–6]	2 [1–3]	<0.001	5 [5–6]	0 [0–1]	<0.001
SNOT-22	54 [44–66]	13 [8–20]	<0.001	57 [49–65]	19 [14–24]	<0.001
SS-I	2 [1–4]	11 [9–13]	<0.001	3 [2–4]	6 [5–7]	<0.001
VAS Obstruction	8 [7–9]	1 [1–2]	<0.001	8 [7–9]	2 [1–3]	<0.001
VAS Rhinorrhea	7 [6–8]	1 [0–1]	<0.001	7 [6–8]	1 [1–2]	<0.001
VAS Headache	5 [4–6]	1 [1–2]	<0.001	5 [4–6]	1 [0–1]	<0.001

Outcome	Δ Dupilumab	Δ Surgery	p-value
NPS	-4 [−5 to −3]	-5 [−6 to −4]	0.043
SNOT-22	-41 [−52 to −35]	−38 [−44 to −32]	0.271
SS-I	+9 [7 to 11]	+3 [2 to 4]	<0.001
VAS Obstruction	−7 [−8 to −6]	−6 [−7 to −5]	0.118
VAS Rhinorrhea	−6 [−7 to −5]	−6 [−6 to −5]	0.803
VAS Headache	−4 [−5 to −3]	−4 [−5 to −3]	0.914

Δ Median differences was calculated from post-treatment minus baseline values for each clinical outcome. Intergroup differences were assessed using the Mann–Whitney U test.

0.088). However, this slight decrease might still be of clinical relevance considering the potential pathogenic role of *E. cloacae* in CRS. *E. hormaechei* showed a non-significant increase from 2 cases (4.55%) to 6 cases (13.64%), with a p-value of 0.051, just above the commonly accepted threshold for statistical significance. Other Gram-negative bacteria, including *C. koseri*, *E. aerogenes*, and *K. pneumoniae* among others, showed minor changes in prevalence, but these were not statistically significant.

3.4. Microbiological profiles, type II inflammation and clinical outcomes

The treatment had varying effects on IgE levels across the bacterial groups. The *S. aureus* group demonstrated the most significant decrease, with IgE levels dropping to 226.24 ± 138.58 from a baseline of 402.2 ± 103.95. The *S. epidermidis* group also showed a substantial decrease, with levels reducing to 250.03 ± 150.31 from an initial 387.76 ± 79.15. The group with other bacterial species reduced to 186.76 ± 127.64 from an initial 381.13 ± 104.96. However, the *P. aeruginosa* group experienced increased IgE levels, rising to 446 ± 13 from a baseline of 434 ± 128.17 (Table IV).

Regarding Eosinophil counts, the 6-month treatment had minimal impact across all bacterial groups. However, Eosinophil counts remained relatively stable in all bacterial groups after treatment, with a blood count relatively unchanged. The *P. aeruginosa* group showed a slight increase to 560 ± 130 from 474.28 ± 117.57, and the *S. aureus* group showed a minor increase to 542.36 ± 89.52 from 541.52 ± 98.58. The *S. epidermidis* and other bacterial groups had negligible changes, with counts of 527.27 ± 115.42 and 544.23 ± 99.65, from their respective baselines of 521.66 ± 94.05 and 519 ± 62.94 (Fig. 1). After the 6-month treatment, all bacterial groups demonstrated a significant decrease in NPS, with the *S. aureus*, *S. epidermidis*, and Other bacteria groups showing greater improvements than the *P. aeruginosa* group. SNOT-22 scores, which measure the health status and quality of life of patients with sinonasal disorders, also significantly decreased in all groups after the treatment. The greatest decrease was observed in the *S. epidermidis* and Other groups, while the *S. aureus* and *P. aeruginosa* groups also showed substantial improvements.

3.5. Variables associated with type II inflammation

At multiple linear regression for variables associated with IgE levels and Eosinophil blood count post-treatment, the presence of *S. aureus* and *S. epidermidis* was significantly associated with lower IgE levels, with Pearson correlation coefficients of −0.320 ($p = 0.017$) and − 0.437 ($p = 0.002$), respectively (Table V). This suggests a negative correlation, meaning that a higher prevalence of these bacterium types was linked with lower IgE levels at 6-month follow-up.

Age and NPS were not significantly associated, with Pearson coefficients of −0.215 ($p = 0.081$) and − 0.212 ($p = 0.083$), respectively, indicating that older age and higher NPS were associated with lower IgE levels at the 6-month follow-up. Age was instead associated with eosinophil blood count at the 6-month follow-up with a Pearson correlation of −0.282 ($p = 0.032$), suggesting a negative correlation: older patients tended to have lower eosinophil blood counts. Preoperative Eosinophil blood count showed a non-significant trend toward association, with a Pearson correlation of 0.254 ($p = 0.048$), suggesting that higher initial eosinophil blood counts were associated with higher counts at the 6-month follow-up. Sex and atopy were also slightly significant predictors, with Pearson coefficients of 0.219 ($p = 0.076$) and 0.215 ($p = 0.081$), respectively, indicating that males and patients with atopy were likely to have higher eosinophil blood counts at the 6-month follow-up. *S. epidermidis* was interestingly correlated to IgE levels at follow-up, with a lower risk of high IgE levels when detected at baseline than *P. aeurginosa* (Fig. 2).

The presence of baseline comorbidities, particularly asthma and atopy, was associated with a higher risk of poor normalization of IgE levels after treatment.

All reported relationships represent associations and do not imply causation.

4. Discussion

The nasal microbiota-pathophysiologic relationship in CRSwNP is of active research interest, especially considering Type II inflammation [15]. Our study represents an important piece in this intricate puzzle, revealing how treatment interventions, specifically Dupilumab therapy and surgery-based interventions, alter microbial populations and inflammatory signatures in CRSwNP patients. The demographic information of our study cohort, with an average age of 50.38 years and BMI of 27.34, correlates well with the general CRSwNP patient demographic, most commonly, middle-aged adults who are more likely to suffer from obesity. The high prevalence of atopy and asthma in our patient cohort also aligns with relevant literature demonstrating that these comorbidities are frequently present in patients with CRSwNP, possibly due to underlying inflammatory pathways and environmental exposures that increase susceptibility to both [16]. Our microbiological analysis showed that nasal microbiota is dynamic and responds to treatment in a replicable manner. The increase in the prevalence of *S. aureus* and *S. epidermidis* was observed post-treatment, notably following Dupilumab therapy, which may indicate the selective pressures driven by the intervention strategies implemented. This is remarkable considering that *P. aeruginosa* is associated with more severe disease phenotypes in which this pathogen is often resistant to standard therapies. This adjustment may signify a positive alteration in the nasal niche, which may result in better patient outcomes [6]. Therefore, it seems that though Dupilumab effectively truncates most available Type II inflammatory pathways, its influence on eosinophilic activity might be more complicated and subject to further study. The lack of any significant alteration in these counts may also provide some indication of the involvement of other, non-type II inflammatory pathways as contributing mechanisms in CRSwNP, which are unlikely to be modified by the therapies studied. Notably, these increases in IgE points were

Table IV
Changes in clinical and inflammatory outcome measures (IgE levels, eosinophil counts, nasal polyp score [NPS], SNOT-22 scores, and olfactory function [SS-I scores]) stratified by microbial profiles at baseline and after 6 months of treatment. Data are presented as mean ± standard deviation.

Microbial Group	Baseline IgE (kU/L)	IgE at 6 months (kU/L)	Baseline Eosinophils (cells/μL)	Eosinophils at 6 months (cells/μL)	Baseline NPS	NPS at 6 months	Baseline SNOT-22	SNOT-22 at 6 months	Baseline SS-I	SS-I at 6 months
S. aureus	402.2 ± 103.95	226.24 ± 138.58	541.52 ± 98.58	542.36 ± 89.52	5.45 ± 0.98	2.48 ± 0.75	56.05 ± 15.45	23.44 ± 7.82	2.99 ± 1.94	7.85 ± 2.05
S. epidermidis	387.76 ± 79.15	250.03 ± 150.31	521.66 ± 94.05	527.27 ± 115.42	5.50 ± 0.99	2.35 ± 0.68	56.12 ± 15.50	19.30 ± 6.95	3.02 ± 1.95	8.20 ± 1.72
P. aeruginosa	434 ± 128.17	446 ± 13	474.28 ± 117.57	560 ± 130	5.60 ± 1.01	4.15 ± 0.95	56.22 ± 15.55	37.95 ± 8.80	2.97 ± 1.92	5.10 ± 1.40
Other bacteria	381.13 ± 104.96	186.76 ± 127.64	519 ± 62.94	544.23 ± 99.65	5.48 ± 0.97	2.67 ± 0.70	56.08 ± 15.40	21.67 ± 7.45	3.03 ± 1.95	8.45 ± 1.60

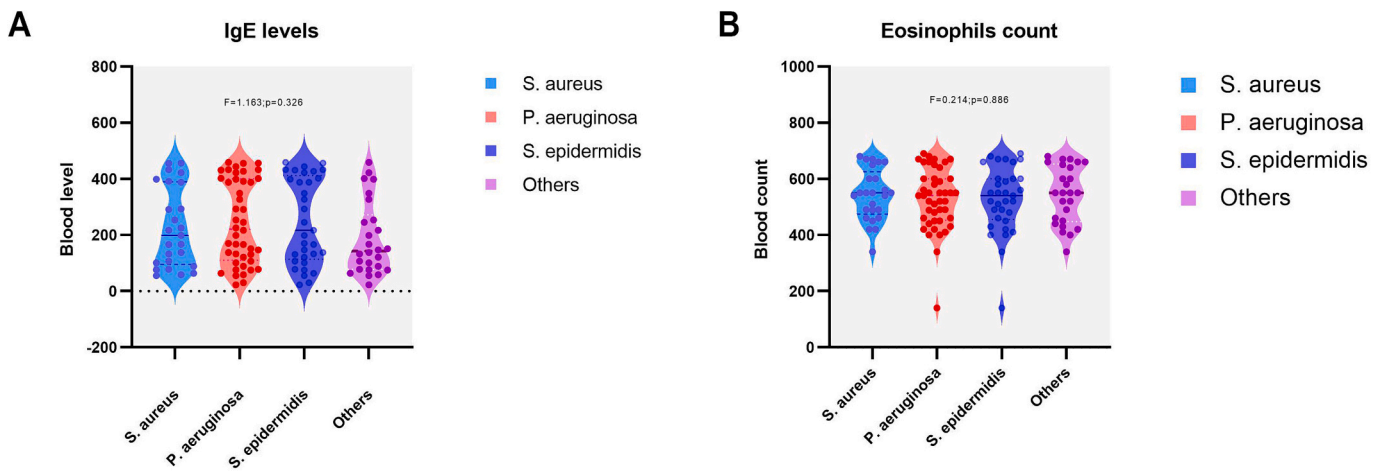

Fig. 1. Violin plots of Ige levels (A) and Eosinophils count (B) according to microbial profiles.
Simultaneously, the 6-month treatment resulted in a significant increase in SS-I scores, with the Other bacteria group showing the highest SS-I score, followed closely by the *S. epidermidis* group. The *S. aureus* and *P. aeruginosa* groups also had lower SS-I improvement.

Table V
Multiple linear regression analysis for preoperative predictors of IgE levels and eosinophil blood count at the 6-month follow-up.

IgE level			Eosinophil blood count	
Preoperative Variable	Pearson	Sign. (one tail)	Pearson	Sign. (one tail)
Age	−0,215	0,081	−0,282	0,032
Sex	−0,165	0,142	0,219	0,076
BMI	0,107	0,246	0,067	0,332
Atopy	−0,027	0,432	0,215	0,081
Asthma	−0,173	0,131	−0,062	0,345
Aspirin intolerance	−0,092	0,276	0,029	0,426
N-ERD	−0,086	0,29	0,071	0,324
IgE level	−0,177	0,126	0,030	0,424
Eosinophil blood count	−0,122	0,215	0,254	0,048
S. aureus	−0,320	0,017	0,059	0,352
P. aeruginosa	−0,098	0,263	−0,131	0,198
S. epidermidis	−0,437	0,002	−0,073	0,319
Others	0,118	0,222	−0,001	0,498
SS-I	0,172	0,132	−0,038	0,404
NPS	−0,212	0,083	0,029	0,426
SNOT22	0,041	0,395	0,061	0,346
VAS Obstruction	−0,072	0,321	−0,173	0,131
VAS Rinorrhea	−0,106	0,248	0,027	0,431
VAS Headache	−0,170	0,134	0,172	0,132

diminished in both groups; however, this increment was less significant in the *S. aureus* group. As IgE is an established mediant of allergic processes, this may indicate a reduction of the atopic trait of CRSwNP [7]. Additionally, the association between *S. aureus*, a bacterium known for its superantigenic properties [17], and IgE levels may drive future insights as the association could allow for targeted treatment of patients with an IgE-dominant phenotype to be treated with Dupilumab. Meanwhile, the evidence of clinical improvement with a dramatic decline in NPS across all bacterial groups further reinforces the potential impact of such therapies to eliminate significant polyp burden due to bacterial accumulation. However, the distal response rates between bacterial groups indicate that nasal microbiota composition may be predictive of treatment responsiveness. The essence is it may change the therapeutic strategies when the profiling of microbiota is a standard tool for pre-treatment evaluation. Focusing on the SNOT-22 and SS-I scores, the broad improvements observed in both favouring post-treatment outcomes highlight the benefit of treatment on patient-reported outcomes and quality of life. Functional and symptomatic improvements are increasingly getting reported from various tertiary centres and serve to highlight the real-world benefits of the intervention and help make a case for continued use and development. This study also has some limitations, although the design and methodology were correct, several potential confounders were not considered. The 6-month follow-up period is sufficient to detect immediate treatment effects, but it is too short to evaluate the durability of these effects or the long-term stability of the treated microbiota. Also, while the study sample size was sufficient to show significant results, it may limit the generalizability of the findings. Additionally, as no placebo control group was included in the study, accounts of the natural course of the disease and spontaneous changes to microbiota over time were neglected. Larger patient cohorts with varying demographics should be included in future studies to determine the generalizability of these results. A longitudinal design in an extended follow-up study would add valuable information on long-term outcomes and the durability of microbiota changes. Further investigation on mechanistic aspects linking specific bacterial profiles, Type II inflammation, and clinical outcomes is required to guide individualized treatment regimens for CRSwNP patients.

5. Conclusions

Our findings demonstrate the complex and dynamic nature of the interplay between the nasal microbiota, Type II inflammation, and treatment outcomes in CRSwNP. The potential predictive value of nasal microbiota composition on treatment response opens new avenues for personalized therapeutic strategies and highlights the need for a deeper understanding of the microbiome's role in CRSwNP pathogenesis. The significant correlations between specific bacterial populations and inflammatory biomarkers after treatment contribute to a growing body of evidence supporting the integration of microbiome analysis into routine clinical practice for managing CRSwNP.

CRediT authorship contribution statement

Gaia Vertillo Aluisio: Visualization, Software, Resources, Data curation, Conceptualization. **Maria Santagati:** Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Giovanna Stilo:** Visualization, Validation, Formal analysis, Data curation. **Mario Lentini:** Writing – review & editing, Funding acquisition, Data curation. **Leigh J. Sowerby:** Writing – original draft, Supervision, Software, Data curation. **Miguel Mayo-Yáñez:** Project administration, Investigation, Data curation. **Ahmad R. Sedaghat:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Investigation, Conceptualization. **Jerome R. Lechien:** Visualization, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Stefania Stefani:** Supervision,

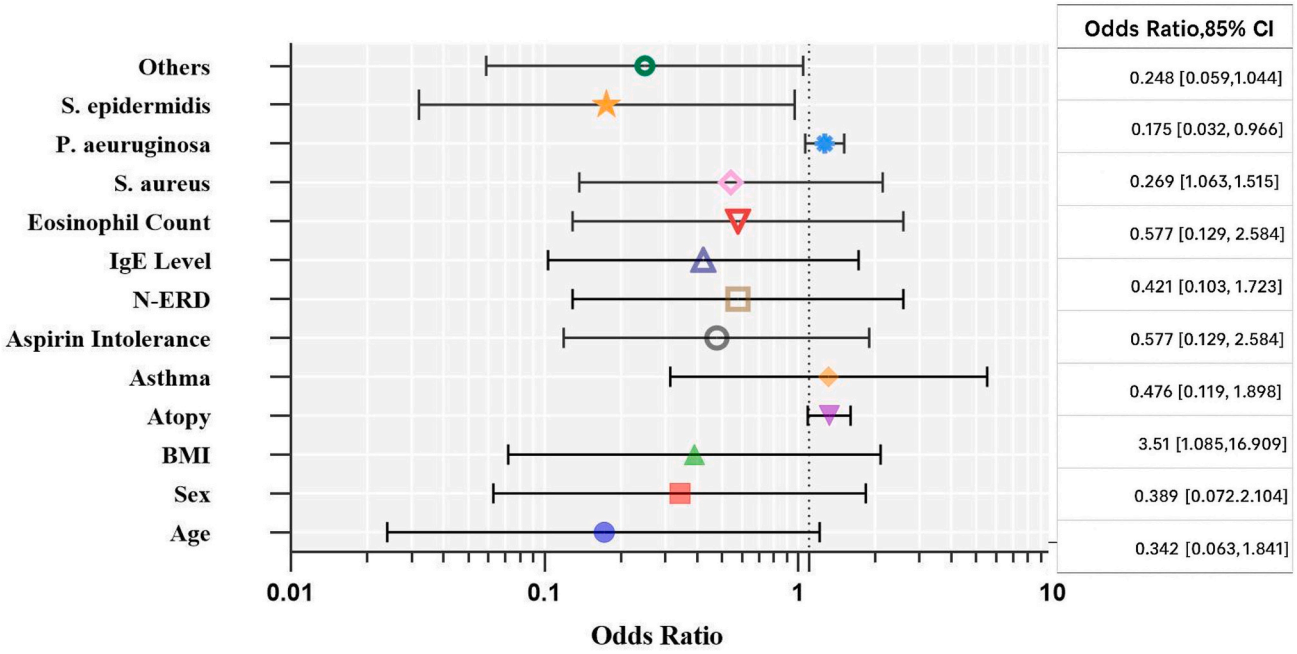

Fig. 2. Odds ratio plot for several variables.

Methodology, Conceptualization. **Igo La Mantia:** Visualization, Supervision, Resources. **Antonino Maniaci:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Ethics approval statement

The study was approved by the Human Medical Research and Ethics Committee of the University of Catania and was conducted under the Declaration of Helsinki (code 24121–21/05/2021).

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amjoto.2026.104786.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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