Ciliary function and sinonasal mucosal cytology in pediatric patients with chronic rhinosinusitis during a year after functional endoscopic sinus surgery*

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Abstract

Background: The objective of the study is evaluation of ciliary function and mucosal cytology after endoscopic sinus surgery in children with chronic rhinosinusitis (CRS).

Methodology: A total of 132 children with CRS who underwent endoscopic sinus surgery, as well as 15 healthy controls were involved in the study. In this follow-up study patients were examined preoperatively, as well as 3, 6, 9, and 12 months after endoscopic sinus surgery. Assessment of ciliary function and sinonasal mucosal cytology was performed using high-speed videomicroscopy. Lund-Kennedy, Lund-Mackay, and sinonasal outcome test 20 (SNOT20) scores were also evaluated.

Results: Total SNOT-20, Lund-Mackay, and Lund-Kennedy values significantly decreased after sinus surgery. In contrast, ciliary function and mucosal cytology only tended to improve after 6 months. 9 months after surgery the number of ciliated cells, ciliary beat frequency, cell viability, and ciliary length were significantly higher than preoperatively. The most significant improvement of ciliary function and cell height was observed 12 months after operation, whereas epithelial dystrophy and neutrophil infiltration were significantly reduced.

Conclusions: Substantial improvement was observed only in a year after surgery, whereas 0 to 3 months after the surgery ciliary function was severely impaired thus predisposing to recurrent sinusitis or other complications.

Key words: sinusitis, nasal mucosa, nasal surgical procedures, paranasal sinus diseases, paranasal sinuses

Introduction

Chronic rhinosinusitis is a common inflammatory disorder of nasal cavity and paranasal sinuses that has significant adverse effects on children's health [(1)]. Chronic rhinosinusitis is defined as the presence of characteristic clinical symptoms including nasal congestion, postnasal drainage, purulent rhinorrhea, low-grade hyperthermia, irritability, and cough during more than 12 weeks [(2)], confirmed by objective investigation using sinonasal endoscopy and computed tomography (CT) [(3)]. Along with the above-mentioned local symptoms pediatric chronic rhinosinusitis may have systemic effects [(4)] altogether resulting in a significantly reduced quality of life [(5)]. Therefore, accurate diagnosis and timely treatment are required. The first-line treatment of CRS involves therapeutic treatment, whereas in case of severe or refractory disease surgical treatment is required [(6)]. Since the 1980s endoscopic sinus surgery has been widely used in management of various nasal pathologies and therapy-resistant chronic rhinosinusitis [(7)] with higher efficiency and safety as compared to open surgery [(8)].

Mucociliary clearance is considered as a primary innate defense mechanism in respiratory system including mucus and surface liquid layers, as well as cilia of ciliary epithelia [(9)]. The latter is

characterized by acquired dysfunction in chronic rhinosinusitis [10], playing a significant role in disease pathogenesis [11]. In turn, improvement of mucociliary clearance may significantly contribute to recovery in CRS [12], thus being considered as a potential marker of treatment efficiency [13].

Sinonasal surgeries were shown to affect mucosal structure and mucociliary clearance [14]. Oppositely, ciliary dysfunction and mucosal inflammation may contribute to FESS failure [15]. The stages of mucosal recovery after FESS include clean cavity (1-2 weeks), mucosal transition (3-10 weeks), and complete epithelization (11-14 weeks) [16]. However, the existing data on timeframes of postoperative mucociliary clearance recovery are highly contradictory, being indicative of 3-4 [17, 18], 6 [19], or 12 months [20] required for substantial improvement. Moreover, data on pediatric CRS patients are insufficient.

Therefore, the objective of the present study was evaluation of dynamic changes in ciliary function and mucosal cytology after FESS in children with CRS using high-speed videomicroscopy in a follow-up hospital-based study.

Materials and methods

Ethics

The protocol of the present study was approved by the Local Ethics Committee (I. I. Mechnikov North-West State Medical University, St Petersburg, Russia). All procedures meet the principles set by the Declaration of Helsinki (1964) and its later amendments (2013). All parents of the examined children signed a Declaration of informed consent prior the inclusion into the study.

Cohort characteristics and study design

A total of 132 children with chronic rhinosinusitis without nasal polyps aged 5-17 y.o. (13.0±3.2 y.o.) took part in the present study. The patients were also characterized by concomitant vasomotor rhinitis (51%), allergic rhinitis (15%), chronic tonsillitis (22%), nasal turbinate hypertrophy (77%), and nasal septum deviation (37%). All patients underwent endoscopic sinus surgery in Department of Otorhinolaryngology of K.A. Raukhfus Children's City Multidisciplinary Clinical Center for High Medical Technologies (Saint Petersburg, Russia) in 2012-2017. The indications for endoscopic surgery included various forms of therapy-resistant chronic rhinosinusitis. In addition, 15 healthy children of the respective age (12.8±2.9 y.o.) were also involved in the current study in order to evaluate the normal values of ciliary function and sinonasal mucosal cytology.

Patients with primary mucociliary dysfunction including cystic fibrosis, primary ciliary dyskinesia, as well as specific genetic syndromes with ciliary dysfunction (Senior-Loken, Alstrom, Bardet-Biedl syndromes) were excluded from the study.

In the postoperative period after FESS all patients obtained standard therapy according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS 2012) [53] and the national guidelines.

Irrigation therapy started from the second day after removal of the nasal tampons and included irrigation of nasal mucosa with a sterile physiological saline for three months. A short course (5-7 days) of antibacterial treatment with amoxycillin-clavulanic acid combination was assigned only in the case of strong indications. All children were administered with topical corticosteroids (mometasone furoate at age specific doses) starting from the seventh say after surgery for three months.

Long-term antibiotic therapy, oral corticosteroids, antimycotics, leukotriene antagonists, immunosuppressants, anti-IgG, anti-IL-5 and any local nasal drops were not used in postoperative management.

In this follow-up study patients were examined preoperatively (time point 0), as well as 3 (point 1), 6 (point 2), 9 (point 3), and 12 months (point 4) after endoscopic sinus surgery. The number of patients who were examined at 0, 1, 2, 3, and 4 points of the follow-up was 132 (100%), 115 (87%), 73 (55%), 86 (65%), and 118 (89%), respectively. In turn, healthy controls were examined endoscopically only at baseline without a subsequent follow-up. Assessment of quality of life, as well as evaluation of ciliary function and mucosal cytology of nasal turbinate were performed at all time points. Computer tomography and endoscopic evaluation of nasal mucosa were performed preoperatively (time point 0) and 6 months after FESS. Subjective evaluation of surgery efficiency by patients and parents was recorded 6 months after FESS.

Endoscopy

Endoscopic sinonasal surgery was performed under general anesthesia with controlled hypotension. Diathermocoagulation (Erbe Elektromedizin GmbH, Tübingen, Germany), hemostatic powder Starsil, and Merocel Kennedy Sinus Pak tampons (Medtronic Xomed, Jacksonville, FL, USA) were used for local hemostasis intraoperatively. Endoscopic manipulations (examination and surgery) were undertaken using Karl Storz equipment including rigid 2.7, 3, and 4-mm endoscopes (0°, 30°, 45°), 18 cv length, Xenon 300 light source, and Telecam SLII video camera (Karl Storz, GmbH & Co. KG, Tuttlingen, Germany). Tricut Blade 4.0 mm, Silver Bullet Blade 4.0, RAD12 Blade 4.0, and RAD 40 Blade 3.5 blades of a Straightshot® M4 Microdebrider powered handpiece (Medtronic, Minneapolis, MN, USA) with a 00 and 45° cutter were used for excision of pathologic mucosa.

Endoscopic examination of sinonasal mucosa included recording of mucosa color, edema, and fluid characteristics with subsequent evaluation using Lund-Kennedy scoring system [21].

Computer tomography

Computer tomography was also performed in order to reveal the presence of cysts, polyps, mucosal edema, liquid, tissue destruction, and/or hypoplasia in sinonasal cavity. The examination

was performed with the use of Somatom Emotion spiral scanner (Siemens Medical Solutions, Erlangen, Germany) with HiRes 0.63 mm and 0.75 mm slice thicknesses. Subsequent evaluation of obtained data was performed using Lund-Mackay scoring system [22].

Evaluation of ciliary function and mucosal cytology

Although saccharin test is widely used for evaluation of mucociliary clearance, this method has several limitations including subjective perception [23] as well as limited use in children [24]. In addition, saccharin test does not allow to assess ciliary beat frequency and cilia morphology. Therefore, ciliary function and mucosal cytology were evaluated using high-speed videomicroscopy that allows precise evaluation of ciliary function [25].

The studied samples were collected from nasal turbinate without mucosal anesthesia. Cell layers were sampled using a brush with its subsequent washing into Eppendorf tube containing 0.5 ml of isotonic saline at 37°C. Subsequently 50 µl of the obtained suspension was subjected to evaluation using Nikon Eclipse E200 microscope (Nikon, Japan) equipped with a high-speed camera Basler (Basler AG, Germany). Three video files (70-100 fps) were recorded per each sample. Video was recorded from the cell layers with an area of not less than 2500 µm2 and intact uniform ciliated epithelial strip >50 µm in length according to the recommendations of other authors [26 – 28] in 2-3 minutes after introduction to a slide. The sample was considered sufficient for analysis if not less than 5 sites for video record were available. MMC Multimeter software (MMCSoft, Russia) was used for morphometric analysis of the obtained video and image files.

Cilia length was evaluated in not less than 10 cells in different fields of view at 400-fold zoom and lateral view. The objective was calibrated with an objective micrometer 0-1 mm/100 (Nikon, Japan). Cell viability was assessed as a period of occurrence of cytolytic changes (disruption of intercellular contacts with subsequent cell layer disorganization and cell swelling). The rate of grainy degeneration and cytoplasm vacuolization was used as a marker of cell dystrophy.

The remaining sample was applied to microscope slide using an automated cytocentrifuge (Cyto-Tek; Sakura, Tokyo, Japan) with subsequent staining with Harris hematoxylin according to Papanicolaou procedure and eosin-methylene blue, following May-Grünwald-Giemsa technique for cytological evaluation [29].

Quality of life assessment

Rhinosinusitis-related quality of life was evaluated using sinonasal outcome test 20 (SNOT-20) [30]. However, due to lack of validation of SNOT-20 in children, future studies should also incorporate other methods of QOL assessment including Sino-Nasal 5 (SN-5) questionnaire [31].

In addition, subjective evaluation using a five-point grading scale (excellent, good, acceptable, no improvement, aggravation) was also recorded both from the patient and the parents.

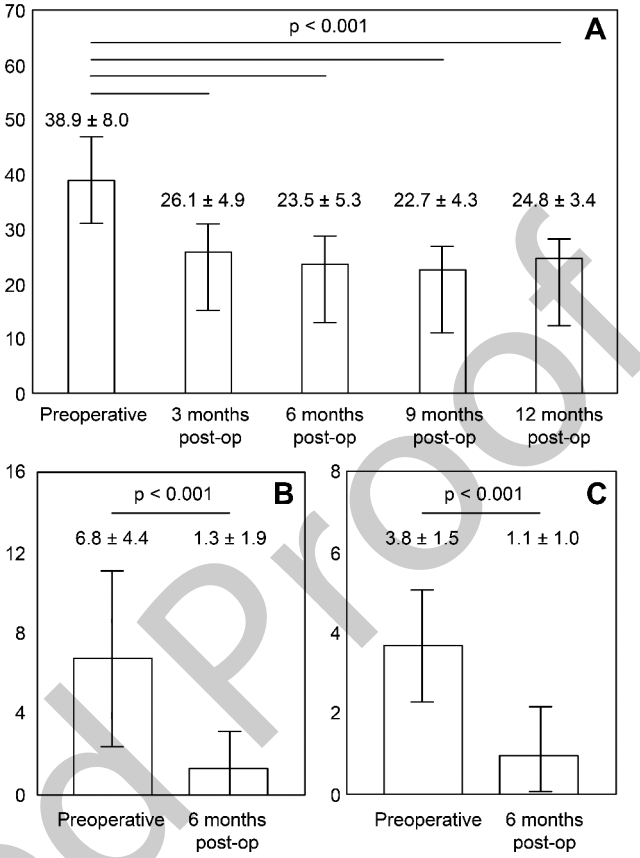

Figure 1. Chronic rhinitis group (N=560). A: number of reported nasal complaints (%); B: prevalence (%) of nasal complaints.

Statistical analysis

Statistical analysis of the data was performed using Statistica 10 software (Statsoft, USA). Quantitative data were expressed as mean values and the respective standard deviations (mean ± SD), whereas qualitative data were provided as percentage of observations (%). Total SNOT-20, Lund-Kennedy, and Lund-Mackay scores were used for statistical processing. Due to follow-up design of the study, dependent group comparisons were performed using Sign test. Significance of the overall trend during the follow-up was evaluated using Friedman analysis of variance (ANOVA) and Kendall coefficient of concordance. Comparison of the control values and pre- and postoperative values in children with chronic rhinosinusitis was performed using one-way ANOVA with Fisher's LSD-test. Multiple linear regression was used for analysis of the association of preoperative values of ciliary function and mucosal cytology with total Lund-Kennedy, Lund-Mackay, and SNOT-20 scores. The level of significance of $p < 0.05$ was used for all statistical analyses.

Results

SNOT-20, Lund-Kennedy, and Lund-Mackay scores

Table 1. Postoperative changes in mucosal morphology and mucociliary clearance in children with chronic rhinosinusitis.

Period	Control	0	1	2	3	4	p trend
		Preoperative	3 months	6 months	9 months	12 months	
Cells with motile cilia, %	85.7 ± 6.3	42.2 ± 34.8 †	44.1 ± 33.9 †	52.5 ± 33.6 †	67.9 ± 27.7 †*	70.9 ± 26.5 *	0.003
Ciliary beat frequency, Hz	8.5 ± 0.8	6.6 ± 4.5 †	4.7 ± 4.4 †*	6.5 ± 4.3 †	7.9 ± 3.2 *	7.6 ± 2 *	0.002
Cell viability, min	29.6 ± 1.1	20 ± 10.6 †	16.8 ± 10.5 †*	23.5 ± 9.1 †	24.8 ± 8 †*	26.1 ± 4.7 *	0.003
Cillia length, µm	6 ± 0.5	4.9 ± 2.6 †	3.7 ± 3.1 †	5.1 ± 2.8 *	5.4 ± 1.9 *	6.3 ± 0.8 *	0.001
Neutrophils (x400 per field of view)	7.9 ± 9.1	15.3 ± 21.6 †	24.3 ± 26 †	16.7 ± 26.2	6.7 ± 9.5	9.9 ± 15.4 *	0.043
Lymphocytes (x400 per field of view)	4.3 ± 4.2	2.8 ± 3.6 †	3.2 ± 4.5	2.1 ± 3.5 †	2 ± 2.4 †	2.5 ± 2.8 †	0.222
Eosinophils (x400 per field of view)	0.1 ± 0.4	0.3 ± 0.8	0.3 ± 0.8	0.2 ± 0.6	0.2 ± 0.5	0.7 ± 1.7 †	0.171
Ciliary beat asynchrony, %		†	†	†	†*	†*	
No	93	29	16	30	57	59	
Mild-to-moderate	7	35	42	39	28	36	
Severe	0	36	42	31	15	5	0.004
Cell dystrophy, %		†	†	†	*	*	
No	87	13	0	4	30	54	
Mild-to-moderate	7	33	52	42	41	32	
Severe	6	54	48	54	29	14	0.002
Relative cell height, %		†	†	†*	*		
High prismatic	87	44	52	71	66	31	
Cuboidal	13	27	30	25	25	70	
Squamous metaplastic	0	29	15	4	9	9	0.290

Quantitative data are expressed as mean ± SD; qualitative parameters are provided as percentages (%), † and * - significant group difference in comparison to the healthy control group and baseline (preoperative) values in children with CRS; p trend (bold) – significance of the time-dependent changes of the parameter

Total Lund-Mackay and Lund-Kennedy scores decreased after the operation by a factor of 5 and 3 in comparison to the preoperative values, respectively (Figure 1). Quality of life assessment using SNOT-20 questionnaire also demonstrated a significant effect of FESS. Specifically, total SNOT-20 values 3, 6, 9, and 12 months after postoperatively were 33%, 40%, 42%, and 36% lower as compared to preoperative values, respectively. The overall trend to a time-dependent decrease in total SNOT-20 values was also significant.

Surgery complications and postoperative exacerbations

87% of patients did not experience any complications. Hemorrhage and synechia occurred only in 4% and 9% cases, respectively. In the postoperative period 9% of children required revision surgery. After the operation 35% of cases did not experience episodes of chronic sinusitis exacerbation. In turn, 26% of examinees had one and two episodes of exacerbation after surgery. 3, 4, and 6 episodes of chronic sinusitis exacerbation in a year after surgery were detected in 4%, 8%, and 2% of patients, respectively.

Subjective evaluation of surgery efficiency

The patients evaluated the results of sinus surgery as excellent (25%), good (46%), acceptable (25%). Nearly similar response was provided by their parents, with 24%, 48%, and 24% reporting excellent, good, and acceptable results, respectively. Only 4% of pediatric patients and 3% of their parents did not report any improvement in the postoperative period, whereas none of examined persons reported disease aggravation.

Ciliary function and mucosal cytology

In contrast to Lund-Mackay and Lund-Kennedy scores and especially SNOT20 values, postoperative improvement of ciliary function and mucosal cytology was delayed (Table 1).

Preoperative (time point 0)

Evaluation of ciliary function and mucosal cytology in children with chronic rhinosinusitis before FESS revealed significant differences in comparison to the healthy controls. Specifically, patients were characterized by a twofold lower number of cells with motile cilia. Ciliary beat frequency, cell viability, and cilia length were 22%, 32%, and 18% lower in children with CRS as compared to control values, respectively. In turn, mucosal cytology in chronic rhinosinusitis was characterized by a nearly twofold increase in neutrophil number, whereas lymphocyte count was shown to be reduced by 35% in comparison to healthy

Table 2. Multiple linear regression analysis of the association between mucociliary clearance and SNOT-20, Lund-Mackay, and Lund-Kennedy scores in children with chronic rhinosinusitis.

Parameter	SNOT-20		Lund-Mackay		Lund-Kennedy	
	β	p	β	p	β	p
Cells with motile cilia, %	-0.248	0.004 *	-0.070	0.481	-0.224	0.027 *
Ciliary beat asynchrony	-0.002	0.976	-0.078	0.348	-0.061	0.463
Ciliary beat frequency, Hz	-0.223	0.002 *	-0.194	0.023 *	-0.118	0.171
Cell viability, min	-0.154	0.058	0.113	0.233	0.086	0.365
Cilia length, μm	-0.031	0.701	-0.142	0.134	0.029	0.761
Cell dystrophy	0.029	0.632	-0.017	0.808	-0.032	0.659
Relative cell height	-0.088	0.151	-0.093	0.190	-0.125	0.081
Neutrophils (x400 per field of view)	0.008	0.888	0.063	0.318	0.052	0.409
Lymphocytes (x400 per field of view)	0.117	0.020 *	-0.018	0.763	0.024	0.680
Eosinophils (x400 per field of view)	0.020	0.696	0.013	0.820	-0.063	0.285
P for a model	< 0.001		< 0.001		0.019	

counterparts. Epithelial dystrophy and ciliary beat asynchrony were both significantly more pronounced in pediatric rhinosinusitis patients than in healthy children.

3 months postoperatively (time point 1)

3 months after the surgery no significant improvement of ciliary function was observed. Moreover, ciliary beat frequency and cell viability were reduced by 29% and 16% as compared to the respective preoperative values.

When compared to healthy controls, the studied parameters of ciliary function remained significantly altered. Neutrophil count was found to exceed the control values by a factor of more than 3. Mucosal dystrophy and ciliary beat asynchrony were still significantly more prevalent 3 months after the operation when compared to the control values.

6 months postoperatively (time point 2)

6 months after the operation (time point 2) cilia length significantly increased by 4% in comparison to the preoperative values. When compared to the respective values obtained 3 months after FESS, the relative number of ciliated cells, ciliary beat frequency, and cell viability were 19%, 38%, and 40% higher. Relative cell height was also significantly higher at time point 2 than at time point 1.

9 months postoperatively (time point 3)

Further improvement of ciliary function and mucosal cytology was observed 9 months after FESS. Particularly, the number of ciliated cells, ciliary beat frequency, cell viability, and ciliary length at point 3 exceeded the respective values at points 0 and 1 by 61%, 20%, 24%, 10%, and 53%, 68%, 47%, 46%, respectively. Ciliary beat synchronicity and cell height were also found to be improved 9 months after FESS (point 3), whereas mucosal dystrophy was significantly reduced when compared to time points 0 and 1. 9 months after surgery the number of neutrophils was twofold lower as compared to preoperative values. When compared to 3 months after operation, at 9 months after FESS children were also characterized by a nearly fourfold decrease in neutrophil number in brush biopsy samples, whereas the number of lymphocytes was 38% lower.

9 months after surgery only the number of cells with motile cilia as well as cell viability remained significantly lower than in healthy subjects by 21% and 16%, respectively. The number of infiltrating neutrophils returned to the control values, with only lymphocyte count being still twofold lower than those in healthy controls.

12 months postoperatively (time point 4)

The most significant improvement in ciliary function and mucosal cytology was observed 12 months after FESS. Particularly, the number of ciliated cells, ciliary beat frequency, cell viability, and ciliary length at time point 4 exceeded the preoperative values by 68%, 15%, 31%, and 29%, respectively. Moreover, 12 months after surgery (point 4) these values were significantly higher than those obtained at point 1 by 61%, 62%, 55%, 70%, respectively. In turn, the number of neutrophils in brush biopsy samples at point 4 was reduced by 35% and 59% when compared to points 1 and 2, respectively.

Ciliary beat asynchronicity and epithelial dystrophy were less prevalent in children 12 months after surgery when compared to time periods 1 and 2. Significant differences, although less pronounced, were observed when the parameters of ciliary function and mucosal cytology observed 12 months after the surgery were compared to those measured 9 months after

the operation. Particularly, percentage of ciliated cells and cell viability at time point 4 exceeded that at point 2 by 35% and 11%, respectively. At the same time, neutrophil count was found to be reduced by 41% as compared to that at point 2 (6 months postoperatively).

It is notable that only 12 months after operation the markers of ciliary function did not differ significantly from the control values. However, the number of lymphocytes was still nearly twofold lower than that in the control group.

Significance of time-dependent changes

Being in agreement with group comparisons, the overall time-dependent improvement of ciliary function was also significant. Particularly, the number of ciliated cells, ciliary beat frequency, cell viability, ciliary length, as well as ciliary beat synchronicity tended to increase postoperatively. In turn, both the number of neutrophils in brush biopsy samples as well as epithelial dystrophy were found to decrease in a time-dependent manner. Neither lymphocyte and eosinophil count, nor relative cell height was significantly changed during a year after FESS.

Association between ciliary function, mucosal cytology, and disease severity

In multiple linear regression models the parameters of mucociliary clearance were significantly associated with disease severity as assessed by endoscopic and computer tomography scores, and quality of life questionnaire (Table 2). Particularly, the number of ciliated cells, ciliary beat frequency, and cell viability were found to be inversely associated with SNOT20 values, whereas the number of lymphocytes in brush biopsy samples was considered as the positive predictor of SNOT20. After adjustment for SNOT20 variability ciliary beat frequency was found to be negatively associated with Lund-Mackay scores. More pronounced associations were revealed between markers of ciliary function and Lund-Kennedy scores. Particularly, in a SNOT20-adjusted model cell viability and cell height were inversely associated with total Lund-Kennedy scores. It is also notable that the overall models incorporating all studied parameters of ciliary function and mucosal cytology were significantly associated with variability of SNOT20, as well as Lund-Mackay and Lund-Kennedy scores.

Discussion

In general, the obtained data demonstrate lack of significant improvement in ciliary function and mucosal cytology 3 months after surgery, whereas within the period from 6 months up to 12 months after the FESS mucociliary function tended to improve. These findings generally correspond to the earlier indications of prolonged sinonasal mucosa recovery in the postoperative period [32].

Although certain studies demonstrated a significant improvement in ciliary function 3-4 months after operation [17, 18], the majority of studies clearly indicate that regeneration of mucosa and mucociliary clearance is not complete.Particularly, ultrastructural analysis of nasal mucosa after ESS demonstrated that despite a relative decrease in saccharin time 12 weeks after the operation [33]. Correspondingly, ciliated cells were rather rare, being indicative of incomplete mucosa recovery [33]. No significant improvement of mucociliary clearance was observed 3 months after experimental FESS in sheep [34]. Experimental study demonstrated that although reepitheliazion occurs 2 weeks after the operation, complete mucosa regeneration requires longer period [35]. In addition, compound cilia and edematous cilia were also observed in regenerated mucosa in association with reduced mucociliary clearance as compared to intact mucosa [36]. In the present study we observed a significant improvement in ciliary function and mucosal cytology only starting from 6 months and up to 12 months after FESS. On the one hand, certain studies also demonstrated significant improvement of mucosal appearance 6 months after surgery. Particularly, a substantial decrease in mucosal edema, inflammation, ulceration, and fibrosis was associated with increased ciliated area, improved ciliary orientation [19], as well as the reversal of basal membrane thickening and epithelial metaplasia 6 months after surgery [37].

On the other hand, further improvement of ciliated epithelium 9 and 12 months after the surgery clearly indicates yet incomplete recovery of sinonasal mucosa 6 months after FESS. Being in agreement with the present findings, it has been stated that complete wound healing after endonasal paranasal sinus surgery lasts for six months, whereas scar remodeling requires more time [38]. Correspondingly, mucosal examination 6 months after endoscopic sinus surgery also revealed pathological findings [39]. 7 months after the operation CBF was also found to be significantly reduced as compared to controls, being not different from the preoperative values [40]. Moreover, mucociliary clearance remained inadequate even 9 months after EES in chronic maxillary rhinosinusitis [41]. It is also notable that even 1 year after surgery patients who underwent FESS successfully were characterized by pseudostratified epithelium, increased number of goblet cells, and reduced number of ciliated cells [20].

In addition to altered mucociliary clearance, the present study as well as the earlier investigations clearly demonstrated persistent mucosal inflammation in the postoperative period [42]. Particularly, in the postoperative period increased rate of macrophage and neutrophil infiltration was observed up to 6 months after surgery [43]. The levels of IL-2, IL-4, IL-17, and IFN-γ in nasal secretions were found to be significantly increased within a period from 4 to 12 weeks after the surgery in comparison to the baseline controls [44]. Another study revealed elevation of IL-6 and IL-8 levels shortly after the operation, being interrelated with collagen I expression [45]. Taking into consideration the role

of inflammation in regulation of mucosal remodeling and mucociliary clearance functioning [10], one can propose that persistent inflammation may contribute to mucociliary dysfunction.

It is also notable that despite a postponed recovery of mucociliary clearance and mucosal morphology, improvement of SNOT20, Lund-Kennedy, and Lund-Mackay scores was observed directly after FESS. This observation corresponds to the earlier data demonstrating lack of association between patterns of mucociliary clearance and postoperative clinical course following endoscopic surgery [28, 46].

The observed association between mucociliary clearance and mucosal morphology and disease severity clearly corresponds to the role of altered mucociliary clearance in pathogenesis of chronic rhinosinusitis [47, 48]. Particularly, it has been also demonstrated that ciliary beat frequency is significantly reduced in CRS [49]. Mucosal infiltration with immune cells was also shown to be associated with disease severity scores.

Impaired mucociliary clearance is associated with the presence of resistant strain bacterial isolates [50] and higher risk of recurrent rhinosinusitis [51]. Therefore, despite a significant clinical improvement, persistent alteration of mucociliary clearance and mucosal morphology clearly indicates high risk of recurrent sinusitis, especially within the period from 0 through 3 up to 6 months after the surgery until a significant improvement is observed. This suggestion corresponds to the earlier indication of importance of close post-operative follow-up for more than 2-4 months in children with chronic maxillary rhinosinusitis undergoing endoscopic sinus surgery [52].

Conclusion

Evaluation of ciliary function and mucosal cytology after FESS in pediatric chronic rhinosinusitis patients demonstrated that despite a significant clinical improvement shortly after surgery, mucociliary clearance remains significantly affected. Substantial improvement was observed only a year after surgery, whereas 0-3 months after the operation mucosal function is severely impaired and the patient is predisposed to recurrent sinusitis or other complications. A close follow-up and improvement of mucociliary clearance are required for 6 months postoperatively. Further studies are required to unravel the mechanisms of post-operative mucosal recovery and their targeted modulations.

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Authorship contribution

SA: Investigation, Methodology, Data curation, Writing - original draft; SK: Conceptualization, Methodology, Validation, Supervision, Writing - review & editing; SAr: Investigation, Formal analysis, Writing - original draft; SB: Investigation, Data curation, Writing - original draft; IA: Investigation, Methodology, Data curation Writing - review & editing.

Conflict of interest

The authors declare no conflict of interest.

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SUPPLEMENTARY INFORMATION

Video 1. Videomicroscopy of mucosal morphology and mucociliary clearance in children with chronic rhinosinusitis in preoperative period (Time point 0). A small number of cells with preserved mucociliary apparatus (from 0% to 10-15%) and low ciliary beat frequency. Lateral view of ciliated epithelium under phase contrast, x400 zoom.

Video 2. Videomicroscopy of mucosal morphology and mucociliary clearance in children with chronic rhinosinusitis at 3 months postoperatively (Time point 1). Lack of visible dynamics in ciliary apparatus activity. Axial view of ciliated epithelium under phase contrast, x400 zoom.

Video 3. Videomicroscopy of mucosal morphology and mucociliary clearance in children with chronic rhinosinusitis at 6 months postoperatively (Time point 2). Increased number of ciliated cells, although alterations of cellular synchronicity and ciliary beat frequency still persist. Axial view of ciliated epithelium under phase contrast, x400 zoom.

Video 4. Videomicroscopy of mucosal morphology and mucociliary clearance in children with chronic rhinosinusitis at 9 months postoperatively (Time point 3). Cells with focal alterations of ciliary apparatus are revealed. Axial view of ciliated epithelium under phase contrast, x400 zoom.

Video 5. Videomicroscopy of mucosal morphology and mucociliary clearance in children with chronic rhinosinusitis at 12 months postoperatively (Time point 4). Complete recovery of ciliary apparatus. Only focal mild alterations of ciliary apparatus may be observed. Axial view of ciliated epithelium under phase contrast, x400 zoom.