

Impact of local anesthesia on ciliary dyskinesia diagnosis by digital high-speed videomicroscopy

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Abstract

Summary: This prospective study investigates the impact of local anesthesia on ciliary function in nasal epithelium. The primary objective was to assess whether lidocaine 2% and naphazoline 0.5% nasal spray alter ciliary beat frequency and pattern in subjects undergoing nasal brushing, aiming to enhance primary ciliary dyskinesia (PCL) diagnosis.

Hypothesis: It was hypothesized that local anesthesia administration would not significantly affect ciliary function in nasal epithelium.

Study Design: A prospective, simple-blind randomized study was conducted between 2020 and 2023. The study employed digital high-speed videomicroscopy to analyze ciliary beat frequency and pattern.

Patient/Subject Selection: A cohort of 38 participants was recruited, consisting of 25 healthy volunteers and 13 referred individuals (including seven diagnosed with PCL). Selection criteria ensured the absence of chronic respiratory diseases, recent respiratory tract infections, or regular use of nasal medications.

Methodology: Participants underwent nasal brushing with administration of lidocaine and naphazoline nasal spray in one nostril and saline in the contralateral nostril. Ciliary beat frequency and pattern were measured using digital high-speed video microscopy. Results: Nasal spray administration did not significantly alter ciliary beat frequency or pattern compared to saline ($p = 0.841$ and $p = 0.125$, respectively). Subgroup analysis revealed consistent results across healthy volunteers, referred patients, and PCD patients.

Conclusion: Local anesthesia with lidocaine and naphazoline spray did not affect ciliary function outcomes. These findings support the safe use of these agents in clinical practice for PCD diagnostic procedures. Further research with larger cohorts is warranted for validation.

KEYWORDS

ciliary beat frequency, ciliary beat pattern, diagnostic, digital high-speed videomicroscopy, local anaesthesia, primary ciliary dyskinesia

1 INTRODUCTION

Primary ciliary dyskinesia (PCD) is a hereditary motile ciliopathy characterized by impaired respiratory cilia motility and/or structure, resulting in compromised mucociliary clearance and significant respiratory and ENT pathologies.^{1,2} Clinically, PCD patients present with recurrent or chronic infections of the upper and lower airway, bronchiectasis, chronic cough, nasal congestion, otitis media, and sinusitis, typically beginning in childhood.^{3,4} Diagnostic complexities likely contribute to underreporting the true prevalence of PCD, suggesting that the actual prevalence is higher than the estimated range of 1 :10,000 to 1 :20,000.⁵ Diagnosis involves a combination of methods such as genetic analysis, nasal nitric oxide measurement, transmission electron microscopy, high-speed video microscopy, and immunofluorescence, with genetic analysis and transmission electron microscopy confirming diagnosis per guidelines.⁶ PCD exhibits considerable heterogeneity, with specific ultrastructural defects and genetic mutations linked to distinct ciliary beat frequency (CBF) and/or ciliary beat pattern (CBP) alterations.⁷

Digital high-speed videomicroscopy (DHSV) is a sensitive and specific tool for assessing ciliary function in PCD,⁸⁻¹¹ examining CBF and CBP.¹²

Controversies surround respiratory epithelium collection conditions, with concerns about anesthetics impacting ciliary function.^{13,14} Research on the effects of anesthetics has been conducted in vitro and in animal models.¹⁵ However, the effects of nasal local anesthesia on CBP alongside CBF remain unexplored.^{15,16} In fact, there are no existing in vivo or in vitro studies at this point that have examined the effect of anesthetics or decongestant molecules on CBP alone or concomitantly with CBF. The exact mechanism by which nasal local anesthesia may interfere with ciliary function is not completely clear.^{15,16} Despite the current precaution for anesthesia-free sampling, lidocaine and naphazoline nasal sprays, commonly used in ENT clinics, offer potential benefits for patient comfort and doctor visualization during nasal brushing. In this prospective singleblind study, we investigated the impact of lidocaine 2% and naphazoline 0.5% nasal spray on 38 subjects undergoing nasal brushing, with CBF and CBP as co-primary endpoints. The study addresses a gap in real clinical settings, examining whether nasal decongestant and anesthesia alter ciliary beating.

2 MATERIAL AND METHODS

2.1 Study design

Respiratory ciliated epithelial samples were obtained from the middle turbinate of 25 healthy volunteers and 13 patients referred to a PCD diagnosis center. A cytological brush was utilized, with local anesthesia (lidocaine 2%) and decongestant (naphazoline 0.5%) nasal spray applied in one nostril and saline in the contralateral nostril (control side). The procedure began with the application and brushing of the nostril exposed to the saline solution. Subsequently, the lidocaine and naphazoline spray is applied to the contralateral nostril, followed by a 3-min waiting period before brushing. Nasal brushings were performed by two trained and experienced physicians within the PCD diagnostic center of the University Hospital of Liège. At the end of the two brushings, each healthy volunteer was able to assess on a pain scale ranging from 0 (no pain) to 10 (worst possible pain) the difference between the two brushings. Exclusion criteria for healthy volunteers included chronic respiratory diseases, family history of PCD, respiratory infections

within the previous four weeks, regular use of nasal or inhaled medications within 24 h, or active smoking. Referred patients were excluded if they had a respiratory infection in the previous four weeks or used nasal or inhaled medications within 24 h. Patients were categorized as PCD when either TEM or genetic analysis was positive. This observational study received approval from the ethics committee of the University Hospital of Liège (2020-220), and written consent was obtained from all subjects before their involvement.

Nasal brushing samples were placed in 2 ml of medium 199 (Thermo Fisher, Waltham, MA, USA) supplemented with a 1% penicillin/streptomycin antibiotic solution and 1% amphotericin B antifungal solution (Thermo Fisher, Waltham, MA, USA). Video sequences of ciliated beat edges will be recorded using an inverted microscope with a 100x oil immersion interference contrast objective (Axio Vert.A1, Zeiss, Oberkochen, Germany) and a high-speed video camera (CrashCam Mini 1510, IDT Innovation in motion, Pasadena, CA, USA), at a frame rate of 500 per second. For video sequences acquisition, 60 μ L of respiratory ciliated edges in medium 199 were placed under the microscope, and the temperature was regulated at 37°C using a heated box (Ibidi, Gräfelfing, Germany) and a microscope lens heater (Tokai Hit, Fujinomiya, Japan). Temperature control was ensured before each recording using a temperature probe for adjustment, as previously described.¹⁷

Ciliary beat recordings were performed at 37°C after nasal brushing under saline and local anesthesia conditions. Under anaesthesia conditions, additional recordings were conducted at 37 °C 1h and 3h after sample collection.

For ciliary functional analysis (CFA), only normal edges or edges with minor projections,¹⁸ measuring at least 50 μ m in length, were recorded. Specifically, cilia free of mucus and those exhibiting a sideways profile were selected for analysis within these edges. CFA was evaluated from a minimum of three high-quality edges meeting the above criteria for each time and condition. Nasal brushing samples that did not permit CFA at HO (for saline and local anesthesia condition), HI and H3 were excluded.

2.2 Ciliary functional evaluation

To manually assess CBF, cilia or groups of cilia exhibiting a sideways profile were identified, the number of frames required to complete 5 beat cycles was counted and converted to CBF through a simple calculation.¹² A maximum of 10 manual CBF measurements were calculated from each ciliated beating edge.¹⁹ Ciliated edges that did not allow a minimum of 5 CBF measurements along the edge were excluded from CFA.¹⁹ If immotile cilia were observed, a CBF of 0 Hz was recorded.¹⁹ For each sample, the mean CBF was calculated at each time-point (0 H, 1 H, 3 H) and under the two experimental conditions (saline or local anesthesia).

The precise trajectory traveled by an individual cilium or group of cilia during a complete beating cycle was compared to normal CBP, observed through DHSV.^{12,20} Each cilium or group of cilia used for manual assessment of CBF was categorized as normal or abnormal CBP.¹⁹ Subsequently, the proportion of normal CBP within the sample was calculated for each time-point (0 H, 1 H, 3 H) and experimental conditions (saline or local anesthesia).

2.3 Statistical analysis

This prospective single-blind study evaluated the effect of lidocaine 2% and naphazoline 0.5% nasal spray on CBF and CBP. Quantitative variables were presented as median and interquartile range from the 25th percentile to the 75th percentile [P25-P75] while qualitative variables were characterized by frequency and percentage. Paired Mann-Whitney *U*-test compared lidocaine-naphazoline administration to saline for CBP and CBF. The evolution of CBF and CBP was analyzed immediately (TO), 1 h (T 1) and 3 h (T3) after lidocainenaphazoline nasal spray using Kruskal Wallis test. Statistical analyses were conducted using STATA software. Results were considered significant at a 5% uncertainty level ($p < .05$).

3 RESULTS

Nasal brushing samples were analyzed with and without local anesthesia in 38 subjects (25 healthy volunteers and 13 patients referred to a PCD diagnosis center). Of the referred patients, 6 were non-PCD cases, while 7 were diagnosed with PCD. Demographic data are summarized in Table 1.

The median age of the total population was 28.5 years [22.0-37.0]. Healthy volunteers had a median age of 30.0 years [27.0-36.5], which was slightly higher compared to the referred patients ($p = 0.03$). The referred patients showed a wider age range, with non-PCD cases having a median age of 15.0 years [12.0-45.0] and PCD cases having a median age of 14.0 years [12.0-47.0]. There was no age difference between PCD and non-PCD cases among the referred patients' group ($p = 0.772$).

Regarding gender, females represented 52.6% of the total population, including 56% among healthy volunteers, 66.7% among nonPCD-referred patients, and 28.6% among PCD-referred patients.

In terms of tolerance to brushing, the results indicated that pain levels, assessed on a pain scale, were 4.0 [3.5-5.0] in the nostril exposed to saline and 3.0 [2.0-3.0] in the nostril exposed to 2% lidocaine and 0.5% naphazoline. Results are expressed by the median with the range extending from the 25th percentile to the 75th percentile. This result demonstrates that nasal brushing, following exposure to a combination of naphazoline and lidocaine, offers better tolerance to sampling compared to exposure to a saline solution.

TABLE 1 Demographic characteristics and analysis of participants exposed to saline and lidocaine/naphazoline nasal spray in healthy volunteers and referred patients.

Demographic data			p-value Mann- Withney U	
N	Total population	38		
	Healthy volunteers	25		
	Referred patients	13		
	Non PCD	6		
	PCD	7		
Age, years, median [P25-P75]	Total population	28.5 [22.0-37.0]		
	Healthy volunteers	30.0 [27.0-36.5]		0.03
	Referred patients	15.0 [12.0-45.0]		
	Non PCD	14.0 [12.0-47.0]		0.772
	PCD	20.0 [12.0-43.0]		
Sex, % Female Male	Total population	20 (52.6%) 18 (47.4%)		
	Healthy volunteers	14 (56.0%) 11 (44.0%)		
	Referred patients	6 (46.2%) 7 (53.8%)		
	Non PCD	4 (66.7%) 2 (33.3%)		
	PCD	2 (28.6%) 5 (71.4%)		

Note: Results are expressed as median [P25-P75]. Median were compared with Mann-Whitney U test.

Measured CBF and percentage of normal CBP with lidocainenaphazoline and with saline are summarized in Figure 1 and Table 2. Overall, the administration of nasal spray did not result in significant alterations in CBF and percentage of normal CBP ($p = 0.841$ and $p = 0.125$ respectively). The median CBF was 14.52 [12.10-15.76] Hz in the Lidocaine-Naphazoline epithelium compared to 14.63 [12.82-15.63] Hz in the saline epithelium ($p = 0.841$), while the median CBP was 81.5 [50.5-94.3] % in the Lidocaine-Naphazoline epithelium versus 89.3 [51.5-100.0] % in the saline epithelium ($p = 0.125$). In subgroup analysis, CBF and CBP measures were higher in healthy volunteers compared to referred patients (non-PCD-referred patients and PCD-referred patients), as anticipated. Nasal spray administration did not induce changes in CBF analysis for healthy volunteers, non-PCD-referred patients, and PCD-referred patients (Table 2). Although percentage of normal CBP exhibited a slight decrease in lidocaine-naphazoline (88.0 [77.0-98.0] %) compared to saline (96.0 [89.3-100.0] %) in healthy volunteers only ($p =$

0.028), this difference did not attain clinical relevance, as both conditions had normal percentages. No significant differences were observed when comparing healthy volunteers' percentage of normal CBP in the saline condition to the administration of local anesthesia after 1 h ($p = 0.147$) and after 3 h ($p = 0.976$). Nasal spray administration did not induce changes in the percentage of normal CBP analysis for non-PCD-referred patients and PCD-referred patients (Table 2).

The temporal evolution of CBF and CBP is outlined in Table 3 and Figure 2, demonstrating stability over time after lidocainenaphazoline administration ($p = 0.906$ and 0.271 , respectively).

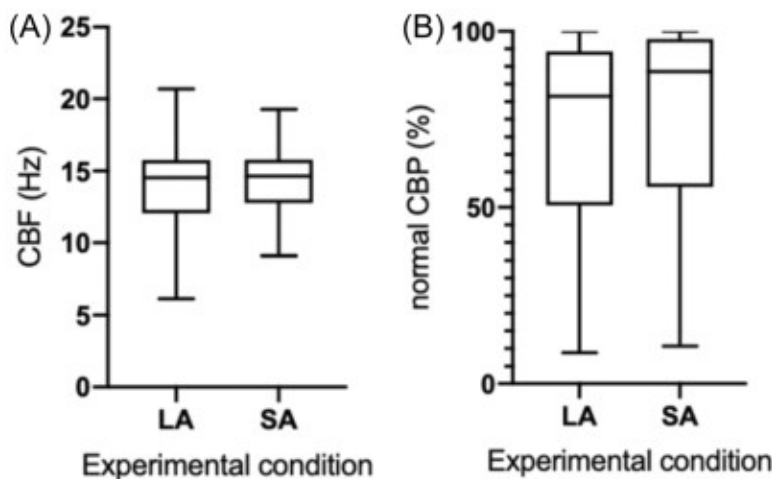


FIGURE 1 Effects of Local Anesthesia on CBF (A) and CBP (B) in the total population, including referred patients (non-PCD referred patients and PCD-referred patients) and healthy volunteers. No significant difference was observed following nasal administration of Lidocaine 2% and Naphazoline 0.5% in terms of CBF and CBP. CBF (Hz), ciliary beat frequency in hertz; Normal CBP (%), percentage of normal ciliary beat pattern; LA, local anesthesia containing lidocaine 2% and naphazoline 0.5%; SA, saline.

TABLE 2 Analysis of ciliary beat frequency in hertz and percentage of normal ciliary beat pattern in lidocaine/naphazoline exposed patients or healthy volunteers compared to saline control.

		Lidocaine/Naphazoline	Saline (control)	p-value Mann-Whitney U test
CBF (Hz)	Total population	14.52 [12.10–15.76]	14.63 [12.82–15.63]	0.841
	Healthy volunteers	15.35 [13.84–15.78]	15.28 [13.48–16.40]	0.904
	Referred patients	11.94 [7.85–15.66]	13.14 [8.97–15.14]	0.588
	PCD patients	8.78 [6.26–11.94]	9.16 [8.62–12.54]	0.974
CBP (%)	Total population	81.5 [50.5–94.3]	89.3 [51.5–100.0]	0.125
	Healthy volunteers	88.0 [77.0–98.0]	96.0 [89.3–100.0]	0.028
	Referred patients	23.0 [8.0–61.0]	39.0 [9.5–72.0]	0.791
	PCD patients	9.0 [0.0–23.0]	11.0 [0.0–39.0]	0.671

Note: Results are expressed as median [P25–P75]. Median were compared with Mann-Whitney U test.

Abbreviations: CBF, ciliary beat frequency; CBP (%), percentage of normal ciliary beat pattern; PCD, primary ciliary dyskinesia.

Results at 0 H, 1 H, and 3 H were not associated with changes in CFA of epithelia having received lidocaine-naphazoline. Regarding subgroup analysis, xylocaine-naphazoline did not change CBF nor the percentage of normal CBP over time in healthy volunteers, in non-PCD-referred patients, and PCD-referred patients (Table 3).

TABLE 3 Temporal analysis of ciliary beat frequency (CBF) and normal ciliary beat Pattern (CBP) in Healthy Volunteers, Referred Patients, and DCP Patients exposed to lidocaine and naphazoline.

		0H	1H	3H	p-value Kruskal-wallis test
CBF (Hz)	Total population	14.52 [12.10–15.76]	14.48 [12.44–16.64]	14.80 [11.66–15.71]	0.906
	Healthy volunteers	15.35 [13.84–15.78]	14.95 [13.04–16.80]	14.91 [13.74–15.75]	0.855
	Referred patients	11.94 [7.85–15.66]	13.20 [7.70–15.22]	12.80 [7.54–15.42]	0.873
	DCP patients	8.78 [6.26–11.94]	7.84 [7.14–12.55]	7.72 [5.70–11.10]	0.891
CBP (%)	Total population	81.5 [50.5–94.3]	87.5 [63.0–95.3]	92.0 [52.8–100.0]	0.271
	Healthy volunteers	88.0 [77.0–98.0]	90.0 [82.50–100.0]	95.0 [90.5–100.0]	0.102
	Referred patients	23.0 [8.0–61.0]	40.0 [11.0–86.0]	30.0 [6.0–84.5]	0.836
	DCP patients	9.0 [0.0–23.0]	12.0 [0.0–40.0]	12.0 [0.0–22.0]	0.897

Note: Results are expressed as median [P25–P75]. Median were compared with Kruskal-Wallis test.

Abbreviations: CBF, ciliary beat frequency; CBP (%), percentage of normal ciliary beat pattern; PCD, primary ciliary dyskinesia.

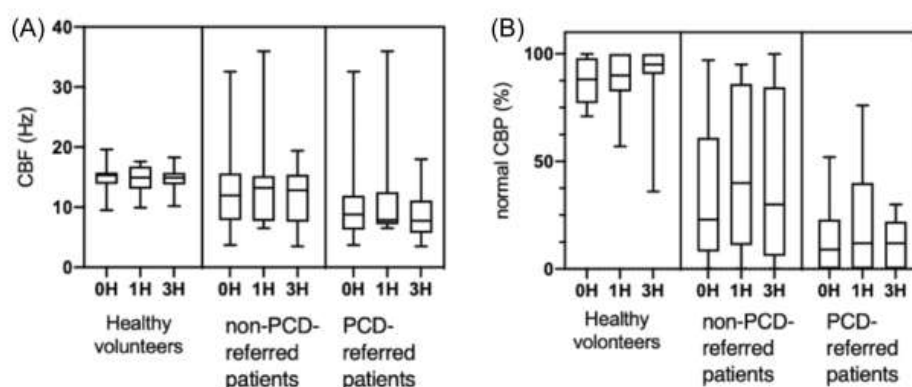


FIGURE 2 Temporal Evolution of CBF (A) and CBP (B) Following Nasal Administration of Lidocaine 2% and Naphazoline 0.5% in Healthy Volunteers and Referred Patients (Non-PCD and PCD). No significant difference observed among the three groups in terms of CBF and CBP. CBF (Hz), ciliary beat frequency in hertz; normal CBP (%), percentage of normal ciliary beat pattern.

4 DISCUSSION

Our findings indicate that the application of local anesthesia (lidocaine 2%) and decongestant (naphazoline 0.5%) through nasal spray during nasal brushing did not cause any significant changes in CBF or CBP in our population. Lidocaine and naphazoline nasal sprays are commonly used in ENT clinic, to allow endoscope insertion and/or minor procedures.²¹ They can enhance patient comfort by mitigating the inconvenience associated with nasal brushing.

In addition, the use of naphazoline as a vasoconstrictor has been shown to improve visibility in anterior rhinoscopy, thereby improving the quality of sampling during nasal brushing on the middle turbinate.

Importantly, our results highlight that this intervention does not compromise the accuracy and reliability of ciliary function analysis, as evidenced by the consistent CBF and CBP across different conditions and time points. Thus, these results support the idea that the administration of a nasal spray, with or without anesthesia, does not appear to impact CBF and CBP.

Our study aligns with the body of evidence suggesting the feasibility and safety of using local anesthesia in various clinical procedures.²² The application of lidocaine for patient comfort and tolerance has been explored in different medical contexts, and our findings extend this understanding to the realm of ciliary function analysis. This study represents the first clinical investigation, to our knowledge, aimed at evaluating ciliary function, specifically CBP and CBF, following the administration of a nasal spray combining a decongestant and a local anesthetic. Previous research on ciliary function, primarily conducted *in vitro* after cell culture, did not assess ciliary beating patterns.¹³ Previous *in vitro* studies have focused on assessing CBF, demonstrating either a decrease or no alteration in CBF following exposure to isotonic saline.^{23,24} Furthermore, Mickenhagen and colleagues found that CBF remained unchanged over a range of naphazoline concentrations from 0.001% to 0.1%.²⁵ Concerning lidocaine exposure, *in vitro* experimentation has revealed that although lidocaine hydrochloride administered *in vivo* before nasal brushing showed no significant change in CBF, incubation of ciliated epithelium *in vitro* with increasing concentrations of lidocaine led to dose-dependent cilio inhibition. Notably, these concentrations were found to be much higher than those encountered in the clinical setting.²⁶

Our study remains consistent with previous research regarding the effect of isotonic saline, naphazoline, and lidocaine on CBF under *in vitro* and *in vivo* conditions.¹³ In addition, we shed more light on the CBP, demonstrating that lidocaine and naphazoline spray employed in clinical settings to smooth sampling had no discernible impact on ciliary function results. Ciliary function did not change over time. This aligns with the broader literature discussing the stability and reproducibility of ciliary function assessments under different conditions.^{19,27,28} Despite being conducted by experienced and trained physicians, the analysis of CBF measurements and CBP remains subjective. The introduction of software tools enabling semiautomatic analysis holds the potential to enhance the precision of results.^{27,28} The utilization of such tools could offer a more standardized and objective approach, reducing the inherent subjectivity associated with manual assessments.

In addition, the results pertaining to the impact of lidocaine and naphazoline spray on a referred patient population, inclusive of those with PCD, necessitate validation through a larger and more diverse cohort. Expanding the study to include a broader patient population not only enhances the generalizability of the findings but also allows for a more comprehensive understanding of the potential effects across various subgroups. This step is particularly crucial in ensuring the robustness and applicability of the observed outcomes to a broader clinical context.

In future research endeavors, the incorporation of semi-automatic analysis tools and the expansion of the study to encompass a larger and more diverse patient cohort could contribute to refining the methodology and strengthening the validity of the findings, ultimately advancing our understanding of the impact of nasal spray administration on ciliary function in clinical settings.

5 CONCLUSION

In conclusion, our study adds valuable insights to the existing literature by specifically addressing the application of local anesthesia and decongestants in the context of nasal brushing for ciliary function analysis. The observed stability in CBF and CBP suggests that the administration of lidocaine-naphazoline, a procedure known for its safety and patient comfort, did not compromise the reliability of diagnostic information.

The implications of these findings extend to clinical practice, where healthcare professionals can confidently use these nasal sprays to improve patient comfort and procedural efficiency. However, further research in larger cohorts is warranted to validate our findings and ensure their generalizability. Overall, our study contributes to optimizing the sampling procedure for PCD diagnostic protocols using DHSV, where patient comfort and adherence to the diagnostic process are crucial considerations.⁷

AUTHOR CONTRIBUTION

Lionel Benchimol, Anne-Lise Poirrier and Celine Kempeneers designed and directed the project; Lionel Benchimol performed the experiments; Lionel Benchimol analysed the data; Lionel Benchimol and Anne-Lise Poirrier wrote the article. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICAL STATEMENT

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committee of the University Hospital of Liège (2020-220).

DATA AVAILABILITY STATEMENT

All data is contained within the article.

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