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Ciliary beat frequency in children with adenoid hypertrophy

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Abstract

Background: Children with adenoid hypertrophy (AH) have impaired respiratory system defense mechanisms, such as mucociliary clearance. We hypothesized that AH negatively affects one of the most important aspects of mucociliary clearance—ciliary beat frequency (CBF) and that adenoidectomy could potentially restore this essential defence mechanism of the airways. This study evaluated the influence of AH and endoscopic adenoidectomy on the CBF of the nasal respiratory epithelium in children.
Methods: This prospective study included 64 children with confirmed AH aged 3 to 18 years and 43 age- and sex-matched healthy controls. Nasal CBF was analyzed using a digital high-speed video microscope and the software application Ciliary Analysis (NI LabVIEW). The preoperative adenoid size was assessed according to Cassano. Clinical symptoms of chronic rhinosinusitis were evaluated using the SNOT-20 questionnaire.
Results: Children with AH had a median CBF of 5.35 ± 1.06 Hz. Six months after surgery, the median CBF was significantly higher (6.48 ± 0.88 Hz; $P < .001$) and reached the values of healthy children (6.37 ± 0.71 Hz; $P = .512$). The size of the adenoid tissue did not correlate with the CBF. No influence of age or gender on the CBF was found. After adenoidectomy, a significant reduction of the mean total SNOT-20 score was recorded ($P < .01$).
Conclusion: Children with clinically symptomatic AH have impaired mucociliary clearance due to decreased nasal CBF. Removal of hypertrophic adenoid tissue normalizes the CBF and reduces the presence of clinical symptoms of rhinosinusitis.

KEYWORDS
adenoid hypertrophy, cilia, ciliary beat frequency, digital high-speed video microscopy, mucociliary clearance

1 | INTRODUCTION

Adenoid hypertrophy (AH) is a common disorder in the pediatric population, especially in preschool-aged children. According to a recent meta-analysis, its prevalence in children and adolescents is 34.46%.[1] Enlarged adenoids cause a posterior nasal obstruction, which leads to typical symptoms, such as mouth breathing, speech anomalies, snoring, and obstructive sleep apnoea syndrome (OSA), as well as recurrent respiratory infections (RRI).[2,3]

Using Andersen's saccharine test, several studies confirmed that prolonged nasal mucociliary clearance time (MCT) in pediatric patients with AH was significantly improved after adenoidectomy or adenotonsillectomy.[4-7] Prolonged mucociliary clearance may be caused either by ciliary dyskinesia (primary or secondary) or by mucus abnormalities. In both cases, it leads to mucus stagnation and an increased risk of respiratory infections.[8] The saccharine test, used in previous studies, evaluates nasal mucociliary clearance without assessing its main components (ciliary motility and the character of respiratory mucus) and requires patient cooperation. Moreover, this method has a high bias caused by subjectivity in its evaluation and interpretation.[9] According to The European Respiratory Society guideline published in 2009, it is difficult to perform this test in children aged less than 12 years and to achieve reliable results and outputs for clinical management and further investigation.[10]

Studies that describe ciliary beat frequency (CBF), one of the most important aspects of mucociliary clearance,[11] and the involvement of its defects in the pathogenesis of RRI in patients with AH, are lacking. We hypothesized that AH negatively affects CBF and that adenoidectomy could potentially improve it. We also hypothesized that CBF defects are associated with symptoms of rhinosinusitis in patients with AH. Therefore, the aim of our study was to evaluate the impact of AH and the effect of endoscopic adenoidectomy on nasal CBF and on symptoms of chronic rhinosinusitis (CRS) in children. We employed digital high-speed video microscopy, a modern method used as the gold standard for the assessment of CBF and beat pattern.[12,13]

2 | MATERIALS AND METHODS

This prospective study was conducted on 64 pediatric patients (34 boys [53.13%]), ≥3 and <18 years of age (mean age 6.65 ± 3.83 years), with confirmed AH, who underwent endoscopic adenoidectomy under general anesthesia, and 43 age- and sex-matched healthy children who acted as controls (23 boys [53.49%]; mean age 7.04 ± 2.43 years) (Table 1). All participants and their legal guardians were well informed about the study and signed an informed consent form. The study was approved by the Ethics Committee of Jessenius Faculty of Medicine, Comenius University (project number: EK 1783/2015).

TABLE 1 Anthropometric parameters in patients with adenoid hypertrophy and in healthy children

	Patients with AH (n = 64)	Healthy children (n = 43)	*P*
Age, y	6.65 ± 3.83	7.04 ± 2.43	.423
Weight, kg	25.75 ± 14.15	27.39 ± 13.18	.568
Height, cm	121.39 ± 21.71	125.06 ± 17.29	.359
Body mass index, kg/m^2	16.32 ± 2.71	16.73 ± 3.07	.537

Abbreviation: AH, adenoid hypertrophy.

2.1 | Subjects

Patients with AH were enrolled in this study at a preoperative clinical visit, at which they were examined by a pediatric otolaryngologist, and a detailed history was obtained from their parents. Adenoidectomy was indicated for RRI (rhinitis, rhinosinusitis, otitis media), in some cases along with clinical symptoms resulting from nasopharyngeal obstruction (snoring, mouth breathing, etc). The exclusion criteria were acute respiratory infections during the previous 4 weeks; the administration of systemic and/or local antibiotics during the previous 4 weeks; diseases affecting nasal mucociliary clearance —congenital (primary ciliary dyskinesia, cystic fibrosis) or acquired (nasal polyps, deviated nasal septum, gastroesophageal reflux disease); positive family history of congenital diseases affecting mucociliary clearance; disorders that may be associated with genetic defects in ciliary structure and function—so-called ciliopathies (situs viscerum inversus, heterotaxy, congenital heart defects, cerebral ventriculomegaly, cystic kidney and liver diseases, skeletal bone defects, polydactyly); the presence of another clinically significant chronic diseases (metabolic, endocrine, oncological). The control group included healthy children in whom AH and other disorders of the ear, nose, and throat were excluded by the pediatric otolaryngologist and who fulfilled the same exclusion criteria as patients with AH.

2.2 | Tests and measurements

Basic identification data (age, gender) and anthropometric parameters (height, weight, body mass index) were recorded. All participants or their legal guardians completed the Sino-Nasal Outcome Test (SNOT-20), a validated self-administered, multiple-choice, 20-item questionnaire that evaluates otorhinolaryngological symptoms, social/emotional consequences, and sleep problems in patients with CRS. The severity of each symptom is scored on a scale of 0 to 5.[14] For each patient, the total score on the SNOT-20 was calculated. Subsequently, the distribution of individual symptoms was assessed, and the mean score was calculated for the sinonasal section (first 10 questions) and for the section related to sleep and emotional symptoms (second 10 questions).

The size of adenoid tissue was determined by endoscopic examination and was categorized according to the degree of nasopharyngeal obstruction described by Cassano (Grade 1: <25%, Grade 2: 25%-50%, Grade 3: 50%-75%, Grade 4: >75%).[15]

2.3 | Evaluation of CBF

The CBF of the nasal respiratory epithelium was analyzed using a digital high-speed imaging workstation that was designed by a team of researchers from Jessenius Medical Faculty in Martin and the University of Žilina (both Slovakia). A detailed description of this method (Utility Model Number 6811)[16] was published in several papers.[17-19] A sample of the ciliated epithelium was obtained from the nose using a cytology brush. Nasal brushing is quick and simple, does not require premedication or local or general anesthesia and can be performed from early childhood.[12] The child was comfortably seated on a chair or on the parent's knee, their head was slightly tilted back and they were encouraged to breathe only through their mouth for a moment. Before brushing, the cytology brush was soaked in physiological saline then inserted deep into the child's nostrils in the medial and slightly caudal direction and rotated gently to collect ciliated cells. Samples of ciliated epithelium were suspended in RPMI 1640 Medium, the temperature was maintained at 20°C and microscope slides were prepared. Ciliated cells were observed using an inverted phase-contrast microscope (ZEISS Axio Scope.A1; Carl Zeiss AG, Germany) with a ×100 objective, while areas with moving cilia were selected. Each microscope slide was examined within the first 10 minutes after its preparation. Ciliated cells with akinetic cilia (damaged during nasal brushing) were excluded from the examination. The movement of cilia was recorded using a digital high-speed video camera (Basler A504KC; Basler AG, Germany) at a rate of 500 frames per second.[8] The recordings were then analyzed by using the software application Ciliary Analysis (National Instruments LabVIEW development environment). Regions with moving cilia were assessed, and the CBF was measured by using specific mathematical algorithms.[18-20] For each participant, 30 different areas with moving cilia were analyzed, of which the median CBF was recorded.

To assess changes in the investigated parameters, patients were contacted 6 months after the endoscopic adenoidectomy. The survey was completed in 55 children (32 boys [58.18%]; mean age 7.19 ± 3.88 years); the remaining respondents could not be contacted or refused to continue the study. Patients were examined by the pediatric otolaryngologist concerning postoperative local conditions, signs of infection and any other abnormalities in the investigated area. The digital high-speed video microscopy with the measurement of CBF was repeated. To avoid secondary ciliary dyskinesia, nasal brushings were performed in children who had not experienced any acute respiratory infection during the previous 4 weeks. Patients or their legal guardians were asked to respond to the SNOT-20 questionnaire again.

2.4 | Statistical analysis

The variables were analyzed statistically using SYSTAT version 11.0 (Systat Software, Inc., CA). Data from both the control and case groups were first tested for normality and equal variance by the Shapiro-Wilk test; all data were normally distributed and recorded as mean ± standard deviation. Descriptive statistical methods were used to describe demographic and anthropometric parameters of patients and controls and the χ^2 test and unpaired Student *t* test were used to establish differences between variables. Differences of selected parameters (CBF, SNOT-20 scores) between patients with AH before and after endoscopic adenoidectomy were assessed using the paired Student *t* test. Differences of selected parameters between the case group and healthy controls were assessed using the independent nonpaired Student *t* test. The Pearson correlation coefficient (r) was used to correlate the association between anthropometric parameters and CBF and also SNOT-20 scores and CBF. Correlation coefficients r = 0-0.19 were considered as weak, 0.20-0.39 as mild, 0.40-0.59 as moderate, 0.60-0.79 as moderately strong, and 0.80-1.0 as a strong correlation. Statistical significance was defined as $P < .05$.

3 | RESULTS

3.1 | CBF in patients with AH before and after endoscopic adenoidectomy

Patients with AH (n = 64) had a median CBF of 5.35 ± 1.06 Hz. There was no significant correlation of CBF with increasing age (r = 0.197; $P = .119$), height (r = 0.173; $P = .171$) or weight (r = 0.121; $P = .339$). CBF did not differ significantly between boys and girls (5.38 ± 1.16 Hz; 5.32 ± 0.94 Hz; $P = .800$). We found no correlation between CBF and the size of adenoid tissue. Median CBF in patients with AH grade 2 (5.47 ± 1.12 Hz) and in patients with AH grade 3 (5.19 ± 0.96 Hz) did not differ significantly ($P = .294$).

Six months after endoscopic adenoidectomy, patients (n = 55) had a significantly higher median CBF (6.48 ± 0.88 Hz) than at the preoperative examination ($P < .001$) (Figure 1A). There was a significant improvement in CBF in all subgroups of patients (Table 2).

3.2 | CBF in healthy controls and in patients with AH

Healthy children, who acted as controls (n = 43), had a median CBF of 6.37 ± 0.71 Hz. As in the group of patients with AH, CBF was not correlated with age (r = 0.125; $P = .424$), height (r = 0.171; $P = .274$) or weight (r = 0.260; $P = .092$). We also did not find a significant difference in CBF between boys and girls (6.43 ± 0.76 Hz; 6.30 ± 0.67 Hz; $P = .536$).

Patients with AH had a significantly lower median CBF than the control group of healthy children (5.35 ± 1.06 Hz; 6.37 ± 0.71 Hz; $P < .001$) (Figure 1B). Six months after surgery, the median CBF was significantly higher and reached the values of healthy children (6.48 ± 0.88 Hz; 6.37 ± 0.71 Hz; $P = .512$) (Figure 1C).

3.3 | Assessment of the SNOT-20 in patients with AH

Patients with AH (n = 64) had a mean total SNOT-20 score of 18.58 ± 11.58. The mean score on the sinonasal section of the SNOT-20 was 13.22 ± 6.76. The mean score on the section related to sleep and emotional symptoms was 5.36 ± 6.94.

Six months after endoscopic adenoidectomy, patients (n = 55) had significantly lower mean total SNOT-20 scores (12.53 ± 11.69; $P < .01$). A significant decrease was also recorded in the mean score for the first 10 symptoms (8.29 ± 6.95; $P < .001$). The mean score for

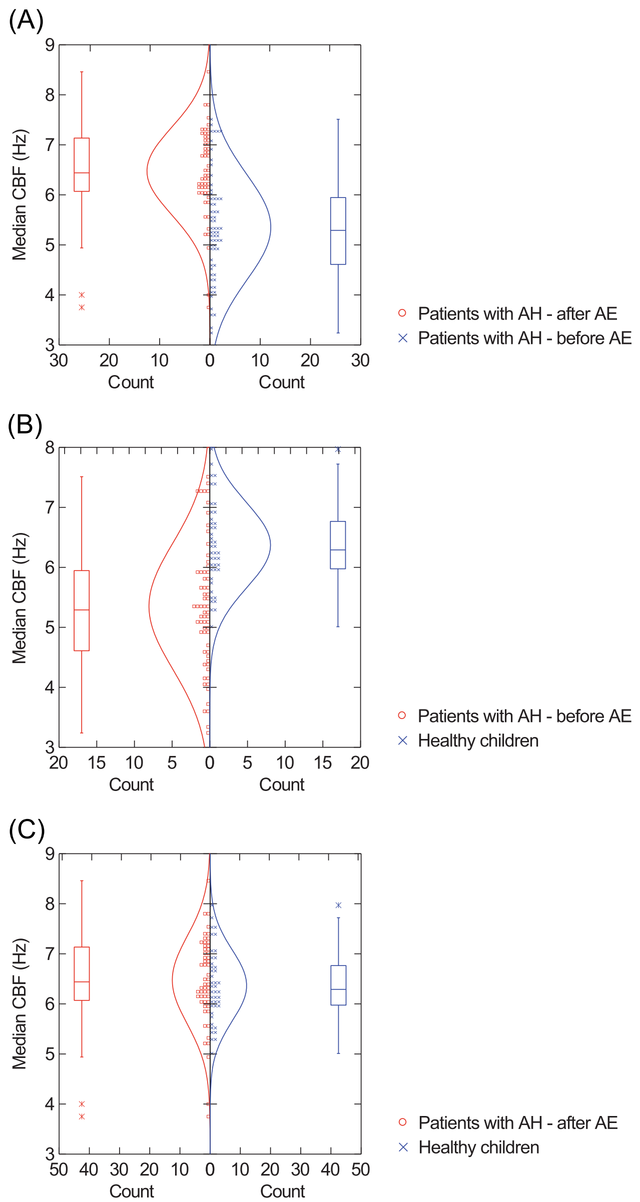

FIGURE 1 Comparison of the median ciliary beat frequency. A, Patients with adenoid hypertrophy before and after endoscopic adenoidectomy: $P < .001$. B, Patients with adenoid hypertrophy before endoscopic adenoidectomy and healthy children: $P < .001$. C, Patients with adenoid hypertrophy after endoscopic adenoidectomy and healthy children: $P = .512$. AE, endoscopic adenoidectomy; AH, adenoid hypertrophy; CBF, ciliary beat frequency [Color figure can be viewed at wileyonlinelibrary.com]

the second 10 symptoms did not change significantly (4.24 ± 6.26; $P = .359$). Compared with the preoperative examination, patients after adenoidectomy had significantly lower scores for symptoms 1, 3, 5, 6, and 7 ($P < .05$). There was no statistically significant difference in scores for other symptoms (Table 3).

We neither found a correlation of the median CBF with the mean total SNOT-20 score in the group of patients before adenoidectomy (r = −0.034; $P = .789$) nor in the group after surgery (r = −0.174; $P = .204$). Median CBF was not correlated with the mean score for the first 10 symptoms, the mean score for the second 10 symptoms or the mean score for individual symptoms on the questionnaire (Figure 2).

4 | DISCUSSION

Mucociliary clearance is one of the most important innate defense mechanisms of the respiratory tract, providing continuous clearance of mucus and protecting the respiratory system against inhaled microbes and irritants. Its efficiency is affected by the viscoelastic properties of mucus as well as by the coordinated movement of respiratory cilia—particularly their beating pattern and frequency.[21] In previous studies, reduced nasal mucociliary clearance velocity (MCV)[5] and higher nasal MCT[6] were observed in children with AH using the saccharine test, while 1 month[4,6,7] and 6 months[5] after adenoidectomy, these parameters were significantly improved and reached the values of healthy children.[6]

In this study, we focused on the evaluation of CBF as one of the main components of mucociliary clearance. We found that patients with AH had a significantly lower median CBF (5.35 ± 1.06 Hz) than healthy children (6.37 ± 0.71 Hz). Six months after endoscopic adenoidectomy, the median CBF was significantly improved (6.48 ± 0.88 Hz), and there was no significant difference in CBF between patients after surgery and healthy controls. Our findings suggest that pediatric patients with AH have an acquired, temporary disorder of CBF, which is improved after adenoidectomy to the level of healthy children. Increased nasal CBF after surgery could explain the postoperative improvement of nasal MCV and MCT recorded in previous studies.[4-7] We suppose that acquired (secondary) ciliary dyskinesia,[22] observed in children with AH, could contribute to the pathogenesis of RRI in these patients by negatively affecting mucociliary clearance and creating a local environment that promotes further development of infection.

Only a few studies were aimed at the evaluation of CBF in patients with AH. Ohashi et al[23] observed that participants with otitis media with effusion (OME) had significantly decreased ciliary activity of the adenoidal surface compared with those with no episode of otitis media or those with otitis media without effusion. All patients had significantly lower ciliary activity compared to that found in normal guinea pigs. Andreoli et al[24] found no significant difference in baseline CBF between children with AH + OSA, AH + chronic otitis media with effusion (EOM) and AH + recurrent episodes of acute otitis media (ROM). After stimulation with isoproterenol, patients with AH + EOM had significantly lower dynamic ciliary responses at 2 and 3 hours. They assumed that chronic inflammation in EOM may result in ciliary dysfunction or that decreased ciliary activity may lead to chronic infection and EOM. While there are limited data on CBF for patients with AH, normal values of nasal CBF in healthy subjects have been published in several studies.[25-27] These values range from 9.6 to 15.3 Hz.[26] Children were found to have significantly higher nasal CBF than adults, with a mean CBF of 12.8 Hz.[25] The median CBF of healthy children in our study was considerably lower (6.37 ± 0.71 Hz). One explanation for these results is that the

TABLE 2 Median ciliary beat frequency in patients with adenoid hypertrophy before and after endoscopic adenoidectomy and in healthy children

	Before AE Median CBF, Hz	After AE Median CBF, Hz	*P*
All patients (n = 64/n = 55)	5.35 ± 1.06	6.48 ± 0.88	<.001
Boys (n = 34/n = 32)	5.38 ± 1.16	6.40 ± 0.83	<.001
Girls (n = 30/n = 23)	5.32 ± 0.94	6.58 ± 0.94	<.001
AH grade 2 (n = 37/n = 31)	5.47 ± 1.12	6.66 ± 0.76	<.001
AH grade 3 (n = 27/n = 24)	5.19 ± 0.96	6.24 ± 0.98	<.001
	Before AE median CBF, Hz	Controls median CBF, Hz	
All subjects (n = 64/n = 43)	5.35 ± 1.06	6.37 ± 0.71	<.001
	After AE median CBF, Hz	Controls median CBF, Hz	
All subjects (n = 55/n = 43)	6.48 ± 0.88	6.37 ± 0.71	.512

Abbreviations: AE, endoscopic adenoidectomy; AH, adenoid hypertrophy; CBF, ciliary beat frequency; controls, healthy children.

environmental conditions under which CBF was evaluated, especially temperature, differed between studies. While Chilvers et al used a heated stage (37°C), we measured CBF at room temperature (20°C). Temperature is among the factors that significantly affect CBF. Between 19°C and 32°C CBF increases linearly, between 32 and 40°C a plateau is reached and above 40°C CBF gradually declines.[28] Jorissen et al[29] evaluated nasal CBF in children and adults at 22°C and found a mean CBF of 7.0 ± 2.6 Hz in biopsies and 8.1 ± 1.3 Hz after ciliogenesis in culture, results which are comparable to ours.

In our study, nasal CBF did not change significantly with increasing age. Most of the previous studies suggested that the CBF tends to decrease with age[25,27,30]; other studies did not confirm this correlation.[29] Our results could be influenced by the fact that only children aged 3 to 18 years were included, while a significant

TABLE 3 Influence of endoscopic adenoidectomy on symptoms of the SNOT-20 questionnaire in patients with adenoid hypertrophy

Symptoms of the SNOT-20	Mean score before AE	Mean score after AE	*P*
1. Need to blow nose	2.13	1.15	<.001
2. Sneezing	1.14	0.80	.075
3. Runny nose	2.59	1.60	<.001
4. Cough	1.56	1.16	.069
5. Postnasal discharge	2.36	1.67	<.01
6. Thick nasal discharge	1.45	0.75	<.01
7. Ear fullness	1.14	0.53	<.05
8. Dizziness	0.11	0.16	.584
9. Ear pain	0.67	0.42	.199
10. Facial pain/pressure	0.06	0.06	.873
11. Difficulty falling asleep	0.59	0.47	.519
12. Wake up at night	0.88	0.62	.229
13. Lack of a good night's sleep	0.59	0.42	.357
14. Wake up tired	0.56	0.42	.400
15. Fatigue	0.58	0.51	.675
16. Reduced productivity	0.34	0.26	.488
17. Reduced concentration	0.42	0.53	.493
18. Frustrated/restless/irritable	0.72	0.58	.418
19. Sad	0.34	0.15	.113
20. Embarrassed	0.33	0.29	.765
Total SNOT-20 score	18.58 ± 11.58	12.53 ± 11.69	<.01
Score for symptoms 1-10	13.22 ± 6.76	8.29 ± 6.95	<.001
Score for symptoms 11-20	5.36 ± 6.94	4.24 ± 6.26	.359

Abbreviations: AE, endoscopic adenoidectomy; SNOT, Sino-Nasal Outcome Test.

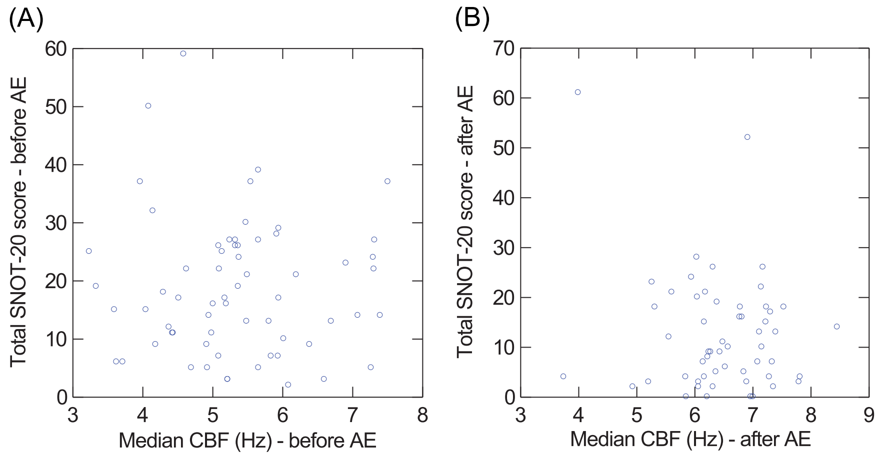

FIGURE 2 Median ciliary beat frequency in correlation with total SNOT-20 score in patients with adenoid hypertrophy. A, Before endoscopic adenoidectomy: r = −0.034; *P* = .789; B, after endoscopic adenoidectomy: r = −0.174; *P* = .204. AE, endoscopic adenoidectomy; CBF, ciliary beat frequency [Color figure can be viewed at wileyonlinelibrary.com]

decrease in CBF was observed in the adult population.[27,30] The impact of gender on CBF was neither confirmed in previous studies[27,30] nor in our study.

Participants in our study were examined only if they had no acute respiratory infection during the previous 4 weeks,[12] but we neither assess the exact time since last infection nor number of infections in the past year, which is a limitation of our study. We examined patients with AH only in certain months of the year (from June to September before adenoidectomy, respectively from January to April after adenoidectomy), therefore we can not clearly assess potential changes of CBF depending on the season of the year. Seasonal allergy and allergic inflammation,[31,32] increased incidence of acute viral respiratory infections in certain months[21,33] as well as other environmental factors (exposure of the airways to cold air, sudden temperature changes,[34] active or passive cigarette smoking[35] or exposure to air pollutants[21]) could also influence the CBF.

Recurrent or CRS is a frequent symptom of AH[2]; however, the role of adenoids in its pathogenesis is not yet fully understood. One of the possible pathomechanisms is that enlarged adenoids cause mechanical obstruction of the nasopharynx with subsequent stasis of secretions, which creates a local environment suitable for the growth of bacteria and development of infection.[4,36] Therefore, we decided to evaluate the influence of adenoid size on nasal CBF in children with AH. We assumed that CBF would be secondarily decreased as a result of stagnant mucus and an altered local environment and that patients with larger adenoids would thus have a slower CBF. The size of adenoid tissue, however, was not correlated with CBF. Although the median CBF in AH grade 2 was slightly higher than in grade 3 (5.47 ± 1.12 Hz and 5.19 ± 0.96 Hz), the difference was not statistically significant. After adenoidectomy, the median CBF improved significantly in both groups. Our results are consistent with a previously published study, in which impaired nasal MCT in AH grade 2 and grade 3 was found to be significantly improved after adenoidectomy. MCT was shorter in patients with AH grade 2 compared with grade 3; however, there was no correlation between adenoid size and MCT improvement.[7]

Although the correlation of sinusitis and the corresponding sinonasal symptoms with the size of adenoid tissue seems to be logical, it has not been confirmed in studies observing adenoid volume,[37] weight[38], and adenoidal-nasopharyngeal ratio.[36,39] In our study, a relationship between adenoid size and CBF was not confirmed, which, together with previous results, suggest that there are most likely other factors causing ciliary slowing and RRI in patients with AH. Several published studies suggest that the adenoids could be involved in the pathogenesis of RRI as a reservoir of pathogenic bacteria impairing mucociliary clearance.[4,36,40] We suppose that hypertrophic adenoid tissue itself may contribute to some extent to the pathomechanism of RRI by causing mechanical obstruction and stasis of secretions, but the adenoid size is probably not the main cause of RRI in these patients.

Adenoidectomy has been shown to be successful in the treatment of RRI in children, especially when medical therapy has failed, as well as in the treatment of OSA.[4,37,41] Already 1 month after surgery, significant improvement of nasal obstruction, rhinorrhoea, cough, postnasal drainage, and headache was observed.[42] Patients had a significantly lower incidence of rhinosinusitis,[43,44] OME and OSA per year.[44] Based on our results of the SNOT-20, we can conclude that adenoidectomy significantly improved the symptoms of CRS in children with AH, especially those related to sinonasal problems. It is important to assess the distribution of SNOT-20 symptoms, because the total score could be elevated in both CRS and OSA patients; however, the two groups differ in symptom distribution pattern—while CRS patients have higher scores on the first section, OSA patients score higher on the second section of the questionnaire.[45] In our study, the median CBF did not correlate with the mean total SNOT-20 score. These results are consistent with the study of Boatsman et al,[46] who found no significant correlation between the total score on the SNOT-20 and nasal MCT in a group of 50 adult volunteers. Impairment of mucociliary clearance is, however, not the only cause leading to the development of the symptoms stated in the SNOT-20, and the questionnaire expresses a subjective assessment of each person's difficulties, which may not objectively reflect the severity of mucociliary clearance or ciliary activity disorder.

In conclusion, we confirmed that children with clinically symptomatic AH have impaired mucociliary clearance due to decreased nasal CBF. After removal of hypertrophic adenoid tissue by endoscopic adenoidectomy, nasal CBF normalized, and clinical symptoms of rhinosinusitis were reduced. To our best knowledge, this is the first study evaluating the influence of AH and endoscopic adenoidectomy on mucociliary clearance using the modern method of digital high-speed video microscopy and assessing the involvement of CBF defects in the pathogenesis of RRI in these patients.

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