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### Title page

#### Review:

#### **Novel findings in immunopathophysiology of chronic rhinosinusitis and their role in a model of precision medicine**

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Highlights in pathophysiology of CRS

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## **Abstract**

Our understanding of the pathophysiology of chronic rhinosinusitis (CRS) is continuously evolving. The traditional description of CRS in terms of two phenotypes based on the presence or absence of nasal polyps belies the underlying intricate immunopathophysiological processes responsible for this condition. CRS is being increasingly recognised as a disease spectrum encompassing a range of inflammatory states in the sinonasal cavity, with non-Type 2 inflammatory disease on one end, Type 2 inflammatory, eosinophil-heavy disease on the other, and an overlap of both in different proportions in between. An abundance in research on the immune mechanisms of CRS has revealed various new endotypes that hold promise as biomarkers for the development of targeted therapies in severe, uncontrolled CRS. The introduction of precision medicine to manage this chronic, complex condition is a step forward in providing individualised care for all patients with CRS. In this review, the latest research on the pathophysiology of CRS with a focus on potential novel biomarkers and treatment options over the last two years are summarised and integrated into a suggested model of precision medicine in CRS.

## **Keywords:**

Chronic rhinosinusitis, immunopathophysiology, precision medicine, endotypes, biomarkers

## 1. Introduction

Chronic rhinosinusitis (CRS) in adults is defined as symptoms of sinonasal inflammation (nasal obstruction/congestion/blockage, nasal drainage, facial pain/pressure/fullness and decreased or loss of sense of smell) for more than 12 weeks, accompanied by objective evidence on nasoendoscopy of purulent discharge, nasal polyps or edema; or radiological imaging showing inflammation or mucosal changes within the sinuses (1, 2). Large-scale epidemiology studies have shown that CRS has a prevalence rate of 11.9% in the United States (3), 10.9% in Europe (4) and 8.0% in China (5), reflecting its disease burden on society.

CRS is conventionally divided into 2 major phenotypes based on objective findings of nasal polyps or lack thereof. The immunologic profile of CRS with nasal polyposis (CRSwNP) is classically associated with a T-helper 2 (Th-2) response resulting in production of cytokines IL-4, IL-5 and IL-13, with downstream tissue eosinophilia (6). CRS without nasal polyposis (CRSSNP) is thought to have a predominantly Th-1 cell response with excess interferon-gamma (IFN- $\gamma$ ) and neutrophilic inflammation (6). However this adage is being challenged by an increasing number of studies that show significant overlap between CRS phenotypes and immunologic profiles, as opposed to a mutually exclusive phenotype-endotype pairing (7-10).

The heterogenous presentation of CRS may be due to its complex pathophysiology, which is probably best summarised as a dysregulated interaction between host (consisting of genetic and immunological factors) and environment (including infection) (11) (**Figure 1**). Hence the differentiation of CRS into two major phenotypes under-appreciates the delicate myriad of mechanisms underlying this disease process. In recent years, much effort has been made to describe CRS in terms of endotypes, each defined by distinct molecular mechanisms and identified by corresponding biomarkers. This may form a meaningful component of precision medicine, which is focused on achieving specific diagnosis, treatment and prognostication. However, for endotyping to be clinically relevant, identification of reliable and easily measured biomarkers that represent unique imprints of the underlying immunologic process is crucial and yet remains a challenge. In this review article, the latest research on the pathophysiology of CRS with a focus on potential novel biomarkers and treatment options over the last two years are summarised and integrated into a suggested model of precision medicine in CRS to demonstrate

its clinical relevance. As we continue to gain more insight into the pathogenesis and with evolving treatment modalities, this model may undergo further modification in due course.

## **2. Precision Medicine in CRS**

Precision medicine is a healthcare model that tailors decision making and treatment to the individual patient (12). The four pillars, or “Ps”, of precision medicine are personalised care, prediction of success, prevention of disease progression or complications and participation of the patient in the therapeutic plan. It is being increasingly recognised as the way forward in improving patient care for those with chronic conditions. In the airway, a prime example of precision medicine is allergen immunotherapy (13, 14). It is tailored to each individual’s sensitisation profile; there are reliable biomarkers to predict success of therapy (15, 16); it potentially prevents development of asthma and sensitisation to new allergens (17-23) and it requires active participation of the patient in his care. This principle of treatment is also being increasingly applied to the treatment of lower airway diseases (24-27). By adapting the model of precision medicine for CRS proposed in the EUFOREA-ARIA-EPOS-AIRWAYS ICP statement, treatment can be stratified into three levels of management as described below (28) (**Figure 2**).

### **2.1 Level 1 management**

The first level of management is applied at the time of diagnosis of CRS. Here, two “P”s - prediction of success of the treatment plan and the patient’s participation in it - are implemented. As per the evidence-based guidelines for treatment of CRS from the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) and International Consensus Statement on Allergy and Rhinology (ICAR), patients are commenced on first-line treatment of anti-inflammatory medication and saline douching (1, 29). Exacerbations of CRS, especially in the presence of purulent nasal discharge and positive bacterial cultures, may benefit from short-term treatment with culture-directed antibiotics (29, 30).

The success of treatment can be predicted based on well-established disease phenotypes. For example, Rouyar et al. noted that loss of smell is related to Th-2 disease, possibly due to the negative impact of the Type 2 inflammatory milieu on the number of immature olfactory neurons (31). CRS phenotypes associated with concomitant bronchial disease are particularly notable for

their challenging nature (32-36). A well-known example is Non-steroidal anti-inflammatory drug-(NSAID) Exacerbated Respiratory Disease (N-ERD), a clinical triad of hypersensitivity to aspirin or other NSAIDs, CRSwNP and asthma, characterised histopathologically by chronic and extensive eosinophilic inflammation of the respiratory tract (37, 38). It may be prudent to educate these patients from the start about the high tendency of nasal polyps to recur, the necessity for close follow-up and early involvement of the respirologists to co-manage them.

Recently, there have been efforts to build upon this library of known phenotypes by using clustering methods to identify various phenotypic subgroups, each with its characteristic phenotype, inflammatory cytokine profile and prognosticated treatment outcome (8, 39-41). The limitations of these studies are that these clusters are heterogenous and tend to vary from study to study. Further cluster analyses based on recognisable clinical and molecular profiling and performed in various populations are required to determine if these cluster phenotypes are consistent and whether they can be applied directly to patient care.

Patient participation in the treatment plan, being one of the pillars in precision medicine, highlights its equality with the other aspects of precision medicine. Increasingly, healthcare systems are employing mobile health technology via mobile applications (apps) to involve patients as active participants in the management of their condition and improve patient empowerment (42). These tools can help monitor disease, triage patients who need further investigations and improve compliance to treatment. They also have immense potential for gathering data for future research and epidemiological studies (43). It is to be emphasised that while active patient participation in his care is introduced here, it is imperative at every level of management in order to optimise treatment outcome.

## **2.2 Level 2 management**

The second level approach is applied to severe CRS that is suboptimally managed with level 1 care. The key “P” introduced here is prevention of progression and complications of disease (44, 45). Extended durations of antibiotic usage is discouraged because of limited benefits and risks such as gastrointestinal disturbances, *Clostridium difficile* colitis and antimicrobial resistance (29). Prolonged exposure of *Staphylococcus aureus* (*S. aureus*) to various antibiotics was found to induce mutations in metabolic genes of about 12% of *S. aureus* clinical isolates in CRS patients, forming antibiotic-tolerant small colony variants (46). Thus at this point, surgery is often proposed

unless contraindicated. In addition, there are roles for additional investigations to assess for immune deficiency and profile blood eosinophils, which is not often included in the initial workup for CRS.

### **2.2.1 Functional endoscopic sinus surgery and analysis of nasal epithelium**

When medical therapy fails, functional endoscopic sinus surgery is usually employed to improve drainage and ventilation of the sinuses and to reduce the disease burden of the nasal polyps. Apart from its effects in the upper airway, sinus surgery has additional benefits of improving asthma control and asthma-specific quality of life in patients with concomitant CRS (with or without nasal polyps) and poorly-controlled asthma (47, 48). Notwithstanding, the outcome of surgery can be variable, with up to 40% of CRS patients having uncontrolled recurrent disease three to five years after surgery (49, 50).

An advantage of surgery that is not often highlighted is the ability to study the histology of the individual's nasal or polyp epithelium, from which much can be gleaned about the disease chronicity and severity, which can in turn prognosticate treatment outcome. The sinonasal epithelium is the first line of defence in the nose, forming a physical barrier against entry of noxious matter, producing the mucus layer and providing mucociliary clearance (51). It is composed of four different cell types: ciliated columnar cells and non-ciliated columnar cells, goblet cells and basal cells, of which the latter have considerable importance due to their regenerative properties (52, 53). Impairment in the sinonasal epithelial barrier results in increased permeability and entry of potentially injurious entities, with a downstream exaggerated inflammatory response (51) (**Figure 3**). With persistent inflammation of the sinonasal mucosa, the epithelium gradually undergoes remodelling, which is seen as abnormal epithelial proliferation, squamous metaplasia, goblet cell hyperplasia, basement membrane thickening, fibrosis and edema on histology (54).

Studies on nasal epithelium in CRS in the last couple of years have shown that epithelial remodelling is contributed by upregulated hippo signalling pathway, abnormal basal cell proliferation, impaired cellular autophagy and abnormal zinc homeostasis. Each of these mechanisms have corresponding markers that could act as potential therapeutic targets in addressing nasal epithelial remodeling. On immunohistochemistry, p63 (a basal cell marker) is observed to be ectopically expressed in multiple cell layers in CRSwNP, implying epithelial

remodelling in nasal polyp tissue (55). Zhao et al. found that Ki67 (a nuclear antibody expressed during all active phases of the cell cycle) was specifically expressed in p63-positive cells and not in goblet or ciliated columnar cells, indicating that cellular proliferation occurred exclusively in these basal cells (56). Hyperplastic and squamous metaplastic epithelium from nasal polyps had significantly increased Ki67-positive and p63-positive cells compared to healthy epithelium; this was even more pronounced in nasal polyps with eosinophilia. Deng et al. noted that the components of the hippo pathway such as Yes-associated protein (YAP), which regulate stem cell self-renewal and tissue regeneration, were abnormally upregulated in CRSwNP (57). In addition, YAP protein expression was positively correlated with epithelial basement membrane thickness and Ki67 expression. Wang et al. reported that eosinophilic and non-eosinophilic nasal polyps demonstrated upregulated interferon-gamma (IFN- $\gamma$ ), which activated autophagy of nasal epithelial cells that formed the epithelial barrier, but was insufficient to degrade autophagy substrates such as p62 and ubiquitinated proteins and thus contributed to apoptosis of nasal epithelial cells (58). Murphy et al. noted that the expression of zonula occludens-1 (ZO-1), a tight junction-associated protein, was reduced in nasal polyp epithelium compared to controls and was associated with increased paracellular permeability. The labile levels of zinc, an essential trace element involved in maintaining epithelial tight-junction barrier and cellular function, were also significantly reduced in nasal polyp epithelium (59). This observation was supported by Ren et al. who found on electron microscopy that remodelled epithelium of nasal polyps had decreased amounts of zinc compared to healthy nasal epithelium (60).

These findings suggest that the presence of extensively remodelled epithelium on histology, substantiated by immunohistochemistry findings of increased positivity for p63, Ki67, YAP and IFN- $\gamma$ , and reduced staining for zinc and ZO-1, signify a dysfunctional epithelial barrier and the patient is likely to require continued medical treatment and close follow-up post-operatively.

### **2.2.2 Blood eosinophils**

Eosinophilic CRS is an emerging term in the literature used to describe CRS associated with a Th2 response that is driven by eosinophilic inflammation, as opposed to the traditional phenotype-based description (61). Eosinophilic CRS is diagnosed by histopathologic findings of eosinophilic infiltration within sinus tissue. While there is no consensus in the literature regarding the

diagnostic criteria, an absolute eosinophil count of >10 eosinophils per high-power field is the generally accepted cutoff value (62, 63). Notwithstanding, a recent meta-analysis reported that a cut-off value of >55 eosinophils per high-power field was highly predictive of disease recurrence following sinus surgery (64).

Fokkens et al. proposed that when initial therapy fails, blood eosinophil counts could be obtained as surrogate markers for underlying Th-2 disease (65). Blood eosinophils were noted to be feasible biomarkers for eosinophilic CRS due to their prominent role in Th-2 disease and are easily obtained especially in the pre-operative setting. The purpose was to strategise treatment and prognosticate disease outcome. In non-eosinophilic CRS, the role of corticosteroids was limited and management options inclined towards saline douches, antibiotics and surgery. In eosinophilic CRS, the emphasis of management was on corticosteroids and surgery, and even so it was recognised that this group was more difficult to treat, with a tendency towards recurrence.

While blood eosinophils are useful indicators for eosinophilic CRS, they are not without limitations. Elevated blood eosinophils is defined as  $>0.24 \times 10^9/L$  or eosinophil ratio  $>4.27\%$  of total white cell count (66). This cutoff value for blood eosinophils predicts eosinophilic CRS with moderate specificity of 78.4% and positive predictive value of 83.0%, but low sensitivity of 70.9% and negative predictive value of 64.5% (66). The cutoff value for eosinophil to total white cell count ratio has a better specificity of 88.5% and positive predictive value of 89.2%, but poor sensitivity of 64.1%. This may be because eosinophil activity at the tissue level may occur independently of elevated blood eosinophils (67). Furthermore, blood eosinophils may be elevated by other systemic conditions such as allergy, autoimmune conditions or adverse drug events not relating to CRS (68). Therefore, blood eosinophil levels should be interpreted with caution, and tissue analysis of eosinophils may still remain the gold standard for diagnosis of eosinophilic CRS.

### **2.2.3 Immune deficiency workup**

In patients with uncontrolled CRS, one may well have to consider the possibility of underlying immune deficiency. The true incidence of immune defects in CRS is unknown, although the incidence of antibody deficiency in refractory CRS is estimated to be about 10% (69). In a study of 1665 adult patients with CRSsNP and had two or more sinus infections per year for three consecutive years, Alpan et al. found that 28% had abnormal serum quantitative antibodies; 38%

had poor vaccine responses or loss of initial response to the polysaccharide vaccines, of which 18% had normal basic immunologic workup; and 67% of patients with abnormal immune phenotyping of T, B, NK and dendritic cells were eventually diagnosed with an immune deficiency (70). In those who had received immunoglobulin replacement therapy, topical and/or systemic antibiotic prophylaxis, the frequency of acute sinus infections was reduced from 3.7 episodes per year to 1.4 per year. This study concluded that abnormalities in each immune compartment could be independent of one another and therefore, the traditional step-wise diagnostic approach could result in significant under-diagnosis of immune deficiencies in CRS.

#### **2.2.4 Virus vaccination**

A viral infection is often the initial insult that kickstarts the process of CRS in certain patients. Viruses are more common in CRS than in the general population. Goggin et al. noted in a study of 288 patients that viral positivity was significantly higher in CRSsNP compared to CRSwNP and controls (71). The virus-positive CRSsNP group was also significantly associated with more severe endoscopic and radiologic sinus disease. The viruses detected were largely rhinovirus, coronavirus and influenza virus, with peak viral detection occurring in spring and winter.

The pathogenesis of viral infection in CRS is unclear, but may be related to the ability of viruses to prime the airway for bacterial infection. Viruses are known to damage the epithelial barrier, reduce tight junction expression, potentiate bacteria-induced histamine release and hamper the innate immune response (72-76). Deficient IFN-beta (IFN- $\beta$ ) responses to viral infection have also been demonstrated in asthma and CRS (77). A lack of early anti-viral activity in patients in CRS may result in more frequent or severe viral infections and pave the way for bacterial invasion, leading to the downstream development of CRS (71). Further research into viral prophylaxis and antiviral therapy could have potential in the treatment of CRS.

### **2.3 Level 3 management**

This approach is reserved for severe, uncontrolled, refractory CRS and requires personalised care - the fourth “P” - in the form of endotype-driven treatment tailored according to the individual’s nasal inflammatory profile. This is usually implemented at tertiary care, if not already done so. Some of the promising new biomarkers in the literature are summarised below (**Figure 4**). It is

worth noting that research efforts are primarily targeted at Type 2 inflammatory endotypes, perhaps reflecting its association with more severe disease and the compelling need for better treatment options for this group.

### **2.3.1 Endotypes**

#### **a) IL-4R $\alpha$**

Overproduction of mucous is considered to be detrimental because it promotes breeding ground for microbes and hyperviscosity impairs mucociliary function (78). In a study by Zhang et al, nasal polyps that had high levels of IL-5 (implying a predominantly Type 2 inflammatory endotype) were found to have significantly higher expression of the mucin genes MUC5AC and MUC5B compared to the IL-5-negative nasal polyp group (79). In addition, expression of the MUC5AC and MUC5B genes was increased by Type 2 inflammatory cytokines IL-4 and IL-13. IL-4 receptor-alpha (IL-4R $\alpha$ ) was also found to be widely expressed in IL-5-predominant nasal polyps. In short, the Th-2 inflammatory pattern is associated with increased expression of IL-4R $\alpha$  receptors and mucin genes in CRSwNP high in IL-5. Anti-IL4R $\alpha$  therapy may be a future strategy for modulating overproduction of mucous.

#### **b) IL-13R $\alpha$ 2**

IL-13 is a pro-inflammatory, pro-remodelling Th-2 cytokine that induces goblet cell hyperplasia, fibrosis, overproduction of mucous and mucociliary impairment (80-82). The effects of IL-13 are mediated through two key receptors: IL-13R $\alpha$ 1 and IL-13R $\alpha$ 2. It is previously thought that IL-13R $\alpha$ 2 has a negative regulatory role as a decoy receptor to reduce IL-13 response (83). Liu et al. found that nasal polyp epithelium had significant increased expression of IL-13, IL-13R $\alpha$ 1 and IL-13R $\alpha$ 2 (82). In addition, expression of IL-13R $\alpha$ 2 but not that of IL-13R $\alpha$ 1 was positively correlated with MUC5AC gene expression, suggesting that IL-13R $\alpha$ 2 was actively involved in IL-13 signalling and contributed to mucous overproduction via upregulation of the MUC5AC gene. Hence IL-13R $\alpha$ 2 could be a potential therapeutic target for modulating mucous production and cilia function.

### c) IL-25

IL-25 plays a crucial role in promoting Th2-biased inflammatory profile in CRSwNP. However, up-regulation of IL-25 in nasal polyp tissue is not a universal finding (84). A review article by Ogasawara et al. noted that most of the studies showing high levels of IL-25 in CRSwNP came from Asian countries including China, Korea and Japan (85-91) with the exception of 1 study conducted in Australia (92). Three studies that showed very low levels of IL-25 in nasal polyp tissue were conducted in Australia, United States and Turkey populations (84, 93-95). The mechanisms of type 2 inflammation may well be different between Asian and Western populations, and further studies are necessary to determine if genetic or environmental factors influence the expression of IL-25 in nasal polyp tissue.

In lieu of these findings, some studies have sub-endotyped CRSwNP into CRSwNP with high or low IL-25 (91, 94). Hong et al. demonstrated that the CRSwNP with high IL-25 group had more severe sinus disease on nasoendoscopy and computed tomography, greater Th-2 response and more severe eosinophilia compared to the CRSwNP with low IL-25 group (91). In another study, administration of anti-IL-25 treatment reduced the number of polyps, thickness of mucosal edema, collagen deposition and infiltration of inflammatory cells in murine models, suggesting that IL-25 may be a viable therapeutic target for treating CRSwNP associated with high IL-25 (87).

### d) IL-33 and *Staphylococcus aureus*

IL-33 is a member of the IL-1 family of cytokines, which are nuclear cytokines basally expressed in structural cells, such as endothelial, epithelial and fibroblast-like cells. IL-33 lacks a signal sequence and is passively released during cellular injury under inflammatory, infective or traumatic conditions. The IL-33 receptor is a heterodimeric complex of Suppression of Tumorigenicity 2 (ST2) and IL-1 receptor accessory protein, which is expressed on various immune cells, including Th2 cells, eosinophils, macrophages, mast cells, basophils and natural killer cells. Therefore the release of IL-33 is recognised as a key activator of innate and adaptive cells that drive Th2 immune responses (96). Recently, IL-33 is also noted for its role in regulating the function of type 2 innate lymphoid cells (ILC), a heterogenous family of cells of lymphoid morphology that produce IL-13 in response to IL-33 stimulation. An increased percentage of ILC and expression of ST2 was noted in ethmoid tissue from CRSwNP (presumed to be associated

with Th2 inflammation) compared with CRSsNP and controls, and ST2-positive ILC were found to be a consistent source of IL-13 in response to stimulation with IL-33 (97).

Kim et al noted in their study of CRS in Asian patients that non-eosinophilic nasal polyp tissue, associated with elevated neutrophil and Th1/Th17 cytokine levels, were associated with significantly elevated expression of IL-33 compared with eosinophilic nasal polyps and controls (98). Moreover, anti-IL-33 treatment administered to a murine model of CRS reduced thickness of oedematous mucosa, subepithelial deposition of collagen and neutrophilic infiltration but not eosinophilic infiltration. It is therefore possible that IL-33 is a key mediator in the pathogenesis of CRSwNP through recruitment of neutrophils.

*S. aureus* is a gram-positive organism that is part of the nasal microbiome in over 30% of the human population with no adverse effects, but is widely implicated in CRS as a pathogen. Apart from its known roles in super-antigen, biofilm and small colony variant formation, *S. aureus* secretes proteases which act as allergens by inducing a specific IgE response. Krysko et al noted that repeated exposure to *S. aureus* proteases triggered the release of IL-33 (99). These findings were supported by Teufelberger et al, who demonstrated that repeated intratracheal exposure to *S. aureus* serine protease-like protein (Spl) -D in a murine model of allergic asthma resulted in increased production of IL-33, eosinophils, bronchial hyperreactivity and airway goblet cell hyperplasia (100). Inhibition of IL-33 with a soluble ST2 receptor significantly reduced this Th2 inflammation but did not affect the production of IgE, suggesting that it did not prevent allergic sensitisation. Further study is required to understand if inhibition of IL-33 could be a promising strategy to inhibit inflammatory changes induced by *S. aureus*.

#### e) **LTD4 in eosinophils**

Eosinophils amplify inflammation and pathogen clearance via release of cytotoxic granules, which contain cytokines, chemokine and lipid mediators. These lipid mediators include cysteinyl leukotrienes, prostaglandins and 15-lipoxygenase (15-LOX)-derived mediators. 15-LOX mediates local production of anti-inflammatory lipid mediators, whereas cysteinyl leukotrienes amplify allergic inflammatory responses. Leukotriene D4 (LTD4) in particular has a strong affinity for cysteinyl leukotriene-1 and promotes bronchial constriction, leaky vasculature and mucous production (101). Miyata et al. noted that eosinophilic LTD4 production was significantly increased in nasal polyp tissue whilst 15-LOX-derived mediators were significantly reduced (102).

Moreover, blood eosinophils of healthy subjects treated with eosinophil activators such as IL-5 also produced a lipid profile similar to that of eosinophilic CRS. This suggested that stimulation with local pro-inflammatory cytokines was important in inducing dysregulated fatty acid metabolism within the eosinophils of eosinophilic CRS. Therefore, LTD4 may potentially be targeted therapeutically to control eosinophilic CRS.

#### **f) Neutrophils**

The role of neutrophils in CRSwNP is relatively understated compared with the conspicuity of eosinophils in Type 2 inflammation. However, neutrophils may play a more important role in the pathophysiology of nasal polyposis than previously thought. While non-eosinophilic (neutrophil predominant) CRS is typically associated with a Th-1/Th-17 skewed inflammatory response and upregulated expression of pro-inflammatory cytokines such as IL-17A, IL-1 $\beta$  and matrix metalloproteinase-9(10), increased neutrophilic infiltration has been demonstrated even in the eosinophilic nasal polyps of both Asian and Caucasian populations (103-105). Neutrophils can be a double-edged sword because on one hand, their ability to phagocytose microbes and generate antimicrobial products such as reactive oxygen species and proteases forms part of the innate immunity; yet these byproducts can produce the undesirable effects of exacerbating tissue damage and inflammation (106). Wang et al. found that one such protease, neutrophil-derived elastase, was a major activator of IL-36 $\gamma$ , a pro-inflammatory cytokine that was significantly upregulated in CRS with and without nasal polyps compared to controls (107). IL-36 $\gamma$  promoted the expression of IL-17A in neutrophils, which in turn, upregulated IL-36 $\gamma$  expression in nasal epithelial cells, leading to a vicious cycle of sustained neutrophilic inflammation in CRS.

In other studies, neutrophils were found to be a major source of transforming growth factor-beta2 (TGF- $\beta$ 2) and oncostatin M, cytokines that induced sinonasal epithelial remodeling and epithelial barrier dysfunction respectively in CRS (103, 105). Arebro et al. demonstrated that three sets of neutrophils existed, stratified according to high or diminished expression of Fc $\gamma$ RIII (CD16) and L-selectin (CD62L) (108). The neutrophil subset CD16<sup>high</sup> CD62L<sup>dim</sup>, thought to be a more activated form of neutrophil with increased ability to phagocytose microbes, was significantly higher in nasal polyps compared to controls. Correspondingly, the neutrophil subset, CD16<sup>high</sup> CD62L<sup>high</sup>, a mature form of neutrophil, dominated in control nasal mucosa.

The presence of neutrophilic inflammation is important as it may implicate treatment outcomes. Wen et al. noted that the presence of neutrophilia in eosinophilic nasal polyp tissue may suggest reduced response to corticosteroids, a mainstay of management of eosinophilic CRSwNP (109). This was corroborated by Ryu et al.'s findings that the cytokine profile of refractory nasal polyps showed increased expression of Th17-associated mediators, B-cell activating factor and IFN- $\gamma$  compared to controls and primary nasal polyp tissue (110).

These findings underscore the important but unsung role of neutrophils in the pathogenesis of CRS. Treatment options that target this pathway may be another potential strategy to address inflammation in this disease.

#### **g) Programmed cell death-1**

Programmed cell death-1 (PD-1) is a negative regulator of T-cell responses. PD-1 is induced by T-cell activation, and interaction with its ligands PD-L1 and PD-L2 inhibits T-cell receptor-initiated events such as cell proliferation and cytokine production (111). PD-L1 and PD-L2 have been implicated in the modulation of allergic disease such as asthma (112). Kortekaas et al. found that nasal polyp tissue had increased T-cells expressing PD-1 in varying degrees, which could be explained by different endotypes of the disease (113). PD-1 expression was more pronounced in patients with concomitant asthma and CRSwNP, and was positively correlated with IL-5 expression. The presence of atopy and use of nasal steroid spray did not significantly affect the expression of PD-1 or its ligands. PD-1 expression could therefore be a useful immunologic marker for disease severity in CRSwNP.

#### **2.3.2 Updates on treatment options**

With increasing understanding of the molecular pathways in CRS, the study of the corresponding pharmacology becomes an ever-expanding and evolving field. Biologics in particular are exciting novel therapeutic strategies that hold great promise in providing targeted therapy for the group of difficult-to-treat, recalcitrant CRS patients. At present, all biologics except Dupilumab are still in phase 2 trials for use in CRSwNP. An exhaustive description of endotype-directed or other new therapeutic options is beyond the scope of this article, and interested readers are directed to some

excellent review articles on this subject (114-116). A succinct update on the evidence with regards to some of the key biologics and the potential role of probiotics is summarised here.

#### **a) Dupilumab**

Dupilumab is a fully human-derived monoclonal antibody that binds specifically to IL-4R $\alpha$ , inhibiting signalling of both IL-4 and IL-13. It is approved in the European Union, USA, Japan and other countries for treatment of inadequately controlled moderate-to-severe atopic dermatitis in adults (117). Dupilumab is recently approved by the United States Food and Drug Administration (FDA) since June 2019 for the treatment of inadequately-controlled CRSwNP based on two pivotal trials, the 24-week SINUS-24 and 52-week SINUS-52, that are part of the Phase 3 LIBERTY clinical trial program (118, 119). These trials evaluated dupilumab 300 mg every two weeks for the duration of the trial with standard-of-care mometasone furoate nasal spray compared to placebo injection plus mometasone furoate nasal spray. Improvement in nasal congestion and loss of smell was observed as early as 4 weeks. At 24 weeks, both trials demonstrated significant improvement in nasal congestion, nasal polyp score, sinus opacification and sense of smell compared to placebo. In addition, the need for surgery in these patients was reduced by 73%. In the 52-week SINUS-52 trial, patients continued to do well through the 52-week treatment period. There was a good safety profile, with the commonest adverse events adverse events being epistaxis (7.7%), injection site reactions (6%), conjunctivitis (2%), arthralgia (3%) and gastritis (2%).

#### **b) Mepolizumab**

Mepolizumab is a humanised IgG1 monoclonal antibody that binds to and blocks the function of circulating IL-5, thus preventing binding of IL-5 to its receptor. It is approved in the European Union and USA for the treatment of severe eosinophilic asthma. In two randomized, double-blind, placebo-controlled trial, Gevaert et al. and Bachert et al. reported that mepolizumab significantly reduced nasal polyp size, with the latter study also showing improved symptom scores and greater reduction in the need for sinus surgery (120, 121). IL-5-targeted therapy appears to benefit mainly eosinophilic-driven diseases of the airway, probably because eosinophils seem to be only partially dependent on IL-5, or that eosinophil-dominated inflammation in the tissue is not exclusively

mediated by eosinophils. However, it is worthwhile noting that as the remaining eosinophils are still able to release toxins that can cause tissue damage, inhibition of IL-5 does not lead to complete cessation of eosinophil function (122). Mepolizumab is currently being evaluated in phase 3 clinical trials for CRSwNP (NCT03085797).

### **c) Omalizumab**

Omalizumab is a humanized monoclonal antibody that binds to constant region 3 (Cε3) of IgE and blocks this protein from binding to its receptor that is located on mast cells and basophils. It is administered subcutaneously and is approved in the European Union and USA for the treatment of severe allergic asthma with high levels of aero-allergen specific IgE (123, 124) and chronic spontaneous urticaria (125-132). Its intranasal application is under investigation for treatment of allergic rhinitis as well (133). There have been two phase II randomised, double-blind, placebo-controlled trials evaluating the effect of omalizumab on CRSwNP. Gevaert et al. observed that omalizumab significantly reduced nasal polyp size, improved radiologic scores and upper and lower airway function in CRSwNP (134). Pinto et al. reported improved SNOT-20 scores but no significant change in polyp size or inflammation on imaging compared to controls (135). Omalizumab is currently undergoing phase 3 clinical trials for CRSwNP (NCT03280537 and NCT03478930).

## **3. Unmet needs and future challenges**

Eosinophilia is increasingly recognised as a prognostic factor for difficult-to-treat CRS. This may be reflected in the trend in some of the literature towards describing CRS using the terms “eosinophilic CRS” and “non-eosinophilic CRS” rather than phenotype-based monikers (64-66, 136-138). While a re-examination of CRS terminology may be fitting, the challenge lies in establishing an acceptable definition of “eosinophilic” which is clinically significant and applicable across various ethnicities and populations.

On the same note, with the growing interest in identifying endotypes of CRS and emerging biologics as exciting novel therapeutic options, it is crucial to critically evaluate the other side of the coin. Well-conducted, unbiased, long-term studies are required to examine issues such as long-

term results, adverse outcomes, cost-effectiveness and refining the target population that would most benefit from this treatment.

#### 4. Conclusion

CRS is being increasingly recognised as a continuum of disease processes, ranging from predominantly non-Type 2 inflammation at one end of the spectrum to predominantly Type 2 inflammatory, eosinophil-heavy disease at the other end, with a mix of both in between, and all of which can present with or without polyps (**Figure 3**). To describe CRS as a dichotomy of CRSsNP and CRSwNP is to oversimplify an extraordinarily complex disease process. The introduction of precision medicine to manage this chronic, complex condition is a step forward in providing individualised patient care. Much progress has been made in defining the molecular mechanisms of various pathophysiological processes to identify potential biomarkers for precision treatment. However, as we consider that biologics work as single mediator antagonists that only affect part of the disease spectrum, more long-term studies are needed to examine the downstream, long-term effects. Endotype-driven treatment will still face multiple challenges before its eventual implementation in daily clinical practice.

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## Figures

**Figure 1.** Chronic rhinosinusitis - the result of complex interactions between infective, immune, genetic and environmental factors.

**Figure 2.** The principles of precision medicine (4 Ps) applied to a proposed treatment algorithm for chronic rhinosinusitis.

**Figure 3.** Putative mechanisms (based on data from this review) of Type 2 and Non-Type 2 inflammation in chronic rhinosinusitis.

**Figure 4.** Summary of novel molecular mechanisms and biomarkers for endotypes of chronic rhinosinusitis in this review.

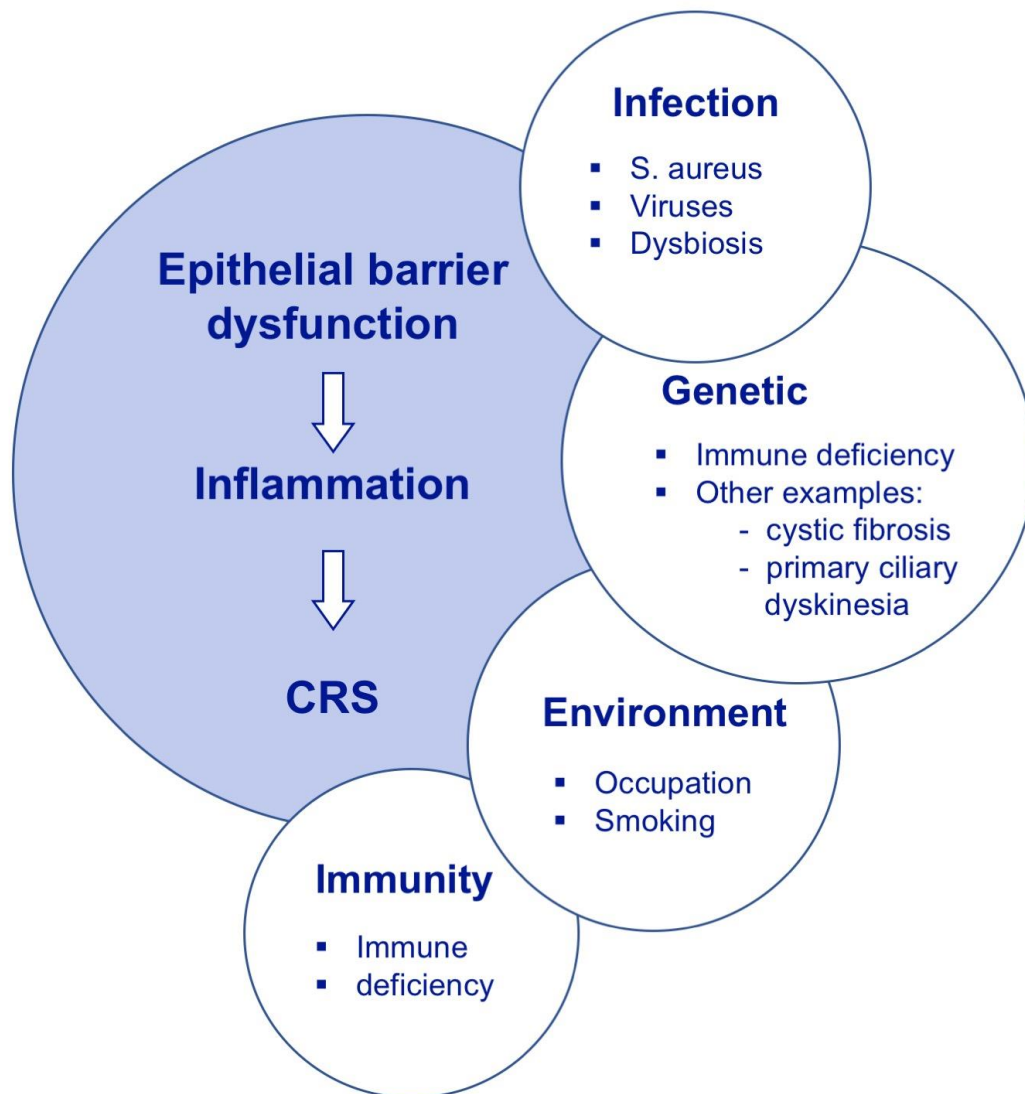


Figure 1\_Wang\_ALL-2019-00535

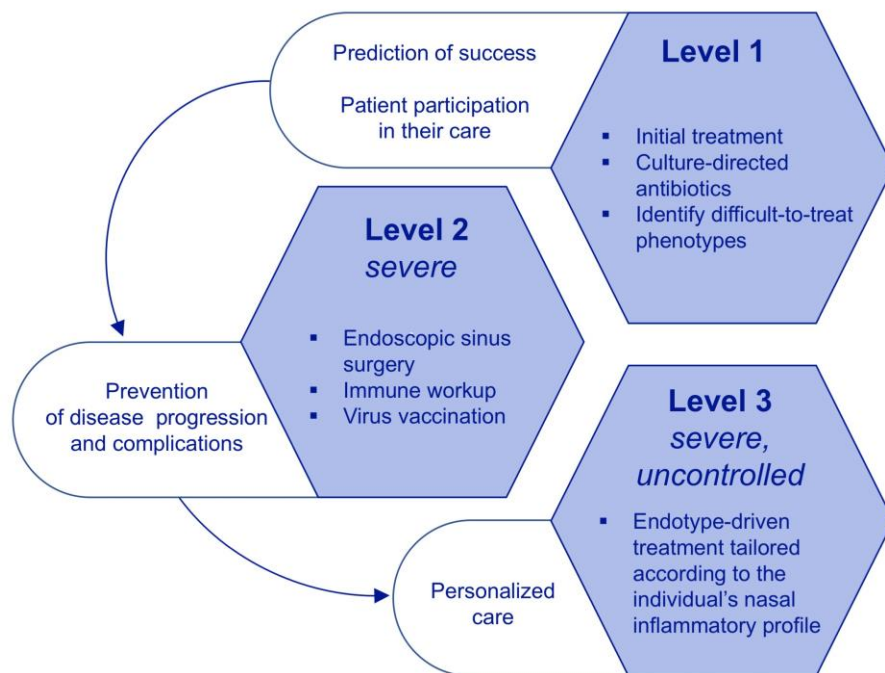


**Figure 2\_Wang\_ALL-2019-00535**

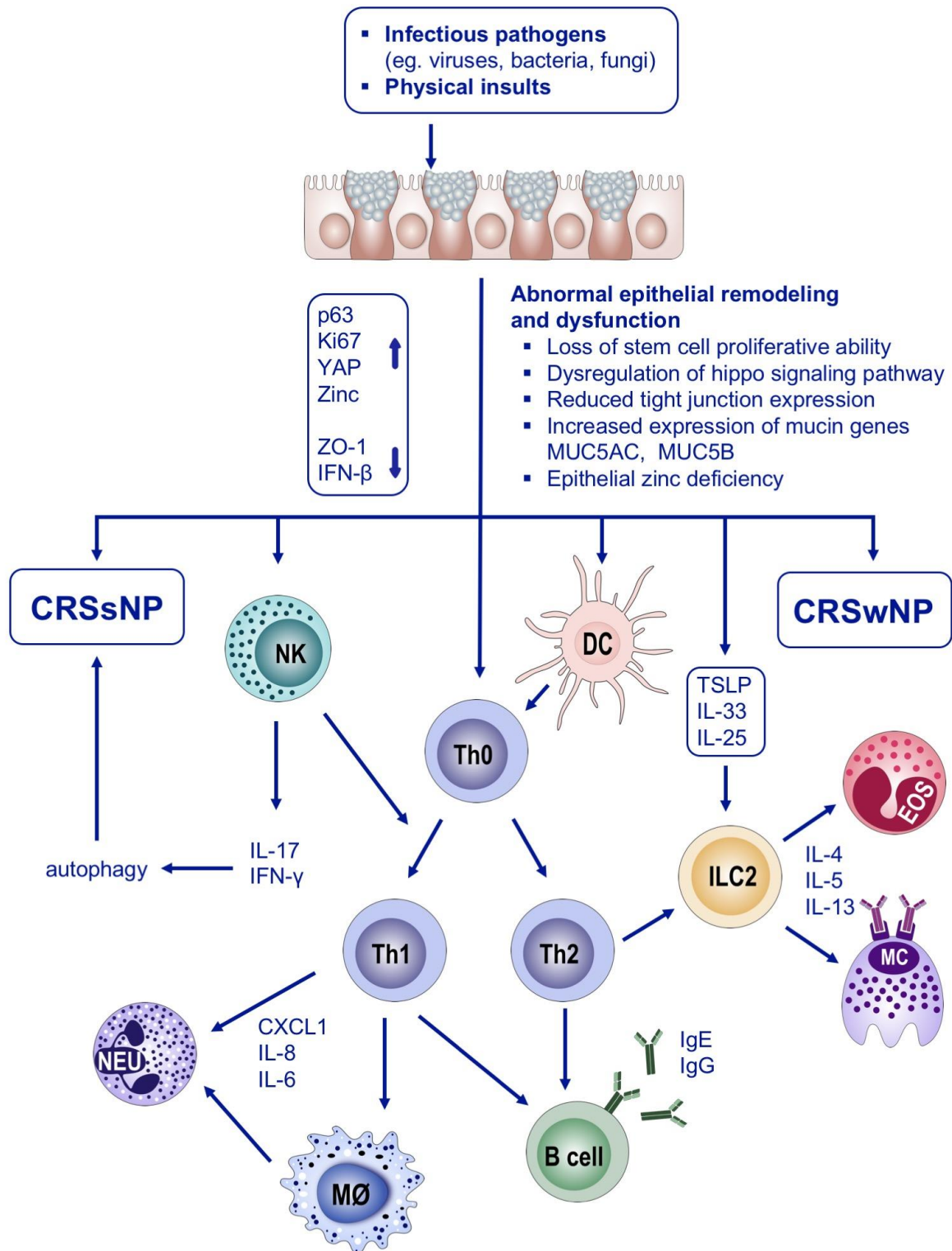


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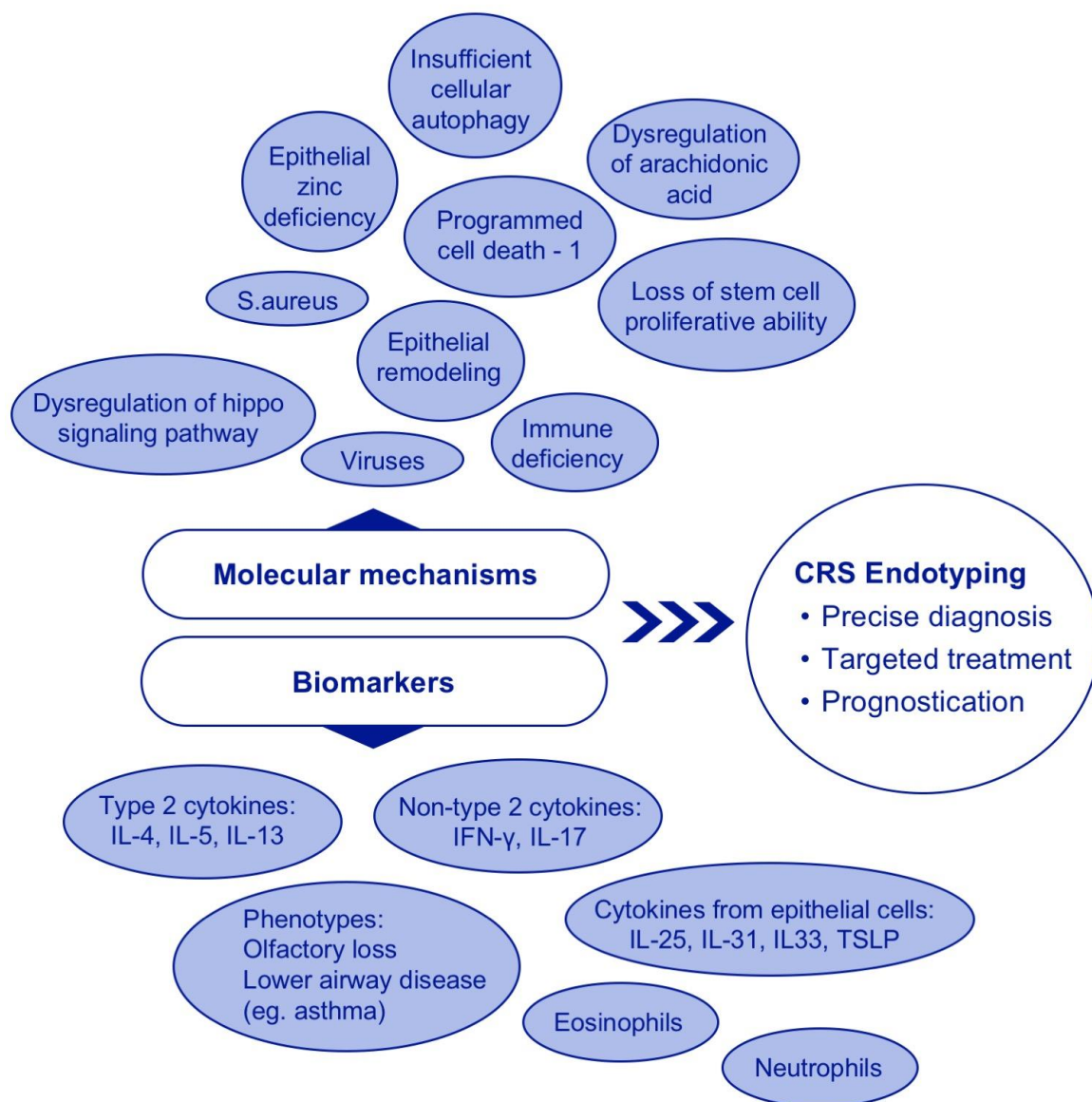


**Figure 4\_Wang\_ALL-2019-00535**