

Personalized Medicine in Chronic Rhinosinusitis Phenotypes, Endotypes, and Biomarkers



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KEYWORDS

• Cytokine • Inflammation • Rhinosinusitis • Phenotype • Endotype

KEY POINTS

- Understanding the molecular basis behind the clinical manifestations of chronic rhinosinusitis (CRS) can potentially provide a more comprehensive and personalized approach to treating individual patients.
- Studies incorporating CRS endotyping have used different variations of biomarkers, resulting in a variable number of disease clusters; however, the studies have largely produced consistent results with respect to the underlying characteristics of identified endotypes.
- The ideal CRS biomarker should be highly specific and have the ability to facilitate diagnosis, differentiate phenotypes and endotypes, indicate treatment responsiveness, and monitor disease control.

INTRODUCTION

Chronic rhinosinusitis (CRS) is now recognized as a heterogeneous disease process with a broad spectrum of clinical presentations and pathogenic mechanisms. Identification of specific drivers of disease has become a focus of CRS research and has the potential to predict outcomes and response to treatment, in addition to encouraging development of new and improved biotherapeutics. With this in mind, it is important to note that definitive algorithms for CRS management have remained elusive and at times highly variable. A more personalized approach to care of the patient with CRS will become imperative going forward, given direct costs to the US health care system of more than 10 billion dollars yearly.^{1–5} Ultimately, meeting this goal will require improved understanding of disease heterogeneity in relation to clinically observable patient characteristics (phenotypes), underlying cellular or molecular

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pathogenic mechanisms (endotypes), and clinically significant outcomes.^{6,7} The present review summarizes recent advancements in personalized approaches in CRS, with a focus on disease phenotypes, endotypes, and potential biomarkers.

PATHOPHYSIOLOGY

Although the pathophysiology of CRS is incompletely defined, current evidence suggests that a combination of host and environmental factors play an integrative role. The following factors are thought to play a role in the cycle of anatomic dysfunction and chronic mucosal inflammation that is commonly observed in CRS:

- **Innate and epithelial immunity:** the upper airways have a unique epithelial barrier characterized by tight and adherens junctions that coordinate to protect underlying immunosensitive tissue from environmental irritants and microbial pathogens.⁸ This epithelial barrier is dysregulated in CRS possibly secondary to pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which can directly affect tight junctions, or due to cytokines such as interferon gamma (IFN- γ) and interleukin 4 (IL-4), which can interfere with barrier protein expression.⁹
- **Mucociliary clearance:** among the most essential functions of the upper airway, mucociliary clearance serves as the first line of defense against airborne particles and microbial pathogens.¹⁰ Ciliated sinonasal mucosa is covered with a mobile mucous layer that lays on a less viscous periciliary layer working together to move foreign particles through the nose and into the gastrointestinal tract.¹¹ A breakdown in mucociliary clearance is characteristic of specific CRS phenotypes, such as cystic fibrosis and primary ciliary dyskinesia, but is also observed in other patients with CRS as well.^{12,13} Certain pathogens, including *Haemophilus influenza*, *Streptococcus pneumoniae*, *S aureus*, *P aeruginosa*, and *Aspergillus* secrete proteases that impair ciliary movement. In addition, hypoxia develops due to excess secretions and alters ion transport, resulting in an ineffective mucous layer and uncoordinated ciliary movement.^{14–17}
- **Tolerance and adaptive immunity:** breakdown of the epithelial barrier enables allergens and microbial antigens to transgress physical barriers, culminating in local tissue inflammation and/or allergen sensitization.⁶ This induces epithelial cells to secrete inflammatory mediators and cytokines that recruit and activate multiple effector cells.^{18(p2),19–21} Microbial pathogens, particulates, and allergens all have the ability to activate antigen-specific T-helper (Th) cells. Multiple subsets of Th and innate immune cells drive inflammation in CRS, including type 1/Th1 cells that produce IFN- γ ; type 2/Th2 cells that secrete IL-4, IL-5, and IL-13; and type 3/Th17 cells that produce IL-17 and IL-22. Immune polarization in CRS is highly variable, and a combination of type 1, type 2, and type 3 immune mechanisms drive the production of specific cytokines and results in a persistent inflammatory state.^{22–24}
- **Microbiome:** the diversity and composition of the sinonasal microbiome plays an integrative role in the health of the respiratory environment. Multiple studies have attempted to isolate specific bacteria linked with the pathogenesis of CRS^{25–28}; however, even with more recent advances in molecular genetics, a specific causative organism has yet to be identified. Nonetheless, current evidence suggests that imbalance (or dysbiosis) within the microbiome, specifically a decrease in microbial diversity, may be a common characteristic of patients with CRS.²⁶ This imbalance can lead to an overabundance of opportunistic pathogens such as *S aureus* and *P aeruginosa* and loss of key commensal organisms.²⁹

PHENOTYPES

Phenotypic classifications in CRS have largely been driven by clinical practice and have remained largely unchanged. Classically, patients have been differentiated primarily by the presence (chronic rhinosinusitis with nasal polyps [CRSwNP]) or absence (chronic rhinosinusitis without nasal polyps [CRSsNP]) of nasal polyps, as this is among the most clinically observable features in most patients. This classification has largely driven treatment approaches, and dichotomization of patients based on polyp status has long been linked to differences in pathobiology and disease mechanisms. CRSwNP has primarily been linked to type 2 inflammation and eosinophilic tissue infiltration, whereas CRSsNP has typically been linked with elevated type 1-associated cytokines.^{30,31} However, this rigid dichotomy is now recognized as overly simplistic, and substantial research now suggests that both CRSsNP and CRSwNP can present with a combination of type 1-, type 2-, and type 3-associated signatures.^{23,32–34} Furthermore, there is evidence to suggest that within phenotypes, particularly among patients with nasal polyps, further subgrouping based on other observable qualities or comorbidities (eg, allergy, asthma, aspirin sensitivity) can be associated with unique disease behavior. This can potentially include local aggressiveness, altered responses to therapeutics, and high recurrence rates after surgical treatment. As such, additional subphenotypes such as aspirin-exacerbated respiratory disease, allergic fungal rhinosinusitis, and cystic fibrosis–associated CRS have been described and are used clinically. Unfortunately, within individual phenotypes there is substantial variability in patient demographics, comorbidities, and clinical responses to therapy that make this approach inefficient.

ENDOTYPES

The heterogeneity and complexity of CRS strongly suggests the need for alternatives to phenotype-based classification systems. In contrast to phenotypes, which are based on easily observable features, endotypes are instead derived from assessment of biomarkers that reflect underlying biological mechanisms.³⁵ Understanding the molecular basis behind the clinical manifestations of CRS can potentially provide a more comprehensive and personalized approach to treating individual patients. Endotyping has advanced treatment paradigms in other heterogeneous inflammatory diseases, particularly in asthma where it has led to improved understanding of treatment-resistant disease, enhanced diagnostic schemes, and facilitated targeting for biological therapies.^{36–38} Inflammatory endotyping has only recently been explored for CRS, but early studies have shown consistent findings and advanced understanding of the disease process. Although endotyping can incorporate any biomarker, most studies have focused on cytokines and inflammatory mediators measured in nasal secretions or sinonasal tissue biopsies.^{32,33} The use of hierarchical clustering and other unstructured statistical approaches allows for incorporation of an infinite combination of biomarkers without any presumed outcome. This allows for patients with similar inflammatory signatures to be grouped into “clusters,” presumably with similar underlying pathophysiologic mechanisms of disease.^{32,33,39,40} To date, studies incorporating CRS endotyping have used different variations of biomarkers resulting in a variable number of disease clusters; however, the studies have largely produced consistent results with respect to the underlying characteristics of identified endotypes. In a landmark European study, Tomassen and colleagues³² used hierarchical cluster analysis using 14 different inflammatory markers to identify putative CRS inflammatory endotypes. They identified 10 distinct clusters characterized by the following:

1. Eosinophilic and Th2-related markers (IL-5 and immunoglobulin E [IgE]),
2. Neutrophilic or proinflammatory mediators or both (IL-1 β , IL-6, IL-8, and myeloperoxidase),
3. Th17/Th22 markers (IL-17A, IL-22, and tumor necrosis factor- α), and
4. IFN- γ .³²

In a smaller cohort study, Divekar and colleagues³⁹ used a similar approach with a commercially available immunoassay of 41 cytokine/chemokines in tissue from 26 patients from a North American population. They identified 3 distinct clusters driven by Th1/Th17-type mediators (IL-17A, granulocyte-colony stimulating factor, IL-8, and IFN- γ), type 2-associated mediators (IL-5, IL-9, IL-13 and eotaxin) and finally a third cluster characterized by elevated growth factors (platelet-derived growth factor and vascular endothelial growth factor). Finally, Turner and colleagues³³ performed hierarchical cluster analysis on 90 patients using 18 different biomarkers measured in mucus to identify 5 to 6 distinct clusters.⁴¹ Individual clusters were primarily characterized by type 2 inflammation, proinflammatory and neutrophilic inflammation, or clusters with minimal to low overall inflammation. All of these studies show similar patterns of inflammatory burden and suggest that type 2-associated inflammation is present in most European and North American patients with CRS but only dominant in a small minority.

In a different geographic demographic, Liao and colleagues⁴⁰ analyzed 246 Chinese patients with CRS and incorporated both inflammatory biomarkers and clinical/demographic variables in the clustering model. Seven distinct clusters were identified. Similar to other published studies, some clusters were characterized by mild inflammatory burden or neutrophilic inflammation, whereas only 20% of patients were assigned to clusters dominated by type 2 inflammation, further highlighting potential geographic differences in disease characteristics. This study also analyzed treatment outcomes with respect to disease clusters and found that “difficult-to-treat” CRS was mainly associated with neutrophilic endotypes and those associated with elevated proinflammatory profiles, whereas a smaller group with severe disease and poor treatment outcomes was associated with the type 2 endotype.

It is important to recognize that endotypic classification is largely a representation of individual inflammatory mechanisms and not a clearly definable entity with a direct biological basis. There is likely a continuum of different types of inflammation within individual patients and thus few patients will be linked directly to a single inflammatory endotype or immune signature. The longitudinal stability of putative CRS endotypes also remains unclear, and it is likely that medical/surgical treatment, environmental factors, geography, and other variables may have significant impacts on endotypic assignment. In support of this hypothesis, a recent study found that most of the patients undergoing endoscopic sinus surgery moved to a different inflammatory endotype postoperatively.⁴²

BIOMARKERS

The term “biomarker” refers to any objective measure that reflects the underlying medical condition of a patient or predicts outcomes to treatment. In CRS management, and for the purposes of this review, biomarkers typically refer to biological molecules measured in serum, tissue, or nasal secretions, which reflect underlying disease pathophysiology and aid in diagnostics and treatment algorithms. In general, an ideal biomarker should be highly specific and facilitate diagnosis, differentiate phenotypes and endotypes, indicate treatment responsiveness, and monitor disease control. Although currently explored biomarkers have some ability to classify

patients, single biomarkers have generally lacked the ability to accurately characterize patients, largely because each biomarker is involved in unique but complex molecular pathways. The use of multiple biomarkers may ultimately have greater potential to clarify such complex pathophysiology and improve predictive power. The following section is a summary of potential CRS biomarkers.

Eosinophils

Eosinophilic mucin, eosinophilia, and elevated IgE levels are part of the type 2 immune response and have frequently been associated with treatment outcomes and extent of disease. Tissue eosinophilia in CRS is thought to be related to more extensive disease and a decreased likelihood of surgical success.^{43–45} Elevated systemic eosinophils may also reflect worse prognosis, increased severity of symptoms, and extensive sinus disease.⁴⁶ Conversely, eosinophilic nasal polyps are generally thought to be more glucocorticoid responsive than their noneosinophilic counterparts.⁴⁷ Because eosinophilia has clear prognostic and treatment implications, substantial effort has focused on minimally invasive approaches to identify these patients. Importantly, the degree of tissue eosinophilia is extremely difficult to determine based on clinical symptoms and cannot be predicted by the presence of asthma or aspirin-exacerbated respiratory disease.⁴⁸ Likewise, phenotypic differentiation of patients with CRS based on polyp status fails to accurately align with presence or absence of eosinophils.⁴⁹ Because significant overlap in clinical phenotypes and symptoms exist among patients with and without eosinophilia, direct measurement of tissue and/or serum eosinophils has generally been required to accurately differentiate patients.

Tissue eosinophilia can most accurately be characterized using tissue histopathology; however, indicators and definitions of eosinophilia have been inconsistent. For example, Kountakis and colleagues⁵⁰ proposed that greater than 5 eosinophils per high power field (HPF) was diagnostic based primarily on the degree of presumed eosinophil activation. In contrast, Soler and colleagues⁵¹ defined mucosal eosinophilia as greater than 10 eosinophils/HPF, which was predictive of less improvement in disease-specific quality of life scores after surgery. Regardless of the precise definition, most patients fall along a continuum of eosinophil infiltration, making precise grouping difficult. As an alternative, peripheral blood eosinophil levels have been investigated as a potential surrogate marker for tissue eosinophilia, as this does not require analysis of surgical specimens or tissue biopsies. In one study by Sakuma and colleagues,⁵² a blood eosinophil percentage of greater than or equal to 6% correlated with tissue eosinophilia. However, various comorbid disorders including allergies, autoimmune diseases, adverse drug reactions, and parasitic infections can alter circulating eosinophil counts, and as such, the utility of blood eosinophil counts as a biomarker remain limited.

Finally, the presence of eosinophils alone does not indicate eosinophilic inflammation or activation. Biomarkers that include byproducts or molecules secreted by eosinophils and key eosinophil-associated cytokines may potentially be more indicative of underlying eosinophil activation.²⁴ This approach may be superior to histologic approaches, which can be semiquantitative and subject to both interpreter and intraspecimen variation. Examples of eosinophil markers include eosinophil cationic protein (ECP), major basic protein, eotaxins, total IgE, and *S aureus* enterotoxins (SE-IgE).⁵³

Immunoglobulin E

IgE binds to receptors on eosinophils, basophils, mast cells, and other cell types and can amplify the type 2 immune response. IgE has been investigated as a potential biomarker due to frequently elevated levels of serum and local IgE in patients with

CRS and associated atopy and eosinophilic inflammation.³² Bachert and colleagues³⁵ found that concentrations of total IgE correlated significantly with levels of ECP and IL-5 and thus local eosinophilic inflammation. In a prospective follow-up, high local IgE ultimately predicted the need for revision surgery.⁵⁴ In addition, among patients with nasal polyps, tissue IgE and SE-IgE were identified as risk factors for comorbid asthma.⁵⁵

Type 2 Cytokines

IL-5 is a key cytokine responsible for survival, maturation, and activation of eosinophils within the bone marrow and at sites of inflammation and is a key mediator of type 2 inflammation.⁵⁶ Patients with high local and systemic IL-5 also have a higher risk of having asthma comorbidity.^{55(p5)} Elevated tissue IL-5 is a common characteristic of nasal polyps, with recent studies suggesting high levels in 80% of European nasal polyp cases and 20% to 60% of Asian nasal polyp cases.²² Gevaert and colleagues⁵⁷ found that elevated IL-5 in nasal secretions predicted clinical response to reslizumab, a humanized anti-IL-5 monoclonal antibody. Likewise, Turner and colleagues⁵⁸ found that levels of mucous IL-5, and the related type 2 cytokine IL-13, were associated with greater disease severity and prior surgical intervention.

IL-4 and IL-13 are also essential cytokines that activate the type 2 inflammatory response. They synergistically promote the synthesis of IgE from B cells and stimulate mucous secretion in epithelial cells. In addition to the induction of eosinophilic inflammation, IL-4 plays a role in nasal polyp formation. IL-13 affects epithelial differentiation resulting in decreased ciliation and goblet cell metaplasia as well as increasing hyper-reactivity of the airway and subepithelial fibrosis. Although IL-4 was not found to be a reliable biomarker in CRS in mucous samples, it has been found to be significantly elevated in tissue samples of patients with CRS compared with controls.^{58–60} Turner and colleagues⁵⁸ found that patients with a high IL-13 signature had significantly worse preoperative disease than their low IL-13 counterparts. Delineating which patients have high IL-13 and IL-4 signatures is thought to be of value not only for disease severity but also for patient selection regarding treatment with targeted biologics against these cytokines.

Interleukin 25 and 33

Epithelial-derived cytokines, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, are recognized for their ability to interact with lymphocytes and dendritic cells to promote type 2 inflammation.^{61,62} IL-25, also known as IL-17E, is a member of the IL-17 cytokine family and has been associated with high computed tomographic (CT) scores and elevated blood eosinophil numbers in patients with CRS.⁶³ IL-33 is a member of the IL-1 cytokine family and is likewise a potent inducer of type 2 responses, although underlying mechanisms remain poorly defined.⁶⁴ Expression of IL-33 is elevated in CRSwNP patients with severe and recalcitrant disease.⁶⁴

Periostin

Periostin is a secreted extracellular protein that has emerged as a marker in pathologic remodeling processes and has an important role in wound repair as well as epithelial-mesenchymal transition of cancer cells.⁶⁵ Periostin is produced mainly by epithelial cells in response to IL-4 and IL-13 and has a pivotal role in subepithelial fibrosis. Overexpression of periostin has been found in eosinophilic CRSwNP and may have roles in epithelial damage, extracellular matrix accumulation, and infiltration of inflammatory cells.^{66–68} A recent study by Maxfield and colleagues⁶⁶ established that high serum levels of periostin were strongly associated with the presence of nasal polyps in

patients with CRS. Zhang and colleagues⁶⁷ also found that periostin expression was elevated in active CRS, and decreased after effective treatment, suggesting that periostin could represent an indicator of disease activity and responsiveness to therapy. In addition to being a diagnostic biomarker, periostin may also be a biomarker for responsiveness to omalizumab in eosinophilic nasal polyps and has already been studied as an effective biomarker in asthma.⁶⁹

P-Glycoprotein

P-glycoprotein is an adenosine triphosphate-dependent efflux pump that is constitutively expressed in multiple cell types.⁷⁰ It also functions as an immunomodulator capable of regulating secretion of proinflammatory cytokines from epithelial cells.^{70,71} P-glycoprotein levels are elevated in all CRS subtypes, particularly those with nasal polyps, and higher levels have been associated with worse subjective and objective measures of disease severity.^{70,71} The reported immunomodulatory function of P-glycoprotein coupled with its presence in sinonasal epithelium suggests that it may play an important role in CRS pathophysiology.

Cystatin

Cystatin is a type 2 cysteine protease inhibitor that plays a key role in epithelial barrier function and immunomodulatory processes and enhances eosinophil activation and recruitment. Cystatins may have mechanistic roles in both eosinophilic CRS and allergic rhinitis by interacting with type 2 cytokines, microbial antigens, and fibroblasts and by mediating inhibition of allergen-related histamine release.⁷² Cystatin-1 has been described as being both transiently elevated in seasonal allergic rhinitis and overexpressed in the setting of eosinophilic CRS.⁷³ Similarly, recent studies have associated high cystatin-2 levels with severe and steroid-resistant asthma.³⁶ In a prospective study by Bleier,⁷¹ cystatin-2 mirrored major clinical events in patients with CRSwNP with a significant reduction in levels after surgery and subsequent increases over time, suggesting that it may be closely linked to the underlying disease process.

Nitric Oxide

Nitric oxide (NO) is a ubiquitous inflammatory mediator with multiple physiologic roles. NO in the respiratory environment is a free radical that takes the form of a colorless, odorless gas. Its chemical structure is extremely reactive with a very short half-life of a few seconds and an extremely high diffusion capacity in tissue.⁷⁴ As a result of its extreme reactivity and its direct and indirect effects, NO is involved in several regulatory functions, such as vasodilatation (as a result of smooth muscle relaxation), neuronal transmission, and inhibition of platelet aggregation.^{74–76} In the nasal airways, it is constitutively produced from epithelial cells lining the paranasal sinuses and contributes to local host defense via its bacteriostatic and antiviral properties and through stimulation of ciliary motility.⁷⁷ Exhaled NO is currently used as a marker of asthma control, with lower levels correlating with better disease control.⁷⁸ A frank decrease in NO has been observed in patients with nasal polyposis, potentially due to sinus ostial occlusion and failure of gaseous NO in the sinuses to reach the nasal cavities.^{78,79} There is also an inverse correlation between nitric oxide levels and the severity of sinus disease as assessed by clinical and/or CT findings.⁸⁰ After surgical or medical treatment, NO levels often improve in parallel with other clinical markers, suggesting a possible role as a less invasive marker of disease status.^{79,80}

Taste Receptors

Recently, genetic polymorphisms in bitter taste receptors have been recognized as potential biomarkers in CRS. Bitter taste receptors are G protein-coupled receptors expressed by upper airway ciliated cells and stimulated by gram-negative bacteria such as *P. aeruginosa*.⁸¹ When bitter taste receptors on ciliated cells are stimulated, there is a downstream antimicrobial NO response, which results in direct bacterial killing and increased ciliary beat frequency. The receptor's function is dictated by specific genetic polymorphisms with some variants resulting in decreased function, making patients more susceptible to sinonasal infections and biofilm formation.⁸² Because taste receptors are expressed both on the tongue and in the upper airway, the patient's genotype can be assessed by a taste test that elicits ratings of bitter and sweet taste intensity on a continuum.⁸¹ Patients with CRSsNP have been found to be significantly less sensitive to a broad bitter taste receptor agonist, whereas patients with CRSwNP and CRSsNP were found to be sensitive to sucrose, a sweet receptor agonist.⁸³ Thus, it may be possible to use bitter and sweet taste tests as biomarkers to evaluate CRS disease status.^{29,84} A genotype of the bitter taste receptor TAS2R38 was also found to predict surgical outcomes in patients with CRS without nasal polyps.⁸⁵

Adaptive Immunity

B cells are integral to humoral immunity and, along with plasma cells and T cells, are increased in nasal polyp tissue.⁸ In addition to their ability to produce antibodies that contribute to disease pathogenesis, B cells can function as antigen-presenting or regulatory cells and produce a variety of cytokines and chemokines that can influence inflammation. Tissue levels of IgG₁, IgG₂, IgG₄, IgA, IgE, and IgM are all increased in nasal polyp versus healthy sinonasal tissue.⁸⁶ This increase in antibodies was only shown locally with normal levels of circulating antibodies in the peripheral blood of the same patients. This suggests that B-cell antibody production in patients with CRSwNP is not a systemic response but rather driven by a stimulus within the local sinonasal inflammatory environment. Because of this increase in local B cells and antibodies in CRSwNP, studies have evaluated whether this elevation in immunoglobulins could be due to autoreactive B cells producing antibodies against self-tissue.⁸⁷ Tan and colleagues⁸⁷ showed a statistically significant increase in autoantibodies within nasal polyp tissue compared with tissue from patients with CRSsNP as well as nondiseased tissue in patients with CRSwNP. Specifically, increases in IgG anti-dsDNA were associated with disease severity and revision surgery, indicating a possible marker for recalcitrant disease.

TREATMENT IMPLICATIONS

With different pathomechanisms underlying endotypes of CRS, a single-treatment approach is unlikely to be universally successful in all patients. Effective management of this heterogeneous disease will ultimately require a more personalized approach based on disease pathophysiology and endotypic status that can help drive medical or surgical care. For example, glucocorticoids have been found to be most effective in patients with eosinophilic nasal polyps, while being largely ineffective in patients with noneosinophilic or neutrophilic disease.⁸⁸ Conversely, long-term use of macrolide antibiotics seems to be most effective in patients with neutrophilic inflammation,⁸⁹ whereas doxycycline, which has effects on matrix metalloproteinases and IgE levels, seems to be better suited for patients with type 2 eosinophilic inflammation.^{89,90} Of immediate importance, the rapid introduction of humanized monoclonal antibodies that target type 2 inflammation have hastened the need to subtype patients based on their

inflammatory signature and predict treatment responses. Recent identification and characterization of inflammatory CRS endotypes has improved understanding of CRS and highlighted a need for continued research into precise mechanisms of disease. Ultimately incorporating personalized approaches that incorporate phenotypic and endotypic characteristics will likely become an essential part of clinical practice.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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