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New therapies for uncontrolled severe chronic rhinosinusitis with nasal polyps

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Abstract

Over the last two decades, there has been a growing comprehension of the pathophysiological processes underlying chronic rhinosinusitis (CRS). This progress has resulted in a shift from phenotyping to endotyping, refocusing attention from eosinophilic inflammation to a more comprehensive consideration of type 2 immunity. Furthermore, conventional perspectives on ventilation and drainage have been replaced by the mucosal concept, influencing therapeutic approaches. The use of endotypes, which are identified based on pathophysiological characteristics, enhances our understanding of the diverse manifestations of CRS. This, in turn, improves the accuracy of diagnosis and informs more effective treatment strategies.

Recently, in relation to the underlying immunopathological profile, three types of CRS have been identified, namely: Ty1, Ty2, and Ty3. These types are concurrently expressed and variably combined, evident in both CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). It should be noted that within the context of CRSwNP, type 2 inflammation is the most common variant in the Western population, with an increase in eosinophils, IgE, interleukin (IL)-4, IL-5 and IL-13.

The conventional treatment approach includes the utilization of local and systemic corticosteroids, and/or sinonasal surgery. However, these strategies might be associated with recurring instances, potentially requiring further surgical revisions. *" Difficult-to-treat patients "* often exhibit more severe conditions, requiring elevated systemic corticosteroid doses and/or undergoing multiple sinonasal surgeries. Literature findings indicate that biologic agents, targeting specific key effectors of type 2 inflammation, may serve as an additional therapy for individuals with severe and uncontrolled CRSwNP, resulting in significant improvement across various outcomes. Therefore, the importance of disease endotyping has progressively grown over the years.

The correct definition of the target patient, the type of biologic drug to be used and the timing of intervention are crucial to ensure personalized therapy and optimize the cost/effectiveness of the treatment.

Randomized controlled trials (RCTs) have shown significant improvements in both objective and subjective parameters. However, the results of these RCTs are not necessarily applicable to daily practice.

The aim of this retrospective study is to assess the therapeutic impact of biologics in patients with CRSwNP within a real-world context. Patients treated with one of the AIFA-approved biologics (dupilumab, omalizumab and mepolizumab) since April 2023 with at least 9 months of follow-up

were included in the study. Changes in Sinonasal Outcome Test 22 (SNOT-22) and Nasal Polyp Score (NPS), as well as subjective changes in sense of smell, changes in serum total IgE levels and total eosinophil counts, comorbidities, discontinuation or change in monoclonal antibody and adverse events were assessed. Thirty-three patients were included in the study.

All agents produced significant improvements in polyp size, symptom severity and subjective olfactory assessment. The monoclonal antibody had to be changed in no patients. No serious adverse events occurred during treatment initiation and follow-up. This analysis showed a moderate superiority of dupilumab, particularly in terms of rapid onset of action, in improving signs, symptoms and quality of life in CRSwNP.

Biological treatments emerge as a promising option for patients with severe refractory CRSwNP. The efficacy of biologics is evident in randomized controlled trials and real-world clinical settings, demonstrating substantial improvements in the evaluation of nasal polyps and related symptoms. Moreover, the incidence of complications associated with biologics in this context is rare.

Advances in statistical methodologies, head-to-head comparative trials, and comprehensive real-life studies are essential to establish more definitive conclusions and elucidate the precise roles of individual biologic agents.

A Stefania e Francesco

1. BASIC CONCEPT OF CHRONIC RHINOSINUSITIS

In line with the guidelines established by the expert committee of the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS), rhinosinusitis is characterized as the inflammation of the nose and paranasal sinuses. This condition presents with two or more symptoms, with one of them being either nasal blockage/obstruction or nasal discharge. Additional symptoms may include facial pain/pressure, and a decrease or loss of smell, or both[1]. Acute rhinosinusitis (ARS) is clinically defined as symptoms lasting less than 12 weeks with complete recovery. Recurrent acute sinusitis (RARS) is characterized by experiencing four or more rhinosinusitis episodes per year without persistent symptoms between episodes.

Chronic rhinosinusitis (CRS), the focus of this study, is distinguished by persistent symptoms lasting for a minimum of 12 weeks on most days without complete resolution. The exacerbation of chronic sinus disease symptoms, followed by a return to baseline, usually after intervention with systemic antibiotics and/or corticosteroids, is defined as acute exacerbations of chronic rhinosinusitis (AECRS). Given the significant potential for false-positive and false-negative results, it is not advisable to rely solely on subjective criteria for a definitive diagnosis. Nasal endoscopy and sinus computed tomography (CT) are essential to ensure accuracy in the diagnostic process[2].

The idea of rhinosinusitis became widely recognized after the pioneering studies by Stammberger, who demonstrated the pathophysiological importance of sinus ostia through endoscopy[3]. "Rhinosinusitis" first appeared in 1997, as inflammation of the paranasal sinuses rarely occurs without concurrent rhinitis; indeed, the nose and sinuses are anatomical structures that are contiguous and share innervation and vascular supply.[4] The intimate pathogenetic connection between rhinitis and sinusitis was subsequently supported by tomographic documentation, emphasizing the nearly constant correlation between infectious rhinitis and sinusitis [5]. In a limited number of instances, like those involving dental issues or iatrogenic sinusitis, there is a reversal of this pathway where activities within the sinus cavity result in subsequent inflammation of the nasal passages. In few patients, CRS may develop as a secondary outcome of intrinsic inflammatory conditions within the mucosa, even in the presumed absence of external stimuli (e.g., sarcoidosis, eosinophilic granulomatosis with polyangiitis) or in conjunction with specific host genetic factors, such as cystic fibrosis, or systemic immunodeficiency[6].

From an epidemiological viewpoint, CRS is a significant health problem and affects 5% to 12% of the general population[7]. In Europe, chronic rhinosinusitis is estimated to affect about 10.9% of the

population, with a regional variation ranging from 6.9% to 27.1%[8,9], according to data from the Global Allergy and Asthma European Network (GAL2EN) in collaboration with EPOS. It's worth noting that these estimates are not consistently supported by objective evidence of sinus inflammation, raising the possibility of overestimation. Research indicates that only 2% of individuals with self-diagnosed CRS were confirmed to have the condition. Another study found that up to 30% of individuals reporting subjective symptoms lacked radiological evidence of CRS.[10] As mentioned earlier, to ensure the accuracy of CRS diagnosis, guidelines recommend radiographic or endoscopic evidence[11]. The negative impact of chronic rhinosinusitis on an individual can be profound, causing significant debilitation[12]. Patients with CRS can experience a complex range of symptoms beyond those related to the nose and paranasal sinuses. Key symptoms that decrease QoL include a loss or reduction of smell and sleep disturbances, which in turn may have further physical and mental health consequences. Patients who experience anosmia report reduced enjoyment of food, difficulty cooking, difficulty assessing personal hygiene, and failure to smell smoke and rotten food[13]. Over 75% of patients with CRS experience reduced sleep quality. Sleep impairments include snoring, fragmented sleep, daytime sleepiness, and obstructive sleep apnea[14]. All these disorders can lead to reduced concentration, frustration, sadness, depression, anxiety, phobia, hopelessness, and embarrassment. The considerable prevalence and chronicity of CRS contribute to substantial costs, encompassing direct healthcare resource utilization and lower productivity at work or absenteeism (indirect costs), with data indicating that the primary financial burden is associated with these indirect costs[15,16]. Understanding these indirect costs is crucial from a socioeconomic perspective, particularly considering that a significant portion of CRS patients falls within the working-age range (30-50 years). The latest evidence from the United States suggests an increase in CRS costs, with the latest 2017 report estimating indirect costs at approximately $20 billion for CRS in general. Additionally, the total direct costs of CRS in the US are on the rise, estimated to be between $10 and $13 billion annually[17]. In Europe, there is a scarcity of data regarding CRS costs. Lourijsen et al., in their report conducted in the Netherlands, highlighted that the economic burden of CRS is at least €1501 per patient per year for direct medical assistance and €5659 per patient per year due to indirect costs[18].
It is important to emphasize how the economic burden of CRS is higher compared to other chronic diseases such as severe asthma ($7,261) and chronic migraine ($5,775) [figure 1-1]; this cost indicates that CRS, especially if recurrent, has a significant impact on the QoL of patients and becomes a significant expense for society[12]. Lately, public health has shown a growing interest in the cost of illnesses and the impact of therapies and interventions on these costs, influencing the choice of procedures and treatments for CRSwNP. Consequently, ENT specialists need to be aware of the impact that indirect costs have on healthcare expenses.

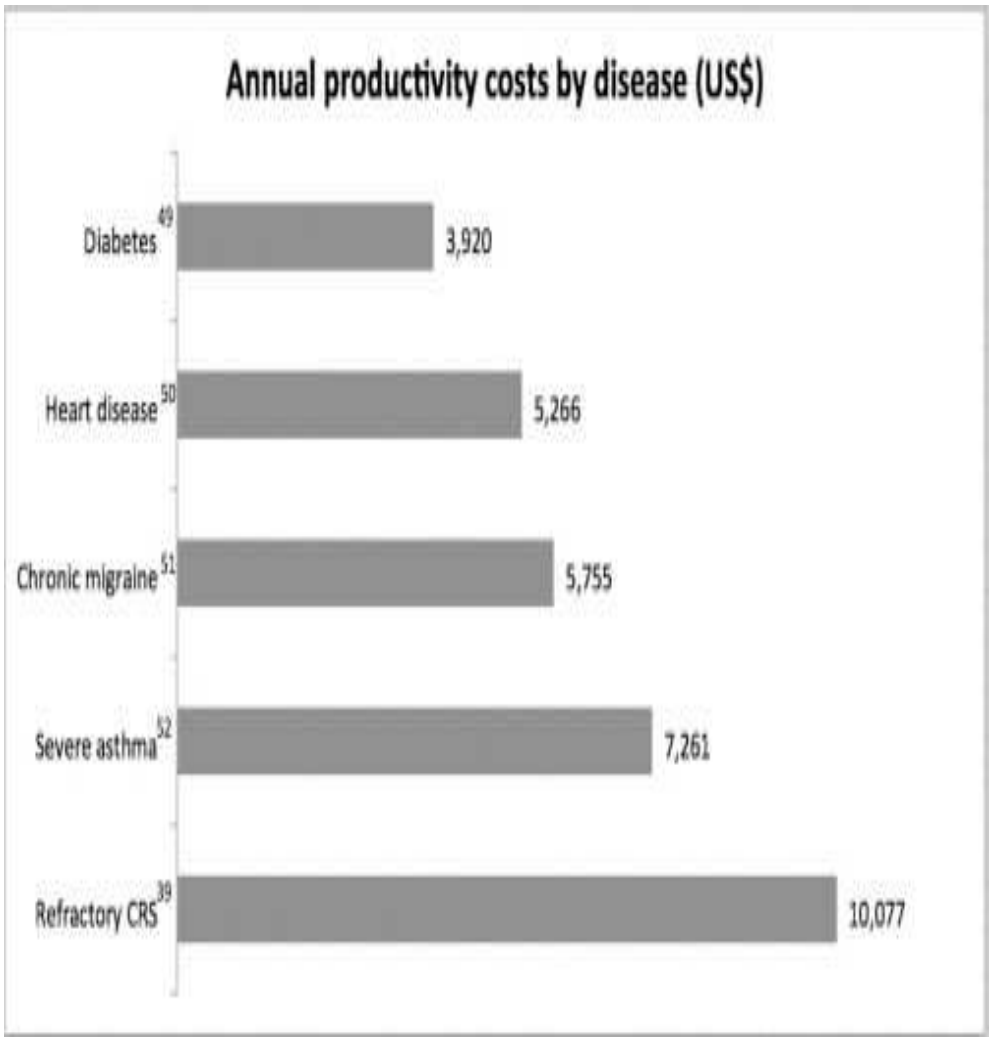

Figure 1-1 Annual productivity loss of various diseases.

"Heart disease" includes acute coronary syndrome, heart failure, and atrial fibrillation.

[Adapted from DeConde AS. et al., 2016]

2. FROM PHENOTYPES TO ENDOTYPES OF CHRONIC RHINOSINUSITIS

CRS is a heterogeneous disease, with multiple presentations and a variety of cellular and molecular pathophysiologic mechanisms[19]. Indeed, despite CRS being studied for about 40 years, it still represents a hot and debated topic in rhinology today. Therefore, the umbrella term 'Rhinosinusitis' is often used to indicate several clinical pictures that, on the contrary, are very different from each other and require completely different considerations and therapies[20].

Currently, it is commonly accepted that the same phenotype expression can recognize different pathogenic mechanisms, just as different phenotypes can be an expression of the same mechanism[21]. This is the reason why it seems useful to keep in mind the concepts of phenotype and endotype. Nevertheless, the definition of phenotypes and endotypes is often confused in the scientific community. In essence, phenotypic classifications depend on observable traits of a disease, while endotypic classifications are rooted in the fundamental pathophysiological mechanisms [22].

In the current times, as there is a growing necessity to justify therapeutic outcomes, clinicians and researchers have started reevaluating the diagnostic criteria for CRS. It became evident that these criteria were inadequate in explaining the diverse nature of the disease. Seeking to address this limitation, there has been a gradual shift towards a different viewpoint, recognizing that the clinical phenotype is essentially the visible manifestation of a much broader complexity in understanding CRS[23].

2.1 PHENOTYPE OF CHRONIC RHINOSINUSITIS

CRS can be phenotypically classified on the presence or absence of nasal polyps (NPs), severity (mild vs. moderate vs. severe), duration (acute vs. chronic), response to conventional therapy, mucus color, nature of triggering events, presence of peripheral specific IgE, recurrent occurrences, and the presence of complications[22]. However, the most common classification considers only the presence or absence of nasal polyps (CRSwNP and CRSsNP), and treatment primarily refers to this subdivision[24].

Another type of subclassification considers the presence or absence of common comorbidities (asthma or allergies); other subclassifications take into account less common conditions such as Aspirin Triad (AERD, NERD), Eosinophilic Granulomatosis with Polyangiitis (EGPA), Granulomatosis with Polyangiitis (GPA), Allergic Fungal Rhinosinusitis (AFRS), sinonasal sarcoidosis, and CRS with immunodeficiency.[25].

In clinical settings, CRSwNP is often linked to obstructive symptoms, olfactory dysfunction, a positive response to steroids and a need for multiple surgeries[26]. In contrast, CRSsNP is more frequently associated with obstruction in the osteomeatal complex, dental issues, and/or bacterial infections[27]. Early recognition, especially in Western countries, highlighted the association of nasal polyps with tissue and peripheral eosinophilia. Additionally, nasal polyp tissues commonly exhibit heightened levels of type 2 inflammatory biomarkers, including IL-4, IL-5, and IgE. CRSsNP, on the other hand, was associated with neutrophilia and elevated levels of IFN-γ and IL-8. Despite the suggestive nature of polyp status concerning inflammatory endotypic features, it lacks a solid foundation in underlying pathophysiology, limiting its value in prognosis and therapeutic development[28]. The previously rigid classification based on inflammatory characteristics related to polyp status is now recognized as overly simplistic. Regardless of polyp status, it is not uncommon to observe coexisting endotypes 1 (IFN-γ, IL-12), 2 (IL-4, IL-5, IL-13), and 3 (IL-17, IL-22)[29].

In recent years, the therapeutic failure (38-50 %) and the diverse range of manifestations in rhinosinusitis, have demonstrated that, in CRS, a treatment based solely on phenotypes may not always be adequate to achieve optimal healing and control [23]. A previous study showed that oral prednisone treatment did not obtain positive responses in all patients with CRSwNP[30]. In the same way, only CRSsNP patients with low IgE levels or with neutrophilic inflammation benefited from long-term low-dose macrolide treatment.[31].

The classic dichotomy between CRSwNP and CRSsNP is too simplistic to explain complex diseases

with mixed pathophysiologies such as CRS. As a result, the traditional therapeutic approach based on such a simple phenotypic classification contributes to imprecise disease control.

Drawing inspiration from pulmonary diseases, particularly asthma, there has been an effort to investigate the immunopathological mechanisms (endotype) underlying CRS[32].

2.2 ENDOTYPE OF CHRONIC RHINOSINUSITIS

Phenotypic classifications categorize patients with CRS based on similar disease behavior or presentation, but they do not account for differences in the underlying disease mechanisms.

Over three decades ago, numerous histopathologic studies on tissue from individuals with CRS indicated a frequent association of elevated eosinophil levels with CRSwNP, while elevated neutrophil levels were commonly linked to CRSsNP[28,33,34]. This led to the categorization of CRSwNP and CRSsNP as "eosinophilic" and "neutrophilic" diseases, respectively. These classifications may oversimplify the complex nature of CRS while recent research suggests a more nuanced understanding of the underlying inflammatory processes in these conditions[25].

However, this simplistic dichotomy has been debunked, and it is now acknowledged that both CRSwNP and CRSsNP can manifest with eosinophilia, neutrophilia, or a combination of both, alongside a complex infiltration of other inflammatory cell types[28].

In a cohort of CRS patients undergoing surgical treatment, approximately 40% of those with CRSwNP exhibited either a neutrophilic or a combined neutrophilic/eosinophilic infiltrate. The same authors observed isolated neutrophilia in about 20% of patients with CRSsNP. Interestingly, elevated levels of eosinophils and neutrophils weren't present in approximately 20% of patients[35]. In the pulmonary field, this not well-characterized paucigranulocytic phenotype is associated with steroid insensitivity and airway hyperresponsiveness[34]. Klingler et al. described an "untypable" endotype that might be compatible with this paucigranulocytic inflammatory phenotype[35]. Furthermore, understanding inflammatory phenotypes in CRS is made more complex by the notable geographic differences observed between patients in Asia and those in Western countries. This variation is particularly noticeable in Asian patients with CRSwNP, who often exhibit significant tissue neutrophilia but frequently lack the eosinophilia observed in many Western patients[36].

The endotyping of CRS is a recent advancement. In 2013, a collaborative effort between the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology resulted in a PRACTALL document, published in the Journal of Allergy and Clinical Immunology [22]. This document underscored significant limitations in understanding the causes of CRS due to a lack of knowledge about pathomechanisms, impeding progress in its treatment. Even though clinically relevant phenotypes for CRS have been defined based on observable characteristics and treatable traits, such as the presence or absence of nasal polyps, it became evident that these phenotypes could arise from various underlying pathomechanisms. This realization prompted the

exploration of innovative and relevant differentiation through endotypes, grounded in biomechanistic principles. Preliminary research identified that CRSwNP was associated with elevated tissue levels of IgE and IL-5 while CRSsNP showed elevated levels of INF- γ and IL-8 [37,38]. These cytokine patterns were subsequently associated with a rise in T cell quantities and their activation, adding to a more profound comprehension of the inflammatory diversity and endotypes in CRS [39].

The preliminary studies encountered evident constraints, mainly because there was no single inflammatory mediator or biomarker that could comprehensively characterize individual phenotypes of CRS. Moreover, there was evident heterogeneity in T-cell polarization and the patterns of cytokine expression[28]. The first analysis of CRS endotypes, primarily based on measurements of cytokines and mediators in the sinus mucosa and secondarily related to clinical traits, was published in 2016[29].

In this study, Tommasen et al. identified 10 distinct endotypes, demonstrating their varying association with the phenotypic presence of asthma or nasal polyposis. The identified endotypes were additionally classified into three groups, distinguishing them by high IL-5 levels, low IL-5 levels, or the absence of IL-5.Remarkably, the groups characterized by elevated IL-5 concentrations were primarily comprised of individuals with CRSwNP who exhibited IgE responsiveness to Staphylococcus aureus enterotoxins. Even though this study did not explicitly establish a direct link between endotypes and the results, it signifies the initial effort to pave the way for future research on how biomarkers can precisely define a specific endotype, potentially guiding distinct therapeutic interventions for each (figure 2-1).

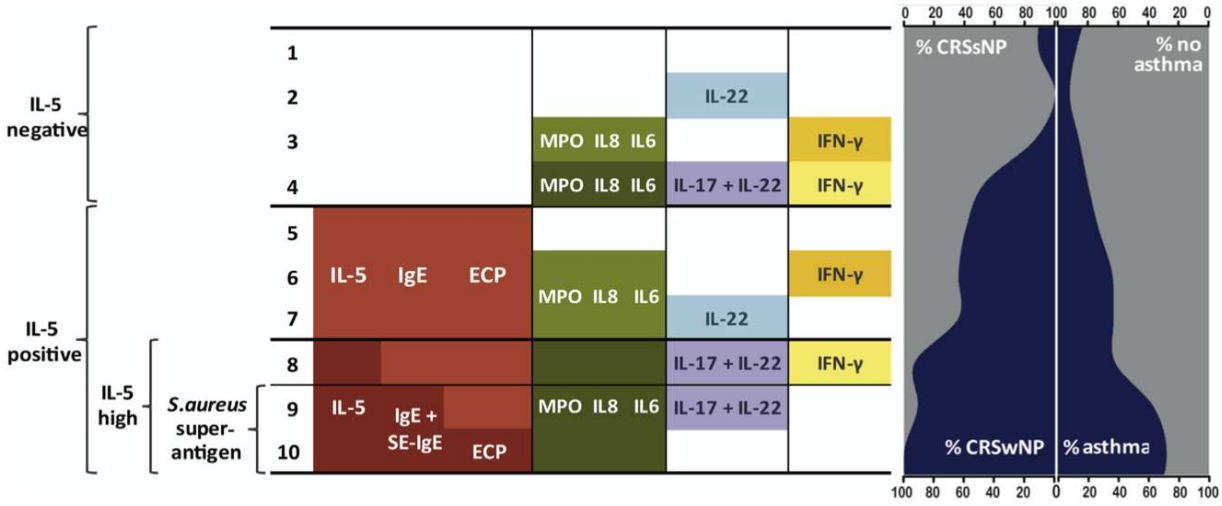

Figure 2-1 Inflammatory endotypes of CRS related to cluster analysis of biomarkers

Graphic representation illustrates clusters in relation to cytokine patterns. Additionally, it depicts the distribution of CRSsNP versus CRSwNP and asthma. Horizontal lines denote clusters grouped based on IL-5, SE-IgE, and characteristics related to CRSwNP and asthma. In the context of cytokines, white represents a lack of heightened concentration, light shades indicate a moderate increase, and dark shades signify a substantial increase in concentration. [Reprinted from Tomassen P et al., 2016]

Recently, attention has turned towards the genetic analysis of CRS tissues, enabling the identification of molecules produced by T lymphocytes of types 1, 2, and 3 (also known as 17)[34].

These types of T cells produce cytokines that influence the inflammatory patterns observed in tissues, referred to as T1, T2, and T3, and Tun (indicating "untypable").

The T1 endotype is defined by a predominant expression of the T1 cytokine IFN-γ, released by Th1 cells, NK cells, cytotoxic T cells and group 1 innate lymphoid cells (ILC1s). The T2 endotype is distinguished by T2 cytokines (IL-4, IL-5, and IL-13), originating from Th2 cells, mast cells, ILC2s, and eosinophils. Finally, the T3 endotype is identified by T3 cytokines (IL-17A and IL-17F) produced by Th17 cells and other cell types.

In some cases, individuals with either CRSsNP or CRSwNP display elevated levels in all three endotypes (T1,2,3sNP and T1,2,3wNP).

From an endotypic standpoint, the distinction between CRSsNP and CRSwNP is far from clear-cut, with 64% of patients with CRS falling outside of distinct T1 and T2 endotypes and displaying a mixed T1/T2 or an alternative endotype, suggesting that there is truly a spectrum of CRS endotypes. This parallels the known clinical heterogeneity of CRS with purely nonpolypoid CRSsNP and purely polypoid CRSwNP at opposite ends of the spectrum[40].

2.3 INFLAMMATORY ENDOTYPES AND THEIR RELATIONSHIP TO PHENOTYPIC CLASSIFICATIONS

Several studies have recognized a broad spectrum of CRS endotypes, ranging from as few as 3 to as many as 10 (table 2.1)[26,29,36,41,42]. While these studies provided strong evidence linking inflammatory endotypes to distinct clinical presentations in CRS, caution is advised in their interpretation. However, it is important to interpret such results with caution due to certain limitations related to the number of participants, the distribution of study populations, biomarkers used for cluster identification, or the approach used in grouping. Similarly, the uniformity across studies concerning the phenotypic and demographic features within each presumed endotype indicates considerable importance in both clinical and scientific contexts.

Author	Year Published	Population and Sample Size	Endotypes Studied	Associated Prognostic Value
Tomassen et al.[11]	2016	173 CRS patients undergoing endoscopic surgery and 89 controls	10 clusters grouped as IL-5 negative, IL-5 positive, and IL-5 high	1. Most IL-5 negative endotypes had a CRSsNP prominence with lower incidences of asthma 2. IL-5 positive clusters tend to have a mixed phenotype 3. IL-5 high clusters were predominantly made up of CRSwNP and had a higher incidence of comorbid asthma
Turner et al.[10]	2018	90 CRS undergoing endoscopic sinus surgery	6 clusters, 2 with low inflammation and 4 with high inflammation	1. Low inflammation clusters were heterogenous and associated with low CT scores, good olfactory function, and longstanding improvements in SNOT-22 scores one year postoperatively 2. 2 clusters with dominant Type 2 inflammatory markers were associated with CRSwNP, comorbid asthma, and temporary improvement in SNOT-22 scores after surgery 3. 2 clusters with nondominant Type 2 inflammatory markers were associated with CRSsNP and mixed polyp statuses respectively, mild to moderate disease, less comorbid asthma, and temporary improvement in SNOT-22 scores after surgery
Liao et al.[58]	2018	246 CRS patients and 16 controls	6 clusters	1. High eosinophilia was associated with severe disease and high proportions of difficult-to-treat CRSwNP 2. High neutrophilic inflammation was associated with older patients with high proportions of difficult-to-treat CRSwNP while moderate neutrophilic inflammation with IL-8 was associated with severe CRSwNP and poor treatment outcomes 3. IL-10 dominant disease was associated with mild CRSwNP with good treatment outcomes
Stevens et al.[71]	2019	121 CRSsNP and 134 CRSwNP patients	3 endotypes defined as T1, T2, or T3 dominant inflammation	1. T1 dominant disease is heterogenous and is more common in females 2. T2 dominant disease is associated with nasal polyps, asthma, and smell loss 3. T3 dominant disease is heterogenous and is associated with increased presence of pus and purulent nasal discharge
Nakayama et al.[70]	2021	8 white CRSwNP patients from the US, 9 Japanese patients with CRSwNP born and raised in Japan, and 8 control patients	2 CRSwNP clusters defined as non-Type 2 inflammation and eosinophilic, Type 2 dominant inflammation	1. CRSwNP with non-Th2 inflammation is associated with lower asthma comorbidity 2. CRSwNP with eosinophilic, Type 2 dominant inflammation is associated with high asthma comorbidity

Table 2-1 Overview of Key Studies Establishing Chronic Rhinosinusitis Endotypes

[Adapted from Chapurin N. et al., 2022]

Stevens et al.[42] examined sinus tissue from 255 patients with CRS (including CRSsNP and CRSwNP) and defined T1, T2, and T3 disease based on the expression of IFN-g, eosinophilic cationic protein/Charcot Leyden Crystal galectin, and IL-17a, respectively, at a concentration that was greater than the 90th percentile of the expression observed in control sinus tissue. In this study 87% of polypoid CRS had evidence of T2 inflammation and 62% had only T2 inflammation. However, T2 inflammation had also emerged as the predominant inflammatory endotype in nonpolypoid CRS . In fact, in this study 55% of nonpolypoid CRS cases exhibited signs of T2 inflammation, with 34% exclusively demonstrating evidence of T2 inflammation (figure 2-2).

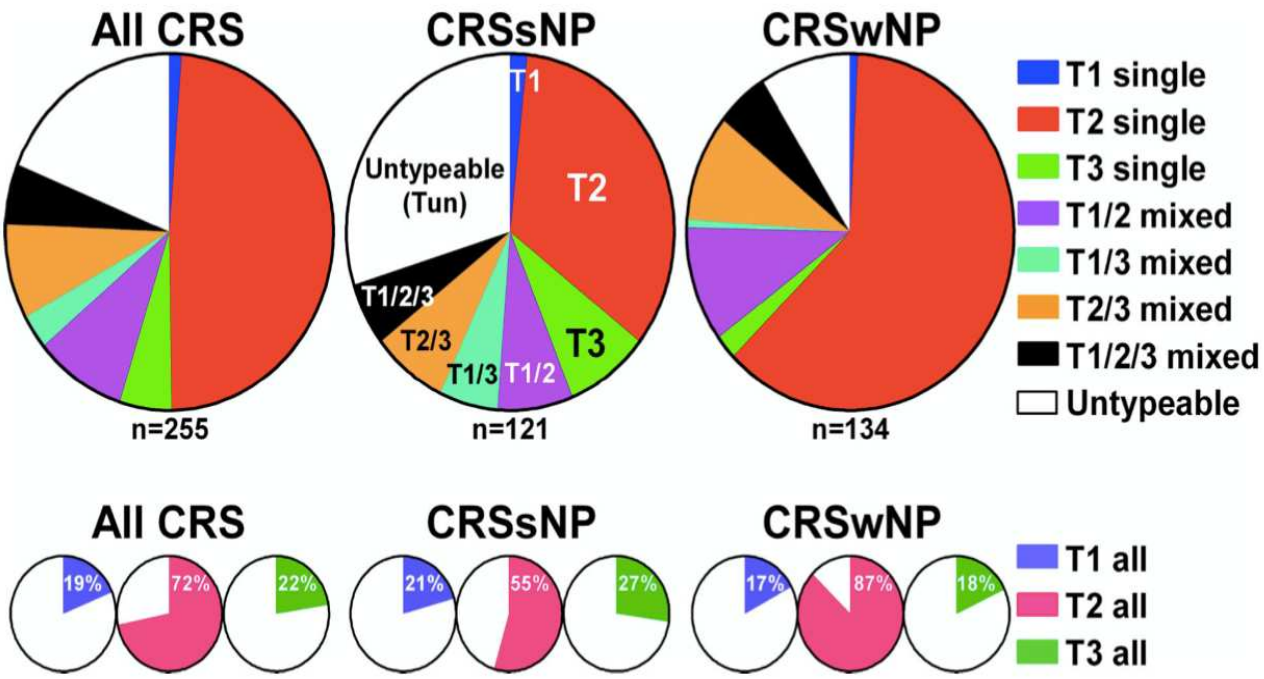

Figure 2-2 Patterns of inflammatory endotypes in CRS.
[Adapted from Stevens W.W et al., 2019]

Moreover, the existence of type 2 inflammation, irrespective of polyp status, demonstrated a higher likelihood of being linked with asthma, loss of smell, and surgical evidence of allergic mucin. Conversely, individuals with T2 inflammation were less inclined to report symptoms such as rhinorrhea, cough, and pus. In addition, smell loss was strongly associated with the T2 endotype and significantly higher in eosinophilic CRS regardless of polyp status. The loss of smell, independent of the presence or absence of nasal polyps, supports the concept that eosinophilic CRS may give rise to neuropathy, potentially mediated by the expression of eosinophil-derived neurotoxin[43] (Table 2-2). Similar to T2 inflammation, the T1/T3 endotype predicted phenotype regardless of nasal polyp status. Consequently, T1 inflammation was linked to symptoms such as rhinorrhea, sinus pressure/pain, headache/migraine, fatigue/fever, and cough—complaints were indicative of the presence of an IFN-γ high or IL-17 high state. The single T1 CRS endotype was rare (1.8%) but, more frequently, it is present as mixed endotypes (T1/T2 or T1/T3). Additionally, in alignment with the capacity of IL-17 to instigate neutrophilic inflammation, a T3 high endotype predicted complaints related to purulent drainage. Furthermore, 26% of the subjects in this study exhibited more than one endotype and generally displayed the anticipated clinical characteristics associated with each other. In conclusion, from the results obtained, it is evident that the previous dogma that NPs can adequately predict the inflammatory endotype can no longer be accepted.

	CRSsNP % (n=121)	CRSwNP % (n=134)	p value
Age (y), median (range)	38 (19–74)	45 (20–76)	0.071
Sex (female)	**57.0**	35.1	**< 0.001**
Atopy *	43.7	**64.3**	**0.002**
Asthma	29.8	**44.0**	**0.019**
Current smoker	4.1	5.2	0.681
Nasal congestion/obstruction/blockage	89.3	94.0	0.172
Rhinorrhea/Post nasal drip/Nasal drainage	**84.3**	69.2	**0.005**
Purulent nasal drainage	19.8	19.5	0.954
Sinus pressure/pain	**73.6**	54.9	**0.002**
Headache/Migraine	**25.6**	14.3	**0.023**
Fatigue/Fever/Feel poor	**14.9**	5.3	**0.010**
Smell loss/Reduced taste	33.9	**72.2**	**< 0.001**
Ear fullness/pain/popping	17.4	13.5	0.399
Eye watering/itching	8.3	6.8	0.651
Cough	**24.8**	8.3	**< 0.001**
History of FESS (> 2)	12.4	**30.6**	**0.001**
Intra-Operative Pus #	15.0	10.3	0.271
Allergic mucin #	2.7	**11.9**	**0.007**

The p value was determined by the Chi-square test.

* CRSsNP (n=119) and CRSwNP (n=112).

CRSsNP (n=113) and CRSwNP (n=126).

Table 2-2 Clinical characteristics of patients with CRSsNP and CRSwNP

[Adapted from Stevens W.W et al., 2019]

3. IMMUNOLOGICAL MECHANISMS OF CHRONIC RHINOSINUSITIS

Typically, an immune response can be categorized into three inflammatory endotypes based on a distinct signature profile. This profile includes distinct signaling molecules, various types of immune cells, and specific physiological functions [44]. Currently, CRS mainly includes two endotypes, namely type 2 and non-type 2 (type 1 and type 3/17) endotypes (figure 3-1).

Type 1 inflammation is identified by the predominant expression of cytokines such as interferon (IFN) γ and IL-12. On the other hand, type 2 inflammation is linked to increased production of cytokines like IL-4, IL-5, and IL-13. Additionally, a more recent discovery introduces a new inflammatory pattern, termed type 3 (or type 17) inflammation, characterized by elevated levels of IL-17 and IL-22.

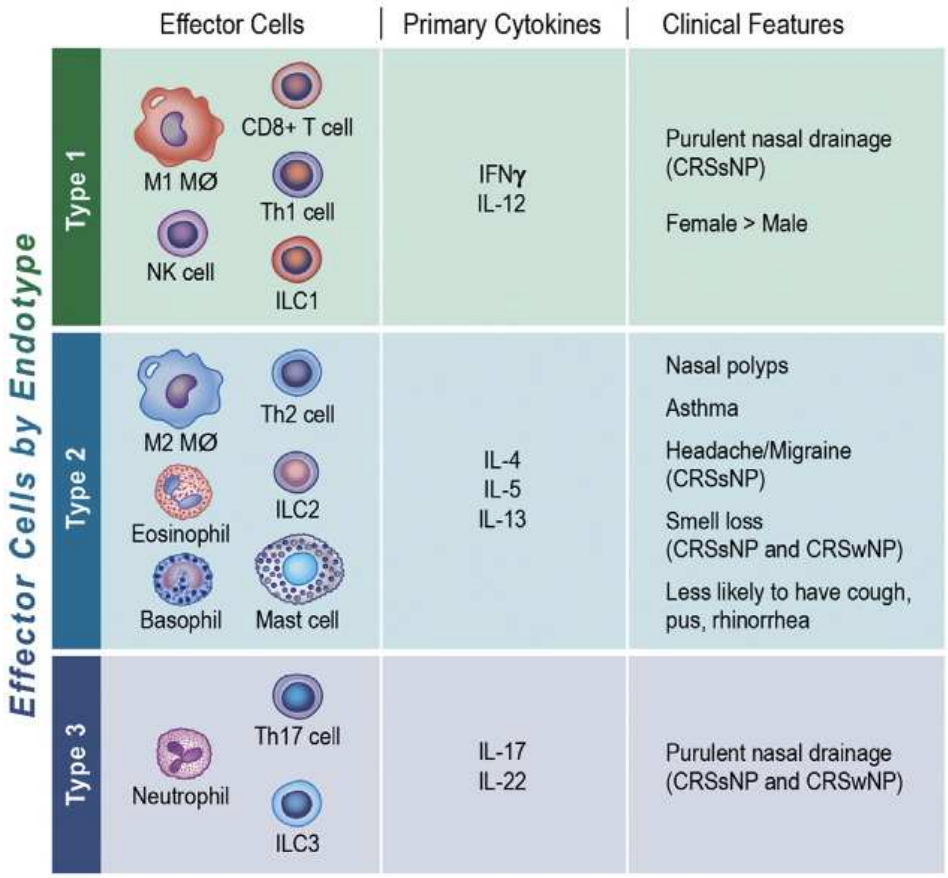

Figure 3-1 Summary of the key cytokines influencing T1, T2, and T3 endotypes, including the cells responsible for primary cytokine production and the effector cells mobilized to the tissue and subsequently activated.

[Adapted from Staudacher AG. et al., 2020]

In previous studies, particular subgroups of CD4+ T cells, known as TH1, TH2, and TH17, were recognized as contributors to the distinct cytokine patterns observed in type 1, type 2, and type 3 inflammatory endotypes, respectively. However, other cell types, including macrophages and components of the innate immune system, may also demonstrate a type 1, type 2, and/or type 3 inflammatory profile. Type 2 inflammation is notably associated with eosinophils, basophils, and mast cells, whereas type 1 inflammation is linked to natural killer cells and CD8+ T cells. Type 3 inflammation, on the other hand, involves neutrophils. Type 1 inflammation primarily plays a role in safeguarding the host against intracellular microbes and viruses. Type 2 immune responses contribute to defense against parasitic infections and are traditionally associated with allergic diseases. Lastly, type 3 inflammation is mainly perceived to defend against external bacteria and fungal infections.

3.1 TYPE 2 IMMUNE RESPONSES AND CHRONIC RHINOSINUSITIS

Historically, type 2 immunity is initiated by allergens or parasitic infections, marked by the transformation of naïve CD4+ T cells into Th2 effector cells. This process is commonly linked with IgE production, eosinophilia, and the activation of mast cells. The keystone cytokines in type 2 immune response, called "type 2 cytokines", include interleukin (IL) 4, IL-5, IL-9, and IL-13.

Recently, an increasing amount of evidence has demonstrated that allergens can activate a Th2 response beside specific IgE involvement and that several other environmental stimuli can trigger the same pattern of inflammation[45]. In light of the emerging pathogenic role of epithelial dysfunction and innate immunity, even type 2-inflammation should be considered no more as a consequence of allergenic stimuli only but the result of a complex crosstalk between airway epithelium, innate and adaptive immunity. In fact, in this pathological context, the dysfunction of the airway epithelium plays a pivotal role. This is triggered by factors that provoke damage, such as airborne allergens, pollutants, smoking, and infections caused by viruses and bacteria. These elements cause harm to epithelial cells in sinus cavities , leading to the release of innate cytokines known as alarmins. Included in these are thymic stromal lymphopoietin (TSLP), interleukin-25 (IL-25), and interleukin-33 (IL-33). These signaling molecules function as early activators of both innate and adaptive immune responses, playing a role in the development of type 2 inflammation.Danger signals (alarmins) directly stimulate ILC2, while TSLP produced by epithelial cells induces an upregulation in OX40L expression on dendritic cells. This series of events supports the conversion of naïve T cells into type 2 T helper (Th2) cells[46]. Th2 cells, ILC2, and type 2 cytotoxic T (Tc2) cells work together to coordinate eosinophilic inflammation through the generation of type 2 cytokines.

Type 2 cytokines are also generated by various cell types, including memory T cells located in tissues (Trm), T follicular helper cells specialized in antibody responses (Tfh2 and Tfh13 cells), as well as eosinophils, mast cells and basophils (Figure 3-2).

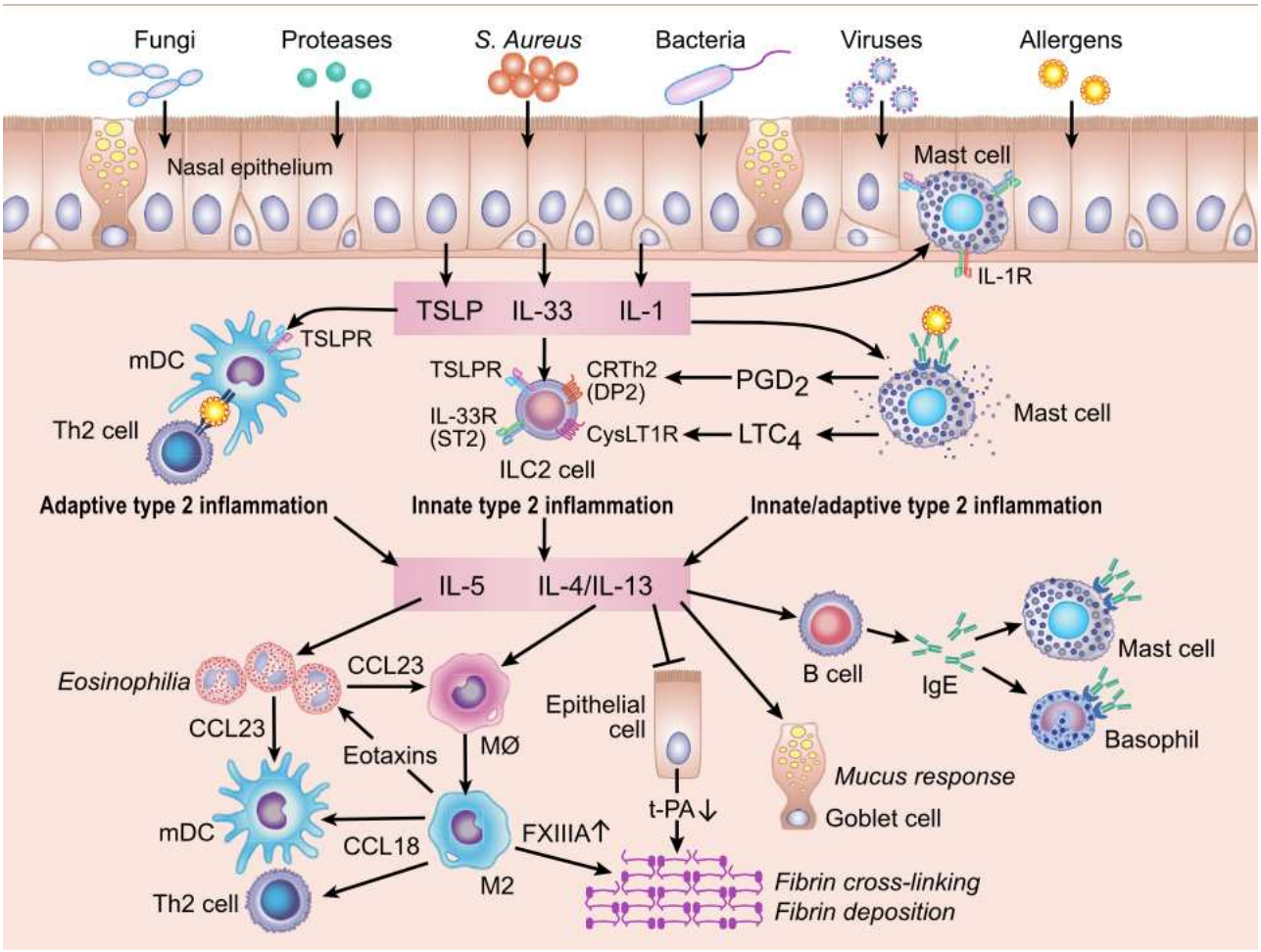

Figure 3-2 Proposed mechanism for amplification of type 2 inflammation in eosinophilic CRSwNP.

Various factors, including fungi, proteases, S. aureus, bacteria, viruses, and allergens, can trigger the activation of nasal epithelial cells, leading to the production of epithelial-derived cytokines TSLP, IL-33, and IL-1. These cytokines, in turn, activate three types of immune cells in nasal polyps (NPs): TSLP stimulates myeloid dendritic cells (mDCs), promoting the differentiation of naive CD4+ T cells into Th2 cells, contributing to adaptive type 2 inflammation; TSLP and IL-33 stimulate innate lymphoid cells type 2 (ILC2s), inducing the production of type 2 cytokines, contributing to innate type 2 inflammation; mast cells, present in the epithelium and mucosal area in NPs, are activated by TSLP, IL-33, and IL-1 to produce IL-5 and IL-13, contributing to innate type 2 inflammation.

Additionally, Ag/IgE/IgER complexes on mast cells induce degranulation, leading to the production of IL-5 and IL-13, further contributing to adaptive type 2 inflammation. Production of PGD2 and LTC4 from mast cells also stimulates ILC2s. IL-5 promotes eosinophilia, and eosinophils produce CCL23 to recruit macrophages and myeloid dendritic cells (mDCs). IL-4/13 activation of macrophages, B cells, and epithelial cells induces eosinophil and Th2 cell recruitment, remodeling, and IgE-mediated reactions. M2 macrophages contribute to the process by producing eotaxins and CCL18, recruiting eosinophils, myeloid dendritic cells (mDCs), and Th2 cells.

[Adapted from Kato A., 2015]

3.2 INTERLEUKIN-4 AND INTERLEUKIN-13

Interleukin-4 and IL-13 are the signature cytokines of the type II inflammatory response. IL-4 and IL-13 are produced by many cell types, including activated Th2 cells, ILC2s, basophils, eosinophils, mast cells, natural killer T cells, and macrophages[47].

IL-4 and IL-13 act on two receptors, R1 and R2. Receptor R1 is solely stimulated by IL-4 and is composed of two chains, IL-4Rα/Yc. On the other hand, receptor R2 is formed by the chains IL-4Rα/IL-13Rα1, meaning the two receptors share a common chain, IL-4Rα. R1-type receptors are found on hematopoietic cells such as B lymphocytes, T lymphocytes, monocytes, eosinophils, and fibroblasts. They are activated only by IL-4, leading to the heterodimerization of the two chains, followed by the phosphorylation of JAK1 and JAK3 and subsequently, the dimerization of STAT-6, which overregulates the GATA-3 transcription factor overseeing the development of the Th2 response. The R2 receptors can be activated by both IL-4 and IL-13 and are found on epithelial cells, smooth muscle cells, fibroblasts, monocytes, and activated B lymphocytes. In this case, JAK1-2 is phosphorylated, leading to the dimerization of STAT-6, resulting in translocation into the nucleus and transcription of a series of genes that initiate a complex series of biological events. This particular receptor conformation is responsible for the fact that IL-4 and IL-13 can have common or specific activities; the prevalence of the effect depends on the different quantitative and spatial distribution of receptors and the local production of IL-4 and IL-13[48].

IL-4 determines and facilitates the maturation and multiplication of naïve T-helper (Th) cells, guiding them toward a Th2 cell phenotype. This pivotal function of IL-4 is enhanced by its capacity to suppress the immunomodulatory activity of regulatory T (Treg) cells, which typically inhibit the development of Th2 lymphocytes in healthy individuals. Additionally, both IL-4 and IL-13 actively support an adaptive Th2 response by activating B cells and locally generating immunoglobulins, particularly IgE. The connection of adjacent IgE molecules bound to their high-affinity receptors (FcεRI), prompted by allergens (cross-linking), initiates the IgE-dependent degranulation of mast cells and basophils. This event contributes to the escalation of type 2 airway inflammation by increasing the production of IL-4 and IL-13 [49]. IL-4 and, to a lesser extent, IL-13 are further involved in recruiting eosinophils from the circulatory system to the tissues. These cytokines, in fact, enhance the expression of both VCAM-1, facilitating the adhesion of eosinophils to the endothelium, and chemokines such as eotaxin-1 and eotaxin-3, promoting the migration of cells from the vessel to the

inflamed tissue. Eosinophils also express IL-4Rα, and its stimulation could contribute to eosinophilia[50].

IL-13 prompts an increase in goblet cell numbers and triggers the synthesis of mucin 5AC (MUC5AC), a glycoprotein that elevates the thickness of bronchial mucus, hindering the mucociliary clearance of nasal epithelium.

Further contributions of IL-13 to airway remodeling include its biologic actions resulting in the stimulation of collagen deposition, fibroblast proliferation, sub-epithelial fibrotic thickening, and activation of the epithelial-mesenchymal trophic unit.

These anatomical changes are, at least partially, initiated by the IL-13-dependent induction of transforming growth factor-β1 (TGF-β1). This activation subsequently stimulates the production of fibronectin and collagen by both epithelial and mesenchymal cells, contributing to the observed anatomical consequences [51]. IL-13 and IL-4 have the capability to prompt the differentiation of monocytes/macrophages into M2 macrophages. These M2 macrophages, in turn, produce coagulation factor XIII-A (FXIII-A). FXIII-A initiates an excessive accumulation of fibrin through the cross-linking of fibrin strands and triggers the activation of antifibrinolytic pathways by binding with α2-Plasmin inhibitor (α2-PI, also referred to as a2 antiplasmin) on fibrin. Simultaneously, Th2 inflammation reduces the levels of tissue plasminogen activator (t-PA), causing a compromised generation of plasmin. Consequently, this impaired fibrinolysis contributes to the retention of fluid and the onset of edema in polyps.

Furthermore, both IL-4 and IL-13 act directly at the level of the epithelial barrier, decreasing the integrity of intercellular junctions. This promotes the passage of allergens into deeper layers of the mucosa, where they meet antigen-presenting cells (APC), leading to a continuous activation of the immune response[52]. It is evident how IL-4 and IL-13 contribute synergistically to the maintenance and amplification of type 2 inflammatory processes.

3.3 EOSINOPHILS AND INTERLEUKIN -5

Eosinophils, constituting up to 6% of nucleated cells in the bone marrow, play a role in various cellular processes across vertebrates. Their involvement in inflammatory responses includes the release of inflammatory mediators, contributing to tissue damage. Increased numbers of eosinophils are detected in both the tissue and peripheral blood of individuals affected by eosinophilic diseases.
The Local Immunity and/or Remodeling/Repair (LIAR) hypothesis, initially proposed by Lee et al.[53], suggests a beneficial role for eosinophils in homeostasis. Eosinophils play diverse roles, including involvement in blood glucose regulation, adipose tissue accumulation, tissue development and remodeling, muscle and liver repair, regulationof physiological processes in the domains of epithelial, neuronal, and microbiome functions, coupled with immunoregulation.
The maturation and development of eosinophils within the bone marrow are orchestrated by essential cytokines such as IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3. Notably, IL-5 assumes a pivotal role in guiding the differentiation, migration, activation, and survival of eosinophils.
IL-5 is predominantly produced by CD34+ progenitor cells, Th2 lymphocytes , group 2 innate lymphoid cells (ILC-2),mast cells and invariant natural killer T cells. Additionally, eosinophils exhibit the capability to release IL-5 in an auto/paracrine fashion. Upon release from the bone marrow, eosinophils are attracted to tissues through chemokines like eotaxin-1 (CCL11), -2 (CCL24), and -3 (CCL26). Initiation of eosinophil activation is crucial for orchestrating effective responses to stimuli. This activation can be triggered by various mechanisms, including those induced by IL-5, IL-3, and GM-CSF, culminating in the release of mediator products.
The development, differentiation, proliferation, and activation of eosinophils are primarily influenced by IL-5, which additionally hinders the apoptotic demise of these cells.
IL-5 transmits signals through IL-5 receptors (IL-5R), consisting of transmembrane α and β chains. However, IL-5 can also bind to a soluble form of the IL-5Rα chain, influencing IL-5R signaling. Consequently, the responsiveness of eosinophils to IL-5 depends on the relative expression of transmembrane and soluble IL-5Rα, which, in turn, is influenced by eosinophil activation status, development, and the eosinophil's location, whether in blood or tissue. Eosinophils in the tissue exhibit a reduced level of transmembrane IL-5Rα expression and show diminished sensitivity to IL-

5 compared to eosinophils circulating in the periphery [54]; this could explain the larger reductions in peripheral blood versus tissue eosinophils seen with anti-IL-5 therapy in patients with eosinophilic disease[55,56]. Notably, soluble IL-5Rα expression in blood and tissue eosinophils is increased in patients with CRSwNP, with further increases in patients with comorbid asthma[54]. Hence, although the intrinsic levels of soluble IL-5Rα may not be sufficient to entirely block IL-5 activity, the heightened concentrations found in eosinophilic diseases could potentially be efficacious. It is crucial to acknowledge, however, that despite the elevated levels of IL-5Rα, the significantly increased IL-5 in CRSwNP compared to healthy controls might not necessarily lead to more pronounced regulation [38]. After being activated, eosinophils produce and release a myriad of factors, including granule proteins, enzymes, cytokines, lipids, growth factors, chemokines, and oxidative products, all contributing to type 2 inflammatory cascade. Moreover, activated eosinophils also secrete cytotoxic granule proteins, primarily playing a role in protective immunity but posing significant toxicity at high concentrations, thus contributing to tissue damage and remodeling [57].

The essential elements found in granules released by eosinophils, such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin, and eosinophil peroxidase, are predominantly situated in the matrix of specific granules, excluding MBP. These proteins showcase cytotoxic and non-infectious characteristics owing to their ribonuclease (RNase) activity. This contributes to detrimental outcomes, including tissue damage, heightened mucus production, alterations in airway structure, and the initiation of inflammatory processes [58].

Eosinophils serve as the primary source of cysteinyl leukotrienes (LT), promoting edema and nasal congestion by inducing vascular leakage, vasodilation, airway remodeling (including fibroblast proliferation, collagen deposition in the subepithelial lamina reticularis, and smooth muscle hyperplasia), and recruitment of inflammatory cells.

Eosinophils have been observed to generate both anti-inflammatory prostaglandin E2 (PGE2) and pro-inflammatory prostaglandin D2 (PGD2) in individuals with CRSwNP. However, there is a reduction in the levels of PGE2, while the levels of PGD2 show an increase in CRSwNP[59].

Following IL-5 stimulation, eosinophils exhibit the production of extracellular DNA traps (EETs), consisting of a network of DNA fibers and granule proteins. These traps play a role in capturing and eliminating pathogens[60].

In individuals with CRSwNP, EETs are primarily located in subepithelial regions where the epithelial barriers are compromised. This phenomenon leads to the capture and containment of S. aureus[61]. Moreover, EETs are significantly present in the mucus of those with eosinophilic CRS, amplifying the viscosity of the mucus. Notably, the generation of EET is strongly linked to the severity of chronic rhinosinusitis, irrespective of the presence of nasal polyps. EET formation plays a pivotal role in the

deposition of CLC (Charcot-Leyden Crystal), which represents the crystallized state of galectin-10[62]. CLCs are found abundantly in the mucosa and mucus of patients with both CRSsNP and CRSwNP and are associated with type 2 inflammation. Recent discoveries have revealed that CLCs go beyond being just a degradation product of eosinophils; they impact the epithelial barrier and sustain neutrophilic inflammation in CRSwNP. Notably, the generation of EETs is is intricately linked to the severity of chronic rhinosinusitis, irrespective of the presence of NP.

The creation of EETs plays a pivotal role in depositing CLC, the crystallized version of galectin-10. These crystalline formations are prevalent within the mucosal lining and secretions of individuals experiencing CRSsNP and CRSwNP , correlating with type 2 inflammation. Recent findings propose that CLCs go beyond being a mere byproduct of eosinophil degradation; they influence the epithelial barrier and play a role in sustaining neutrophilic inflammation in CRSwNP.

EET formation plays a crucial role in the deposition of CLC, the crystallized form of galectin-10. These crystals are rich in the mucosa and mucus of individuals with both CRSsNP and CRSwNP, and they are associated with type 2 inflammation. Recent discoveries indicate that CLCs have a broader impact beyond being a mere degradation product of eosinophils; they influence the epithelial barrier and contribute to sustaining neutrophilic inflammation in CRSwNP. Amplified migration of neutrophils, triggered by CLCs, has been documented both in laboratory settings and mouse experiments. This correlation has been substantiated in the tissue of individuals with severe type 2 CRSwNP. CLCs further contribute to inflammation by provoking neutrophilic inflammation and activating the NLRP3 inflammasome upon absorption by macrophages. This process culminates in inflammation driven by IL-1β.

It is significant to acknowledge that only the crystallized form of galectin-10 triggers these pro-inflammatory effects in CRSwNP; conversely, soluble galectin-10 demonstrates anti-inflammatory effects[63]. Additionally, an inverse correlation exists between heightened CLC gene expression levels in CRSwNP patients and olfactory threshold, suggesting a potential link between CLCs and olfactory dysfunction prevalent in CRSwNP compared to CRSsNP[43,64]. Eosinophils produce substances that cause inflammation and contribute to changing tissues. These substances include TGF-α, TGF-β1, fibroblast growth factor, vascular endothelial growth factor, IL-13, and IL-17. As a result, the balance of the extracellular matrix is disrupted, leading to tissue changes and fibrosis. Specifically, TGF-β is important in this process as it stimulates the multiplication of fibroblasts and increases the production of collagen fibers and glycosaminoglycans (Figure 3-3).

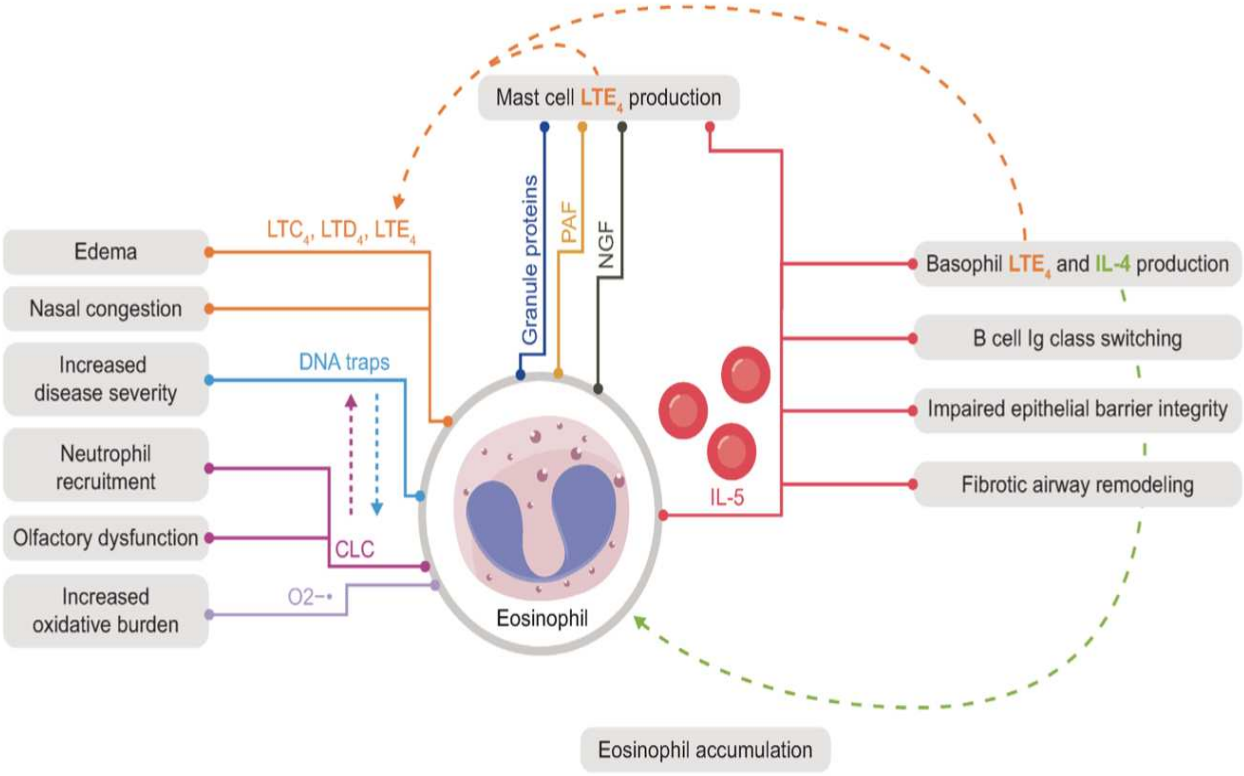

Figure 3-3 The role of IL-5 and eosinophils in the pathophysiology of CRSwNP

CLC, Charcot–Leyden crystals; CRSwNP, chronic rhinosinusitis with nasal polyps; DNA, deoxyribonucleic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; ILC2, group 2 innate lymphoid cell; LTC4, leukotriene C4; LTD4, leukotriene D4; LTE4, leukotriene E4; NGF, nerve growth factor; O2−•, superoxide radical anion; PAF, platelet-activating factor

[Adapted from Gevaeret et al ., 2021]

3.4 NON-TYPE 2 IMMUNE RESPONSES AND CHRONIC RHINOSINUSITIS

The "non-type 2" endotype in CRS can be more precisely classified into type 1 and type 3/type 17 endotypes, considering the distinctive inflammatory cytokine profiles (see figure 3-4).

The type 1 immune response is classically seen with intracellular microbes and viruses. The presence of viruses is particularly associated with worse objective endoscopic and CT sinus scores in CRSsNP, although interestingly, not in CRSwNP[65]. This endotype is characterized by a prevailing presence of interferon-gamma (IFN-γ), mainly generated by Th1 cells, natural killer cells (NKs), and type 1 innate lymphoid cells (ILC1s)[25]. The stimulation of macrophages by Th1 cells leads to the release of IFN-γ, establishing a unique hallmark of type 1 inflammation.

FN-γ prompts programmed cell death in nasal epithelial cells and enhances the activity of neutrophils[66]. Type 1 inflammation may contribute to the persistent nature of the disease and is generally more prevalent in individuals with CRSsNP[67].

Invasion of nasal epithelia triggers the secretion of IL-6, IL-8, Tumor Necrosis Factor α (TNFα), and various chemokines by nasal epithelial cells. Toll-like receptors (TLRs) are capable of recognizing pathogens or foreign substances by interacting with structures known as pathogen-associated molecular patterns (PAMPs)[68]. Various structures, including DNA, RNA, chemical products, or physical structures, form part of these PAMPs, which are unfamiliar to the local immune system. When PAMPs bind to the ligand-binding domain of TLRs, it initiates downstream signal transduction, activating the production of inflammatory cytokines and chemokines. The interactions between PAMPs and TLRs have been demonstrated to stimulate the production of IFN-γ and IL-8[39]. Interleukin 8 attracts neutrophils to the region, where they release additional cytokines such as IL-1β, IL-6, IL-8, and myeloperoxidase (MPO), an enzymatic substance discharged by neutrophil granulocytes. IFN-γ, released by epithelial cells upon recognizing pathogens, guides the differentiation of CD4+ T cells towards Th1 maturation. Th1 cells orchestrate the type 1 inflammatory response by generating IFN-γ and IL-2. Meanwhile, the epithelial secretion of IL-6 directs the differentiation of CD4+ T cells toward the production of Th17 and Th22. Th17 cells go on to secrete IL-17 and IL-22, while Th22 cells secrete IL-22 alone[69]. IL-22 is known to stimulate production of antimicrobial peptides and mucin 1 in an inflammatory environment. In response to different markers, increased mucus production is seen in type 2 and non-type 2 inflammation.

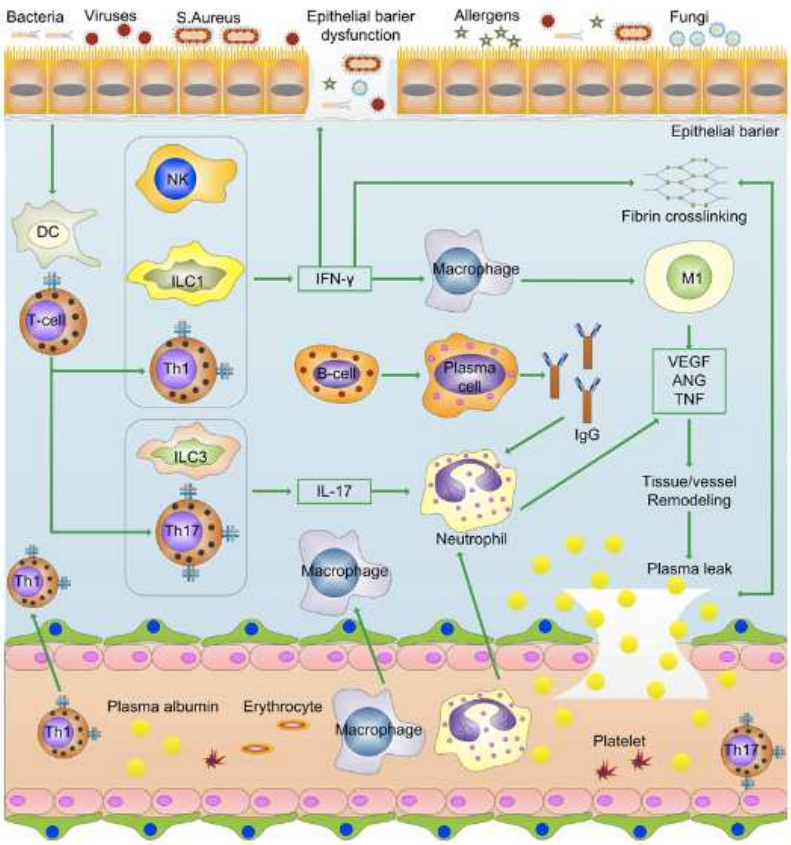

Figure 3-4 Inflammatory pathways in non-type 2 inflammation in CRS

Non-type 2 immune responses can be subdivided into type 1 and type 3/17 inflammation. Epithelial responses to environmental stimuli drive Type 1 inflammatory mechanisms, promoting the differentiation of Th1 and Th17 cells. This type of inflammation is characterized by an elevated expression of the Th1 cytokine IFN-γ, produced by Th1 cells, NK cells, and ILC1s. Additionally, Th1 cells activate macrophages to release IFN-γ.

On the other hand, Type 3 inflammation is triggered in response to extracellular bacteria and fungi, stimulating epithelial cells to produce osteopontin. Osteopontin further activates DCs, leading to the induction of Th17 cell differentiation. Th17 cells and ILC3s contribute to chronic inflammation by producing cytokines such as IL-17 and IL-22.

Following the activation of both Type 1 and Type 3 responses, there is an observable recruitment, activation, and proliferation of neutrophils in the tissue. This inflammatory process is associated with increased vascular permeability and initiates tissue remodeling. Ang, angiopoietins; DCs, dendritic cells; IgG, immunoglobulin G; IFN-γ, interferon-γ; IL, interleukin; ILC1s, type 1 innate lymphoid cells; ILC2s, type 2 innate lymphoid cells; ILC3s, type 3 innate lymphoid cells; M1, type 1 macrophage; NKs, natural killer cells; Th1, T helper type 1; Th3, T helper type 3; Th17, T helper type 17; Treg, T regulatory; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor.

[Adapted from Cui NA et al ., 2023]

However, induction of hypoxic microenvironments can perpetuate inflammatory processes in both responses[70]. Epithelial cells, upon recognizing pathogens, secrete IFN-γ, guiding the differentiation of CD4+ T cells towards Th1 maturation. Th1 cells orchestrate the type 1 inflammatory response by producing IFN-γ and IL-2. Simultaneously, the epithelial secretion of IL-6 directs the differentiation of CD4+ T cells towards Th17 and Th22 production. Th17 cells release IL-17 and IL-22, while Th22 cells specifically release IL-22[69]. Notably, in an inflammatory setting, IL-22 triggers the synthesis of antimicrobial peptides and mucin 1. Nevertheless, the creation of hypoxic microenvironments has the potential to sustain inflammatory processes in both scenarios[70].

In CRSsNP, there is an observed increase in Tregs compared to healthy individuals. On the other hand, in CRSwNP, there is a decrease in Tregs[71]. Tregs tend to be upregulated in a type 1 immune environment, where IL-2 produced by Th1 cells is crucial for Treg maturation.

Tregs are pivotal for immune regulation as they suppress the functions of both Th1 and Th2 cells. Additionally, they contribute to immune balance by producing the anti-inflammatory cytokine IL-10. They also produce TGF-β, which belongs to the cytokine superfamily and is suggested to play a significant role in tissue remodeling in CRSsNP[72]. TGF-β plays a role in initiating and promoting the growth of fibroblasts, as well as increasing the production of the extracellular matrix[73]. This contributes to the restructuring of the airway lining, leading to noticeable symptoms in individuals with CRSsNP[72]. Furthermore, TGF-β encourages the transformation of CD4+ T cells into Th17 cells and supports the maturation of Tregs. The significance of Tregs in CRS remains uncertain because, although they have the ability to decrease inflammation through the production of IL-10, they may also play a role in reshaping the airways and causing fibrosis due to TGF–β production.

Type 3 inflammation is provoked by extracellular bacteria and fungi, prompting the generation of osteopontin by epithelial cells[74]. Osteopontin then activates dendritic cells, fostering the differentiation of Th17 cells that release cytokines such as IL-17, IL-22, and IL-23.

This process results in the recruitment, activation, and proliferation of neutrophils, constituting the type 3 immune responses[75]. Neutrophils, possessing innate immune capabilities, phagocytize microorganisms and generate antibacterial products. However, the inflammatory factors they release can exacerbate tissue damage and the inflammatory response.

Previous studies have identified that elastase, released by neutrophils, acts as the primary trigger for IL-36γ. This, in turn, amplifies the production of IL-17A in neutrophils, establishing a recurring cycle of persistent neutrophilic inflammation in CRS. The research conducted by Wang et al.[76] emphasized neutrophil-secreted elastase as the primary instigator for IL-36γ activation. IL-36γ, subsequently, promotes the upregulation of IL-17A in neutrophils, creating a recurrent sequence of enduring neutrophilic inflammation in CRS.

In their study, Kim et al.[77] observed that administering anti-IL-33 treatment to a mouse model of CRS resulted in a decrease in the thickness of edematous mucosa, sub-epithelial deposition of collagen, and neutrophil infiltration. Interestingly, there was no significant impact on eosinophil infiltration. This leads to speculation that IL-33 could potentially emerge as a crucial mediator in the pathogenesis of CRSwNP, particularly through the recruitment of neutrophils[78].

Klingler et al.[34] discovered that type 3 CRSsNP is marked by various immune cells, including Th17 cells, B cells, dendritic cells (DCs), M1 macrophages (type 1 macrophages), and neutrophils. There is speculation that the presence of this diverse array of immune cells suggests a potential association between type 3 CRSsNP and bacterial infection. Li et al.[79] observed a noteworthy increase in the Th17/Treg (T regulatory) ratio and elevated IL-6 levels in both eosinophilic CRS with Nasal Polyps (eCRSwNP) and non-eosinophilic CRS with Nasal Polyps (non-eCRSwNP). This suggests that IL-6 might play a role in regulating the functionality of Th17 and Treg cells, as well as influencing the Th17/Treg ratio, contributing to the pathogenesis of CRSwNP.

in conclusion, neutrophils can actively contribute to the pathophysiology of CRS through enhanced proteolytic activity, involving elastase and cathepsin G, or via NETosis (neutrophil extracellular traps)[80]. Activated neutrophils exhibiting elevated CD16 and reduced CD62L expression release their DNA in a mesh-like configuration, forming traps recognized as NETs. NETs play a crucial role in sequestering and eliminating bacteria, fungi, and viruses. Recent findings indicate that double-stranded DNA (dsDNA) released during NETosis may directly contribute to pathogenesis by inducing a type-2 immune response[81]. In the mucus of patients with CRSwNP, NETs—alongside EETs (eosinophil extracellular traps) and CLCs—were identified to enhance mucus viscosity, resulting in plug formation, impeding mucociliary clearance, and eventually causing airway damage[82].

4. A NEW ERA IN THE TREATMENT OF SEVERE UNCONTROLLED CHRONIC RHINOSINUSITIS with NASAL POLYPS

The general therapeutic approach for CRS involves a combination of medical treatment and surgery, with surgery being considered in cases where medical interventions fail to alleviate persistent symptoms[22]. The recommended treatment for NPs includes nasal saline irrigation, nasal corticosteroids, and, in severe cases, oral corticosteroids[83]. Corticosteroids, owing to their anti-inflammatory properties, are the most effective option for managing both CRSwNP and CRSsNP. Nevertheless, topical intranasal corticosteroids exhibit greater efficacy in treating CRSwNP compared to CRSsNP[84]. Oral corticosteroids, administered over 2 to 4 weeks, provide temporary relief by reducing NP size and CRS symptoms; however, the optimal dosage and duration remain undetermined. Development of optimal delivery strategies for nasal and sinus diseases is still uncertain[1].

A multitude of alternative strategies, lacking substantial or any supportive evidence, have been suggested. These interventions can be grouped into various categories, including immunotherapy, pharmacological treatments (such as antihistamines, leukotriene receptor antagonists, and 5-lipoxygenase inhibitors), topical and oral medications (including antifungal drugs), non-pharmacological methods (such as large-volume irrigations, sinus ostia massage, and air purifiers), and dietary interventions[6,85].

As CRS is not primarily an infectious process, the predominant treatment does not involve antimicrobial therapy. However, certain antimicrobials, such as macrolide antibiotics and tetracyclines, have been identified for their anti-inflammatory properties. These medications may exert their influence on the disease through nonantimicrobial mechanisms[86]. Specifically, macrolide antibiotics have demonstrated the capacity to inhibit the production of IL-8 and tumor necrosis factor-α, downregulate the NF-κB signaling pathway, and suppress neutrophil functions. Consequently, these antibiotics have been proposed for the treatment of CRSsNP. However, the current information does not recommend using antibiotics regularly for individuals with CRSwNP[87].

Surgery is usually recommended when sinus problems persist despite trying different medications. However, it's not always clear who would benefit most from surgery, and some patients may need more than one procedure. For those with CRSsNP, the chances of the problem coming back after surgery are low. But for those with CRSwNP, the likelihood of the issue returning after surgery is higher, reaching up to 60% within a year of treatment. Studies have found that factors like asthma,

eosinophilia, aspirin-exacerbated respiratory disease, and atopy are important reasons for treatment not working well and the problem returning, suggesting a specific type of inflammation89.

In the case of CRSsNP, the chances of the condition recurring after effective sinus surgery are low. Conversely, in the case of CRSwNP, there's a higher likelihood of the condition recurring after surgery, ranging from 38% to 60% within 12 months of follow-up[88]. Studies investigating reasons for treatment failure and recurrence have identified critical clinical risk factors like bronchial asthma, eosinophilia, aspirin-exacerbated respiratory disease, and atopy. These factors collectively point to a specific type of inflammation profile known as the type 2 inflammation endotype[89].

The extent of surgery remains a topic of discussion. According to a recent study, completely removing the diseased mucosa from the paranasal sinuses (reboot approach) in a specific group of patients with severe type 2 CRSwNP significantly reduces the recurrence of nasal polyps for 30 months after the operation, compared to the current approach that spares the mucosa in type 2 inflammatory CRSwNP[90].

Considering surgery earlier after diagnosis appears to be better for patients, resulting in improved surgical outcomes and lower risk of developing asthma compared to waiting for a longer time (beyond 5 years) while relying on medications[89]. However, it's unfortunate that a significant number of patients still experience symptoms or nasal polyps returning even after trying various treatments like systemic corticosteroids and surgeries multiple times. This suggests that the usual treatments may not work for everyone.

"Difficult-to-treat" patients are those who don't achieve an acceptable level of control, even with the right medical and surgical treatments. For these individuals, the only option in recent years has been to undergo multiple ESS, with a growing risk of complications and a decreasing duration of symptom relief between surgeries[91]. The success of targeting specific immunologic mediators in asthma using biologics has sparked interest in applying a similar approach for CRSwNP[92]. Consequently, the introduction of biologics is rapidly and widely changing how CRSwNP is treated.

4.1 BIOLOGICAL TREATMENTS

Over time, the growing number of severe refractory patients experiencing CRS has prompted the exploration of innovative therapeutic approaches. Furthermore, the limited understanding of the pathogenic mechanisms underlying CRS has historically led to inappropriate utilization of pharmacological interventions (such as antibiotics and extensive steroid usage) and surgical procedures (incomplete surgeries), thereby contributing to the prevalence of uncontrolled cases. Specifically, EPOS criteria classify CRS patients into controlled, partially controlled, or uncontrolled categories, wherein the status is objectively determined by factors such as symptom reduction, clinical mucosal appearance, adverse events, the necessity for systemic medication, and the requirement for functional endoscopic sinus surgery[1,93] (Table 4-1).

EPOS 2020: Assessment of current clinical control of CRS (in the last month)

	Controlled (all of the following)	**Partly controlled** (**at least 1** present)	**Uncontrolled** (**3 or more** present)
Nasal blockage[1]	Not present or not bothersome[2]	Present on most days of the week[3]	Present on most days of the week[3]
Rhinorrhoea / Postnasal drip[1]	Little and mucous[2]	Mucopurulent on most days of the week[3]	Mucopurulent on most days of the week[3]
Facial pain / Pressure[1]	Not present or not bothersome[2]	Present on most days of the week[3]	Present on most days of the week[3]
Smell[1]	Normal or only slightly impaired[2]	Impaired[3]	Impaired[3]
Sleep disturbance or fatigue[1]	Not present[2]	Present[3]	Present[3]
Nasal endoscopy (if available)	Healthy or almost healthy mucosa	Diseased mucosa[4]	Diseased mucosa[4]
Rescue treatment (in last 6 months)	Not needed	Need of 1 course of rescue treatment	Symptoms (as above) persist despite rescue treatment(s)

[1] Symptoms of CRS; [2] For research VAS ≤ 5; [3] For research VAS > 5; [4] Showing nasal polyps, mucopurulent secretions or inflamed mucosa

Table 4-1 Assessment of current clinical control of CRS.

[Adapted from Fokkens WJ et al ., 2020]

Even with recommended treatments, about 40% of CRS patients still have discomforting symptoms, even after procedures like functional endoscopic sinus surgery[1,94].

Within the context of CRS, patients classified as "difficult-to-treat" are those who face challenges in achieving a satisfactory level of control, even after undergoing appropriate surgery, receiving intranasal corticosteroid treatment, and completing up to two short courses of antibiotics or systemic corticosteroids in the past year[1]. Historically, the only recourse for these patients has been to undergo multiple repetitions of ESS, carrying an elevated risk of perioperative complications and a progressively shorter duration of symptom relief between surgeries. Identifying an uncontrolled patient involves persistent symptoms, visible endoscopic signs of mucosal swelling, and cumulative use of systemic medications for more than three months in a year. Information from various studies consistently shows that approximately 20% of individuals undergoing ESS with concurrent medical therapy exhibit unsatisfactory responses, leading to the need for a revision surgery [19,88]. Recent research suggests a higher rate, estimating that around 40% of CRS patients remain uncontrolled 3-5 years after undergoing endoscopic sinus surgery (ESS). [94]With the advent of disease-modifying therapies, specifically biological antagonists, for the treatment of severe asthma, and considering the common co-occurrence of asthma and CRSwNP, it was a natural progression to assess the influence of these novel treatments on nasal polyps and their effects on patients' quality of life.

This progression subsequently opened the door for additional investigations to confirm the viability of these treatments as a potential therapeutic choice for CRS [95].

The effectiveness of biological agents in treating asthma is extensively documented in literature[96]. Numerous trials[97] have demonstrated both subjective and objective improvements in patients with CRSwNP, whether or not they have asthma, along with a favorable safety profile. Due to these findings, biologic agents have been suggested as an additional treatment option for patients with CRSwNP.

In Italy, 3 monoclonal antibodies (mAb) are approved by the Italian Agency for the Drugs (AIFA) for the treatment of severe uncontrolled CRSwNP in clinical practice: dupiluman (Anti-IL-4/IL-13) omalizumab (anti-IgE) and mepolizumab (anti-IL5).

4.1.1 Anti-IL-4/IL-13: dupilumab

Dupilumab, administered through subcutaneous injection, is a fully human monoclonal antibody of the IgG4 subclass. It specifically targets the IL-4Rα subunit nit. Through the binding and blockade of this subunit, dupilumab inhibits IL-4 and IL-13, which are the main instigators of type 2 inflammatory diseases in humans. These conditions include asthma, atopic dermatitis, and CRSwNP (Figure 4-1).

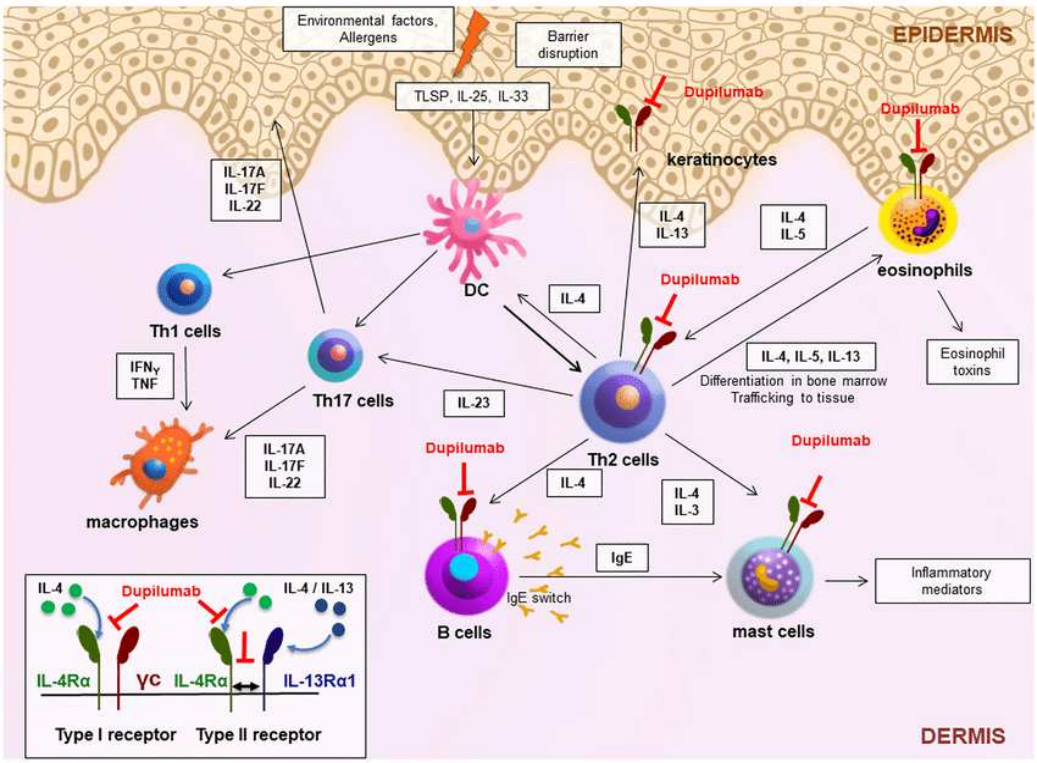

Figure 4-1 Mechanism of dupilumab action.

Dupilumab inhibits both the binding of IL-4 to the IL-4Rα subunit, shared by type I and type II receptors, and the dimerization of type II receptor subunits, as illustrated in the diagram. The cells targeted by dupilumab in CRS include those with type I receptors found on B cells, T cells, dendritic cells, granulocytes, and keratinocytes. Additionally, type II receptors are present on Langerhans cells, monocytes, and macrophages. There is a suggested hypothesis that the inhibition of Th2-mediated pathways by dupilumab may lead to an augmentation of Th1/Th17 polarization. DC, dendritic cells; Th, T helper

[Adapted from Kychygina A et al ., 2022]

In a phase II clinical trial conducted by Bachert et al.[98], dupilumab was evaluated in patients with CRSwNP who showed no response to intranasal corticosteroids (INCS). Sixty patients were enrolled, and they were randomly assigned to receive either biweekly subcutaneous injections of dupilumab or a placebo. The group treated with dupilumab showed a significant decrease in nasal polyp size, which was the primary endpoint of the study. This reduction became clinically evident starting from the fourth week of treatment.

Following this, the phase 3 trials SINUS-24 and SINUS-52[99] demonstrated the efficacy and safety of subcutaneous dupilumab administered at a dosage of 300 mg every 2 weeks, in comparison to a placebo, for individuals with severe CRSwNP that remained uncontrolled with standard care (including intranasal corticosteroids, prior systemic corticosteroid use, and/or surgery). Patients experienced notable improvements across all primary and secondary measures at both the 24-week and 52-week intervals.

Significantly, treated patients exhibited substantial improvements compared to those in the placebo group concerning the severity of nasal congestion/obstruction, nasal polyps score (NPS), sinus opacification, and loss of smell. As for the two primary endpoints, NPS and NCS, remarkable improvement was evident as early as the fourth week of treatment. The University of Pennsylvania Smell Identification Test (UPSIT) score demonstrated a notable enhancement starting from the second week of observation, persisting until the conclusion of the treatment period in both studies for all endpoints.

Concerning the sense of smell, a significant shift from anosmic to non-anosmic status was observed in 62% of patients undergoing dupilumab treatment. Furthermore, the administration of dupilumab resulted in a notable decrease in the dependence on systemic corticosteroids and the requirement for revision surgery in comparison to the placebo group. Validating the mode of action of dupilumab, an examination of biomarkers in individuals subjected to dupilumab treatment within the SINUS-52 study demonstrated a uniform reduction in the levels of total serum IgE, periostin, TARC, and plasma eotaxin-3 at both the 24-week and 52-week marks. Additionally, there was a decline in the concentrations of ECP, total IgE, eotaxin-3, and IL-5 in nasal secretions at week 24. Additionally, in the SINUS-24 study, discontinuing dupilumab at week 24, in contrast to the placebo, led to a diminishing efficacy across all observed endpoints up to week 48.

Similar patterns have been observed with real-world use; a recent a Phase IV real-life, observational, multicenter study[100], support the effectiveness of dupilumab as an add-on therapy in patients with severe uncontrolled CRSwNP. The study demonstrated a reduction in polyp size and improvements in quality of life, symptom severity, nasal congestion, and sense of smell in 96.9% of patients at the 12-month mark, based on the EPOS 2020 criteria.

In conclusion, data from the literature[101,102] strongly support the benefits of integrating dupilumab into the routine standard of care for patients with CRSwNP. This innovative approach effectively targets the full range of clinical manifestations associated with the disease, including frequently linked type 2 lower airway comorbidities.

Dupilumab achieved a significant milestone by becoming the first biologic to receive approval from the Food and Drug Administration (FDA) on June 26th, 2019, specifically for the treatment of adults grappling with inadequately controlled CRSwNP. Subsequently, the European Medicines Agency (EMA) provided a favorable opinion on dupilumab on October 26th, 2019, endorsing its use as an additional therapy with INCS for adults dealing with severe CRSwNP. This recommendation is especially beneficial for those individuals for whom systemic corticosteroids and/or surgery fail to provide sufficient control over the disease.

In Italy, the Italian Agency of Drugs (AIFA) granted approval for dupilumab on December 9th, 2020, for adult patients with severe CRSwNP (assessed by NPS ≥ 5 or a SNOT-22 score ≥ 50) who do not achieve adequate disease control with systemic corticosteroids and/or surgery, in addition to background therapy with INCS.

4.1.2 Anti-IgE antibody: omalizumab

Omalizumab, introduced in the US in 2003, is a monoclonal antibody with proven efficacy for both adults and pediatric patients experiencing severe allergic asthma. Specifically designed to address IgE-mediated diseases, its mechanism involves reducing the concentration of free IgE in both blood and tissues[103].

Omalizumab was designed to bind strongly to free IgE, effectively preventing allergen-specific IgE from attaching to FcεRI. This binding reduces the levels of free IgE, leading to a decrease in the number of FcεRI receptors on mast cells, basophils, and antigen-presenting cells[104]. By doing so, Omalizumab inhibits mast cell degranulation, which is responsible for vasodilation with tissue edema, leakage of serum proteins, extravasation of leukocytes, smooth muscle contraction (bronchoconstriction), and pruritus. Additionally, it hinders the binding of IgE to the low-affinity IgE receptor (FcεR2) found on B and T lymphocytes, macrophages, and eosinophils, preventing the activation of these cells by allergens and, consequently, reducing chronic inflammation[105].

Omalizumab does not directly activate mast cells or basophils, as it does not bind to cell surface IgE[106]. Additionally, Omalizumab exhibits extra anti-inflammatory effects by inducing eosinophil apoptosis and downregulating inflammatory cytokines such as IL-2 and IL-13[107]. Due to its diverse potential mechanisms for limiting Type 2 inflammation, Omalizumab has been explored not only in asthma but also in CRSwNP (figure 4-2).

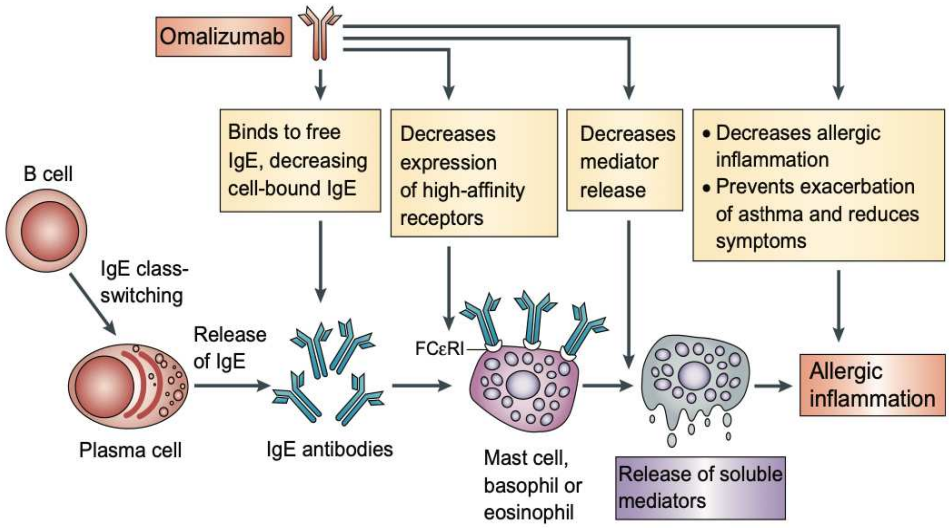

Figure 4-2 Mechanisms of action of omalizumab

[Adapted from Thomson N.C et al ., 2012]

Omalizumab demonstrated efficacy in reducing nasal polyp size and improving symptoms in two randomized, placebo-controlled, phase 3 trials (POLYP 1 and 2) involving patients with CRSwNP who had an inadequate response to nasal corticosteroids[108]. In these trials, omalizumab (administered subcutaneously at doses ranging from 75-600 mg every 2 or 4 weeks, adjusted based on pre-treatment serum IgE levels and body weight) was compared to a placebo in patients with severe CRSwNP that was not controlled with standard background therapy using INCS. Both POLYP 1 and 2 achieved their coprimary endpoints, showing significant reductions in NPS and average daily NCS from baseline at week 24.

Furthermore, secondary endpoints, including the SNOT-22, average daily sense of smell, postnasal drip, runny nose, and the UPSIT, demonstrated significant improvement. Moreover, a diminished requirement for surgical intervention by week 24 (NPS of ≤ 4 and a significant improvement in SNOT-22) was noted in 19% of individuals treated with omalizumab compared to 3% in the placebo group in POLYP-1, and 17% versus 3% in POLYP-2. The improvements in NPS, NCS, and SNOT-22 were consistent among patients with CRSwNP, with or without comorbid asthma. Combined safety data from POLYP 1 and 2 exhibited comparable profiles between omalizumab and placebo. Adverse reactions aligned with those observed in clinical trials involving patients with moderate to severe allergic asthma or chronic spontaneous urticaria. Following these trials, patients participated in an open-label extension study, where they either continued omalizumab or switched from placebo to omalizumab for 28 weeks. They were subsequently monitored for an additional 24 weeks after discontinuation of omalizumab[109]. This extended study revealed a gradual but further improvement in polyp and congestion scores from week 24 to week 52, suggesting that the complete benefits of treatment might not be fully realized until after 6 months. Conversely, upon discontinuation of treatment, NPS, NCS, and SNOT-22 progressively worsened, although they never returned to pre-treatment levels.

Omalizumab received FDA approval for the treatment of CRSwNP on November 30th, 2019. Furthermore, the EMA issued a positive opinion on omalizumab on July 7th, 2020, for use in Europe.

4.1.3 Anti-IL-5: mepolizumab

Mepolizumab is a completely humanized IgG1/k-class monoclonal antibody designed to selectively bind to interleukin-5 (IL-5). This specific binding prevents IL-5 from engaging with its receptor alpha (IL-5Rα), ultimately inhibiting downstream activities. As a result, the proliferation, differentiation, activation, and survival of eosinophils are blocked[110]. Because of its high-affinity binding, mepolizumab appears to avoid interference with other cytokines and exhibits a favorable safety and tolerability profile.

Mepolizumab has shown various clinical advantages across eosinophilic diseases. In severe eosinophilic asthma, it is linked to a decreased risk of exacerbations, enhanced symptom control, and improvements in health-related quality of life[111]. Additionally, in eosinophilic granulomatosis with polyangiitis, mepolizumab has demonstrated an increased duration of remission and a higher proportion of patients achieving remission[112]. Furthermore, in hypereosinophilic syndrome, mepolizumab is associated with a reduced occurrence of flares[113].

The mepolizumab clinical development plan for CRSwNP includes a crucial Phase III study (SYNAPSE)[114], along with supportive data from Phase II studies[115]. In a Phase II study conducted by Bachert et al.[115], 105 patients with severe bilateral CRSwNP , eligible for surgery under predefined conditions (NPS > 3 or more in one nostril and a VAS > 7), were administered intravenous mepolizumab at a dosage of 750 mg every 4 weeks. The outcomes revealed a substantial decrease in the requirement for surgery and a remarkable enhancement in symptoms when compared to the placebo group. Furthermore, Gevaert et al.[116] assessed intravenous mepolizumab at the same dosage in 30 adults with severe uncontrolled CRSwNP, revealing a significant enhancement in the mean total nasal polyp score in 60% of mepolizumab-treated patients compared to 10% in the placebo group.

The SYNAPSE study[114], a 52-week phase 3 trial, was a randomized, double-blind, placebo-controlled investigation that included subcutaneous administration of mepolizumab at a dose of 100 mg. This study involved 407 adult patients with highly symptomatic CRSwNP that remained uncontrolled despite prior surgery and treatment with INCS. Patients meeting the criteria had undergone a surgical procedure within the last decade, experienced recurring nasal polyps despite standard care, and necessitated nasal polyp surgery (with an overall VAS score exceeding 7 and a NPS of at least 5, with a minimum score of 2 on each side). The results were initially presented at the European Respiratory Society Congress held on September 7-9, 2020[117]. Approximately 71% of the study participants had asthma. The study successfully met its primary endpoints, with 50% of patients on

mepolizumab experiencing a minimum 1-point improvement in the total polyp score. Additionally, 71% of patients showed an enhancement of at least 1 point in the nasal obstruction Visual Analog Scale (VAS) score. Mepolizumab treatment demonstrates clinical benefits, evident in a statistically significant decrease in the number of patients needing nasal surgery. Moreover, there is a notable reduction in the use of systemic corticosteroids and antibiotics. Crucially, from the patients' standpoint, there is a significant improvement in overall symptoms. This improvement is reflected in statistically significant reductions in symptom VAS scores, encompassing composite and loss of smell, as well as improvements in SNOT-22 scores.

A post hoc analysis[118] of the MUSCA study[111] and a meta-analysis of MUSCA and MENSA trials[119] for severe eosinophilic asthma assessed the occurrence of concurrent nasal polyposis through a combination of physical examination and a thorough review of medical records. The prospective application of mepolizumab in managing CRSwNP was evaluated by analyzing alterations in the quality of life related to sinonasal symptoms during the study durations, using the SNOT-22. In the trials analyzed, nasal polyposis was present at baseline in 19% of patients. For patients with nasal polyposis, the average SNOT-22 score exhibited a decline of 13.7 points from the baseline following 24 weeks of supplementary mepolizumab therapy, as opposed to a decrease of 1.9 points observed in those administered a placebo. Moreover, mepolizumab not only substantially reduced the overall rate of asthma exacerbation but also demonstrated a more pronounced impact in individuals with coexisting nasal polyposis compared to those without. Additionally, the enhancement in the quality of life related to upper and lower airway symptoms was more significant in patients with nasal polyposis than in those without this condition.

In Italy, mepolizumab received approval from the AIFA on March 1st, 2023, for adult individuals with severe CRSwNP. This approval is applicable to those assessed with an NPS ≥ 5 or a SNOT-22 score ≥ 50, and for whom standard therapy with systemic corticosteroids (SCS) and/or surgery fails to achieve sufficient disease control. This approval is contingent on concurrent background therapy with intranasal corticosteroids (INCS).

4.2 SURGERY AND BIOLOGICS

The current treatment strategy for CRSwNP aims to reduce local inflammation and achieve disease control. Primary treatment for CRSwNP includes medical therapy, such as topical intranasal corticosteroids, nasal irrigation, antibiotics, and short courses of oral corticosteroids. ESS plus medical therapy is always reserved for patients who are unresponsive to appropriate medical therapy[120]. Numerous studies have demonstrated that ESS plus medical therapy significantly improves symptoms and health related Qol in patients with CRSwNP[85,121,122]. However, there are still debates whether biologic treatment could be initiated before or after surgical removal of the polyps[123]. In the most recent EPOS update, in collaboration with the EUFOREA expert panel, there is continued support for the recommendation made in the 2020 EPOS to reserve biologics for patients who have undergone sinus surgery[1,85]. This recommendation is based on three main arguments: firstly, the observed positive effects of suitable medical treatment and sinus surgery in a majority of patients with CRSwNP[97]; secondly, the high cost associated with biologics[124]; and lastly, the uncertainty surrounding the optimal duration of biologic treatment and potential long-term risks[125].

ESS typically results in rapid symptom relief, particularly in alleviating nasal obstruction, and it enhances the overall management of the disease achieved through prolonged use of local corticosteroids. Following surgery, the sinuses become more accessible to local treatments, thereby improving disease control through the extended use of INCS; non-recurrence is observed in 60%-70% of cases within a 5-year period[126]. Additionally, the utilization of high-volume corticosteroid nasal irrigation or systemic steroids post-surgery seems to decrease the recurrence rate[123].

Therefore, in cases where a patient has not previously undergone surgery, the consideration of endoscopic sinus surgery is justified. ESS enhances the control of the disease by allowing the distribution of INCS across the entire sinonasal mucosa. Considering this, it may be challenging for patients who have not undergone surgery to achieve complete control with INCS. The scenario differs for CRSwNP patients who have already undergone at least one previous surgery. Specifically, in cases where a simple polypectomy was performed, and the ethmoidal cells were not adequately opened, the option of revision surgery should be discussed with the patient. On the contrary, if the disease remains uncontrolled despite prior appropriate surgery and consistent adherence to INCS, changing to a biologic is advisable. For individuals who have undergone multiple surgeries, resulting in a substantial negative impact on their quality of life and a brief interval of symptom control between interventions, the use of a biologic is recommended, irrespective of the current endoscopic nasal

polyp scores. Similarly, for patients previously treated with surgery who have reported significant complications following ESS, transitioning to biologics is recommended[88].

In patients with CRSwNP who have undergone at least one previous surgery, it is essential to determine the surgical technique employed and assess the appropriateness of the procedure.

This continues to be a topic of discussion, mainly due to the absence of a universally agreed-upon definition for what constitutes "appropriate surgery." Additionally, the available literature lacks precise data on the percentage of cases in which surgery might be considered inadequate for individuals with CRSwNP.

Recently, Reitsma et al.[127] proposed a new score based on post-operative CT examination to define the extent of surgery. This scoring system, known as ACCESS (Amsterdam Classification on Completeness of Endoscopic Sinus Surgery), resembles the Lund-Mackay score (LM) but is based on bony boundaries rather than sinus opacification. The evaluated sites include the ostiomeatal complex, maxillary sinus, anterior ethmoid, posterior ethmoid, sphenoid sinus, and frontal sinus[128]. Each site is assigned a score from 0 to 2: 0 denotes a "functionally open" site that does not necessitate further surgery; 1 indicates that previous surgery addressed the site but was inadequate for ensuring proper opening; and 2 is assigned if the site was not addressed. The ostiomeatal complex can only receive a score of 0 or 2, similar to the LM score. In total, 6 sites per side are assessed, and by summing the scores, the total score can range from 0 to a maximum of 24. A lower total score suggests that comprehensive surgery was conducted for most sinuses, while higher scores indicate a reduced extent of prior surgery. Examining the appropriateness of surgery is very important, especially when the surgical procedure was limited. In such cases, discussing the potential need for additional surgery with the patient becomes pertinent. Regarding the extent of surgery, it is crucial to note that a more conservative and substantially inadequate surgical approach, such as polypectomy, is more likely to lead to disease recurrence after surgery. Numerous studies[129] have indicated that an extended surgical approach may yield superior results compared to traditional FESS. For instance, in a retrospective study, Jankowski et al.[130] demonstrated that the radical procedure (Radical Endoscopic Sinus Surgery - RESS, involving full ESS including Draf IIA frontal sinusotomy and resection of the inferior two-thirds of the middle turbinate) reduced recurrence rates from 58.3% to 22.7% compared to functional ethmoidectomy. Another study reported a significant reduction in the need for revision surgery, from 12.3% to 4.0% at 36 months, with the radical approach compared to FESS[131].

Extensive surgery, commonly referred to as "re-boot" surgery, has been associated with substantial reductions in eosinophilic cationic protein and IL-5 levels in postoperative nasal secretions.

Jonstam K et al.[132] demonstrated that the reboot technique, emphasizing the removal of sinus mucosa down to the periosteum in all affected sinuses, with or without a Draf III procedure, led to reduced relapse rates compared to conventional mucosa-sparing surgery.

However, there is currently insufficient data to establish that more extensive surgery yields better outcomes for patients with CRSwNP [90]. Patients who had experienced surgery within the three years preceding the current procedure exhibited elevated rates of subsequent surgical interventions. In a multiple regression analysis, the duration between prior operations emerged as a more precise predictor of ensuing revision surgery than the presence of asthma. Additionally, the comorbidity of NSAID-exacerbated respiratory disease (NSAID-ERD) emerged as the most robust predictor for the necessity of further surgery, with more extensive surgical procedures being linked to lower revision rates[133]. Consequently, various factors have been considered as potential influences on the recurrence rate: asthma, NSAID-ERD and especially and the failure to consistently follow intranasal steroid therapy are the prevalent factors strongly linked to early recurrence post-surgery[134].

A meta-analysis including 45 studies with 34,220 subjects concluded that the factors associated with increased recurrence rates included allergic fungal rhinosinusitis (28.7%), aspirin-exacerbated respiratory disease (27.2%), asthma (22.6%), prior polypectomy (26.0%), and surgery prior to 2008 (22.7%), when steroid rinses as medical adjunctive treatment were not used in routine clinical practice[95]. Additionally, the authors examined revision rates for the initial surgery compared to subsequent surgeries and identified a significant difference (14.3% vs. 26.4%, respectively). These findings indicate that revision surgery is a risk factor for subsequent procedures. This "aggressive" presentation may signify a more severe underlying endotype of the condition or could involve individuals who do not adhere to medical treatment or follow-up appointments.

Tsuzuki et al.[135] conducted a study aiming to identify potential indicators of CRSwNP advancement after FESS. The participants were divided into eosinophilic (n = 205) and non-eosinophilic chronic rhinosinusitis (n = 76) groups. In the eosinophilic group, multivariate analyses revealed that young adulthood, bronchial asthma, a high computed tomography (CT) score in the pre-operative stage, and the presence of polyps in the frontal sinus during FESS were predictors of disease progression after surgery. In the non-eosinophilic chronic rhinosinusitis group, the pre-operative CT score and the presence of sphenoid sinus polyps during surgery were identified as significant factors in the univariate analyses, although none emerged as an independent factor in multivariate analysis. Ultimately, the authors confirmed that the post-operative course in non-eosinophilic CRSwNP is more favorable compared to eosinophilic CRSwNP. Furthermore, Lou et al.[136] conducted a retrospective analysis involving 387 patients with CRSwNP, covering a period extending beyond 24 months. Their findings validate that the presence of tissue eosinophilia serves as a key indicator of

severity in CRSwNP, exhibiting a correlation with the recurrence of nasal polyps. According to statistical analyses, a cutoff of 27% tissue eosinophils accurately predicted recurrence, with a sensitivity of 96.7% and specificity of 92.5% (area under the curve = 0.969; $p < 0.001$). Likewise, a total count of 55 eosinophils per high power field was indicative of recurrence, demonstrating a sensitivity of 87.4% and specificity of 97.1% (area under the curve = 0.969; $p < 0.001$). Hence, a tissue eosinophil proportion exceeding 27% of total cells or a tissue eosinophil absolute count surpassing 55 eosinophils per high power field may serve as a reliable prognostic indicator for nasal polyp recurrence within the 2-year post-surgery timeframe.

Moreover, De Corso et al.[137] revealed that the inflammatory pattern before surgery may exhibit varying associations with inadequate disease control. In particular, the persistence of neutrophilia was linked to a relative risk (RR) of inadequate control at 3.10 (CI: 1.24-7.71), sustained eosinophilia demonstrated an RR of 8.42 (CI: 2.72-15.12), and CRSwNP characterized by a combination of eosinophilic and neutrophilic inflammation exhibited a RR of 25.11 (CI: 19.41-30.01). They further validated that asthma, allergies, blood eosinophilia, and the ASA triad were robust predictors of inadequate disease control.

Other points discussed in the EPOS/ EUFOREA 2023 update supporting prior surgery before the use of biologics include the limited knowledge of potential risks associated with prolonged biologic treatment compared to ESS.

Endoscopic sinus surgery for chronic rhinosinusitis has been developed for several decades and its safety has been confirmed. A recent meta-analysis[138] suggested that biologic treatment in CRSwNP had a similar incidence of adverse events to placebo groups, which indicated a great safety and tolerability profile of biologics. While the range of adverse events (AEs), whether serious or non-serious, exhibited differences between the ESS cohort and biologics, there was no statistically significant variance in the increased risk. However, given that previous randomized controlled trial (RCT) studies typically had a follow-up period of around 1 year, additional observations are necessary to conclusively determine the long-term safety of biologics in the treatment of CRSwNP.

Another aspect supporting the use of biologics after the failure of surgery, according to recent guidelines[85,86], is the high cost associated with biologics. A real-world study found that around one fifth of the asthmatics discontinued biological therapy considering the heavy financial burden[139].

In the absence of a cost-effectiveness analysis, the preference for biologics, particularly dupilumab, over ESS is driven by its superior efficacy in improving nasal symptoms and enhancing quality of life. Historically designated for cases where ESS has not proven successful, biologics are increasingly being embraced.

A Markov decision-tree economic model, adopting a cohort-style approach, was utilized to compare the cost-effectiveness of treating CRSwNP. The findings indicate that ESS is more cost-effective, with a cost of $50,436 for 9.80 quality-adjusted life years (QALYs), in contrast to dupilumab, which incurs a cost of $536,420 for 8.95 QALYs[140]. Parasher AK et al. highlighted that, even though there is comparable clinical effectiveness, ESS emerges as the more economically efficient standard of care for patients with CRSwNP who do not respond to medical therapy[141].

In the aggregate, biologics like dupilumab are clinically effective alternatives rather than cost-effective strategies. Although a de-escalation strategy with an increased dosing interval could reduce the direct cost and decrease the financial burden, unfortunately, biological drugs are still not a "curative" treatment therapy for CRSwNP, which indicates that continuous biological treatment is still warranted for the maintenance of therapeutic benefits for this disease[142]. Future research is imperative to examine both the direct and indirect costs associated with ESS and biologics. In practical clinical settings, it is essential to incorporate considerations of benefits, costs, and risks into the patient-centered decision-making process when selecting appropriate treatment modalities.

In summary, for patients who have undergone multiple surgeries without achieving sufficient control, experienced significant complications post-ESS, or have coexisting debilitating type 2 conditions like asthma, contraindications to surgery, or a heightened reliance on systemic corticosteroids, transitioning to biologics is highly recommended. Conversely, in instances of inadequate control of sinonasal symptoms following biological treatment, the options of salvage surgery or transitioning to an alternative biologic should be contemplated after a 4-6 month period of therapy[88].

5. CRITERIA TO EVALUATE RESPONSE TO BIOLOGICS

Over time, ENT specialists have realized that the aim of treating a complex and persistent condition like CRSwNP is not necessarily to cure it. Rather, the focus is on managing distressing symptoms, enhancing overall quality of life by improving cognitive, physical and social functions, preventing complications, and minimizing the risk of recurrence after surgery[1].

In EPOS 2012[6], the notion of disease control was introduced for the first time through the statement: "*a disease state in which the patient is symptom-free or experiences non-bothersome symptoms, possibly accompanied by a healthy or nearly healthy mucosa, and requiring local medication only*."

As a result, the evaluation included various criteria: management of the four primary sinonasal symptoms (nasal blockage, rhinorrhea/postnasal drip, facial pain/pressure, smell), assessment of sleep disturbance and/or fatigue, examination of the endoscopic appearance of nasal mucosa, and consideration of medication usage.

Depending on the presence or absence of these factors, patients were classified into categories of well-controlled, partly controlled, and uncontrolled rhinosinusitis.

In 2019, the EUFOREA expert panel introduced criteria for evaluating the response to biologics, focusing on various factors[83]. These criteria encompass reduced nasal polyp size, decreased reliance on systemic corticosteroids (SCS), improved quality of life, enhanced sense of smell, and mitigated impact of comorbidities. Notably, the EPOS adopted these criteria in 2020[1].

Initially, the evaluation of biologic response occurred after 4 months of initiating therapy, and if there was an inadequate response, the treatment was discontinued due to the considerable cost of biologics.

In 2021, the EUFOREA expert panel extended the initial assessment duration to 6 months of treatment and established specific limits for each criterion. The authors specified that treatment continuation should be considered when a distinct improvement in at least one of the following criteria is observed: an increase in smell score by > 0.5, a reduction in nasal corticosteroid (NCS) use by > 0.5, a decrease of 1 point in NPS, a reduction of > 8.9 in SNOT-22, and a decrease of > 2 cm VAS (figure 5-1).

When assessing the effectiveness of biologics, it is important to recognize that clinical measures may not fully capture the patient's experience. Therefore, the authors suggest initiating conversations with the patient to better understand their perceived improvements.

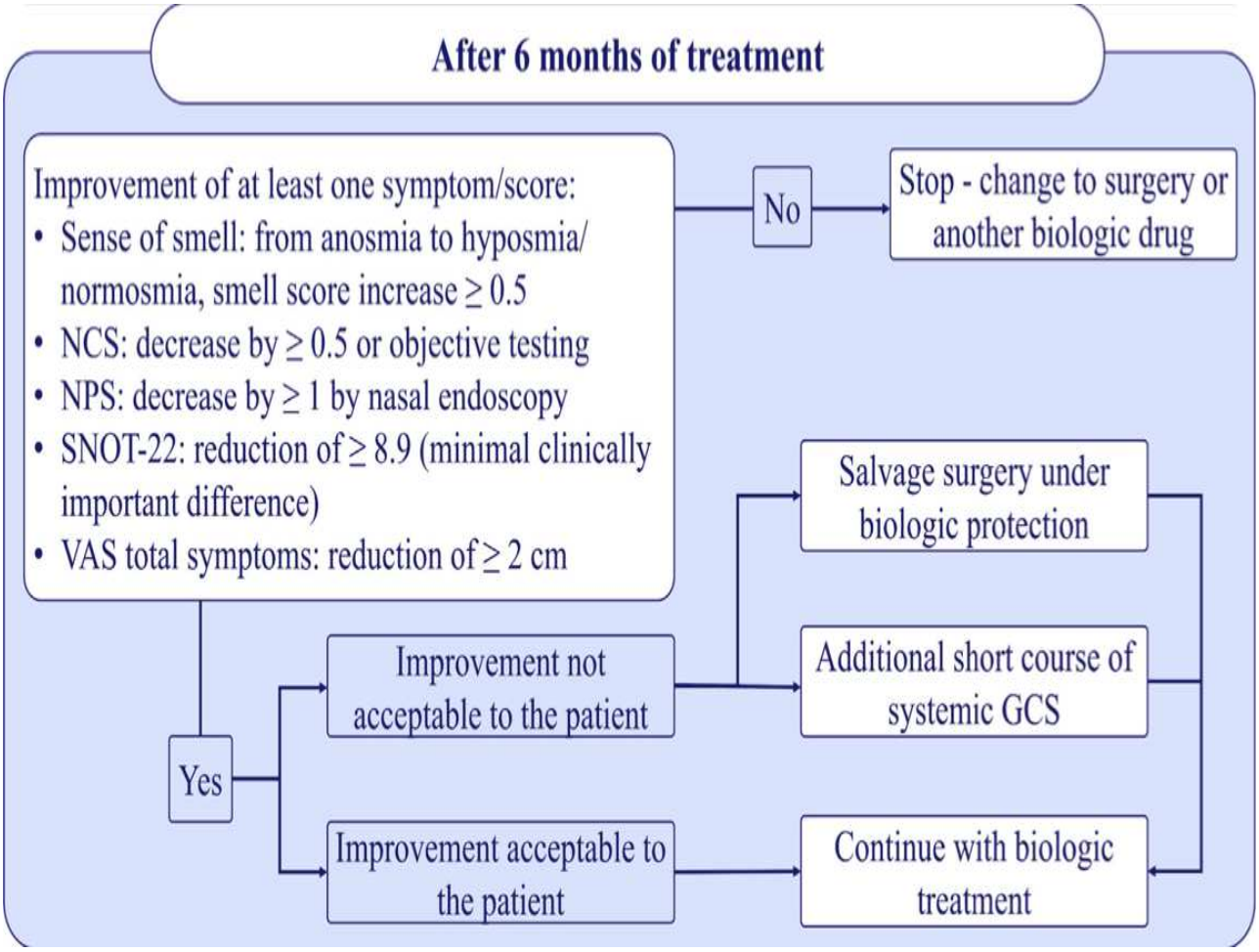

Figure 5-1 Response criteria for biologicals in CRSWNP

[Adapted from Bachert C et al, 2021]

In connection with the improvement in various parameters, the response to biological treatment is classified:

- No response: 0 criteria;
- Poor- Moderate response: 1-3 criteria;
- Good-Excellent response: 4-5 criteria .

If patients show no response to any of the criteria, the biologic should be interrupted and/or substituted, or consideration can be given to planning a revision surgery.

A subset of patients may indeed require surgery, which the authors termed "*salvage surgery under biologic protection,*" although there is limited data regarding the long-term benefits of this approach.

If there are certain improvements (e.g., poor-moderate response), various strategies can be employed, taking into consideration the patient's preferences. Indeed, if patients are willing to accept improvement, even in the case of a minimal response, the treatment should be extended until 12 months, at which point efficacy needs to be reassessed.

To proceed with the treatment, the following conditions must be satisfied: NPS < 4; NCS < 2; VAS < 5; SNOT-22 < 30. If these criteria are not fulfilled, surgery should be contemplated, or an alternative biologic option should be explored.

Adverse effects rarely necessitate discontinuation of biologic treatment. Transient hypereosinophilia is a recognized phenomenon during anti-IL4 alpha treatment, typically occurring between 2 and 6 months into the treatment period[143]. However, if it persists, especially with accompanying symptoms, there is a potential risk of organ damage[144]. As a precaution, it is recommended to monitor blood eosinophil levels at one and three months after initiating biologic treatment for every patient. Patients with elevated baseline eosinophils (>500/mL) and those previously on chronic systemic corticosteroids before starting biologic treatment should undergo more frequent monitoring. Additionally, a comprehensive evaluation of symptoms/signs of vasculitis/hypereosinophilia is advised at each visit. After the initial three months, the monitoring frequency can be adapted based on the blood eosinophil count. Even though there is a lack of precise recommendations on monitoring frequency owing to limited data, a prudent approach implies intervals of approximately 2-4 weeks when blood eosinophils are elevated (above 1500 cells/mL)[86]. If blood eosinophils surpass 3000, contemplation may be given to dose adjustments (to every four weeks) or the administration of a brief course of systemic corticosteroids. Persistent high blood eosinophil levels or symptoms indicative of vasculitis should prompt consultation with an immunologist[145].

Data on biomarkers, other than blood eosinophil levels, for screening hypereosinophilic syndrome are limited, necessitating further research.

In summary, the recent guidelines aimed to standardize the assessment of biologic efficacy and the decision-making process, considering both the results and the patient's comfort and preferences. Future evaluations may be necessary to validate these criteria or establish more stringent indications. Real-life experiences will play a crucial role in supporting this shared decision-making model.

6. MATERIALS AND METHODS

6.1 *Study Design*

The present study is a bicentric, retrospective not for profit study. The study was carried out by the Oto-Rhino-Laryngology Departments of 2 hospitals: Ospedale "Annunziata" of Cosenza and Ospedale "Misericordia" of Grosseto between April 2023 and December 2023. All participants in the study were treated with one of the biologics (dupilumab, omalizumab or mepolizumap) approved in Italy by AIFA for severe uncontrolled CRSwNP and had a minimum follow-up period of 9 months.

6.2 Study Population

The diagnosis of CRSwNP was verified according to the EPOS criteria[1]. Patients exhibiting secondary CRS were precluded from inclusion in the study. Every patient included in the study exhibited evidence of type 2 inflammation, determined by either laboratory results (elevated levels of blood or tissue eosinophils; total IgE > 100) or clinical observations (positive skin prick test for allergies, presence of late-onset asthma, NSAID intolerance).

The administration of monoclonal antibody treatment followed the criteria outlined in the EPOS 2020 guidelines: all patients who were ≥ 18 years of age, presenting bilateral polyposis with a minimum NPS of 2 on each side, having undergone at least one prior endoscopic sinus surgery (ESS), and meeting at least 3 of the following 5 criteria: evidence of Th2 inflammation (tissue eosinophilia > 10/hpf OR blood eosinophilia ≥ 150 cells/mm3 OR total IgE ≥ 100 kU/L), requirement for systemic corticosteroids (≥ 2 courses per year OR ≥ 3 months of low dose systemic corticosteroids) or contraindication to steroids, significantly impaired quality of life (SNOT-22 ≥ 40), substantial loss of smell (anosmic on olfactory test), and a diagnosis of comorbid asthma (asthma necessitating inhaled corticosteroids).In real-world situations, we considered the following conditions as reasons to exclude individuals from treatment: a history of radio-chemotherapy for cancer within the 12 months preceding therapy initiation; ongoing immunosuppressive therapy; concurrent long-term corticosteroid use for chronic autoimmune diseases; and pregnancy or breastfeeding.

The biologics injection were administered by the patients themselves at home according to the therapeutic regimen specified by AIFA; namely, dupilumab 300 mg subcutaneously every 4 weeks,

mepolizumab 100 mg every 4 weeks and omalizumab depending on total IgE and body weight every 4 weeks. The therapeutic approach was complemented by the use of topical intranasal corticosteroid therapy using mometasone furoate nasal spray, administered twice daily, totaling 200 mg per day.
In accordance with the EPOS/EUFOREA 2023 guidelines, the response to treatment was assessed after 6 months from the initiation of therapy, except in cases where adverse events occurred.
If there was no response, the patient received counseling and was presented with the option of revising the monoclonal antibody or undergoing surgery when deemed necessary. In instances of a partial response, the possibility of continuing the treatment was discussed with the patient.
The study adhered to the principles of the Declaration of Helsinki. Informed consent, safeguarding clinical data usage, was obtained from all patients during the initial data collection. The analysis of clinical data was carried out in a completely anonymous manner..

6.3 Primary Outcome Parameters

The primary objectives of the present study were change in quality of life, as assessed by SNOT-22[146] and change NPS [147] as differences vay from baseline to 4 weeks, 3, 6 and 9 months of treatment.
We applied the validated Italian version of the SNOT-22 questionnaire, encompassing a potential overall score range from 0 to 110[148]. This questionnaire comprises 22 queries addressing symptoms specific to rhinosinusitis and aspects related to the quality of life associated with health. Patients were asked to answer the questions at each time in a determined point of data acquisition with reference to the past 14 days, considering both the severity of symptoms and the frequency of occurrence. A change in the score of 8.9 points is considered as a minimum important clinical change (MCID).
The size of nasal polyps was measured using a 0°/30° rigid nasal endoscope and evaluated with the NPS. When evaluating each nostril, we assign scores based on the size of polyps, ranging from 0 (no polyps) to 4 (complete blockage of the nasal cavity). These individual scores are combined to derive the bilateral NPS, with a possible scale from 0 to 8. Improvement in endoscopy is determined by a decrease of at least 1 point in the NPS score.
Another primary objective of this real-world study was to investigate whether monoclonal antibodies could improve olfactory functions. To assess olfactory function, we used the Sniffin' Sticks-12 Identification Test (SSIT-12)[149].
The SSIT-12 is a test designed for olfactory identification, comprising 12 distinct everyday odors. For each odor, the patient is presented with a multiple-choice question featuring four alternative descriptions, and they choose the most fitting answer. This results in a score of 0–12 possible correct

answers, with lower score corresponding to more severe olfactory impairment. We defined a normosmia (SST-12 ≥ 11), an hyposmia (10 > SST-12 > 6), or an anosmia (SST-12 ≤ 6).

6.4. Secondary Outcome Parameters

Various parameters were recorded for each patients , including age, sex, clinical history, the number of sinus surgeries conducted before and during treatment, prior systemic corticosteroid treatments, and the presence of comorbidities such as asthma, intolerance to non-steroidal anti-inflammatory drugs (NSAIDs), and aspirin-exacerbated respiratory disease (AERD). Additionally, the levels of total blood eosinophils and total serum IgE were documented. These factors were identified as potential confounders that could influence the study outcomes.

In patients with comorbid asthma, the Asthma Control Test (ACT)[150] was used (range 5-25). The ACT is a validated questionnaire, that allows the definition of asthma control.

The ACT consists of 5 items related to asthma control: activity limitation, shortness of breath, waking up because of asthma symptoms, use of asthma reliever medication, and a global assessment of control. The ACT items evaluate symptoms in the past 4 weeks and are scored from 1 to 5. The overall score serves to classify asthma control as perfectly controlled (25 points), well-controlled (20–24 points), or poorly controlled (≤19 points). Furthermore, secondary outcomes included the assessment of discontinuation or modification of monoclonal antibody treatment and the evaluation of adverse events.

6.5 Baseline and follow-up visits

At the baseline visit medical history was assessed, including smoking history, presence of comorbidities with a focus on asthma, sensitization to common inhalants, presence of allergy or intolerance to acetylsalicylic acid (ASA) or other NSAIDs and number, type, and timing of sinonasal surgeries. Patients were scheduled for follow-up visit in 4 weeks, 3, 6 and 9 months. At baseline and follow–up, patients underwent endoscopic examination, quality of life assessment, evaluation of nasal obstruction and olfactory assessment, and blood tests for peripheral eosinophilia and total IgE levels. In asthmatic patients ACT was performed at clinical visit. Any adverse events were also documented at each visit.

6.6 Statistical Analysis

Statistical analysis was conducted utilizing Grafpad Prism 9.5.1, software with comparisons between variables conducted through Student's t-tests when considered appropriate. The data are presented as mean ± SD, and statistical significance was determined by * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.0001$.

7. RESULTS

During the observation period, between April 2023 and December 2023, 33 patients (m = 18, f = 16, mean age 44.69 years ± 17.09) with uncontrolled and severe CRSwNP were treated. Patients were followed for 9 months from the start of the treatment.

Fourteen patients were randomly assigned to receive dupilumab (group A), 11 patients received omalizumab (group B), and 8 received mepolizumab (group C). No patient discontinued the study after 9 months of follow-up.

The demographic and clinical baseline characteristics of the entire cohort and the specific characteristics of the three patient groups are shown in Table 7-1. No significant difference in clinical or demographic characteristics were observed amongst the three groups (p-value > 0.05).

All patients had previously undergone ESS, with an average of 2 previous endoscopic surgical procedures (min 1, max 4 , M = 2.06 ± 0.9).

All questionnaires, tests, and clinical examinations showed a progressive and significant improvement from baseline in each of the three of patients' groups treated with the three biologics studied.

Mean pre-treatment SNOT-22 scores were 60.28 ± 3.84 in patients treated with dupilumab (group A), 58.82 ± 3.68 in patients treated with omalizumab (group B) and 57.62 ± 2.59 in patients treated with mepolizumab (group C). The total SNOT-22 score decreased after 4 weeks and decrease progressively after 3 and 6 months of therapy in each group, reaching a total score of 12,07 ± 3.08 in group A, 17.81 ± 2.12 in group B, and 18 .37 ± 2.68 in group C at 9 months of therapy.

In group A the values for the mean changes from baseline from -14.71 at 4 weeks, to -24.71 at 3 month, - 33.64 at 6 months and - 48.21 at 9 months. These differences were significant from 4 weeks to 9 mounths of follow-up (t-test - $p \leq 0000.1$).

Table 7-1 Baseline characteristics of study population stratified by study group.

	TOTAL	GROUP A DUMIPUMAB	GROUP B OMALIZUMAB	GROUP C MEPOLIZUMAB
N patients	33	14	11	8
Age: y	44,69 ± 17.09	47.5 ± 17,19	44,80 ± 17.31	45.3 ± 16.44
sex M, F *n*. (%)	18 (54.5%), 16 (45.5%)	8 (57.1%), 6 (42.9%)	6 (54,.5%), 5 (45.5%)	4 (50%), 4 (50%)
Smoke habits: n. (%)				
smoker—ex smoker	20 (60,6%)	9 (64,2%)	6 (54,6%)	5 (62.5%)
nonsmoker	13 (39,4%)	5 (35.8%)	5 (45,4%)	3(37.5%)
Nasal polypoid surgery				
1	11 (33.4%)	4 (28.6%)	5 (45,5%)	3 (37.5%)
2	12 (36.3%)	6 (42.8%)	4 (36.3%)	2 (25%)
3	7(21.2%)	3 (21.5 %)	1 (9.1%)	2 (25%)
4	3 (9.1%)	1 (7.1%)	1 (9.1%)	1 (12.5%)
Systemic corticosteroid in the last 2 years	33 (100%)	14 (100%)	11 (100%)	8 (100%)
Comorbidity, n (%)				
non	4 (12,1%)	2 (14,2%)	1 (9.1%)	1 (12.5%)
asma	24 (73%)	11 (78.5%)	8 (72.7%)	5 (62.5%)
NSAID intolerance	3 (9.1%)	1(7.1%)	1 (9.1%)	1 (12.5%)
allergic rhinitis	15 (45.4%)	7 (50%)	4 (36.3%)	3 (37.5%)
SNOT-22 total score before therapy	58,9 ± 3.37	60.28 ± 3.84	58.82 ± 3.68	57.62 ± 2.59
VAS nasal congestion before therapy (scale 0–10)	5.4 ± 0.5	5,6 ± 0.6	5.5 ± 0.6	5.3±0.4
Smell Test Score before therapy Sniffin' Sticks Screening 12 (Scale 0–12)	4.5± 2.1	4.8 ± 3.2	4.4 ± 1.7	4.5± 1.3
Baseline Blood eosinophils (n/ml)	462± 46.78	433 ± 24.87	453 ± 52.54	500 ± 62.94
Baseline Blood IgE (IU/ml)	134 ± 50.06	128 ± 40.6	145 ± 65.0	130± 44.6

In group B and in group C the total SNOT-22 score decreased significantly after 3 months of therapy (t-test - $p < 0.0001$) (Table 7-2). Comparing the three groups at 9 months using ANOVA tests and Tukey's multiple comparisons test, we find no statistically significant difference between SNOT-22 values of three groups at 9 months (A vs. B p=0.6632; A vs. C p=0.6458; B vs. C p=0.9942).

SNOT - 22	GROUP A DIPILUMAB Δ(difference compared to T0)	GROUP B OMALIZUMAB Δ(difference compared to T0)	GROUP C MESOLIZUMAB Δ (difference compared to T0)
T0	60.28	58.82	57.62
4 weeks	45.57 (-14.71)	50.81 (-8.01)	50.11(-7.51)
3 months	35.57 (-24.71)	39.80 (19.02)	39.51(-18.11)
6 months	26.64 (-33.64)	28.45 (-30.37)	29.62(-28)
9 months	12.07 (-48.21)	17.81 (-41.01)	18.37(-39.25)

Table 7-2 Change in mean SNOT-22 score over time in each group of treatmentent and difference (Δ) comparated to baseline (TO)

Endoscopic examination at baseline revealed a median for NPS of 5.6 ± 0.6 in group A, 5.5 ± 0.6 in group B and 5.3 ± 0.4 in group C respectively. In group A the mean NPS significantly decreased to 4.6 ± 0.8 after 4 weeks of therapy ($p < 0.0001$) and continued to decrease to 3.9 ± 07, 2.68 ± 0.8 and to 1.8 ± 1.3 after 3, 6 and 9 months of therapy, respectively. After 4 weeks, 71.4 % of the patients improved by 1 point on the NPS and 21.4% even improved by ≥2 points. After 9 months of treatment, 92% of patients treated with dupilumab improved their NPS by at least 1 point, 64.2% by 2 points and 28.5% by ≥ 2 points.

In group B and C the mean NPS did not significantly decrease, going from 4 weeks (group B: 5.3 ± 0,6; group C: 5.2± 0.2) to 3 months (group B : 5.1 ± 0,5; group C: 4.6 ± 0,6) of follow-up (t-test - p > 0000,1); a statistically significant difference was observed after 6 months of therapy in group B (3.6 ± 0.6; t-test - $p < 0.0001$) and after 9 months in group C (3.2 ± 0,6 $p < 0.0001$). After 9 months of therapy, all patients in both groups B and C improved their NPS by at least 1 point, 54% and 63% by 2 points in group B and group C respectively (Table 7-3).

Comparing the three groups at nine months using ANOVA tests and Tukey's multiple comparisons test, we find a significant difference between patients treated with dupilumab compared to both omalizumab ($p<0.0001$) and mepolizumab (p=0.0004), but no statistically significant difference between patients treated with omalizumab and mepolizumab (p=0.9423) [Figure 7-1].

NPS	GROUP A DIPILUMAB	GROUP B OMALIZUMAB	GROUP C MEPOLIZUMAB
T0	5.6 ± 0.6	5.5 ± 0.6	5.3 ± 0.4
4 weeks	4.6 ± 0.8	5.3 ± 0.6	5.2± 0.2
3 months	3.9 ± 0.7	5.1 ± 0.5	4.6 ± 0.6
6 months	2.6±0.8	3.6 ± 0.6	4.4 ± 0.6
9 months	1.9 ± 0.6	3.1± 0.5	3.0 ± 0.5

Table 7-3 Change in mean NPS score over time in each group of treatment.

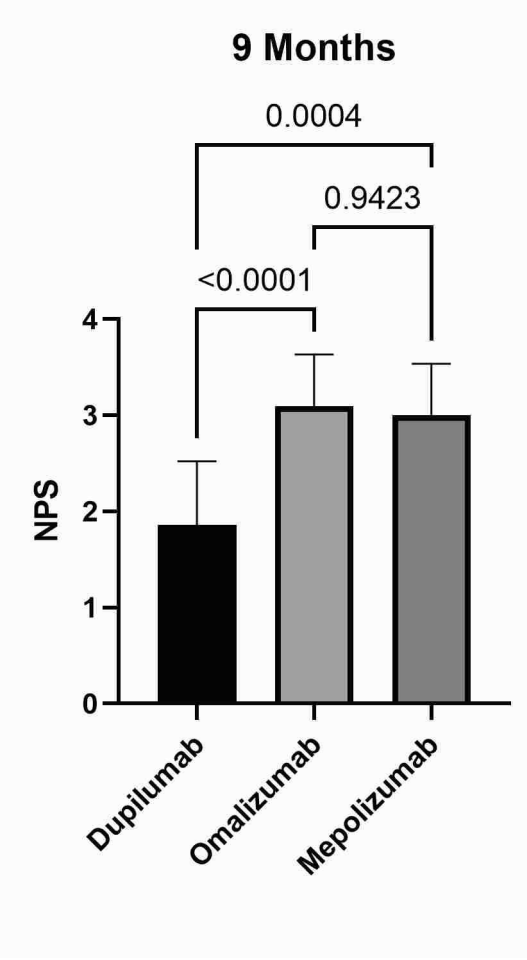

Figure 7-1 Change mean NPS score in each group of treatment afther 9 months of tretmente with standard deviation.

Before treatment, the mean number of odours correctly identified on the SSIT-12 was of 4.8 ± 3.29 in group A, 4.4 ± 1.7 in group B and 4.5 ± 1.3in group C. The number of normosmic, hyposmic, and anosmic subjects for each group is shown in the table 7-4.

	BASELINE	4 WEEKS	3 MONTHS	6 MONTHS	9 MONTHS
GROUP A (14pz)					
- normosmic	1 (7.1%)	1(7.1%)	1 (7.1%)	2 (14.3%)	4 (28.6%)
- hyposmic	4 (28.6%)	6 (42.9)	7 (42.9%)	7 (50.0%)	6 (42.9%)
- anosmic	9(64.3 %)	7 (50.0%)	6 (50.0%)	5 (35.7%)	4 (28.6.%)
GROUP B (11pz)					
- normosmic	0	0	0	0	0
- hyposmic	3 (37.5%)	3 (37.5%)	3 (37.5%)	5 (45.5%)	5 (45.5%)
- anosmic	5 (62.5%)	5 (62.5%)	5 (62.5%)	6 (54.5%)	6 (54.5%)
GROUP C (8pz)					
- normosmic	1(12.5%)	1(12.5%)	1(12.5%)	1(12.5%)	1(12.5%)
- hyposmic	2 (25.0%)	2 (25.0%)	2 (25.0%)	3 (37.5%)	3 (37.5%)
- anosmic	5(62.5%)	5(62.5%)	5(62.5%)	4 (50.0%)	4 (50.0%)

Table 7-4 Change in number and percentage of normosmic, hyposmic, and anosmic subjects over time for each group of treatment

In group A the mean SSIT-12 score before treatment improved significantly from 4.8 ± 3.29 to 5.4 ± 2.91 ($p < 0.0001$) after 4 weeks of treatment, and it continued to improve significantly to 7.71± 3.12 after 9 months of treatment ($p < 0.0001$). The number of anosmic subjects decreased from 64,3 % at baseline to 28.6 % at the end of the 9-month study.

In group B the mean SSIT-12 scores at 4 weeks and 3, 6 and 9 months were 4.5 ±1.81, 4.9± 1.83 , 5.6 ± 1.43 and 6.09± 1.92 , respectively. These results were comparable to data obtained with SSIT-12 in group C which showed a median of 4.6 ±1.71, 4.9± 1.93 , 5.7 ± 1.42 and 6.12±1.42 at the successive intervals. In both group B and group C, the percentage of anosmic subjects changing to hyposmic from 62.5% at baseline was 54.5 % and 50 % at 9 months, respectively.

At baseline, 24 patients (73%) had asthma. In 3 cases (12.5%), asthma was associated with ASA sensitivity, with the subsequent presence of the triad for the diagnosis of NSAIDs-exacerbated respiratory disease (N-ERD). Fifteen patients (45.5%) reported allergies.

Comorbid asthma was monitored using the ACT score (v:5-25). At pretreatment, all asthmatic patients had a score < 20, indicating "not well controlled" asthma (groups A: 15.27 ± 1.66; group B:

15.81±0.78; group C: 15.6 ± 0.8). The mean ACT score improved progressively at each interval, reaching a value indicative of "well - controlled" asthma (score ≥ 20) after 4 weeks in every group (Table 7-5).

	BASELINE	4 WEEKS	3 MONTHS	6 MONTHS	9 MONTHS
GROUP A 11 (78.5%)	15.27±1.66	20.09±1.17	21.08±1.02	21.9±0.89	22.9±0.51
GROUP B 8 (72.7%)	15.81±0.78	20± 0.7	21.85± 1.05	22.1± 0.92	22.75± 0.66
GROUP C 5 (62.5%)	15.6± 0.8	20± 0.63	21.8± 0.4	22.4±0.48	22.8± 0.4

Table 7-5 Change in mean ACT score over time in each group of treatment

At each timepoint, total blood eosinophil counts and plasma IgE levels were measured. For the parameter eosinophilia the mean eosinophil count, in group A it increased from 433 ± 24.87 cells/μL pretreatment to 615± 45.83 cells/μL at the 3 month time point ($p < 0.0001$), but decreased to baseline level 449 ± 31.53 cells/μL at the 6 month timepoint. Plasma IgE levels decreased continuously over the observation period from an initial mean of 128.00 ± 40.6 to 110 ± 33.6 after 4 weeks , 77.60 ± 24.5 after 3 months, 63.90 ± 43.5 after 6 months and finally to 57.75 ± 51,7 after 9 months of therapy ($p < 0.0001$).

In group B, we observed a progressive reduction in mean eosinophil count between the beginning of the treatment (453 ± 52.54 cells/μL) and after 9 months (422 ± 72.44 cells/μL) with a reduction of approximately 7%($p < 0.001$). Alternatively, the total blood IgE data showed an increase from an initial value of 145 ± 65 U/L to 456 ± 66.5 kU/L after 6 months. After 9 months of therapy, total blood IgE levels had slightly reduced, but the value remained higher than the baseline.

In group C, we observed a progressive and statistically significant reduction in eosinophil cell count from a median baseline of 500 ± 62.94 cells/μL to 97 ± 62.94 cells/μL after 9 months of treatment

(p< 0.0001). Median total IgE levels were 131.00 ± 44.6, 118 ± 23.3, 110 ± 56.5, 105 ± 43.7 and 97 ± 62.94 at baseline, 4 weeks, 3, 6 and 9 months, respectively (Table 7-6).

	BASELINE	4 WEEKS	3MONTHS	6 MONTHS	9 MONTHS
GROUP A					
Blood eosinophils (cells/μL), median	433 ± 24.87	470±32.56	615± 45.83	449 ± 31.53	439±33.15
Blood total IgE (IU/ml), median	128.00 ± 40.6	110 ±33.6	77.60±24.5	63.90± 43.5	57.75 ± 51.7
GROUP B					
Blood eosinophils (cells/μL), median	453 ± 52.54	441 ± 62.44	438 ± 25.58	433 ± 52.54	422 ± 72.44
Blood total IgE (IU/ml), median	145 ± 65	155 ± 76.5	245 ± 76.7	456 ± 66.5	356 ± 65.6
GROUP C					
Blood eosinophils (cells/μL), median	500 ± 62.94	450± 23.63	334.8± 86.4	154.4± 0.48	97 ± 62.94
Blood total IgE (IU/ml), median	131.00 ± 44,6	118 ±23.3	110 ±56.5	105 ±43.7	101 ±43.6

Table 7-6 Change in mean total blood eosinophil counts (cells/μL) and plasma IgE levels (IU/ml) over time in each group of treatment

Treatment response was assessed in all patients at 6 months after the initiation of the therapy. Overall, 19 patients had a "good-excellent response" and 12 patients had a "poor-moderate response". Only one patient (group B) had a "no response" (0 criteria out of 5) with stable NPS, no improvement in olfaction and a 9 point reduction in SNOT-22. Detailed disease control using EPOS/EUFOREA 2023 criteria for each treatment group is shown in the table 7-7.

	4 WEEKS Tot Pz (%)	3 MONTHS Tot Pz (%)	6 MONTHS Tot Pz (%)	9 MONTHS Tot Pz (%)
GROUP A (14 pz)				
No response	2 (14%)	1 (7%)	0	0
Poor- Moderate response	6 (43%)	5 (36%)	4 (29%)	2 (14%)
Good-Excellent response	6 (43%)	8 (57%)	10 (71%)	12 (86%)
GROUP B (11 pz)				
No response	8 (73%)	7 (64%)	1 (9 %)	1 (9%)
Poor- Moderate response	2 (18%)	3 (27%)	5 (45,5%)	1 (9%)
Good-Excellent response	1 (9%)	1 (9%)	4 (45,5%)	9 (82 %)
GROUP C (8 pz)				
No response	6 (75%)	5 (62.5%)	0	0
Poor- Moderate response	1 (12.5%)	2 (25%)	3 (37.5%)	2 (15%)
Good-Excellent response	1 (12.5%)	1 (12.5%)	5 (62,5%)	7 (75%)

Table 7-7 Response to biologics according to EPOS/EUFOREA2023 criteria at different timepoints
(N = 33) . No response 0 criteria; Poor- Moderate response 1-3 criteria; Good-Excellent response 4-5 criteria.

No serious adverse events occurred at baseline or during follow-up. One patient reported pain in the site of injection, two reported conjunctivitis, and three reported musculoskeletal pain within the first 3 months of treatment. One patient had a marked eosinophilia of > 1500/μL 6 months after starting dupilumab treatment. In this case, we repeated the blood tests monthly instead of every two months.

8. DISCUSSION

CRS represents a intricate inflammatory condition, and the spectrum of available therapeutic interventions is expanding rapidly.

Traditionally, CRSwNP has been treated primarily through medical interventions, including the use of INCS and nasal irrigations with saline solutions, often supplemented by antihistamines or antileukotrienes. In cases of limited success with local therapeutic approaches, short courses of oral corticosteroids (OCS), with or without antibiotics, may be considered to manage obstructive nasal symptoms and reduce the volumetric size of nasal polyps. When medical interventions prove insufficient for achieving adequate control, the option of endoscopic surgical treatment becomes viable. This approach aims to alleviate nasal obstruction, restore normal ventilation, and enhance accessibility for subsequent local treatments in the future151. Nonetheless, there is a subgroup of patients who do not find relief with OCS and/or surgery, presenting persistent or recurrent disease[151]. These individuals have recently been identified as suffering from "severe uncontrolled CRSwNP "[152]. Considering that the pathophysiology of CRSwNP is influenced by eosinophilic inflammation, which involves the activation of T-helper cell 2 cytokines and the formation of IgE antibodies, biological therapy utilizing monoclonal antibodies, as observed in conditions like asthma or atopic dermatitis characterized by an underlying type 2 inflammatory pathway, can be applied to type 2 CRSwNP as well. This involves targeting specific immune mediators crucial to the underlying inflammatory process, such as inhibiting anti-IL-4/IL-13 signaling (with dupilumab), blocking anti-IL-5 pathways (using mepolizumab or benralizumab), and employing anti-IgE antibodies (like omalizumab)[153].

In our study, starting from April 2023 we initiated the prescription of one of the three currently recommended biological agents (dupilumab, omalizumab, and mepolizumab) in Italy, as approved by AIFA, for patients with severe uncontrolled CRSwNP in routine clinical practice.

The present study confirms the efficacy of dupilumab, omalizumab and mepolizumab in reducing NPS and improving quality of life, sense of smell and control of asthmatic comorbidity, as well as a low number of side effects.

Mean NPS and SNOT-22 at baseline in our study were comparable to the randomized controlled trials (RCTs)[99,108,114]. After 9 months of follow-up, all patients in our study demonstrated significant improvement in NPS and quality of life, comparable to the outcomes observed in RCTs. In fact, in all patients we observed a significant reduction at 9 months, with a median decrease of 2 points (from 1.7 to 2.5) for NPS and 41.5 points (from 39.25 to 43.01) for SNOT-22.

While a direct comparison is limited by the smaller sample size in our study, we observed a more rapid and significant improvement in NPS and SNOT-22 among patients treated with dupilumab.

In their recent meta-analysis, Cai et al.[154] indicated that dupilumab exhibited superior effects compared to placebo in reducing NPS and severity of nasal congestion at follow-ups of 24 weeks and beyond 48 weeks. This trend was followed by omalizumab and mepolizumab.

Moreover, based on the findings of the same meta-analysis, it was revealed that dupilumab is among the biologics that result in the most significant mean difference in the SNOT-22 score from baseline to 24 weeks and at the conclusion of the follow-up period, followed by omalizumab and mepolizumab demonstrating a comparable level of effectiveness.

Similar results were obtained in our series, revealing a mean reduction difference in the SNOT-22 score from baseline after 9 weeks of - 48.21 in the dupilumab group, - 41.01 in the omalizumab group, and -39,25 in the mepolizumab group. Similar findings were obtained by Lipworth et al.[155] through an indirect comparison, indicating that the smallest mean percentage reduction in NPS occurred with mepolizumab at 15% (SYNAPSE) and the largest with dupilumab at 35% (SINUS 24) with omalizumab in between at 18% (POLYP 1). Similar data had been obtained concerning the SNOT-22, with dupilumab being ranked first in terms of SNOT-22 reduction (-21.2 units), followed by omalizumab (-16.12 units), and mepolizumab (-13.7 units).

Moreover, Oykhman et al.[156] in their meta-analysis of 20 RCTs (n = 2530) determined that there is moderate to high certainty evidence affirming dupilumab as the most efficacious biologic agent for reducing NPS and SNOT-22, with omalizumab and mepolizumab following in effectiveness.

In line with previous real-world evidence[100157–160], the benefits of biologics on patients' quality of life and dimensions of nasal polyps were clinically evident at various months. This effect was characterized by a more or even less significant reduction in SNOT-22 and NPS during the follow-up period. Our data demonstrated that dupilumab, compared to omalizumab and mepolizumab, is rapidly effective in severe uncontrolled CRSwNP. More specifically, among our patients treated with dupilumab, a significant reduction in the NPS score and SNOT-22 was recorded at the first assessment after 4 weeks of treatment with continuous improvements which were evident up to the end of treatment in the study.

De Corso et al.[100] in Phase IV real-life, observational, retrospective, multicenter study observed improvement during therapy with dupilumab in NPS score and SNOT-22 after only 2 weeks of treatment. Also Mocellin et al.[161] in 23 patients affected by severe CRSwNP and treated with dupilumab, have demonstrated a quick significant improvement just after 15 days of treatment in both questionnaires and endoscopic evaluation.

In our study, notable enhancements were observed within 3 months in both the omalizumab and mepolizumab cohorts. Conversely, the SNOT-22 scores exhibited marked improvement after 4 weeks for omalizumab and after 3 months for mepolizumab. Lombardo et al.[162] demonstrated real-life clinical improvement in patients with CRSWNP treated with omalizumab, showing positive effects on NPS and SNOT-22 after 16 weeks of treatment. Similar results were observed in the POLYP 1 and POLYP 2 clinical trials. The efficacy of mepolizumab in CRSwNP was assessed in two phase II randomized clinical trials. These trials revealed a decrease in NPS at week 9 and a reduction in SNOT-22 at week 25[116].

Regarding olfactory dysfunction associated with CRSWNP, several clinical trials[99,108,114]and real-life studiesoy[156,160,163] have documented an improvement in olfactory function in patients undergoing treatment with biological drugs.

Oykhman et al.[156], in their meta-analysis, reported that dupilumab appeared to be the best agent in terms of the impact on the sense of smell versus placebo (measured by mean difference on the University of Pennsylvania Smell Identification Test [UPSIT]), followed by mepolizumab and omalizumab. Lipworth et al.[155] reported in their recent ITC that both dupilumab and omalizumab improved the UPSIT score versus placebo. Dupilumab treatment provided better results compared with omalizumab: change in UPSIT score reached 3.9 units from a baseline of 13.2 for omalizumab and 10.6 from a baseline of 14.1 for dupilumab. Moreover, a post hoc responder analysis performed by those authors showed that the proportion of patients with anosmia, treated with dupilumab, decreased by 50% in SINUS-24 and by 49% in SINUS-52, while no change was reported in the placebo group.

In our study focusing on the loss of smell, 64.3% of patients undergoing dupilumab treatment transitioned from anosmic to non-anosmic status. A complete response, defined as a shift from anosmia or hyposmia to normosmia, was observed in 21% of patients after 9 months of treatment.

Our data showed that dupilumab was effective in rapidly restoring olfaction, as evidenced by a significant improvement in the SSIT-12 after just 4 weeks of treatment.

Mullol et al.[164] described that dupilumab produced rapid improvements in the sense of smell, which were evident by day 3 according to daily patient-reported loss of smell. Similarly, De Corso et al.[100], in their real treatment experience with dupilumab, recorded that the improvement in the sense of smell was among the earliest changes observed, becoming evident after only 1 month of treatment and improved progressively throughout the treatment periods. The notable improvement supports the hypothesis that the reduction in olfaction is linked to both a contributing factor from nasal polyps and inflammation of the mucosa in the olfactory cleft[165]. As biologics determine both polyp shrinkage and a reduction in inflammatory neurotoxins, their action on olfactory function is multifactorial;

further studies are needed to assess the role of therapy in the olfactory epithelium renewal after disuse atrophy and/or surgical harm.

Given that the biological agents examined in our study are approved for treating various type 2 inflammatory conditions like atopic dermatitis and bronchial asthma, it's unsurprising that asthma symptoms also ameliorated in individuals with coexisting asthma. This could be demonstrated by the significant reduction in the ACT score in our cohort as well as by another author[166,167].

Certainly, in line with the previous literature, our study demonstrated that 64% of patients experienced improved clinical control of asthma. This improvement was reflected by an advancement of at least one step on the ACT scale.

Patients with coexisting asthma and AERD are recognized for exhibiting elevated recurrence rates and a higher likelihood of refractory disease[140]. In our cohort, all patients had undergone at least one prior surgery. While this isn't mandatory for monoclonal antibody treatment, surgery is presently regarded as a primary intervention for individuals who do not respond adequately to medical treatment[1,85]. Additionally, studies indicate that FESS is currently more cost-effective than biologic treatment[140].

Furthermore, biological treatments have demonstrated effectiveness in reducing various local and systemic markers of type 2 inflammation in patients with CRSwNP, including IgE and eosinophil cells[168].

In our study, patients undergoing dupilumab treatment have shown a temporary rise in blood eosinophils, which resolves spontaneously. This increase is notable within the initial 3 months of therapy (433 ± 24.87 vs 615 ± 45.83), followed by a subsequent return to baseline or even lower values by the end of the treatment period.

Temporary elevations in average eosinophil counts were noted in dupilumab clinical trials (102). Higher mean eosinophil counts in dupilumab versus placebo were observed at week 16 in SINUS-24 (586 vs 434 cells/mL) and SINUS-52 (594.8 vs 434.5 cells/mL), which decreased from week 24 (SINUS-24: 534 vs 444 cells/mL; and SINUS-52: 457.1 vs 439.5 cells/mL) and reached below baseline by the end of 52 weeks of treatment in SINUS-52 (baseline, 447.6 cells/mL; and week 52, 374.6 cells/ mL).

In our cohort, we describe a single case of a patient with an eosinophil count exceeding 1,500 cells/mL, without any collateral effects. Wechsler et al.[143] analyzed eosinophil counts and treatment-emergent adverse events related to eosinophilia across 11 dupilumab clinical trials. This post hoc analysis underscores that temporary rises in eosinophil counts during dupilumab treatment did not compromise effectiveness and were seldom clinically significant. Physicians should make decisions based on individual patient history and baseline eosinophil counts, remaining watchful for signs of

hypereosinophilia. The mechanism by which dupilumab induces transient hypereosinophilia is still unclear. A potential mechanism could be that dupilumab treatment inhibits the trafficking of eosinophils to the tissues, resulting in a transient increase in blood eosinophils. The adhesion of circulating eosinophils to blood vessels is regulated by chemokines and adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1, found on the endothelium. The expression of VCAM-1 is controlled by IL-4. When bound, eosinophils enter the tissue, guided by chemokines like thymus and activation-regulated chemokine, eotaxins, IL-5, and IL-13.2 By blocking both IL-4 and IL-13 signaling, dupilumab may inhibit VCAM-1 expression and the eosinophil migration process. This is supported by the reduced levels of serum eotaxin-3 and thymus and activation-regulated chemokine observed in dupilumab clinical trials[99,169]. Since IL-4 and IL-13 do not regulate eosinophil maturation and release into the blood, the diminished migration of eosinophils into the tissue may result in transient increases in blood eosinophil counts.

In our omalizumab group, we observed a progressive elevation in total IgE levels, with values starting to decline from the 6th month, although they remained higher than baseline levels. The increase in IgE levels during omalizumab therapy is commonly attributed to the potential prolongation of the relatively short half-life of free serum IgE (2-3 days) due to its binding to omalizumab, an IgG1 subclass with a half-life of around 26 days[170]. Nevertheless, another plausible explanation is conceivable: omalizumab could induce cross-linking with IgE situated on the surface of IgE-positive memory B cells, functioning as B cell receptors through its interaction with the Cε3-free regions [171]. These memory cells could be thereby activated, resulting in enhanced IgE production and total serum IgE levels. Though this current study contributes another piece to the jigsaw of understanding the rise in total IgE levels upon omalizumab therapy by showing it to be of polyclonal character, the issue regarding the underlying mechanism leading to the observed IgE rise remains unaddressed and awaits further investigation.

Meier et al.[172] analyzed the therapeutic outcome of 3 biologics, including omalizumab, in patients with CRSwNP in a real-life setting and sought to identify predictive biomarkers for successful treatment; however, their study indicated that eosinophil counts, total IgE, ECP, and IL-5 levels in the blood were not linked to success rates. This could be explained by the fact that nasal polyps are local lesions, so local IgE may play a more important role in nasal polyps than serum IgE. Given that blood IgE does not reflect tissue IgE concentrations and nasal polyp disease is not necessarily driven by specific aeroallergens[173], it follows that blood IgE as a biomarker for T2-CRS may not be clinically relevant[174].

Numerous studies [175] have consistently affirmed the evident and reproducible impact of mepolizumab in reducing circulating blood eosinophils. Our findings indicate a substantial decrease from baseline

blood eosinophils (500 ± 62.94 cell/μL) to post-treatment with mepolizumab (9 months) blood eosinophils (97 ± 62.94 cell/μL, $p < 0.001$), reflecting an 80.6% reduction.

In the Phase III SYNAPSE trial, the subcutaneous administration of mepolizumab at a dosage of 100 mg led to a decrease in the incidence of surgery and corticosteroid use, along with symptom improvement in this population. Additionally, it resulted in a reduction in blood eosinophil counts when compared to the placebo group. Nevertheless, the reduction in blood eosinophil levels may not necessarily correspond with the simultaneous decrease in the clinical and endoscopic impact of sinonasal disease.

In a retrospective study, Chan et al.[176] observed that individuals with uncontrolled Severe Eosinophilic Asthma (SEA) and concomitant CRSwNP exhibited positive responses to mepolizumab concerning asthma control and the reduction of blood eosinophils. However, their CRSwNP condition persisted and, in some instances, continued to deteriorate.

However, the data from the majority of published RLSs assessing the effectiveness of mepolizumab treatment in patients with Severe Eosinophilic Asthma (SEA) and comorbid CRSwNP are in accordance with the results observed in RCTs in terms of the clinical control of CRS. [160,175,177].

Concerning the efficacy of biologic treatment in patients with CRSwNP, various guidelines are currently referenced [1,83,84].

In a recent survey conducted in Italy[178], the evaluation of treatment response during the first year of follow-up revealed interesting trends. All respondents, as a consistent practice, utilize quality of life assessments through the SNOT-22. Interestingly, only one-third (34%) of the participants incorporate NPS in their assessments. Olfactometric evaluations, a crucial aspect in assessing sinonasal disease, are conducted by a majority of respondents, accounting for 53%. On the other hand, a minority of participants opt for specific pulmonary function tests, with 5% utilizing Fractional Exhaled Nitric Oxide (FeNO) tests and 17% employing spirometry.Among ENT specialists, 80% assess eosinophilia through blood counts, while 39% perform blood chemistry tests.

In our study, therapeutic outcomes were monitored over the nine months of treatment, following the most recent EPOS/EUFOREA 2023 update[84].

Overall, 57,5% and 81% of patients had a good/excellent response at 6 and 9 months respectively.

Our data are like those reported by Haxel et al.[179], who conducted a real-world study evaluating 70 consecutive patients with CRSWNP treated with dupilumab (49 pz) and omalizumab (21pz). A response ranging from moderate to excellent was observed in over 90% of patients undergoing the therapy, with no discernible difference in the overall response between the two treatments. To the best of our knowledge, there is currently no specific survey that comprehensively evaluates the real-life multidisciplinary response to mepolizumab treatment.

In their real-world study, Gallo et al.[160] studied the effectiveness of mepolizumab in 43 asthmatic patients with coexisting CRSwNP. Patients were categorized based on improvements in both SNOT-22 and NP scores. Overall responders were those showing improvement in both aspects, while partly responders exhibited benefits in either clinical or endoscopic scores. Patients with no improvement in any aspect were considered non-responders. Combining clinical and endoscopic effects, the study revealed that 47% of patients (20/43) were overall responders, while 53% were partly (13/23) or non-responders (10/23).

Regarding the timing of response we documented that the group of patients treated with dupilumab exhibited a super-early response. In fact, after just 4 weeks of treatment, 43% of the patients exhibited a 'very early' good response, and this percentage continued to increase at each subsequent assessment, reaching 71% at 6 months. These findings are in line with the results published by De Corso et al (103), who described that 43.0% of their cohort exhibited an excellent response after 1 month of treatment with dupilumab and defined those patients as “early responders”.

Similar data have also been described by Mocellin[161]; in their real-life study involving 23 patients with CRSWNP treated with dupilumab, 47% of patients reached an excellent response (considering EPOS criteria) after 6 months of therapy. Moreover, this type of response was observed in 28.6% of patients at 15 days of follow-up and in 45.0% one month after the first injection of dupilumab. We believe that a rapid response is important to understand the course of the disease and also because it encourages the patient to continue the therapy as well, reducing the risk of abandonment or poor compliance.

Only one patient receiving omalizumab treatment showed no improvement at 6 months. The patient was given the choice between undergoing salvage surgery or making a shift from omalizumab to dupilumab. The patient opted for surgery given the limited improvements achieved and a hesitancy to believe that another biologic could alleviate their symptoms. At baseline, his NPS was 7, and SNOT-22 was 61. Biopsies from his initial surgery revealed no tissue eosinophilia. His baseline eosinophil count was 215/μL, total IgE was 198 IU/mL, and he has no known history of asthma. Considering these factors, his CRSwNP might be attributed to type-1 inflammation, potentially explaining the lack of improvement in NPS. This aligns with a prior study[180] where the authors concluded that the patient with the least favorable response to therapy (only a 9-point reduction in SNOT-22, stable NPS and LKS, meeting only 2 criteria for response according to EPOS2020 had laboratory tests not indicative of type 2 inflammation at baseline. This underscores the critical importance of meticulous patient selection and the necessity for biomarkers.

Finally, we analyzed the side effects that occurred during the 9-month treatment period.

No severe adverse events occurred during the initiation of the treatment or the follow- up period. All adverse events that occurred were mild and subsided without further treatment, so safety was comparable to other published studies.

Currently, there has been limited research on the long-term adverse events of biologics due to insufficient follow-up period. Dupilumab and mepolizumab showed no serious safety concerns for more than 3 years in the treatment for other diseases [181,182]. However, a recent analysis based on real-world data reported that omalizumab may be associated with a significantly increased risk of malignant tumors[183]. Therefore, the use of biologics for long-term treatment should be carefully considered until further studies confirm their long-term safety.

Our study has certain limitations. Firstly, the retrospective nature of the study may introduce inherent biases. Secondly, the relatively small sample size limited our ability to establish statistical significance for all the outcomes examined, leading to significant findings only within a subset of our results. However, we aimed to provide preliminary real-world results regarding biological treatment. Presenting the clinical improvement rate following biological treatment should be suitable for this goal. Thirdly, the 9-month follow-up period is quite limited and does not allow for the evaluation of the biologic's efficacy at 1 year, as recommended by numerous guidelines[1,84]. This is due to the inclusion of mepolizumab in our study, which was approved for use in Italy from March 2023. Nevertheless, we assessed the initial follow-up after 6 months. We plan to continue with a multicenter national study that will provide data on a much larger set of patients with longer follow-up times.

9. FUTURE DIRECTION

Precision medicine represents an innovative approach to advancing healthcare.

Precision medicine refers to the "*ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology or prognosis of those diseases they may develop, or in their response to a specific treatment"*[184].

Implementation of the principles of precision medicine into the management of upper airway diseases like CRSwNP is a major task for the next decade. Endotype-driven treatment is an important component of precision medicine especially in patients with uncontrolled severe disease. Monoclonal antibodies could be a potential new treatment when we can find the patients with the phenotype and endotype that will benefit most from these treatments. The ability to predict which patients will respond favorably to a certain monoclonal antibody will be a key issue in achieving cost-effectiveness. Ideally, we should be able to discriminate these patients early in the disease and treat them early to prevent multiple surgeries in the years to follow and potentially also to prevent the development of lower airway disease. There is still a lack of head-to-head studies to provide evidence of the real-world effectiveness of different biologic drugs.

Anticipating further investigations in the coming years, we aspire to the development of an internationally recognized consensus guideline regarding the utilization of specific biological agents in the management of CRSwNP.

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