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REVIEW

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Latest advances in chronic rhinosinusitis with nasal polyps endotyping and biomarkers, and their significance for daily practice

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Abstract

The term chronic rhinosinusitis (CRS) comprises of an assortment of diseases that share a common feature: inflammation of the sinonasal mucosa. The phenotype classification of CRS, based on the presence of polyps, has failed to offer a curative treatment for the disease, particularly in refractory cases. Chronic rhinosinusitis with nasal polyps (CRSwNP) remains a challenging entity. Researchers have made efforts trying to characterize subtypes of the disease according to the endotypes, which are delineated by different immunological pathways, using biomarkers. Even if the inflammatory processes controlling CRSwNP are not fully understood, data suggested that the disease associated with a type 2 inflammatory mechanisms can be also linked to the type 1 or type 3 pathomechanism, being highly heterogeneous. Biomarkers for CRSwNP are proposed, such as: eosinophil count, cytokines, metalloproteinases, bitter and sweet taste receptors, and the nasal microbiome. For endotyping to be clinically applicable and simply determined, biomarkers referring to the intrinsic biomolecular mechanism still need to be found. Precision medicine is becoming the new standard of care, but innovative therapies such as biologics may be rather challenging for the clinicians in their daily practice. This new approach to CRSwNP implies patient selection and a simple algorithm for deciding the right treatment, easy to implement and adjust. Our review points out the ongoing new research on the pathophysiology of CRSwNP, biomarkers and treatment opportunities. It allows clinicians to keep abreast of current evidence-based knowledge and to individualize the management of CRSwNP, especially in refractory cases.

Keywords: chronic rhinosinusitis, nasal polyps, phenotype, endotype, refractory cases.

Introduction

The definition of chronic rhinosinusitis (CRS) has been updated to include an assortment of various diseases, which all lead to the inflammation of the sinonasal mucosa [1]. Their prevalence has been estimated by comprehensive epidemiology studies as almost 12% of the population in the USA and about 10% of Europeans, and 5–12% worldwide [2].

CRS has a negative impact on patients' quality of life (QoL), not only due to the discomfort of specific symptoms (chronic rhinorrhea, nasal obstruction, facial pressure, and hyposmia), but also due to central dysfunction manifested as fatigue and loss of sleep, which may further cause cognitive impairment, or depression [3–5]. Some studies have reported poorer health utility values in CRS patients than in patients affected by Parkinson's disease, end-stage renal disease requiring dialysis, or heart disease necessitating percutaneous procedures [6].

The classification of CRS disorder based on phenotype refers to clinical manifestations, whereas the classification based on endotype refers to the critical underlying pathophysiological mechanisms. According to phenotype, CRS has been clinically defined as chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP) [2, 7]. CRSwNP occurs in fewer than 25% of CRS cases but it is clinically more challenging, as it tends to be more severe, recurrent, and associated with other comorbidities. In some groups of patients, the disease is unresponsive to currently established treatments including intranasal glucocorticosteroids (GCSs), oral GCSs or endoscopic sinus surgery (ESS). These cases are also known as difficult-to-treat or poorly controlled rhinosinusitis [8–11].

Regarding endotyping, several studies reported that CRSwNP is more generally associated with a type 2 inflammation and an increase in interleukin (IL) activity, like IL-4, IL-5, IL-13, and immunoglobulin E (IgE). The same type 2 endotype is present in different CRS clinical subgroups, such as: allergic fungal rhinosinusitis (AFRS), central compartment allergic disease (CCAD), and eosinophilic CRS (ECRS). Furthermore, CRSwNP has additional clinical traits which associate it with other general disorders, such as asthma, aspirin exacerbated respiratory disease

(AERD), and other subgroups. Recent research has revealed that these clinical subgroups may be controlled by an analogous type 2 pathomechanism [12, 13]. While type 2 inflammation is present in 80% of patients with nasal polyps which obviously leads to more severe clinical expressions of the disease, in 20% of the patients other, non-type 2 mechanisms may be involved, such as type 1 and type 3 [7, 12, 13].

Even if the inflammatory processes controlling CRSwNP are not yet fully understood, significant recent progress suggests that the disease comprises distinctive subtype groups and is highly heterogeneous [14]. However, for endotyping to be clinically applicable, predictable and simply determined biomarkers referring to the intrinsic biomolecular mechanism still need to be found [15]. Consequently, CRS research in this particular area has greatly intensified in recent years, and many different biomarkers for nasal polyposis have been identified: eosinophil cytokines IL-4, IL-5, IL-13, IL-25, IL-33, metalloproteinases, bitter or sweet taste receptors and nasal microbiome [16, 17].

Precision medicine is becoming the new standard of care, but innovative therapies such as biologics may be rather sophisticated for clinicians in their daily practice. This new approach to CRSwNP, for instance, would need a simple method of selection and an algorithm for deciding the right treatment, easy to implement and adjust [18, 19].

Our review points out the ongoing research on the pathophysiology of CRSwNP and focuses on new, promising biomarkers and treatment opportunities. It allows clinicians to keep abreast of current evidence-based knowledge and to individualize the management of CRSwNP cases, especially in refractory cases.

Clinical phenotyping – recent advances

All clinicians encounter CRSwNP cases in which the disease is difficult to treat or refractory, meaning that patients complain of persistent or recurrent symptoms even after maximal medical therapy and surgery [8, 11]. In particular, type 2 immune reactions across all clinical phenotypes of the disease seem to be more recalcitrant with many relapses, and associated with other pathological condition [10, 20].

With the latest advances in biomarker identification, as well as the introduction of biologics as newly approved treatment options, the patients' endotype should, ideally, be identified at the first clinical visit in order to arrange for optimal therapy. In this manner, physicians would have the opportunity to prescribe targeted therapies, prevent under- and overtreatment, and minimize the need of successive surgery or repeated series of oral GCSs with modest results [12]. Some authors stated that a type 2 immune response in a patient can be identified from clinical data even without blood or serum biomarkers. For instance, in CRSwNP associated with late-onset asthma and/or non-steroidal anti-inflammatory drug-exacerbated respiratory diseases, the probability of a type 2 endotype is 100%. The same applies to patients having undergone one or more effective courses of oral GCS in the previous year or surgery with tissue eosinophilia at the histological examination [12]. So, it came out the idea that analyzing multiple baseline clinical variables can be group phenotypes of CRSwNP to detect the refractory forms of disease and predict the response to the treatment [2].

Currently, patients with CRSwNP are clinically classified based on polyp status in endoscopic grading and imagistic scores using the Lund–Mackay system. This scoring system is based on the total sum of the individually graded fullness of the sinuses on computed tomography (CT) imaging [11]. Recent studies have shown that patient-reported outcome measures need to be considered as well and in addition to more classical traits to accurate phenotype the patients. Of the many sinus-specific QoL questionnaires available, the total sino-nasal outcome test-22 (SNOT-22) has been proven the most effective [2, 11].

In one prospective, multicenter cohort study, the phenotypic subgroups of 382 CRS patients who had failed initial medical therapy were identified considering 103 clinical variables. This was done using an unsupervised clustering method by which the patients were grouped into clusters indicated by the data, without any hypothesis [21]. The authors described five distinct clusters of patients based on age, severity of patient recorded scores, depression, and fibromyalgia. The results concluded that CT and endoscopy differed partially between clusters and common clinical measures inclusive of polyp/atopic condition, previous number of surgeries, brief smell identification test and asthma did not show differences among clusters. The conclusion of the study was that the only three variables needed to correctly stratify the patient clusters were in this order, productivity in the past 90 days, age, and the total score of SNOT-22 [21]. In a subsequent study by the same authors, these three variables were used to predict outcomes after surgical therapy. The results pointed to surgery as being the most beneficial for the cluster of patients with the worst endoscopy scores and the lowest sinus-specific QoL, general QoL, more sleep-related problems, and productivity loss [22]. It is known that these cases usually belong to the refractory CRSwNP.

Further research in this area is necessary and the practical, clinical relevance of emerging results remains to be seen. New biomarkers are expected to work as prognostic predictors and help clinicians customize treatment options.

Structured histopathological examination – advantages and limitations for current practice

CRSwNP can be diagnosed by the presence of nasal polyps visible in the middle meatus. The appearance of polyps needs to be documented by endoscopic examination as pale, gray masses in the nasal cavity (Figure 1, A and B).

These should be distinguished from other polypoid changes in the sinuses and/or middle meatus that can occur in CRS variants [11, 23]. Even though the precise pathways involved in the formation of nasal polyps are still to be outlined, it is now generally recognized that the sinonasal mucosa of patients from Western countries is characterized by type 2 eosinophilic inflammation and its chronic remodeling results [24].

Histologically, CRSwNP features basal membrane thickening, less glandularity, predominantly edematous stroma with albumin deposition and infiltrated predominantly by eosinophils and pseudocyst formation, compared with CRSsNP in which increased fibrosis is predominantly, neutrophilia and globed cell hyperplasia are present (Figures 2–7).

Since eosinophils are the main inflammatory cells in many nasal polyp tissues, they have long been considered a certification of type 2 inflammation and potentially responsible for the etiopathogenesis and prognosis of the disease [25, 26]. Further research demonstrated a large fluctuation in the number of eosinophils observed in nasal polyps, with many unpredictable and regional asymmetries. Some reports showed that 20–75% of nasal polyp samples might be non-eosinophilic compared with specimens collected from CRSsNP subjects that may display a very high eosinophilia infiltration in the tissue [27]. Likewise, it is known that up to 50% of CRSwNP individuals in East Asia showed no eosinophilic inflammation [28].

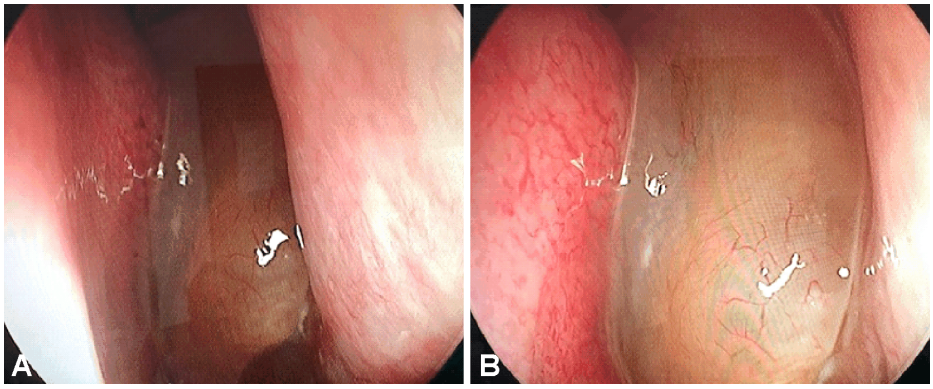

Figure 1 – ***(A and B) Images of nasal polyps during nasal endoscopy, filling the entire nasal cavity, Lund–Kennedy score 4.***

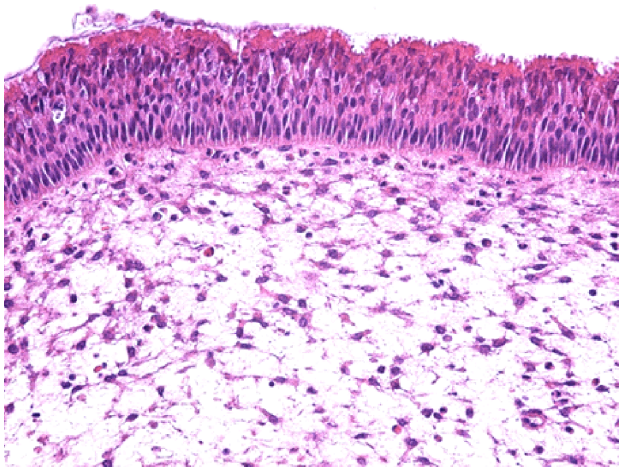

Figure 2 – ***Nasal mucosa: ciliated, pseudostratified, respiratory epithelium with myxoid stroma (HE staining, ×200).***

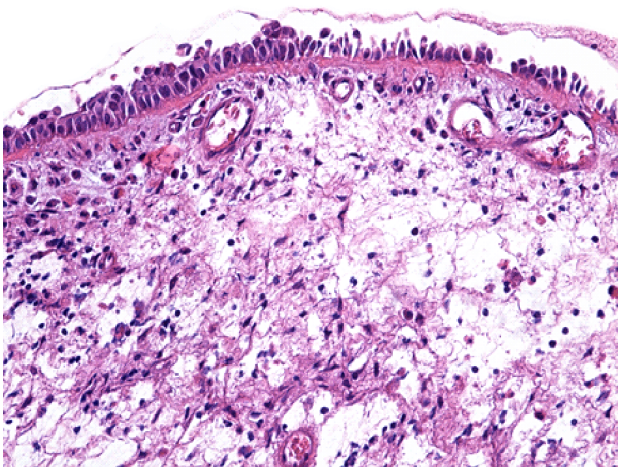

Figure 3 – ***Nasal polyp area with profound epithelial erosions (up to the basal layer), thickening of the basal membrane and myxoid stroma with moderate inflammatory infiltrate, fibroblasts, collagen fibers and congested blood vessels (HE staining, ×200).***

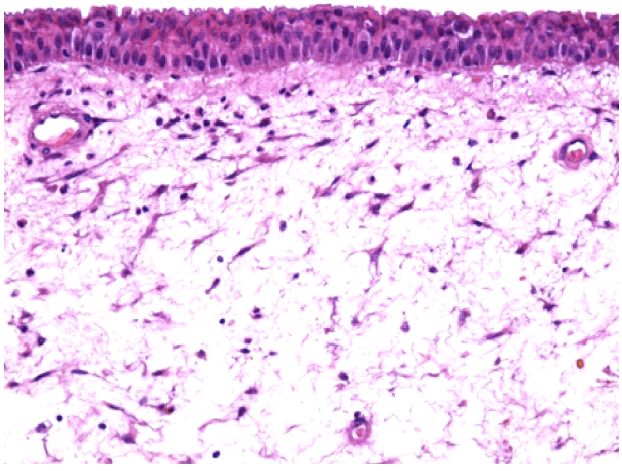

Figure 4 – ***Polypoid area with incomplete squamous epithelial metaplasia (HE staining, ×200).***

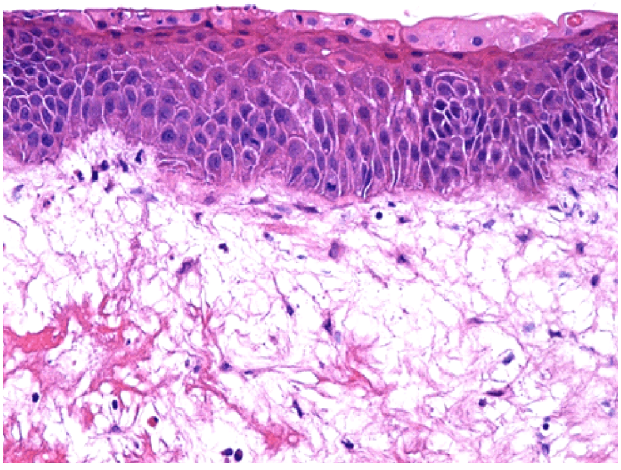

Figure 5 – ***Nasal polyp with complete epithelial metaplasia (HE staining, ×400).***

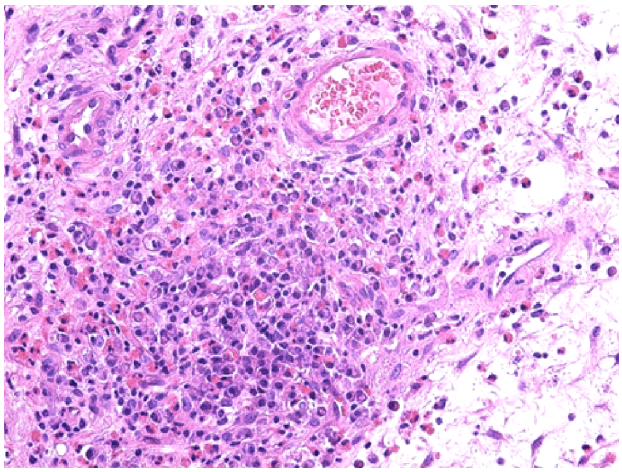

Figure 6 – ***Nasal polyp area with strong lymphocytic, plasmocytic and eosinophilic infiltrates in the stroma (HE staining, ×200).***

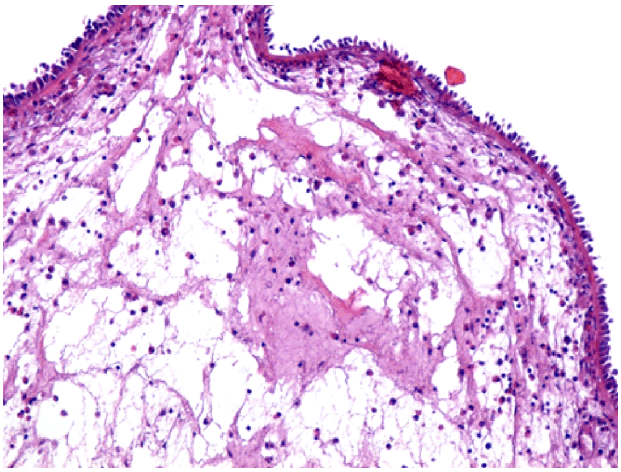

Figure 7 – ***Nasal polyp swollen myxoid stroma with pseudocystic aspect (HE staining, ×200).***

Regardless of these variations, the eosinophilic-abundant status of polyp tissue has been linked to increased patients' distress, as well as to an increased risk of revision surgery. Therefore, it has been concluded that a structured histopathological (HP) examination of polyp samples can be a very useful objective method to assist clinical decision-making [29–31].

In 2012, Snidvongs *et al.* [29] emphasized the importance of supplementing the classical HP examination, commonly used only for malignancy exclusion, with a more standardized report of inflammation profile of the surgical biopsies. Their report focused on the status of tissue inflammation considering the overall degree of inflammation, tissue eosinophilia, neutrophilic infiltrate, inflammatory cell predominance (lymphocytic, lympho-plasmacytic, eosinophilic, lymphohistiocytic, neutrophilic, others), basal membrane thickening, edema in the sub-epithelial space, hyperplastic/papillary change, ulceration of the mucosal lining, squamous metaplasia, fibrosis, and mucin. The results revealed a connection among high eosinophilic inflammation in the sinus mucosa and bad prognosis.

Following studies used structured HP reporting looking to distinguish histological markers in order to stratify subtypes and discern the puzzling pathophysiological mechanisms in CRS [31–33]. The association of HP findings with clinical features of CRS and CRS categories was investigated recently using 99 patients and 13 variables [31]. Even though the degree of inflammation between CRSsNP and CRSwNP was almost the same, the study showed a statistically significant increase in the number of eosinophils per high-power field (HPF) in patients with CRSwNP [31]. SNOT-22 scores were not connected with any histological parameters, but higher Lund–Mackay scores (LMS) correlated with high in-tissue eosinophilia and eosinophilic aggregates. Thus, a high number of eosinophil aggregates was proven a key link to recalcitrant forms of disease unconcerned by the CRS subtype [31]. Another study performed on patients who have gone through functional endoscopic sinus surgery (FESS) for CRS with numerous recurrences found that tissue eosinophil aggregates seem be the most important predictive factor for increased doses of intranasal GCS requirements after sinus surgery to control the outcomes in a more favorable manner [32].

The HP aspect of the polyp specimens was typified by Donell *et al.* [33] in primary *vs.* recurrent CRSwNP patients undergoing ESS. The authors did not find any disparity between primary and recurrent CRSwNP for eosinophilic infiltrated tissue, but in recurrent CRSwNP cases mucin was quite often inclined to presents eosinophil aggregates compared to primary CRSwNP. The results indicated that eosinophilic aggregates defined as a minimum of two distinct aggregates of at least 10 cells each/HPF can be a predictor of eosinophilic stimulation. This supports previously published results and suggests that eosinophilic aggregates could work as a possible marker for recalcitrant CRSwNP cases [31, 32].

Other recent surveys continue to attempt characterizing different disease endotypes based on structured HP examination, trying to find correlations between the pathogenetic mechanism and clinical outcomes. Marino *et al.* [34] emphasized that, besides tissue eosinophilia known to be related to CRSwNP, the analysis of cellular infiltrates, such as lymphocytic and lymphoplasmacytic inflammatory predominance, may be useful for differentiating CRS traits, the amount of inflammation, and its consequences. Their work confirmed others' results for CRS endotyping and the fact that CRS varies even between subjects with nasal polyps [35, 36]. A recent review explored the impact of high tissue eosinophilia and related biomarkers at the structured HP examination as an indicator of disease recurrence in CRSwNP. The authors concluded that the cutoff value for tissue eosinophilia must be adapted to the specificity of the disease according to geographic and ethnic differences [37].

The latest developments in the field of CRSwNP show the need for diagnostic criteria informed by cluster analysis of clinical outcomes. Biomarkers such as eosinophilic cationic protein and IL-5 are promising in terms of clinical diagnostic and predictive tools for typing and prognosis. Recently, Brescia *et al.* [38] proposed a classification of different CRSwNP cases based on structured histopathology and discriminant analysis. They

described four distinct histotypes of CRSwNP patient clusters: patients with limited fibrosis (cluster 1), patients with hyperplastic/papillary changes, fibrosis and neutrophil infiltration possibly as a response to ulceration (cluster 2), patients with fibrosis (cluster 3), and patients without neutrophilic infiltration but with hyperplastic/papillary changes and fibrosis in almost all cases (cluster 4). Even though the study did not find an association between these distinctly identified clusters and clinical outcomes, likely due to the limited number of cases, future larger case series may allow histotyping CRSwNP patients and find prognostic factors [38].

Recent CRSwNP research has revealed that a structured HP report of tissue samples biopsies, even if usually adapted by healthcare providers, can provide valuable prognostic markers and helps clinicians target therapies more specifically for refractory cases of CRSwNP.

In practice, however, there are a few challenges. One is related to the number of eosinophils that should define tissue eosinophilia. Various reports have suggested different threshold values for tissue eosinophilia, ranging from >5 eosinophils/HPF to >120 eosinophils/HPF [39–41], most recommending >10 eosinophils/HPF [33, 42]. The same is also suggested as a reference value in the new *European Position Paper on Rhinosinusitis* (EPOS) Guide [11]. At the same time, the Guide advises that an additional distinction must be made between cases with 10–100 eosinophils/HPF and those with >100 eosinophils/HPF [11]. According to a very recent meta-analysis, high tissue eosinophilia is commonly linked to the prognosis and severity of disease, and a threshold value of >55 eosinophils/HPF has the highest sensitivity for predicting the likelihood of ECRS recurrence [43].

A second source of difficulty concerns the technique used for detecting tissue eosinophilia by quantifying the eosinophils, *i.e.*, number/HPF (400×). This approach has been criticized by some authors for being too laborious and subjective for consistent application in clinical settings. One weakness of the procedure is that the pathologist has to assess the ratio of eosinophils (RE) to other infiltrative inflammatory cells in tissue (which include neutrophils, lymphocytes, plasma cells, etc.) in 10 random HPFs, and there could be significant differences between various HPFs, resulting in sampling errors and inaccurate overall eosinophil counts in the total sample [42, 44]. A recently published study showed an improved method of tissue eosinophilia evaluation based on artificial intelligence (AI). The authors developed an AI CRS evaluation method called AI CRS evaluation platform (AICEP) $RE_{slide-predict}$ in order to diagnose CRSwNP rapidly and accurately *via* deep artificial learning and whole-slide imaging (WSI). Although the results were promising, the method still requires improvements and multicenter testing [44].

Despite the practical difficulties of mainstreaming the classical method for structured HP evaluation, the approach remains effective. Some skilled pathologists have proposed to scan the slides in a panoramic view, and the semiquantitative scores to be tested to every variable at higher magnification after the densest areas have been identified. Finally, it is worth noting that nasal mucosa remodeling due to inflammation is an active process, so the patient's nasal HP aspect can transform over time, including after medication or surgery [35].

Cytokine signatures

Cytokine signatures are extremely valuable in CRSwNP endotyping, having a greater diagnostic value than eosinophilia or even the presence of actual nasal polyposis [16].

CRSsNP is characterized by a T-helper (Th) 1 or Th17 inflammatory neutrophilia phenotype, expressing transforming growth factor-β (TGF-β), ILs (IL-6, IL-8, or IL-17) and type I interferons (IFNs).

CRSwNP are characterized with increased expression of thymic stromal lymphopoietin (TSLP) and type 2 inflammatory cells, such as type 2 innate lymphoid cells (ILC2s) which produce ILs (IL-4, IL-5, IL-13, IL-25, IL-33) that defines a Th2 microenvironment [45].

ILC2s activate B- and T-cell activity. Type 2 cytokines mentioned earlier, produced by Th2 cells, mast cells and ILC2s are believed to control the inflammation in allergic patients [46].

IL-5 induces eosinophilia and its presence can be defining for a different endotypes of CRSwNP with a severe manifestation; IL-13 activates macrophages, B-lymphocytes and epithelial cells (mucus production with IL-4). Epithelial-derived cytokines, such as IL-25, IL-33 and TSLP, are an integral factor in the pathogenesis of CRSwNP and asthma should be treated as biomarkers for CRSwNP. Elevated eosinophil counts in the tissue are linked to an upregulated IL-25 expression and to also an increased CT score, thus showing the possibility of being use as a sensitive biomarker [45, 47].

Epithelial cells are a physical barrier which initiate and regulate innate and adaptive responses through tissue cytokines like TSLP, IL-25 and IL-33. They contribute to the regulation of immunity and inflammation induced by different exogenous or endogenous stimuli or different pathogens, local traumas, allergens, proinflammatory and type 2 cytokines [48]. The IL-17 family of cytokines includes IL-25. It promotes type 2 inflammation with eosinophilia. IL-25 is secreted by Th2 cells, mast cells, eosinophils and epithelial cells. Th2 cells, basophils and ILC2s express as a receptor IL-25R and increases the production of other cytokines in these cells. The latest discovered member of the IL-1 subgroups is IL-33, a cytokine linked to type 2 inflammation. It is increased in nasal polyps with eosinophilia and the production of IL-33 is controlled by the innate immunity. IL-33 is secreted by immune cells (macrophages and dendritic cells) and epithelial cells [49]. Memory Th2 cells, basophils, mast cells, natural killer T (NKT) cells and ILC2s express an IL-33R cytokine that induces IgE production in B-cells from polyps. TSLP is an IL-7 like cytokine and induces in innate and adaptive type 2 inflammation. TSLP seems to be an important contributor of type 2 immunity in CRSwNP with the type 2 endotype [50].

The Th1 and Th2 classification is useful but is an oversimplification of the status in nasal polyps. Nasal polyps in 85% of the cases show a high concentration of IL-5, but there is a concomitant increase in expression

of IL-17 and IFNγ that is usually more attributed to a Th1 process [27].

A recent study has demonstrated that CRS may be better defined as an inflammatory process that evolves with time and with non-mutually exclusive and variable immunologic markers [16].

Anti-cytokine agents are used as asthma treatments and under intense development, and there seem to be numerous links to the mechanisms that produce Th2 inflammation in cases of CRS. Asthmatic patients with a Th2 phenotype and eosinophilia have been shown to benefit from IL-5 antagonist therapy [51].

Dupilumab, a recently approved treatment for polyposis is an anti-IL-4 receptor antibody which interferes with the action mechanism of both IL-4 and IL-13 and has been also used in cases of atopic dermatitis and asthma and has shown efficacy in treatment as long as there is a type 2 inflammatory disease and not confused with non-type 2 [52].

The inflammatory signature can efficiently predict the response, so patient selection and prior testing for therapy is crucial [53]. An IL-25 blockade has been shown by the murine studies to reduce polypoid lesions and edematous inflammation. The testing was done at cellular and mucosal level [16].

In a study by Ryu *et al.* (2019) [54], Th1 and Th17 were shown with an increased activity level in all the cases of refractory CRS. Refractory CRSwNP with Th2 inflammation showed higher levels of eosinophilic activity with increased marker levels and heightened autoantibody counts. Inhibition of these pathways are shown through this study that they may offer a new treatment strategy for CRSwNP, for refractory cases in particular [54].

The involvement of the matrix metalloproteinases (MMPs) in CRSwNP

MMPs and the tissue inhibitors of these MMPs (TIMPs) are the most relevant units of the proteolytic system. This system plays a part in the reshaping of the extracellular matrix (ECM). A plethora of studies already investigated the role of MMPs and TIMPs in the etio-pathogenesis of nasal polyps in CRSwNP. Multiple types of MMPs have been studied but the most common ones associated with CRSwNP are MMPs -2, -7 and -9 [55].

Of all MMP subtypes, MMP-9 is the most frequently cited up-regulated metalloproteinase associated with the construction of nasal polyps [28, 56–58]. MMP-9 is not usually found in tissues that are healthy; an increased concentration of the MMP-9 appears in chronic inflammatory diseases. Several studies showed MMP-9 increase in autoimmune disorders and chronic obstructive pulmonary disease (COPD) [59, 60]. The action mechanism of MMP-9 was demonstrated to be the separation of type IV collagen fibers, those fibers being the main constituent of the basement membrane, with the basement membrane acting as a foundation for the blood vessels and the mucosa covering the sinonasal cavity [61–63]. Because of the increased concentration of MMP-9, the blood vessels in the sinonasal mucosa start to leak and in turn lead to a stromal edema. The edema, in turn, facilitates the formation of nasal polyps [64, 65].

In 2018, Suzuki *et al.* [56] demonstrated that a certain protein deacetylase called sirtuin 1 (SIRT1) negatively affects the expression of MMP-9. They found lower SIRT1 levels in the lungs of patients with COPD and the activation of this deacetylase could point to a new therapy for chronic disease featuring high concentrations of MMP-9. After this study, the link between MMP-9 and SIRT1 pathway and activity in nasal epithelial tissue was proven and they found a significantly increased concentration of MMP-9 in patients with CRSwNP compared to control subjects and patients with CRSsNP. Moreover, they identified an inverse correlation between MMP-9 and SIRT1 levels in cases with either CRSwNP or CRSsNP, but not in the control subjects. Understanding the negative effect that SIRT1 has on MMP-9 levels could lead to the discovery of new treatment options. In CRS, topical treatment with SIRT1 activators (*e.g.*, resveratrol) could control the MMP-9 levels and become a new therapeutic class for these patients [56, 64].

MMPs have also been associated with certain unhealthy habits, such as smoking. We know that MMP-9 is increased in smokers suffering from various diseases in which inflammation plays an important role, such as COPD [66]. Because of the similar pathogenesis of inflammatory diseases and CRSwNP, we may assume that smoking could be a risk factor for CRSwNP by enhancing the MMP-9 in the sinonasal mucosa. In fact, smoking restriction and passive smoking avoidance could be valuable strategies in the prevention of polyposis recurrence [67]. Children exposed to passive tobacco smoke were found to have a higher MMP-9 concentration and activity in the nasal secretions compared to a control group of children not exposed to passive smoking. These findings support the same idea that tobacco smoke can alter the inflammatory response of the sinonasal mucosa by enhancing the MMP-9 levels and leading to a higher recurrence of the nasal polyps. This is yet another argument highlighting the important role of smoking cessation as a valuable therapeutic step, this time in addressing nasal polyposis.

Another metalloproteinase strongly related to CRSwNP is MMP-2, which has similar effects to MMP-9, plays a significant role in degradation at the ECM level and affecting type IV collagen fibers. These collagen IV fibers play a crucial role in the physiology of the ECM and basement membrane of the sinonasal mucosa [68]. Can *et al.* [69] found increased concentrations of MMP-2 in nasal polyps. It can thus be assumed that, if MMP-2 is higher than normal in the sinonasal mucosa, a nasal polyp will be formed in the following period.

In contrast to MMP-9, MMP-2 is more present in the surface epithelium of the recurrent and non-recurrent nasal polyps (MMP-9 is significantly increased in the gland of the sinonasal mucosa) (Figure 8, A and B) [70].

The enzyme-linked immunoassay (ELISA) test which is usually used to determine MMPs, has the disadvantage that it makes a quantitative determination, therefore in an unpublished study we decided to use zymography method to determine the expression of the pro-MMP-2,

pro-MMP-9 from polyp tissue and normal mucosa samples. The result was that we could detect the active forms of MMP-2 and MMP-9, which cannot be detected by ELISA method.

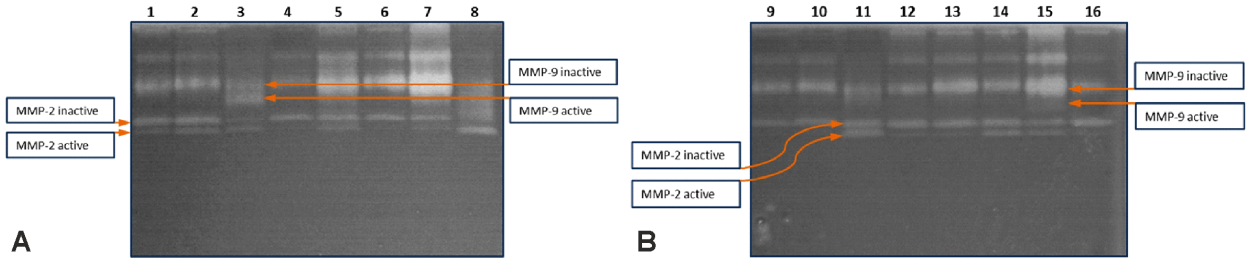

Figure 8 – ***(A and B) Zymography method for MMP-2 and MMP-9 determination from normal mucosa samples (1, 3, 15) and nasal polyps' tissue. Proteolytic activity appears as clear lysis strips on an intense and uniform blue background. The quantification was done by densitometry, the proteins being confirmed with a specific marker. MMP: Matrix metalloproteinase.***

Combined with MMP-2 activity on the sinonasal mucosa, TIMP-1 plays a major role as well: greater MMP-2/TIMP-1 ratio correlates with the harshness of nasal polyposis. Thus, selective blockage of MMP-2 secretion and stimulation of TIMP-1 levels could represent a different option for CRSwNP patients. Corticosteroids, as seen in the study published by Yigit *et al.* [71], lower the tissue MMP-2 concentration and the tissue TIMP-1 concentration, holding a fixed MMP-2/TIMP-1 ratio. The corticosteroid effect on nasal polyposis could be enhanced by adding a TIMP-1 up-regulator, such as genipin, to the therapy [72].

MMP-related treatment for CRSwNP

There are multiple treatment options that influence the MMP concentration in the nasal polyps [73]. One of the most important for CRSwNP is the administration of corticosteroids in a variety of ways (oral/intravenous, transnasal nebulization, intranasal spray) to help reduce the symptomatology in nasal polyposis. Zhang *et al.* [73] studied the effect corticosteroids have on MMPs depending on the administration route. The collagen deposition in the ECM of the polypoid tissue was found increased after corticosteroid treatment regardless of which route of administration (oral/transnasal nebulization/intra-nasal spray). MMP-2 and MMP-9 concentrations were lower after Budesonide inhalation suspension and were also lower after oral Methylprednisolone, but the MMP levels remained constant after treatment with Budesonide nasal spray. In line with the MMP-2 and MMP-9 concentration decrease, TIMP-1 and TIMP-2 were significantly increased in the nasal polyp tissue after treatment with oral and inhalator corticosteroids but remained unaltered after treatment with corticosteroid nasal spray. Therefore, corticosteroids are an effective way of lowering MMP-2 and MMP-9 levels and are also effective in increasing TIMP-1 and -2 levels. The most effective administration routes for corticosteroid therapy for MMP down-regulation and TIMP up-regulation seem to be transnasal nebulization with Budesonide inhalation suspension and Methylprednisolone taken orally [70].

As mentioned before, topical treatment with SIRT1 activators such as resveratrol could control the MMP-9 levels and help alleviate nasal polyps' formation in CRSwNP. There has been further research regarding SIRT1 expression and its effect on nasal polypogenesis. For instance, Lee *et al.* [64] studied SIRT1 activity in mice. They found that transgenic mice treated with specific substances that normally induce polypoid lesions of the nasal mucosa had no mucosal changes when they were administered these substances compared to the wild-type mice, which had marked nasal polypoid lesions. Moreover, in this study, the effect of resveratrol was also analyzed in transgenic and wild-type mice. The authors found that resveratrol instillation in wild type mice resolved the polyp formation problem after the mice were administered ovalbumin (OVA) and staphylococcal enterotoxin B (SEB) substances (OVA and SEB were the nasal polyps-inducing drugs used in this specific study). Considering such results, SIRT1 activators represent a new potential therapeutic class for CRSwNP treatment and their effect on MMP-9 down-regulation should be taken into consideration.

What is the microbiome and why is it important for CRSwNP?

In almost in all areas of the body there is a microbiome of different species that maintain homeostasis. Such is the case of sinuses, where there is a complex microbiome characteristic to the upper respiratory system, and which modulates the inflammatory and immune response. Research in the field is showing that a richer and balanced number of living organisms in the sinuses correlate to a better prognostic with fewer relapses [11].

Taking notice of this fact opens up an entire treatment paradigm. Until recently, there was extensive guidance toward sterilizing the sinonasal cavities, but now the trend is to preserve the commensal flora. The latest advances in molecular biology shows that a plethora of molecules produced at the epithelial level helps with the regenerative processes. Also, the roles of different species are gradually being discovered, such as the example of *Bacteroides* and *Fusobacteria* being classified as opportunistic bacteria that develop when there is poor ventilation in the sinuses [2]. Also, high numbers of patients were found to be colonized with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacteria that produce enzymes like lysozyme, S100 proteins and β-defensins, which can disrupt the natural function of the epithelium [16].

These theories are still in their infancy and the related studies available have included relatively small cohorts, partly due to the expenses involved. Further research is needed to confirm and expand on the initial findings. Also, there is a lack of patient reported outcomes which, in the case of CRS, is paramount because of the high variation in physical and psychological symptoms.

Genomic classification of CRS

Taste 2 receptors (TAS2Rs) are bitter taste receptors from the family of transmembrane G protein-coupled receptors, widely used as study points for therapy and testing. These receptors being also at the level of the taste buds of the tongue can offer a bitter taste sensation when stimulated and that is where they get their name. Extraoral bitter taste receptors are studied because of their implications in numerous pathophysiological interactions.

Bitter taste receptors are easy to test in a clinical setting, and in refractory cases they can be a useful tool for clinicians. This is a relatively new, but very promising field of study. The knowledge, before initiating treatment, that a certain case can have poorer outcomes can influence the way the treatment is tailored and administered.

To illustrate, taste 2 receptor member 38 (TAS2R38) is a bitter taste receptor that has been studied recently, with results showing the capability of sensing Gram-negative bacteria at the sinonasal ciliated epithelium level. Patients that show homozygote function from TAS2R38 can be accurately tested clinically and show good outcomes because of the different modulation of the innate immune system in antimicrobial response. The TAS2R38 protein has two versions, active and inactive, because of two common polymorphisms. The most active variant is the homozygote functional form (20%), which also yields the best results. The heterozygote TAS2R38 is moderately active (50%), and the patients with the worst outcomes tend to have the homozygote inactive TAS2R38 (30%). These clinical findings seem to be valid for CRSsNP and less so for CRSwNP, some studies even showing no significant impact on outcomes [2, 16].

Periostin is another molecule of interest in this field. This protein is produced when the cystatin 1 (*CST1*) gene is activated, and fibroblasts produce it during the Th2/ eosinophilic inflammation process. Levels of periostin can be measured using the ELISA test and, by being an integral part of the eosinophil function, it can become useful in refractory cases or in future treatments as a targeted, highly specific molecule. For clinicians, the capability to interact directly with the inflammatory cascade through different proteins and biochemical paths is a great choice if the diagnostic is well established. In the case of periostin, this test is applicable only for the type 2 endotype, as pathways differ for non-type 2 [74].

Epigenetics and CRSwNP

During our lifetime, our deoxyribonucleic acid (DNA) undergoes numerous changes due to the effects that time and the environment exert. The field of epigenetics in CRS is aimed at studying specific genes that mutate and produce different outcomes. DNA methylation in specific genes has been shown to significantly influence the disease, both in CRSsNP and CRSwNP. With the study of the polymerase chain reaction, bisulfite sequencing and reverse transcription-quantitative polymerase chain reaction becoming increasingly accessible, it seems four genes with altered hypomethylation in nasal polyp tissue have a direct impact over the progression of the disease. These are keratin 19 (KRT19), nuclear receptor subfamily 2 group F member 2 (NR2F2), a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 1 (ADAMTS1), and zinc finger protein 222 (ZNF222). Research on nasal polyps has linked them to larger quantities than normal in polypoid degenerated mucosa compared to normal [2, 75].

Precision medicine benefits directly from such findings, with extremely specific treatments becoming more and more common. Because of the complicated nature of the subject, further development is slow but promising. The prospect of using clustered regularly interspaced short palindromic repeats (CRISPR) to optimize the treatment according to individual patient needs is worth pursuing.

New approaches in CRSwNP treatment

The new guidelines of treatment emerging lately are based on diagnosing endotypes (type 2 *versus* non-type 2 CRS). Non-type 2 patients respond well to classical treatment, including low dose macrolides, where we take advantage of the anti-inflammatory particularities of the treatment [76], and classical FESS, where we take into account the low rate of recurrences.

When it comes to type 2 inflammation, novel treatments are being discovered with relative speed and should be considered. In these cases, treatment can now include biologics and reboot surgery, two new and innovative solutions to an age-old problem [77].

Biologic therapy is a technique of using human monoclonal antibodies, targeting and blocking specific IL-4 or IL-5 activity. These have been used for some time and with great benefits to treat other inflammatory conditions (*e.g.*, asthma and atopic dermatitis) [77]. Although the drug candidates are many (*e.g.*, Reslizumab, Mepolizumab, Benralizumab and Omalizumab), only Dupilumab (with the commercial name Dupixent) has been approved by the Food and Drug Administration (FDA) in case of recurring CRSwNP. Newly published studies are showing an improvement not only regarding recurrence, but even in regaining olfactory sense and improving overall QoL [78].

However, this treatment also has certain noteworthy drawbacks resulting from the fact that it is new and that doses should be administered at every six months to one year throughout the patient's life: it is extremely expensive and its long-term side effects have not been studied well enough.

Reboot is a new type of approach to classical FESS surgery which aims to maximally remove all sinus mucosa, allowing the preserved healthy nasal mucosa to cover the inside of the sinus by re-epithelialization. Some studies reported improvements from 45% relapse in two years in classical FESS to just 17% relapse. Further investigating should be carried, but the results are promising [79].

Last but not least, because CRS involves an inflammatory physiological chain, prevention may be obtained by making healthy lifestyle choices, such as improving diet [80] or ceasing smoking, which are known to improve outcomes and space out recurrence.

Conclusions

Etiopathogenesis of CRSwNP remains a very hot topic in both clinical and fundamental research but still represents an equation with many unknowns. A deeper understanding of the complicated molecular pathogenic mechanism of CRSwNP could enable individualized treatments for the patients based on the endotype of their disease, characterized by specific biomarkers. It became evident that the proper management of cases of CRS, specifically patients with severe and recurring CRSwNP, increases in complexity, but current progress in research had already provided valuable information for clinicians to recognize possible indicators for type 2 immune reactions even starting with patient's history data. A more complex classification can be achieved using three simple available data categories (SNOT-22 score, lost productivity, and age). A good collaboration with the pathology department, providing a structured HP examination of the surgical biopsies offers supplementary information regarding the severity of the disease. Other molecular markers recently identified such as inflammatory cytokines or MMPs will be essential in choosing the individualized treatment and predicting the evolution of the disease. Also, novel developments in genomics revealed that an easily accessible taste test may anticipate the results after FESS. Microbiomics and CRS, although new as a concept, have already demonstrated that diverse ecosystems, including the nasal sinus are associated with improved surgical outcomes. New approaches in treatment, such as reboot surgery and biological therapy, compared to FESS and intranasal corticosteroids, offer a chance for remission of the disease and a better outcome. With the advent of endotyping, our decision-making ability will improve, making possible the stratification of patients for the appropriate treatment of uncontrolled cases. These are a part of the initial strides that have been made in the particularly elaborate concept of CRS.

Conflict of interests

The authors declare that they have no conflict of interests.

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