



Direct measurement of upper airway inflammation in children with chronic rhinosinusitis: implications for asthma

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Purpose of review

Chronic rhinosinusitis (CRS) is a common heterogeneous multifactorial disorder affecting children and adults. The exact mechanism is not completely understood. In this review, we aim to summarize our recent findings regarding etiologies and pathogenesis of CRS in children and review recent studies investigating inflammatory patterns in the upper airways in children and adults with CRS.

Recent findings

There are only a few small studies measuring upper airway inflammation in children with CRS. These studies demonstrated more toward eosinophilic and T-cell driven inflammatory pattern. Cytokine patterns in upper airways seem to correlate with asthma symptoms in children with CRS. Adult studies demonstrate eosinophilic and Th-2 (T helper-1) driven inflammation in CRS with nasal polyps and Th-1 (T helper-2) driven inflammation in CRS without nasal polyps.

Summary

Current knowledge about the characteristics of tissue inflammation in upper airways in children with CRS is limited. More studies are needed to better understand the pathogenesis and better define the subgroups of CRS. The findings of such studies will lead to identifying the biological targets to treat this condition.

Keywords

biomarkers, children, chronic, cytokines, inflammation, rhinosinusitis

INTRODUCTION

Chronic rhinosinusitis (CRS) in children is a heterogeneous and multifactorial disorder. Its pathogenesis is not well understood which contributes to the challenges in diagnosis and treatment of this common condition. Assessment of the type of the tissue inflammation will help understand the mechanism, define the subclasses, and subsequently identify the treatment options. This review focuses on recent advances in understanding the pathophysiology of CRS by tissue analysis in children. We will also review recent such studies in adults because of the limited number of reports in the pediatric population.

CHRONIC RHINOSINUSITIS IN CHILDREN

CRS is a heterogeneous and multifactorial disorder. There are differences in etiologies and pathogenesis between pediatric and adult CRS. For example, CRS with nasal polyps (CRSwNPs) is a common subgroup

in adults but is rare in pediatric CRS where the adenoids are one of the major players (Fig. 1).

Etiologies and pathogenesis of chronic rhinosinusitis in children

Although CRS has been actively investigated for several decades, its cause remains unclear. This suggests that more than one factor contribute to the development of CRS. A recent large population study [1^{••}] confirmed familial risk in children with

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Curr Opin Allergy Clin Immunol 2016, 16:18–23

DOI:10.1097/ACI.0000000000000237

KEY POINTS

- CRS is a complex disorder with multifactorial etiologies and different phenotypes in children and adults.
- Pediatric CRS seems to differ from adult CRS with regard to etiology, pathogenesis, and distribution of subgroups.
- Studies suggest Th2-driven inflammation in allergic and nonallergic CRS in children. However, more distinct classification is needed to better understand the inflammatory patterns.
- Cytokine patterns in upper airways seem to correlate with asthma symptoms in children with CRS.

CRS. Another large study [2[¶]] on 598 patients implicated two airway epithelial potassium channels as novel susceptibility loci in contributing to the pathogenesis of CRS. A large retrospective study [3] demonstrated that 26.9% of children with CRS had allergic rhinitis, 12.3% had an immunologic disorder, 4.1% had cystic fibrosis, and 0.2% had primary ciliary dyskinesia. According to a recent review, based on current evidence, bacteria harbored within adenoid biofilms contribute to pediatric CRS, even in the absence of obstructive adenoid hypertrophy and, adenoidectomy can be considered as a first-line surgical treatment for patients who failed medical therapy [4].

An increasing number of studies suggest that impaired mucosal defense, such as physical barrier defects, innate immune dysregulation, and inappropriate adaptive immune system activation may play

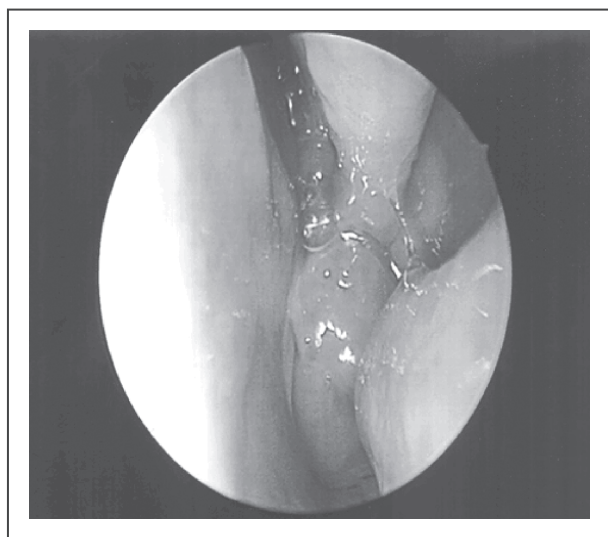


FIGURE 1. Enlarged adenoid obstructing the posterior choana.

role in development of CRS in response to environmental factors and microbes [5,6].

Infections in chronic rhinosinusitis

Although the role of infections in acute rhinosinusitis is well known, it is not established in CRS. Recent reports in adults demonstrated a correlation between type of intramucosal bacteria and type of CRS and inflammatory pattern. For example, a higher quantity of *Staphylococcus aureus* was demonstrated in nasal polyp tissues from adult patients with aspirin exacerbated respiratory disease [7]. Subsequently, gram-positive bacterial colonization was demonstrated in IL-5-positive nasal polyps, whereas gram-negative bacteria were shown in IL-5-negative nasal polyps [8]. The same group later demonstrated that the cytokines for CRS without nasal polyps (CRSsNPs) were typically Th1 driven, whereas CRS with eosinophilic nasal polyps was Th2 driven. In addition, CRS with neutrophilic nasal polyp expressed increased amounts of IFN- γ and/or IL-17 or none of those. They further showed that the presence of *S. aureus* was associated with CRSwNPs, where IL-5 and specific IgE to staphylococcal enterotoxins were present and with nasal polyps from cystic fibrosis patients. Patients with CRSsNPs who had TNF- α > 20 pg/ml in their nasal tissue had increased incidence of mucosal presence of *Pseudomonas aeruginosa* [9[¶]].

A recent study [10] in children with CRS reported normal flora in 41.5%, coagulase negative *Staphylococcus* in 22%, and *Propionibacterium* in 19.5% of the children. More similar studies are needed to identify the role of bacterial infections in children with CRS.

Fungi, especially *Alternaria* species, have been shown to be important sources of antigens that drive CRS. Earlier studies demonstrated fungi and eosinophilic mucin in most patients with CRS, as well as fungi in healthy study participants. In-vitro studies showed that lymphocytes from patients with CRS, but not control study participants, respond to fungal antigens and produce IL-5 [11]. However, later studies showed that the involvement of fungi in CRS was overestimated [12].

Diagnosis of chronic rhinosinusitis in children

Diagnosing CRS in children is often challenging because children present more subtle and nonspecific symptoms than adults, and common childhood disorders, such as frequent upper respiratory tract infections, adenoid hypertrophy, and allergic rhinitis can be difficult to distinguish from CRS.

A recent study [13] in 275 children with a clinical diagnosis of CRS (confirmed by nasal fibroendoscopy in 228 of the children) demonstrated that among the five symptoms (nasal obstruction, nasal discharge, cough, facial pain, and halitosis) nasal discharge was the strongest predictor for CRS. The probability of CRS was 60%, 93%, and virtually 100% with children with three (without rhinorrhea), four, and five symptoms, respectively. In this study, 51 children had only two symptoms. These findings support the challenges in clinical diagnosis of CRS in children. Invasive procedures (such as nasal endoscopy) and radiologic imaging are needed to confirm the diagnosis. However, they may not be readily available or the child may not be cooperative. In addition, radiologic imaging is not a preferable diagnostic test in children. Many times the patients are empirically treated with antibiotics and/or topical or oral steroids.

Defining inflammatory patterns by assessing biological markers will provide more insight into the pathophysiology of different types of CRS. Biomarkers can also be helpful to make accurate diagnosis and predict the response to treatments and they may reveal potential targets to develop specific therapies.

UPPER AIRWAY INFLAMMATION IN CHRONIC RHINOSINUSITIS

Inflammation of upper airways can be evaluated by histopathological examination (i.e. identification of inflammatory cells, thickening of basal membrane, and presence or absence of goblet cells) (i.e. eosinophils vs. neutrophils), detection of molecular components of the immune system as well as expression of pro and anti-inflammatory cytokines in the sinus and adenoid tissues, in nasal tissues obtained by nasal scraping, and in rhinonasal lavage.

Cellular inflammation

Earlier studies demonstrated predominantly lymphocytic inflammation in sinus tissues of children with CRS and eosinophilic inflammation in adults with CRS [14]. Coffinet *et al.* [15] undertook immunohistopathological studies to define the further cellular characteristics in maxillary mucosal biopsies from children and adults. They showed that children with CRS had increased CD8⁺ T lymphocytes (cytotoxic T lymphocytes), MPO (myeloperoxidase; neutrophils), and CD68 (monocytes/macrophages) cells ($P < \text{or} = 0.03$) and a trend toward more CD3⁺ lymphocytes (total T lymphocytes) and CD4⁺ T lymphocytes (helper T lymphocytes) cells ($P = 0.06$) in their epithelium and more CD20+

lymphocytes (B lymphocytes), kappa⁺ and lambda⁺ (plasma cells), MPO⁺, and CD68⁺ cells ($P < \text{or} = 0.05$) and a trend toward more CD4⁺ cells ($P = 0.06$) in their submucosa compared with adult control study participants (Table 1) [15,16²²,17–20].

Inflammatory cytokines

There has been increasing number of studies published every year investigating the characteristics of mucosal inflammation in different subgroups of CRS. Some of such studies are summarized in Table 1. Our group recently reported one such study in pediatric CRS with and without allergies and with and without asthma. We demonstrated that TNF- α levels were higher in the sinus tissues, and epidermal growth factor, eotaxin, fibroblast growth factor-2, growth-related oncogene, and platelet-derived growth factor-AA were higher in adenoid tissues in all children with CRS (without allergy and asthma) compared with the controls [16²²]. This was the only study investigating the sinus and adenoid tissue inflammatory cytokines in children with CRS. Another study in children with CRS looked at the cytokine profile in adenoid tissues and demonstrated that levels of soluble IL-2 receptor, transforming growth factor- β 1, MMP-2 and 9 (matric metalloproteinase), and TIMP-1 (tissue inhibitor of metalloproteinase) were significantly higher in adenoid tissues in patients with CRS. Also the eosinophilic cationic protein level was higher in patients with severe CRS than in those with mild to moderate CRS [17].

As there are some similarities between pediatric CRS and adult onset CRS, studies on CRS in adults may provide some insight into pathogenesis of the same entity in children.

In the literature, many times CRS was clinically and histopathologically divided into several subgroups. Clinical subgroups were usually classified based on presence or absence of nasal polyps, asthma, allergies, and peripheral eosinophilia. Different inflammatory markers were demonstrated in different subgroups.

In one of the earlier studies, 15 cytokines from the Th1, Th2, and Th17 subsets in the sinonasal secretions were analyzed in adult patients with acute rhinosinusitis, CRSwNPs, and CRSsNPs. All 15 cytokines were significantly higher in all three groups compared with the controls and IL-5 levels were higher in patients with CRSwNPs [21]. Later higher eosinophils and IL-5 levels were demonstrated in CRSwNPs by many investigators.

Another group looked at the cytokine profiles and other markers in adult patients with CRSwNPs who underwent one or more nasal surgeries. They

Table 1. Recent studies evaluating tissue inflammation using different biomarkers in patients with chronic rhinosinusitis

Reference	Biomarker (tissue)	Pediatric vs. adult (n)	Phenotype	Results
Coffinet <i>et al.</i> (2009) [15]	Cellular markers (sinus)	Children (19) and adults (5)	CRS (adults with CRSsNPs)	Children had increased CD8 ⁺ , MPO ⁺ , and CD68 ⁺ cells and a trend toward more CD3 ⁺ and CD4 ⁺ cells in their epithelium and more CD20 ⁺ , kappa ⁺ and lambda ⁺ , MPO ⁺ , and CD68 ⁺ cells and a trend toward more CD4 ⁺ cells in their submucosa compared with adult study participants with CRSsNPs
Anfuso <i>et al.</i> (2015) [16 ^{***}]	40 cytokines (sinus and adenoids)	Children (28)	CRS with and without asthma	TNF- α levels were higher in sinus and epidermal growth factor, eotaxin, fibroblast growth factor-2, GRO, and PDGF-AA were higher in adenoids in all CRS patients, these levels were higher in CRS with asthma patients compared with nonasthmatic children with CRS
Shin <i>et al.</i> (2009) [17]	12 cytokines (adenoid)	Children (16)	CRS	Levels of sIL-2R, TGF- β 1, MMP-2, MMP-9, and TIMP-1 were significantly higher in adenoid tissues in CRS. ECP level was higher in patients with severe CRS than in those with mild to moderate CRS
Van Zele <i>et al.</i> (2014) [18]	6 cytokines; IgE; sIgE to <i>S. aureus</i> enterotoxin (sinus)	Adults (36)	CRSsNPs with one or more surgeries	IgE, specific IgE to <i>S. aureus</i> enterotoxin, ECP, and IL-5 were significantly increased in recurrent CRSsNPs at the first surgery
Lam <i>et al.</i> (2013) [19]	mRNA expression of 4 cytokines (sinus)	Adults (37)	CRSsNPs and CRSNPs	IL-25, IL-33, eotaxin-3 levels were elevated in CRSsNPs. Increased eotaxin-3 level was associated with poorer CT and endoscopic scores. Increased IL-25 was associated with poorer CT scores and raised eosinophils
Xia <i>et al.</i> (2015) [20]	7 miRNAs (sinus)	Adults (40)	CRSsNPs and CRSsNPs	In all CRS miR-125b, miR-155, and miR-146a are upregulated, miR-92a, miR-26b, miR-181b were downregulated. miR-125b and miR-155 are upregulated in CRSsNPs

CRS, chronic rhinosinusitis; CRSsNPs, CRS without nasal polyps; CRSsNPs, CRS with nasal polyps; CT, computed tomography; ECP, eosinophilic cationic protein; GRO, growth-related oncogene; MPO, myeloperoxidase; PDGF-AA, platelet-derived growth factor-AA; TIMP-1, tissue inhibitor of metalloproteinase.

demonstrated that tissue levels of IgE, specific IgE to *S. aureus* enterotoxin, eosinophilic cationic protein, and IL-5 were significantly increased in recurrent versus nonrecurrent CRSwNPs at the moment of the first surgery [18].

Lam *et al.* [19] reported association between characteristics of tissue inflammatory cytokines and disease prognosis in adults with CRS and demonstrated that CRSwNPs predicted elevated IL-25, IL-33, and eotaxin-3 levels. In this study, increased eotaxin-3 correlated with poorer computed tomography and endoscopic scores. Increased IL-25 correlated with poorer computed tomography scores and raised serum eosinophils. No association with reactive airway disease, atopy, and symptom measures were found in this study.

MicroRNAs

MicroRNAs (miRNAs) are small noncoding RNA molecules that are one of the major epigenetic regulatory mechanisms that can mediate posttranscriptional silencing of the target genes. There are over 1000 miRNAs in the human genome (<http://www.mirbase.org/index.shtml>). They play a role as regulators of gene expression in many processes including cell proliferation, differentiation, apoptosis, organ development, and signal transduction. Abnormal miRNA expression may result in various disorders including cancers and changes in inflammatory response. The first study [22] on miRNA expression profiles in CRS was published in 2012 and showed distinct expression profiles in CRSwNPs and in eosinophilic CRSsNPs. In this study, the only upregulated miRNA in eosinophilic CRSwNPs, miR-125b, was shown to target Eukaryotic Translation Initiation Factor 4E-binding protein 1(4E-BP1) in airway epithelial cells. Through suppressing 4E-BP1, miR-125b enhances the expression of type I interferon which positively correlates with IL-5 mRNA levels and eosinophilic inflammation in sinus mucosa. A recent study [20] also showed upregulation of miR-125b as well as miR-155 in CRSwNPs.

Other markers

A recent study demonstrated increase arginase activity in CRSsNPs and this was associated with lower levels of nasal fractional exhaled nitric oxide. On the other hand, patients with CRSwNPs showed significant inducible nitric oxide synthase messenger RNA upregulation with concomitant higher levels of oral and nasal fractional exhaled nitric oxide suggesting that fractional nitric oxide is a valid marker for differentiating CRS phenotypes

[23]. Another recent study [24] in adults demonstrated elevated $\alpha 7$ nAChR protein levels in CRS with or without nasal polyps suggesting that the cholinergic system is involved in the inflammatory response of CRS.

CHRONIC RHINOSINUSITIS AND ASTHMA

Association between CRS and asthma has been demonstrated in clinical and epidemiologic studies [25–27]. A large epidemiological study also demonstrated this association to be even stronger in patients who have both CRS and allergic rhinitis. In addition, CRS in the absence of nasal allergies was positively associated with late onset asthma [27]. Despite this knowledge the pathophysiologic mechanism underlying this association has not been well understood. This may be because of the lack of studies assessing the inflammatory markers in the upper and lower airway mucosa. One of the earliest studies measuring upper airway inflammation in children with CRS and asthma demonstrated a significant increase in IL-4 and significant decrease in IFN- γ in rhinosinusal lavage in allergic and nonallergic children with asthma and CRS. Allergic study participants also showed significant increase in TNF- α [28]. Tosca *et al.* [29] looked at the change in levels of IL-4 and IFN- γ in rhinosinusal lavage fluid in children with asthma and CRS after treatment with a 14-day course of oral antibiotics and intranasal steroids and a 10-day course of oral steroids. They demonstrated a significant decrease in IL-4 and significant increase in IFN- γ in allergic study participants and significant decrease in IL-4 and nonsignificant increase in IFN- γ in nonallergic study participants after the treatment.

Our group recently demonstrated that children with CRS had significantly higher levels of TNF- α in the sinus tissue and significantly higher levels of epidermal growth factor, eotaxin, fibroblast growth factor-2, growth-related oncogene, and platelet-derived growth factor-AA in adenoid tissues [16^{***}]. These levels were higher in children who also had asthma. These findings support the hypothesis that upper airway inflammation is associated with lower airway disease.

CONCLUSION

CRS in children is a heterogenous disease with multiple etiologies and is an active area of research. Studies suggest genetic, anatomical (e.g. adenoid hypertrophy), and environmental factors (e.g. microbes and allergens) in development of CRS in children. The exact pathogenesis is still not well understood perhaps partially because of not well

defined clinical subgroups. Biological markers are needed to better classify the subtypes (endotypes) of CRS to define distinct phenotypes and their molecular pathways. Recent studies in adults promise toward improving understanding of the pathophysiology of CRS. More such studies are needed in children to fill in the gaps in our knowledge and, hopefully, to lead to advances in treatment of these patients.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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