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Otolaryngology -- Head and Neck Surgery 2011 144: 85
DOI: 10.1177/0194599810390443

The online version of this article can be found at:
http://oto.sagepub.com/content/144/1/85

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What is This?
Pediatric Chronic Rhinosinusitis Histopathology: Differences and Similarities With the Adult Form

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No sponsorships or competing interests have been disclosed for this article.

Abstract
Objective. To compare the histopathology and immunohistochemistry of pediatric and adult chronic rhinosinusitis (CRS).

Study Design. Cross-sectional study.

Setting. University-affiliated hospital.

Patients and Methods. Inflamed sinus-mucosal samples of 16 children (mean age, 11.6 ± 2.9 years) with refractory CRS who underwent endoscopic sinus surgery were studied. Twenty-nine diagnosis-matched adults served as controls. Study analysis covered sinus computed tomography (CT) scores, general pathologic features, eosinophil and T-lymphocyte population, and thickness and integrity of the epithelium.

Results. Children had a lower CT score than adults did (P = .005). The inflammatory response of the children, which differed greatly from that of adults, was dominated by cellular infiltration of the lamina propria with chronic inflammatory cells and fibrosis (8/16 had extensive fibrosis); eosinophils were scanty. Adult CRS was characterized by polypoid mucosa and eosinophilia (type A) or glandular hyperplasia (type B). Extensive fibrosis was shown in adult type-B patients (7/13). Assessment of eosinophils in the lamina propria showed marginal statistical significance between children and adults (P = .065). This difference was accentuated when pediatric and adult type A were compared (14.6 ± 25.3 vs 121.5 ± 174.2 cell/mm²; P = .043). Complete epithelial shedding was less significant in children (9.4% ± 8.2% vs 25.4% ± 15.1%; P < .001). The number of lamina propria and epithelial T lymphocytes was similar.

Conclusions. The marked differences in the inflammatory response of children and adults with CRS may attest to different pathophysiologic pathways. The significantly reduced epithelial shedding in children is probably associated with diminished tissue eosinophilia. Extensive fibrosis was found in half of adult type-B patients; similar findings were found in children.

Keywords
eosinophils, epithelial integrity, fibrosis, immunohistochemistry, sinus mucosa

Accepted June 29, 2010; revised October 11, 2010; accepted October 19, 2010.

Acute rhinosinusitis is a common childhood disease, affecting about 5% to 10% of children with upper respiratory tract infections. This superimposed acute bacterial infection usually resolves after a course of antibiotics supplemented with adjuvant therapy. Chronic rhinosinusitis (CRS) is inflammation of the sinuses that endures for more than 3 months despite treatment. This ailment is mostly diagnosed in children with predisposing factors (eg, allergic rhinitis, cystic fibrosis, anatomic abnormalities) and is associated with significant decrement of quality of life.

Contrary to children, the pathogenesis and histopathology of adult CRS, which is classified into 2 distinct types that are characterized by eosinophilia and polypoid changes or glandular hyperplasia, is well documented. Recently, it has been argued that the inflammatory response in young children differs from that of adults, showing less eosinophils, more lymphocytes, thinner epithelium and basement membrane, and fewer submucosal mucous glands.

We aimed to further elucidate the qualitative and quantitative histological and immunohistochemical characteristics of pediatric

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 CRS and gain a better understanding of its inflammatory mechanisms. Differences and similarities with the adult form are discussed.

Materials and Methods

Study Population and Tissue Preparation

Sinus mucosa samples were retrieved from 16 consecutive pediatric patients (12 boys and 4 girls; age range, 7-16.8 years; mean age, 11.6 ± 2.9 years). Archival sinus mucosa samples retrieved from 29 adult patients (16 men and 13 women; age range, 21-75 years; mean age, 48.5 ± 14.7 years)4 served as controls. All patients had a variety of symptoms including colored nasal discharge, postnasal drip, bothersome cough, nasal obstruction, facial or frontal pain or pressure, halitosis, hyposmia, and earache and were eligible for the study if they had refractory CRS and failed conventional medical management for a period longer than 3 months. In both groups, no previous endoscopic sinus surgery (ESS) was documented. The use of systemic steroids or intranasal steroid sprays was stopped at least 4 weeks before surgery. ESS was performed between May 2001 and January 2008. This prolonged time period for data collection reflects the treatment policies for children with CRS and thus may explain the relative rarity of pediatric sinus mucosa samples over this time. The study protocol was approved by the Institutional Review Board of Meir Medical Center.

The sinus-mucosal tissues of both groups were subjected to standard histological processing and immunohistochemical staining.4 Representative sections were stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS), Masson trichrome, and reticulin.

Preoperative Evaluation

Flexible nasal endoscopic examination was performed as part of routine preoperative evaluation, demonstrating purulent or mucopurulent material in the middle meatus, frontal recess, or sphenoid recess. The extent of sinus involvement was assessed by coronal computed tomography (CT) and scored by the Lund-Mackay staging system14 in a blinded manner.

Qualitative and Quantitative Assessment

To obtain a general impression of the typical histopathology, the H&E sections were examined with a light microscope under low-power magnification (40×). PAS staining was used to demonstrate secretion of mucus by submucous glands and goblet cells. H&E (under standard and polarized light microscopy), Masson trichrome, and reticulin staining served to detect collagen fibers.

The H&E sections were used to separately count the number of eosinophils in the lamina propria (LP) by an eyepiece of the microscope containing 10 × 10 square reticules (the length of the reticule at 400× magnification was 0.2 mm, and the total surface area corresponded to 0.04 mm² [0.2 × 0.2 mm]). Twenty-five reticules were counted in each specimen and yielded the number of eosinophils in 1 mm² of the LP (0.04 mm² × 25 = 1 mm²). The same measures were used for counting immunohistochemical stained sections of T lymphocytes residing in the LP. Given that preliminary observation disclosed that epithelial eosinophils were too scarce, their count was relinquished. T lymphocytes in 1-mm length of the epithelium were counted along five reticules (0.2 mm × 5 = 1 mm) under the same magnification. For analysis purposes, adult samples were divided according to dominant features to type A, characterized by polypoid mucosa and eosinophilia, or type B, characterized by glandular hyperplasia.4 Standard stereological and morphometric methods15 were used to measure the relative proportion of mucous/serous subepithelial glands. A reticule composed of 10 × 10 squares corresponding to a 4-mm² surface area (2 × 2 mm) at magnification 40× was superimposed on the LP. The type of glands appearing at the upper-left intersection of each square was recorded, allowing 100 hits on each sample. The number of each type was divided by the total number of glands found in the sample, and its percentage was calculated.

A semiquantitative guide for estimating the extent of fibrosis was formulated and rated as follows: none, limited with small and scattered areas of fibrosis, and extensive with wide areas of fibrosis. Additional measurements were used for comparing the thickness and the integrity of the epithelium, which included the length of the basement membrane (1) covered with intact pseudostratified ciliated columnar epithelium, (2) covered with a single layer of basal cells (ie, indicates partial epithelial denudation), and (3) devoid of epithelial cells (ie, indicates complete epithelial denudation). The total length of the basement membrane was measured by adding the length of the corresponding areas of each of these 3 measurements. The numerator for calculating the relative proportion (ie, percentage) of the basement membrane covered by each of these lengths was the sum of the lengths of the corresponding areas, and the denominator was the total length of the basement membrane (eg, the relative proportion for A was A/[A + B + C]). The length of the reticule at magnification 100× was 0.8 mm.

All samples were analyzed in a blinded fashion by a trained observer (G.B.). Data verification was performed by a second blinded observer (T.K.), and consensus was reached in cases of disagreement.

Statistical Analysis

The independent t test was used to compare CT scores, patients’ age with/without fibrosis, number of eosinophils, LP and epithelial T lymphocytes, epithelial thickness and integrity, and the relative proportion of mucous/serous subepithelial glands of both groups. Data comparing the number of eosinophils, not fitting normal distribution, were analyzed with the nonparametric Mann-Whitney rank test. The Fisher exact test served for comparisons of the prevalence of comorbidities (asthma and polyposis). Measurements were expressed as means, with standard deviation in parentheses. Results were considered significant at two-tailed P < .05.

Results

Patient Characteristics

All patients had persistent clinical symptoms, endoscopic findings, and radiologic scores compatible with CRS. A comparison of the prevalence of comorbidities, which was based on admission history forms, showed a near-significant difference between...
 pediatric and adult control patients for polyposis (3 of 16 [18.8%] vs 15 of 29 [51.7%], respectively, $P = .055$) and a significant difference for asthma (nil [0%] vs 10 [34.5%], respectively, $P = .005$). None of the 3 children with nasal polyps had cystic fibrosis. Three children had adenoidectomy before undergoing ESS, and 7 had their adenoids removed at the time of ESS. Three children (18.8%) had documented allergy to house dust mite, of whom 1 also had allergy to grass and another to cockroaches. Six of 29 adults underwent allergic evaluation, of whom 3 had allergy to house dust mite and the other 3 had negative skin-prick tests to 20 common airborne allergens. In the remaining patients, no further data on a positive or a negative history of allergy were indicated. The extent of sinus disease was significantly greater for adults than for children (Lund-MacKay CT score: 13.4 $\pm$ 6.1 vs 8.1 $\pm$ 4.6, $P = .005$).

**Tissue Source**

Most of the pediatric samples (11/16, 68.8%) were retrieved from the ethmoid sinus. The remaining 5 were removed from the uncinate process (n = 4) or from the maxillary sinus (n = 1). One of each of the ethmoid sinus and the uncinate process samples also included the mucosa surrounding the opening of the maxillary sinus. All adult samples were removed from the ethmoid sinus; 18 of 29 also included maxillary sinus mucosa, and 1 also included frontal sinus mucosa.

**Qualitative and Semiquantitative Findings**

There were different histological patterns of pediatric and adult sinus mucosa. The inflammatory response of the pediatric group was dominated by cellular infiltration of the LP with chronic inflammatory cells, mostly lymphocytes, plasma cells, and macrophages. Wide areas of fibrosis were detected throughout the LP in half of the children, with another 2 displaying limited fibrosis (Figure 1A); 6 had no fibrosis. Fibrosis was not associated with age (mean age, 11.4 $\pm$ 3.1 years for patients with extensive fibrosis and 11.7 $\pm$ 3 years for those with limited fibrosis or none, $P = .859$). Fibrosis was documented by H&E, Masson trichrome, and reticulin staining. The H&E sections were evaluated by regular (Figure 2A) and polarized light microscopy showing strong birefringence of collagen fibers (figure not shown). This process was also demonstrated by Masson trichrome (figure not shown) and by reticulin staining, showing marked formation of coarse dark submucosal collagen fibers (Figure 2B). Eosinophils in children were scanty, and the epithelial layer appeared intact.

By contrast, the adult sinus mucosa was characterized by polypoid mucosa with edematous loose connective LP infiltrated with a lower density of chronic inflammatory cells and high concentration of eosinophils (type-A patients, n = 16; Figure 1B) or with abundant submucosal glands (type-B patients, n = 13; Figure 1C). Patches of complete epithelial shedding were observed in both types. Fibrosis was absent in 7 type-A patients or was found in limited and scattered areas of the LP in another 7; however, in 7 of 13 type-B patients, fibrosis was found in wide areas of the LP adjacent to regions of glandular convergence. Of note is that the typical pediatric form described herein was mainly maintained in the younger ages (7 to $\leq$13 years), whereas in part of the older children (>13 to 16 years), it was more heterogeneous and displayed polypoid changes characteristic of the adult form.

**Quantitative Findings**

More eosinophils were found in adults than in children. This difference was significantly stressed when the comparison was drawn between pediatric and adult type-A patients.
However, the number of LP and epithelial T lymphocytes, the proportion of submucosal serous and mucous glands, and the thickness of the epithelium in children were similar to those in adults. Measurements of epithelial integrity indicated that adults with CRS are at a higher risk of developing complete epithelial denudation than children. The proportion of intact areas and areas partially devoid of epithelial cells did not differ significantly between the groups (Table 1).

**Discussion**

The diagnosis of pediatric and adult CRS is currently based on clinical, endoscopic, and radiologic parameters. The substantial differences found herein between both populations in the general histopathological features and the extent of sinus disease raise a fundamental question as to whether the cause and the pathophysiological process, which lead to a common symptom complex in different age groups, represent a similar disease. At present, it seems there is no definite answer to this question. Our data showed that while the pediatric form is characterized by greater cellularity of chronic inflammatory cell infiltrate, the adult form is characterized by both polypoid changes with edema of the LP and glandular hyperplasia. The exception to the rule was the older children with features typical to both forms. The population of eosinophils and the proportion of complete epithelial denudation were also significantly different. Given that the extent of sinus disease was also significantly different, it can be concluded that the inflammatory reaction of children and adults is qualitatively and quantitatively different. Others also found significant differences in the population of various inflammatory cells including eosinophils, neutrophils, lymphocytes, and macrophages and in the amount of mucous glands, the epithelial height and intactness, and the basement membrane thickness.10-12 The marked histopathological differences between pediatric and adult CRS were attributed to varied underlying mechanisms or alternatively to progression, over a prolonged period of time, from the pediatric form that is characterized by less eosinophil infiltration to the adult form. These 2 forms may coexist within the spectrum of pediatric CRS: one with transient CRS and the other destined to evolve into persistent, adult-like CRS.11 Indeed, this progression toward the adult-like form was observed in the older children, whose inflammatory reaction displayed features common to both forms with greater cellularity, fibrosis, and polypoid changes with eosinophilia. Recently, it was postulated that the inflammatory mechanism in young children with CRS is distinct from that of the helper T-lymphocyte type 2 and eosinophil-mediated mechanisms characteristic of adult CRS.12 Factors related to maturing of the immune system and enlargement of the sinuses also separate adult CRS from pediatric CRS, with allergy, air pollution, gastrointestinal reflux, daycare settings, and enlarged tonsils and adenoids being predisposing factors for children.16

Despite common assumptions,10 we showed that the extent of fibrosis was not associated with the patient’s age but rather with histopathological features, demonstrating wide areas of fibrosis in half of the children with greater cellularity of chronic inflammatory cell infiltrate. A similar extent of fibrosis was shown in adult type-B patients characterized by glandular hyperplasia. However, fibrosis was missing or limited to scanty areas of the LP in most of adult type-A patients characterized by polypoid mucosa. This unexpected pathologic collagen deposition in children, which is part of chronic wound healing,17 was documented by a number of staining methods. Similar to other inflammatory changes such as eosinophil infiltration, which was found in only part of the samples,18 the inconsistent form of expression of this specific inflammatory involvement of childhood CRS does not diminish its significance and implies that the fibrotic responses and the disease itself are heterogeneous. It was also suggested that bacteria, fungi, viruses, and tissue inflammatory responses may contribute differently to the final state of the mucosa.19 Although there is a lack of adequate clinical corroboration for this finding in children, it is known that fibrosis may complicate other pediatric diseases such as cystic fibrosis and pulmonary and bone abscesses.20 Of note is that transforming growth factor-β1 and tumor necrosis factor-α (TNF-α), which are cytokines known to activate collagen deposition by fibroblasts, are found in the sinus mucosa of patients.
with CRS.21,22 The fact that fibrosis can be blocked by TNF-α inhibitor23 is encouraging and might have therapeutic value for CRS patients.

Our findings of a significantly greater extent of sinus involvement and sinus mucosal eosinophilia in adults than in children may explain the significantly greater epithelial denudation shown in this group and account for the toxic effects of eosinophil granule major basic protein and eosinophil cationic protein on the respiratory epithelium, causing the epithelium to slough off.7,24 One suggestion for this difference in the general histopathological picture and in the eosinophil population might be our unbalanced proportion of asthmatics. Nevertheless, there is evidence to suggest that neither the atopic state nor the presence of asthma is the sole contributor for these differences. Significantly fewer eosinophils and major basic protein-positive cells were found in a group of children with a considerable proportion of inhalant allergy and/or asthma.11 Others also found no correlation between the extent of sinus involvement and degree of tissue eosinophilia or allergic status of asthmatic, nonasthmatic, and cystic fibrosis children with CRS.18

The thickness of the mucosal epithelium retrieved from our pediatric ethmoid sinus and uncinate process was very similar to that retrieved from the maxillary sinus of very young and young children with CRS.11 However, while we did not find a significant difference in this respect between children and adults, others reported a significantly thicker epithelial layer in adults than in children.17 Interestingly, previously published data on the thickness of the epithelium of adults with normal inferior turbinate25 were consistent with ours and others studying childhood CRS (54 µm, 50.7 µm, and 51 µm, respectively).11 This may attest to the tendency of the epithelium to remain unchanged regardless of age, site, or health status.

Similar to previous data on LP T lymphocytes,10,12 their population was not significantly different between children and adults. Furthermore, compared with normal adults, children with CRS had a significantly increased population of helper T lymphocytes situated in the LP sinus mucosa.26 A comparison of sinus tissues of children treated preoperatively with antibiotics and intranasal corticosteroids for CRS with a control group of 3 children with choanal polyp or facial pain syndrome evinced an indistinguishable mononuclear cell subset composition for both groups.27 Nevertheless, this issue warrants further research.

The study is limited by the lack of a pediatric normal control group. To our knowledge, because of the rarity of research material and ethical restrictions to date, no adequate normal controls were used for this purpose. We are aware that CRS patients may be affected by different comorbidities, which can act as confounding factors in the evaluation of the above-mentioned parameters and may represent another possible limitation of this study. Yet it should be stressed that all of our pediatric and adult patients had frank CRS, which was confirmed clinically, endoscopically, and radiologically, and the different frequencies of their comorbidities does not necessarily distinguish them from each other in this respect. The difference, however marginal, in the prevalence of polyposis is not surprising as it is commonly found in adults with CRS, but it is relatively rare in childhood CRS and should raise the suspicion of cystic fibrosis. In addition, instead of being described as distinct and separate entities, asthma and allergic rhinitis are better described as a continuum of inflammation involving one common airway, one disease.26 It was also shown that CRS is common in patients with asthma and allergic rhinitis and is a significant trigger of asthma in children and adults29; thus, upper respiratory tract infections can provoke hyperresponsiveness of the lower airway. However, uncertainty exists as to whether the severity of upper respiratory tract infections and with it CRS are related to more severe asthma.30

### Conclusions

There are more differences than similarities between sinus mucosal tissues of pediatric and adult CRS, with marked differences in the inflammatory response of both groups, attesting to different pathophysiological pathways of disease in both populations. Further significant differences exist in the

| Table 1. Histopathological and Immunohistochemical Data of the Pediatric and the Adult Patients With CRS |
|----------------|------------------|------------------|------------------|
|               | Pediatric CRS (n = 16) | Adult CRS (n = 29) | PValue |
| LP eosinophils, cell/mm² | 14.6 ± 25.3        | 67.4 ± 141.4      | .065  |
| Subepithelial glands, % | Muco 53.2 ± 20.3   | 50.6 ± 18.5       | .67   |
| Subepithelial glands, % | Serous 46.8 ± 20.3 | 49.4 ± 18.5       | .67   |
| Subepithelial glands, % | Intact 51.4 ± 12.7 | 48.5 ± 9.7        | .429  |
| Subepithelial glands, % | Partial denudation 35.8 ± 26.5 | 32.8 ± 16.7 | .695  |
| Subepithelial glands, % | Complete denudation 9.4 ± 8.2 | 25.4 ± 15.1 | <.001 |

Abbreviations: CRS, chronic rhinosinusitis; LP, lamina propria.

aBoth types (A and B) of adult CRS histopathology.

bThe median (interquartile range) of LP eosinophils of children was 4.00 (1.25-23.5) and that of adults was 33.50 (4.75-172.5) cell/mm² (nonparametric Mann-Whitney rank test).
magnitude of tissue eosinophilia and in the extent of complete epithelial shedding. This increase in adult epithelial shedding probably results from secreted eosinophil cationic protein released by activated eosinophils. Similar to the process found in adult type-B patients, substantial fibrosis was found in half of our pediatric patients.

Acknowledgments
We thank Dr. Mario Cordova, MD (Department of Pathology, Meir Medical Center), for helpful discussion of the fibrotic process in pediatric CRS; Professor Arnon Goldberg, MD (Allergy and Clinical Immunology Unit, Meir Medical Center), for sharing his knowledge with us on the common airway disease theory; Nava Jelin, MA (Statistical Service, Meir Medical Center), for statistical consultation; and Rachel L. Berger, BA (Kfar Saba), for writing and editing assistance.

Author Contributions
Gilead Berger, study design, data collection, data analysis, writing; Tatiana Kogan, data analysis; Miki Paker, data collection; Sivan Berger-Achituv, writing; Yaniv Ebner, data collection, data analysis.

Disclosures
Competing Interests: None.
Sponsorships: None.

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