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Analysis of Respiratory Ciliary Function on Nasal Epithelium: Validation of a Clinical Diagnostic Protocol Using Digital High-Speed Videomicroscopy

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Table des matières

List of abbreviations

- ABPA: Allergic BronchoPulmonary Aspergillosis
- AI: Artificial Intelligence
- ALI: air-liquid interface
- AOM: acute otitis media
- ASL: Airway Surface Liquid
- ATS: American Thoracic Society
- CaCC: Calcium-activated Chloride Channels
- cAMP: cyclic Adenosine MonoPhosphate
- CBF: Ciliary Beat Frequency
- CBP: Ciliary Beat Pattern
- CF: Cystic Fibrosis
- CFA: Ciliary Functional Analysis
- CFTR: Cystic Fibrosis Transmembrane Conductance Regulator
- cGMP: cyclic Guanosine MonoPhosphate
- chOME: chronic Otitis Media with Effusion
- CRS: chronic rhinosinusitis
- CRSwNP: Chronic RhinoSinusitis with Nasal Polyps
- DHSV: Digital High-Speed Videomicroscopy
- ENaC: Epithelial sodium Channel
- ERS: European Respiratory Society
- FEV1: Forced Expiratory Volume in one second
- GDMCC: Genetic Disorder of Mucociliary Clearance Consortium
- HRCT: High resolution computed tomography
- HSVM: High-Speed Videomicroscopy
- HSVMA: High-speed video microscopy analysis
- IDA: Inner Dynein Arm
- iNOS: Nitric Oxide Synthase
- LCI: Lung Clearance Index

- MCC: MucoCiliary Clearance
- MRI: Magnetic Resonance Imaging
- NO: Nitric Oxide
- NTM: non-tuberculous mycobacteria
- ODA: Outer Dynein Arm
- OME: otitis media with effusion
- PCD: Primary Ciliary Dyskinesia
- PCL: PeriCiliary layer
- PICADAR: "PrImary CiliAry DyskinesiA Rule"
- ROM: Recurrent Otitis Media
- SCD: Seconday Ciliary Dyskinesia
- TEM: Transmission electron microscopy
- TTN: Tachypnea of The Newborn

Summary

Primary ciliary dyskinesia (PCD) is a rare, genetically inherited disorder that impairs the function of motile cilia in the respiratory tract, leading to chronic respiratory problems and a range of related complications. The accurate diagnosis and effective management of PCD remain challenging due to the variability in clinical presentations and the limitations of existing diagnostic methods.

One of the key challenges in diagnosing PCD is the absence of a definitive "gold standard" test. This necessitates the integration of thorough clinical examination with multiple diagnostic tools, including genetic analysis, nasal nitric oxide (nNO) measurement, transmission electron microscopy (TEM), high-speed video microscopy (HSVM) after cell culture, and immunofluorescence. Although ciliary videomicroscopy using digital high-speed videomicroscopy (DHSV) is a valuable tool for visualizing and assessing ciliary motion, it is not recognized as a standalone diagnostic method by the European Respiratory Society (ERS) or the American Thoracic Society. This is primarily due to two persistent issues: the lack of an internationally standardized protocol for DHSV, leading to variable reference values and diagnostic criteria across different centers, and the difficulty in accurately determining the sensitivity and specificity of DHSV in the absence of a universally accepted reference standard. While previous studies have shown that DHSV is an effective diagnostic tool for PCD, its accuracy may be compromised when evaluated against incomplete or non-optimal reference standards.

In the first part of this work, we focused on understanding and improving clinical management of PCD patients by examining diagnostic approaches, treatment strategies, and the impact of tailored interventions and multidisciplinary care on patient outcomes. We also addressed challenges in early diagnosis and assessed the effectiveness of current interventions.

The second part of our study investigated the impact of a common local anesthetic used in ENT procedures on the accuracy of PCD diagnosis, specifically its potential to alter

ciliary beat frequency (CBF) or ciliary beat pattern (CBP) during DHSV assessments. Since anesthetics are often used to enhance patient comfort, we aimed to assess whether their use compromised diagnostic integrity.

The third part of our research examined the feasibility of performing ciliary sampling under general anesthesia without altering ciliary function. This is particularly relevant for adapting ciliary diagnostic procedures for patients, especially pediatric, who require general anesthesia in ENT settings. We aimed to determine whether PCD diagnosis remained accurate under these conditions.

Finally, we studied ciliary function in chronic rhinosinusitis with nasal polyps (CRSwNP) patient who had undergone multiple treatments, including biotherapy. While not directly related to PCD, this research is relevant since PCD patients often develop nasal polyps. This study assessed how various treatments affected ciliary function in CRSwNP patients, providing insights that could inform management of both PCD and CRSwNP.

In summary, although ciliary videomicroscopy has demonstrated high sensitivity and specificity for diagnosing PCD, it is not yet included in international PCD diagnostic guidelines. This is largely due to a lack of standardization in the test protocols and reliance on suboptimal reference standards. Standardizing the key steps of the ciliary videomicroscopy protocol is crucial for developing an universally accepted diagnostic method.

The primary aim of this thesis was to evaluate different sample collection methods in real clinical settings, involving both patients from the CHU of Liege PCD center and healthy volunteers, with the goal of establishing ciliary videomicroscopy as a reliable diagnostic tool for PCD.

1. Introduction

1.1 Human respiratory tract

The development of the lower respiratory tract begins around day 22 of embryogenesis and proceeds to form essential structures such as the trachea, lungs, bronchi, and alveoli. The respiratory system originates from the primitive gut tube, the precursor to the gastrointestinal tract. This gut tube, derived from the endoderm, forms during the early embryonic period when the embryo undergoes lateral folding. Around the fourth week of development, a respiratory diverticulum emerges from the proximal foregut portion of the primitive gut tube. Initially, the respiratory diverticulum remains continuous with the foregut; however, this arrangement is not compatible with functional respiratory and digestive systems. The formation of the tracheoesophageal septum, a longitudinal ridge, separates the two structures, enabling them to function independently and support life. The respiratory diverticulum then bifurcates into two buds, which develop into the left and right primary bronchi. These primary bronchi continue to proliferate and give rise to secondary and tertiary bronchi, contributing to the branching architecture of the bronchial tree.

Functionally, the respiratory system is divided into two key components: the conducting portion and the respiratory portion. The conducting portion is responsible for conveying, moistening, and warming the air as it enters the body, preparing it for gas exchange in the lungs. The conducting portion is also responsible for cleaning the inhaled air from particles, playing a critical role in trapping and removing inhaled debris. Gas exchange itself occurs in the respiratory portion.

The "respiratory zone," which extends from the alveolar ducts to the alveoli, is primarily responsible for gas exchange, allowing oxygen necessary for cellular respiration to be absorbed while carbon dioxide (CO2), a byproduct of metabolism, is expelled(1,2).

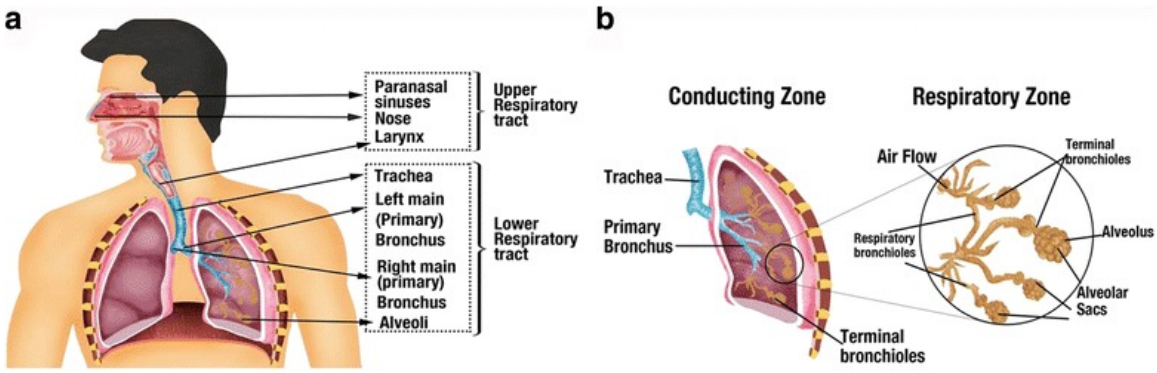

Figure 1: Representation of the human respiratory system divided anatomically by the upper and lower respiratory tract (a) and functionally by the conducting zone and the respiratory zone (b). Reproduced from Bhagirath et al., Cystic fibrosis lung environment and Pseudomonas aeruginosa infection, BMC Pulmonary Medicine 16, 174 (2016), https://doi.org/10.1186/s12890-016-0339-5. © The Authors 2016. Distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). (3)

Structurally, the respiratory system is divided into the upper and lower tracts (2). The upper respiratory tract includes the nasal cavity, oral cavity, pharynx, and their associated structures. The lower respiratory tract comprises the trachea, bronchi, bronchioles, and alveoli.

The upper respiratory tract forms alongside the skull during development. Most facial and neck structures arise from the pharyngeal arches, which are composed of an outer ectodermal layer and an inner epithelial layer derived from the endoderm(4). The formation of the nose follows a tightly coordinated sequence of events, occurring between embryonic weeks 4 and 8, corresponding to Carnegie stages 13-19 (4). Development of the external nose and nasal cavities begins with the appearance of nasal placodes, which emerge from ectodermal thickening in the frontonasal process.

These placodes gradually invaginate to form nasal pits, which eventually become the primitive nostrils and nasal cavities (4). Notably, while most of the respiratory tract (e.g., pharynx, trachea, bronchi, lungs) originates from the endoderm, the nasal cavity—being the starting point of the respiratory system—develops from ectodermal tissue (4).

Given the constant exposure of the lungs to pathogens, particles, and pollutants in inhaled air, multiple defense mechanisms are essential for preserving respiratory health. These include physical barriers like the mucociliary clearance (MCC) system, the immune response, and the cough reflex. As bacteria, viruses, fungi, allergens, and air pollutants enter the upper respiratory tract, they are captured by the mucus layer lining the nasal cavity, preventing deeper penetration into the respiratory system.

MCC, in particular, is a crucial first line of defense, involving the coordinated action of mucus and cilia lining the airway epithelium to trap and clear out harmful particles and microorganisms from the lower respiratory tract (5–7). The airway surface liquid, which coats the respiratory mucosa, in composed of an upper gel layer (mucus) and a lower layer (pericilary fluid). Most airway surface liquid is produced by submucosal glands, and their stimulation inscreases airway surface volume, promoting more effective MCC. However, viral or bacterial infection, allergen exposure, medication or primary mucus disorder can change liquid production and impact mucociliary function. These factors, along with ciliary dyskinesia, can significantly impair MCC.

In the study of PCD, understanding the structure and function of human cilia and the respiratory ciliated epithelium is vital. Although cilia were discovered over a century ago, significant advancements in our understanding of their ultrastructure and function have been made in recent years, deepening our insight into their critical role in respiratory health and disease.

In the following sections, we will explore the detailed structure of the human respiratory epithelium by examining the various cell types that compose it, and we will further explain its function through the mechanism of mucociliary clearance.

1.1.1 Histological features

The mucosa of the upper and lower airways shares several common histological features, including ciliated respiratory epithelium, the lamina propria with submucosal glands, and a basement membrane composed of type IV and V collagen (4). However, there are notable histological differences between the upper and lower airways. In the lower

airways, smooth muscle is physiologically present and plays a role in airway tone; during inflammation, airway remodeling leads to smooth muscle hypertrophy, contributing to bronchoconstriction(4,8). In contrast, the nasal mucosa of the upper airway lacks smooth muscle (except in blood vessels), and remodeling due to allergic inflammation or chronic rhinosinusitis often results in the formation of polyps(9,10). These polyps arise from the overgrowth of fibroblasts in the lamina propria, causing epithelial protrusions and basement membrane rupture (4).

Additionally, the vascular systems of the upper and lower airways differ significantly. The lamina propria of the nasal mucosa contains exchange, resistance, and capacitance vessels, while the lower airway lacks both resistance and capacitance vessels (4). In the nasal mucosa, the resistance vessels regulate blood flow throughout the capillary network, and their constriction can trap blood in large capacitance vessels like sinusoids, leading to upper respiratory tract congestion (4).

The respiratory epithelium of the lower airways serves as the first line of defense for the lungs, providing both a physical barrier and a variety of protective functions. This epithelium undergoes a gradual transition from pseudostratified ciliated epithelium in the proximal airways to simple cuboidal epithelium in the bronchioles (2,7). It is composed of several distinct cell types, each contributing to the maintenance of lung health and the defense against inhaled pathogens, dust particles, and pollutants by performing antimicrobial, regulatory, and pro-inflammatory functions, with various cell types synthesizing and secreting a range of products, including antimicrobial proteins, mucus, cytokines, nitric oxide (NO), and others enzymes… (7,11,12).

The key cell types within the respiratory epithelium include:

- **Basal Cells**: Located at the base of the epithelium, basal cells act as progenitor cells responsible for the regeneration and repair of other epithelial cell types. Their abundance progressively decreases from proximal to distal airways. (Figure 2) (5)
- **Suprabasal Cells**: Positioned just above the basal layer, suprabasal cells serve as intermediate progenitors during epithelial regeneration. Although mentioned for their functional significance, not shown in Figure 2.
- **Club Cells**: Found predominantly in the bronchioles, club cells exhibit anti-inflammatory and immunomodulatory properties. They also contribute to epithelial

repair by differentiating into ciliated cells through physiological transdifferentiation (not shown in Figure 2). (5)

- **Goblet Cells**: Interspersed among ciliated cells, goblet cells secrete mucins. Mucins are large glycoproteins that form the mucus layer essential for trapping inhaled particles and pathogens. Their number tends to increase in chronic airway diseases (**Figure 2**). (5)
- **Ciliated Cells (Multiciliated Cells)**: These are the most numerous epithelial cells in the conducting airways, accounting for approximately 50–80% of the epithelium. Equipped with motile cilia, they ensure effective mucociliary clearance by propelling the mucus layer toward the pharynx. They are labeled as "ciliated cells" in Figure 2.
- **Serous Cells**: These secretory epithelial cells contribute to the composition of the airway surface liquid by releasing watery secretions enriched with antimicrobial proteins such as lysozyme and lactoferrin. Located mainly in the submucosal glands, they play a central role in the innate immune defense (Figure 2). (5,6)

These cells work together to maintain the protective barrier of the respiratory epithelium (figure 2).

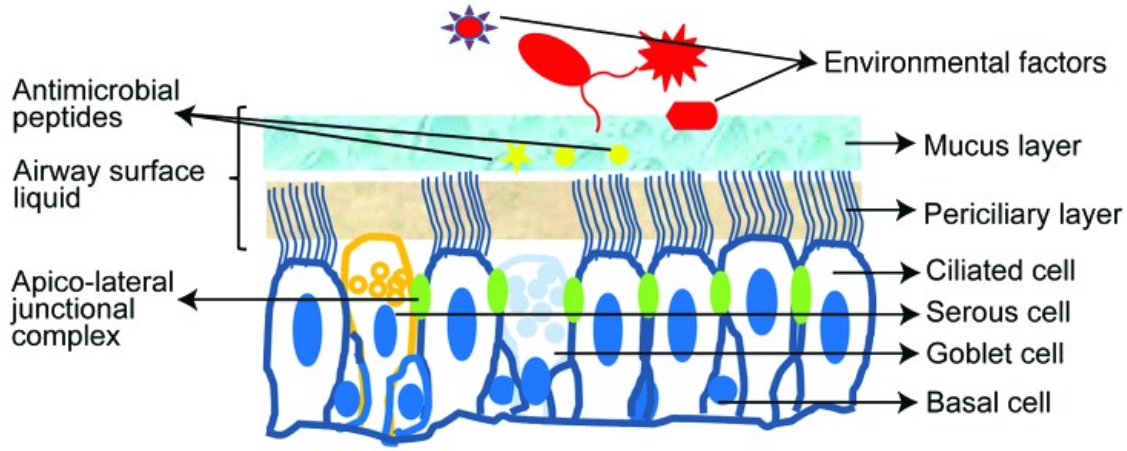

Figure 2: The main cellular components of the human respiratory tract. Reproduced from: Ganesan S, Comstock AT, Sajjan US. Barrier function of airway tract epithelium. Tissue Barriers. 2013;1 (4):e24997. © Informa UK Limited [2013], reprinted by permission of Informa UK Limited, trading as Taylor & Francis Group, https://www.tandfonline.com . (2)

The barrier function is enhanced by tight junctions and adhesion junctions, which regulate permeability and control the passage of substances through the epithelial layer (2). Additionally, the epithelium secretes antimicrobial products that actively combat inhaled pathogens, further reinforcing its role as a protective barrier(2,13,14).

Overall, the airway epithelium is a dynamic and complex tissue, with each cell type playing a specialized role in maintaining respiratory health. Its ability to provide physical, immunological, and biochemical defenses makes it an essential component of the lung's protective mechanisms.

1.1.2 Mucociliary clearance (MCC)

Mucociliary clearance (MCC) is a vital defense mechanism within the respiratory system, functioning to continuously clear mucus and trapped particles, including inhaled pathogens, from the lower respiratory tract (1,7). This process relies on a highly coordinated interaction between several key components: the airway surface liquid (ASL), which consists of a mucus layer and an underlying periciliary layer (PCL), and the cilia present on the apical surface of respiratory epithelial cells (1,7). These components work together to transport mucus towards the oropharynx, where it is either swallowed or expectorated, thereby maintaining lung sterility and preventing chronic infection.

For optimal MCC, several factors must be in place: a sufficient density of ciliated cells, an appropriate ciliary beat frequency, a coordinated ciliary beat pattern, and an optimized mucus layer (1). The **ASL**, which comprises the mucus layer and the PCL, is not only essential for the physical movement of mucus but also for the biochemical defense of the lungs.

The ASL is slightly hypotonic and contains various antimicrobial agents, such as immunoglobulin A, lysozyme, lactoferrin, and defensins, which help neutralize inhaled pathogens before they can reach the epithelial surface (7,15,16). The volume and composition of the ASL are tightly regulated by the airway epithelium through active ion transport processes, ensuring that the mucus remains hydrated and maintains its viscoelastic properties (15,17). Proper hydration of the mucus is crucial because it affects

its ability to flow: dehydrated, highly viscous mucus can impede ciliary movement, while overly hydrated mucus can lose its adhesive properties, making it less effective at trapping particles (7,15).

The **mucus layer** serves as the first line of defense, acting as a physical barrier that captures inhaled particles, pathogens, and environmental toxins (18). This mucus layer should have a favorable composition or concentration of mucins, making it more efficient at trapping harmful inhaled substances and facilitating their effective removal by the cilia (1) .The mucus is primarily composed of water (95%), along with proteins, salts, and glycoproteins known as mucins, which account for 2-3% of its composition (7). Mucins, produced by goblet cells and submucosal glands, are high-molecular-weight glycoproteins that endow mucus with its viscoelastic properties, crucial for trapping debris and facilitating its movement by ciliary action (1,19). There are two main types of mucins in the respiratory tract: free-secreted mucins (e.g., MUC5AC and MUC5B), which contribute to the mucus gel layer, and membrane-bound mucins (e.g., MUC1, MUC4), which form part of the PCL.

MUC5AC is primarily produced by goblet cells in the proximal airways, while **MUC5B** is produced by submucosal glands and, in the distal airways, also by goblet cells (19,20). The proportion of these mucins can vary significantly depending on the respiratory condition (20). For example, MUC5AC is more prevalent in the mucus of asthmatic patients, whereas MUC5B dominates in healthy individuals and those with cystic fibrosis (CF) (19,20). The balance and regulation of these mucins are critical for maintaining the appropriate viscoelastic properties of mucus, which in turn affects its ability to be effectively cleared by ciliary action and coughing (20). Figure 3 depicts the structural and functional alterations of the mucus barrier and mucociliary clearance observed in healthy airways compared to those seen in obstructive airway diseases.

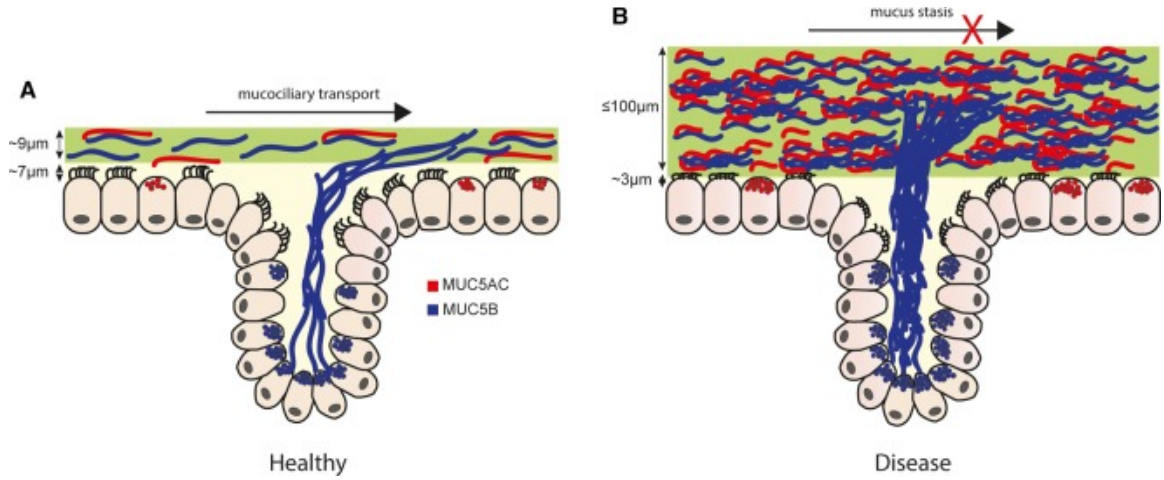

Figure 3 **(A)** In healthy airways, the protective mucus barrier is primarily composed of MUC5B (blue), with a smaller proportion of MUC5AC (red), which traps inhaled particles and microbes. MUC5B bundles are depicted emerging from the submucosal glands. Proper airway hydration ensures the mucus gel maintains suitable rheological properties, enabling effective mucociliary clearance (MCC). **(B)** In obstructive airway disease, increased mucin concentration leads to collapse of the periciliary layer (PCL), compression of the cilia, and a resulting impairment or cessation of MCC. Reproduced from: Ridley C, Thornton DJ. Mucins: the frontline defence of the lung. Biochem Soc Trans. 2018 Oct 19;46 (5):1099–106.© 2018 The Author (s) permission conveyed through Copyright Clearance Center, Inc .(19)

Beneath the mucus, the **periciliary layer (PCL),** a thin polyanionic gel that lies beneath the mucus, is essential for the optimal functioning of the cilia. The PCL's depth, approximately 7 µm, matches the length of the outstretched cilia and provides a lubricated environment that allows the cilia to beat freely (1). The maintenance of this precise depth is critical; if the PCL is too shallow, the cilia can become trapped in the overlying mucus, whereas if it is too deep, the cilia may fail to propel the mucus efficiently (7). This balance is regulated by the transport of ions, particularly sodium (Na+) and chloride (Cl-), which creates osmotic gradients that control the movement of water across the airway epithelium, thereby influencing the volume and composition of the ASL (19,20). Key ion channels involved in this regulation include the cystic fibrosis transmembrane conductance regulator

(CFTR), calcium-activated chloride channels (CaCC), and the epithelial sodium channel (ENaC) (20). CFTR is a protein encoded by the *CFTR* gene that functions as a chloride and bicarbonate ion channel located on the apical membrane of epithelial cells, particularly in the airways, pancreas, intestines, and sweat glands (21,22). In healthy individuals, CFTR maintains airway surface hydration by regulating the movement of chloride and bicarbonate ions, which is essential for proper mucociliary clearance and epithelial homeostasis (23). In patients with CF, mutations in the *CFTR* gene lead to the production of a dysfunctional or absent CFTR protein. This impairs chloride transport, resulting in dehydrated, viscous mucus that obstructs airways and predisposes individuals to chronic infection and inflammation (24,25).

Respiratory ciliated cells, the predominant cell type in the airway epithelium, are highly specialized for MCC. These cells are characterized by their cylindrical or pyramidal shape, with a height of approximately 20 μm and a width of 7 μm. Each ciliated cell has an apical surface covered by around 200-300 motile cilia, each about 0.2-0.3 μm in diameter and 6-7 μm in length (though shorter in the smaller airways) (13). The cilia beat in a coordinated, wave-like manner to transport mucus along the airway surface towards the pharynx (5,6). The ratio of ciliated cells to secretory cells is approximately 5:1 in healthy airways, but this ratio can decrease in chronic airway diseases such as asthma, chronic obstructive pulmonary disease (COPD), and CF, where ciliary function is often compromised (2). Number of cilia per ciliated cell may also be reduced, further impairing mucociliary clearance (26).

In conditions where mucociliary function is impaired, such as in PCD or CF, the consequences are severe. Dysfunctional cilia or abnormal mucus composition can lead to ineffective mucus clearance, resulting in the accumulation of mucus, obstruction of the airways, and increased susceptibility to recurrent respiratory infections. These diseases highlight the critical importance of maintaining the integrity of MCC mechanisms for overall respiratory health (16).

In summary, MCC is a sophisticated and highly coordinated process involving multiple cellular and molecular components. It serves as a crucial defense mechanism for the respiratory system, protecting the lungs from inhaled pathogens and particles. The delicate balance between mucus production, ciliary activity, and ASL composition is essential for the effective clearance of mucus, and disruptions in any part of this system can lead to significant respiratory challenges (figure 4).

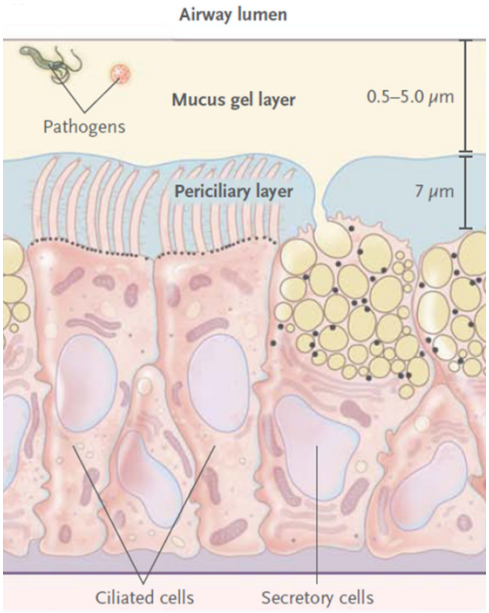

Figure 4 : Illustration of a distal bronchus, depicting the delicate balance and interaction between ciliated cells and the airway surface liquid, including the mucus gel layer and periciliary liquid layer, which are crucial for effective mucociliary clearance. Reproduced with permission from: Fahy JV, Dickey BF. Airway Mucus Function and Dysfunction. New England Journal of Medicine. 2010;363 (23):2233–2247. *Copyright © 2010, Massachusetts Medical Society* .(18)

1.2 Cilium

1.2.1 General description

Cilia are conserved, hair-like structures that extend from the surface of cells into the extracellular environment and are present on nearly all cell types throughout the human

body (12,26,27). These organelles, first identified over a century ago, are integral to a variety of physiological processes. They are involved in cellular movement, the propulsion of fluids like mucus and water, embryonic development, sexual reproduction, and the maintenance of cellular signaling and homeostasis (12,28,29). Cilia serve as both motors, driving fluid movement, and as sensory antennae, detecting changes in the surrounding environment.

Traditionally, cilia have been classified into two categories based on their structure and movement capabilities (Figure 5): motile cilia, characterized by a "9+2" arrangement of microtubules (nine peripheral microtubule pairs surrounding two central microtubules), and typically present in multiple copies on a cell; and non-motile or primary cilia, which have a "9+0" arrangement (lacking central microtubules) and usually appear as a single projection per cell (30). However, this binary classification has been found to be limiting, as there are overlapping features and functions among different cilia types. Cilia are found across many eukaryotic organisms, including plants and animals, and are vital for the normal functioning and development of human tissues and organs (6).

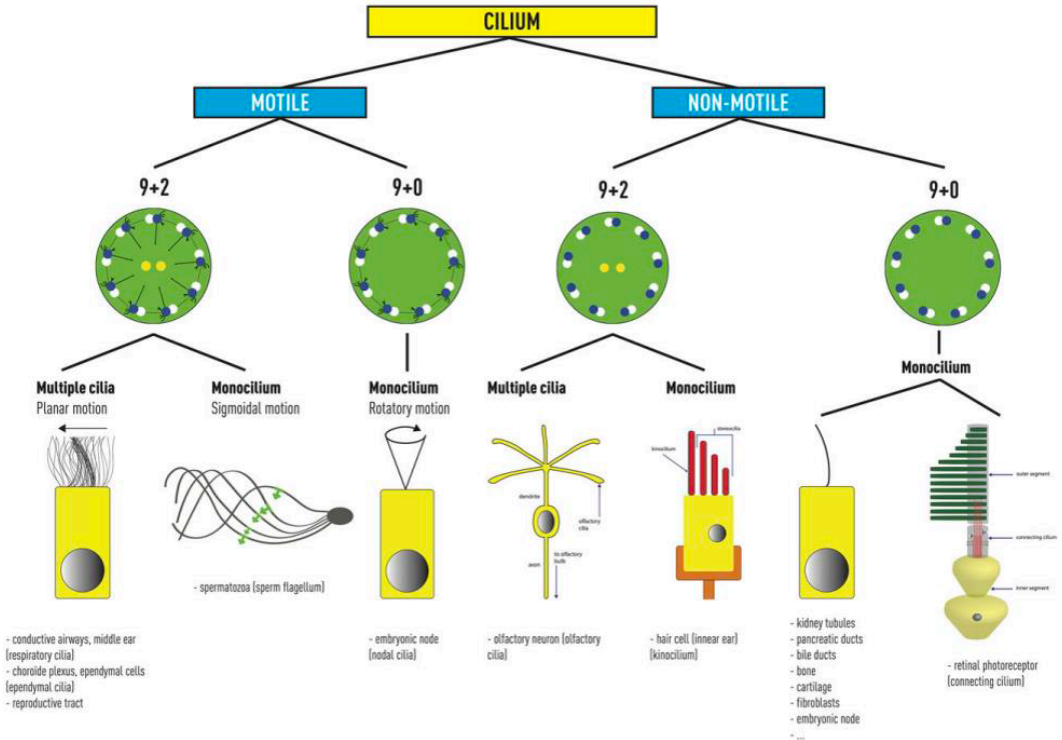

Figure 5: Actual classification of human cilia based on motility (motile vs. non-motile), structural composition ('9+2' vs. '9+0'), and number on the apical surface (monocilium vs. multiple cilia). Reproduced from Kempeneers C, Chilvers MA. To beat, or not to beat, that is question! The spectrum of ciliopathies. Pediatric Pulmonology, 2018, 53 (8):1122–1129. Copyright © 2018 With permission from John Wiley and Sons. (29)

Motile cilia can be further subdivided based on the structure of their axoneme and the number of cilia present on the cell surface (29). Multiple motile cilia, which possess a "9+2" axoneme structure, are typically found on the apical surface of epithelial cells in the respiratory tract, middle ear, and female reproductive system, as well as on ependymal cells lining the brain's ventricles (29). These cilia move in coordinated waves, propelling fluids such as mucus or cerebrospinal fluid. Monocilia, which also have a "9+2" structure, exhibit a sigmoidal, three-dimensional motion and are essential for sperm motility, as seen in sperm flagella (29). Additionally, some monocilia possess a "9+0" structure and perform a clockwise rotational motion, crucial for establishing left-right asymmetry during embryonic development, as observed in nodal cilia on the embryonic node cells (29).

Non-motile cilia, in contrast, are found on nearly all cell types, including epithelial cells in renal tubules and non-epithelial cells like chondrocytes, photoreceptor cells in the retina, and neurons (12,28,29,32). These cilia lack dynein arms, rendering them immobile, and can exhibit either a "9+2" or "9+0" structure (12,29,33–35). Initially regarded as vestigial structures with little clinical significance, non-motile cilia have recently been recognized for their critical role as cellular sensory antennae (12,34). They are involved in various signal transduction pathways essential for development and homeostasis, such as the Hedgehog and Notch pathways, which play key roles in embryogenesis and cell differentiation.

The activation of the Hedgehog pathway begins when Hedgehog ligands bind to Patched (Ptch) receptors on the cell membrane, triggering a signaling cascade (36,37). This cascade leads to the activation of Smoothened (Smo), which then translocates into the non-motile cilium (37,38). Once inside, Smo facilitates the conversion of the Full-Length Glioma-Associated Oncogene (GliFL) into its activator form, Glioma-Associated Oncogene Activator (GliA). GliA acts as a transcription factor by inhibiting the Suppressor

of Fused (SuFu), thereby enabling its entry into the nucleus where it regulates the transcription of Hedgehog-targeted genes. In the absence of Hedgehog ligands, Ptch inhibits Smo from entering the cilium, resulting in the phosphorylation and cleavage of GliFL into its repressor form, GliR. Upon nuclear translocation, GliR suppresses the transcription of genes targeted by the Hedgehog pathway(38). Similarly, the Notch signaling pathway, which is crucial for cell-to-cell communication, involves the Notch receptor located on the non-motile ciliary membrane (36,38). When ligands such as JAGGED or DELTA, found on the membrane of adjacent cells, bind to the Notch receptor, it undergoes cleavage, releasing the Notch Intracellular Domain (NICD)(36). This domain then translocates to the nucleus, where it interacts with the transcription factor CSL, initiating the transcription of Notch target genes (38).

These cilia also participate in other significant signaling pathways, including WNT, mTOR, Hippo, and RTKs, underscoring their importance in maintaining cellular functions and overall organ health (38).

Before delving into the detailed structure and functions of cilia, it is important to first understand their development, or ciliogenesis, which begins early in embryogenesis and is crucial for the proper functioning and organization of various tissues and organs.

1.2.2 Ciliogenesis

Ciliogenesis is the complex and specialized cellular process through which cilia and their associated basal body structures are formed. This process involves the activation of specific genes that are crucial for cilia formation and function, while simultaneously repressing genes associated with the cell cycle. This repression is necessary to halt cell replication temporarily, thereby redirecting cellular resources towards the formation of cilia and ensuring the process proceeds efficiently. (39)

Ciliogenesis can be broadly divided into four main stages (40):

1- **Centriole Generation**: Centrioles serve as the foundational structures for cilia formation. This process, known as centriologenesis, can occur via two distinct pathways: the centriolar pathway and the acentriolar pathway. The centriolar pathway, which relies on existing centrioles to produce new ones, plays a minor role in ciliogenesis, contributing to roughly 10% of the centrioles required (41). The acentriolar pathway, on the other hand, is the primary mechanism for centriole generation in multiciliogenesis (42). This pathway involves the aggregation of fibrous granules derived from ribosomes, leading to the formation of deuterosomes, which then orchestrate the creation of new centrioles, or procentrioles (43).
2- **Migration of Centrioles to Form Basal Bodies**: Once centrioles are duplicated, they migrate towards the apical region of the cell, guided by the intracellular cytoskeleton (43). Upon reaching the apical region, these centrioles align perpendicularly to the cell surface and anchor to the apical plasma membrane, thus transforming into basal bodies (43). This process is facilitated by structures such as alar sheets.
3- **Elongation of the Cilium**: Cilia begin to elongate from the basal body once it is anchored to the plasma membrane. This elongation involves the addition of α- and β-tubulin molecules to the microtubules, with the axoneme being constructed from the distal end. Intraflagellar transport (IFT) plays a critical role in delivering the necessary components to the growing cilium (44,45).
4- **Formation of Basal Body Accessory Structures**: The final stage of ciliogenesis involves the development of basal body accessory structures, such as alar sheets, basal feet, and striated rootlets (43). These structures are crucial for stabilizing and positioning the cilium on the apical surface of the cell (40). The basal foot aligns with the direction of ciliary beating to ensure coordinated movement, while the striated rootlets extend from the basal body into the cell, contributing to the structural integrity of the cilium (46).

Figure 6 provides a visual overview of the multiciliogenesis pathway in airway epithelial cells, highlighting the transition from basal progenitor cells to multiciliated cells. Club cells are depicted as an intermediate secretory stage capable of

differentiating into multiciliated cells. The figure further illustrates the migration of centrioles to the apical surface, basal body formation, and ciliary elongation, as well as the assembly of accessory structures essential for ciliary function.

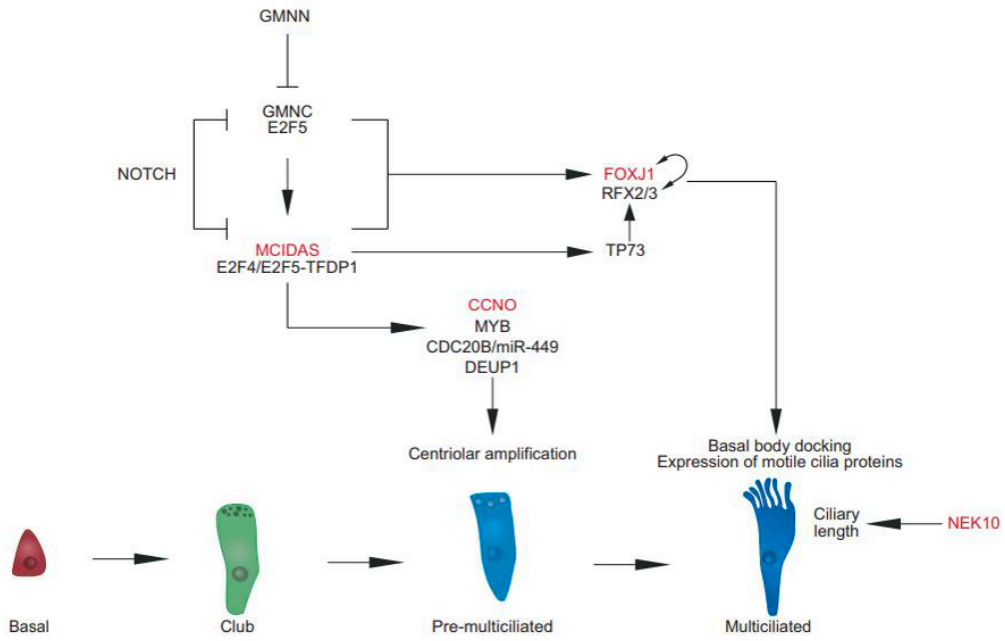

Figure 6: Illustration of the differentiation pathway of airway epithelial cells, starting from basal cells, which serve as progenitors. Club cells appear as an intermediate secretory stage capable of differentiating into multiciliated cells (MCCs). The process involves activation of key transcription factors such as GMNC and MCIDAS for centriole amplification, followed by FOXJ1-mediated ciliogenesis. Reproduced from Legendre M, Zaragosi LE, Mitchison HM. Motile cilia and airway disease. Semin Cell Dev Biol. 2021 Feb;110:19–33. Copyright © 2021 with permission from Elsevier. (5)

Ciliogenesis is orchestrated by a tightly regulated network of signaling pathways and transcriptional controls that ensure the proper differentiation of basal cells into multiciliated cells (38). Two key regulatory mechanisms are involved in this process: the Notch signaling pathway and the GEMININ signaling pathway (GMNN) (5,39).

The Notch signaling pathway plays an early and decisive role in lineage specification by suppressing the differentiation of basal cells into secretory cells and promoting their commitment toward the multiciliated cell fate.

When Notch signaling is low or inhibited, this allows the expression of transcription factors **GMNC** and **MCIDAS**, which are essential initiators of the multiciliogenesis program (5,47–49).

In parallel, the cell cycle inhibitor GEMININ (GMNN) also regulates the timing of ciliogenesis. GMNN represses the activity of GMNC and MCIDAS during the cell cycle to prevent premature initiation of cilia formation (5,50,51). As GMNN levels decrease at the end of the cell cycle, GMNC is released from inhibition and activates MCIDAS, leading to the expression of downstream genes such as CCNO and FOXJ1, which control centriole amplification and motile cilia assembly (5,50,51).

Together, these pathways converge to activate genes that also regulate ciliary structure and function, including RFX2, RFX3, and **TP73**, which modulate ciliary length and motility (39,52,53). Additionally, NEK10, a kinase involved in ciliary length control, ensures proper cilium size; mutations in NEK10 lead to shorter cilia without affecting their number (54). In contrast, mutations in key ciliogenesis genes such as MCIDAS**,** FOXJ1, and CCNO can result in a complete loss or severe reduction of motile cilia at the apical surface of airway epithelial cells (55–57).

1.3 Structural dynamics and functional mechanisms of respiratory motile cilia

Respiratory ciliated cells are specialized, columnar-shaped cells that taper towards the surface and connect to the basement membrane at their basolateral pole(58). These cells, measuring approximately 20 µm in length and 7-2 µm in width (from surface to base), play a crucial role in clearing mucus from the lower respiratory tract through the highly coordinated beating of cilia (58). Additionally, intercellular junctional complexes, such as

tight junctions and adherens junctions, contribute to the barrier function of the respiratory epithelium by providing mechanical cohesion, regulating paracellular permeability, and secreting antimicrobial products(59).

Traditionally, ciliated cells were considered terminally differentiated and incapable of self-renewal, with basal cells—anchored to the basement membrane—acting as progenitors due to their proliferative and multipotent differentiation capacity(60). However, recent research indicates that other cell types, such as Clara cells, can also proliferate and differentiate into ciliated and goblet cells in the distal airways(61). Moreover, ciliated cells have been observed to dedifferentiate after injury, spread, and transdifferentiate into other epithelial cell types, thereby playing a significant role in the regeneration of the bronchial epithelium (62). Normally, the proliferation rate of ciliated cells is low, less than 1% over 24 hours, but it can increase to 17% following injury, with epithelial repair typically completed within 2 to 3 weeks (63).

Each ciliated cell has 200-300 cilia projecting from its luminal surface (58). These cilia vary in length, ranging from 6-7 μm in the trachea to 2-4 μm in smaller airways, with a relatively constant diameter of 0.2-0.3 μm (58). The cilia are densely packed in the central region of the cell, decreasing towards the edges. Surrounding each cilium are six microvilli, which may regulate transepithelial fluid and electrolyte transport (57,58). At the tip of each cilium, there are 3 to 7 claw-like projections that may assist in propelling mucus during ciliary beating (58).

1.3.1 Ultrastructure of respiratory motile cilia

The respiratory motile cilium is an essential and highly conserved organelle, with its structure consistent across species (12,64–66). Comprising more than 250 polypeptides, the cilium plays a crucial role in maintaining respiratory health (12,64–66). The core of the motile cilium is the axoneme, a cylindrical assembly characterized by a "9+2" microtubule arrangement—nine peripheral microtubule doublets (MTDs) encircle a central pair (CP) of microtubules

(Figure 7-A). This axoneme is covered by a specialized ciliary membrane that is continuous with the cell's plasma membrane but distinct in its composition (67). The axoneme's microtubule-associated structures, including inner and outer dynein arms (IDAs and ODAs) and radial spokes (RSs), are organized into a precise, repeating pattern every 96 nm along its length (Figure 7-B). This repeating unit includes four ODAs, six single-headed IDAs, one double-headed IDA (I1/f), three RSs, and one nexin-dynein regulatory complex (N-DRC), also known as the nexin link (65,68–70).

The **basal body** of the cilium, derived from centrioles involved in cell division, anchors the cilium to the epithelial cell and orchestrates the process of ciliogenesis(67) (Figure 7-C). This structure consists of nine triplet microtubules, each composed of an A-tubule, a B-tubule, and a C-tubule (67). The A- and B-tubules continue from the basal body into the axoneme, forming the peripheral MTDs, while the C-tubule stops short within the basal body, providing space for critical structures involved in ciliary motility and intraflagellar transport (IFT) (67). The basal body is connected to the ciliary membrane via transition fibers, which also serve as docking sites for the IFT machinery (67).

The **transition zone** is a critical area where the triplet microtubules of the basal body transition into the doublet microtubules of the axoneme (67). This zone acts as a selective barrier, regulating the molecular composition of the cilium by allowing the entry of only specific, targeted ciliary proteins (67). It is characterized by Y-shaped structures that connect the nine peripheral MTDs to the overlying ciliary membrane, maintaining the structural integrity and composition of the cilium (67).

The **axoneme** itself is a highly organized structure, where the peripheral MTDs are more complex, consisting of a complete A-tubule, made up of 13 protofilaments, and an incomplete B-tubule, composed of 10 protofilaments(1,5,7,71–73) (Figure 7-D). The B-tubule utilizes part of the A-tubule's wall to complete its structure. In the midwall area of the MTDs, the protein tectin, an intermediate filament, may serve as an adhesive, binding the A and B-tubules together and potentially regulating the length of the cilium.

The **dynein arms** are essential for the motility of the cilium. These large multimeric protein complexes contain several heavy, intermediate, and light chains, and are mechanochemical ATPases that generate the sliding motion of microtubules, which

underpins ciliary beating (67). The ODAs are primarily responsible for generating the force required for ciliary motion, while the IDAs, although also involved in microtubule sliding, contribute to the complexity of the ciliary beat, with different isoforms playing distinct roles in shaping the ciliary waveform (1). Dynein arms are attached to the A-tubule of each peripheral MTD and extend toward the adjacent B-tubule, where they undergo cycles of attachment, retraction, and release, driven by ATP hydrolysis. Within the 96 nm repeating unit of the axoneme, ODAs are composed of dyneins β and γ, and IDAs include several different dyneins (a-e, g), each contributing to the fine-tuning of ciliary movement(74).

Radial spokes (RSs) and the **nexin-dynein regulatory complex (N-DRC)** are critical for regulating dynein activity and, consequently, the ciliary beat and waveform(72). RSs are multipolypeptide structures that link each peripheral MTD to the CP, forming a radial scaffold within the cilium (72). These spokes consist of a cylindrical stalk attached to the A-tubule of the peripheral MTD and an RS head that interacts transiently with the CP(71,72,75). The RSs are organized into triplets (RS1, RS2, and RS3) within each 96 nm repeating unit, with RS1 and RS2 being structurally identical and symmetric, while RS3 differs. The RSs contain signal transduction pathways that relay information from the CP to regulate dynein activity (75) (Figure 7-F).

The **N-DRC** is a large, complex structure that connects the A-tubule of one peripheral MTD to the B-tubule of the adjacent MTD (72,75). It plays a key role in restricting the relative motion between adjacent MTDs, facilitating the attachment of IDAs, and transmitting signals from the CP and RSs to the dynein arms, thereby modulating dynein activity and ensuring coordinated ciliary movement

(Figure 7-F).

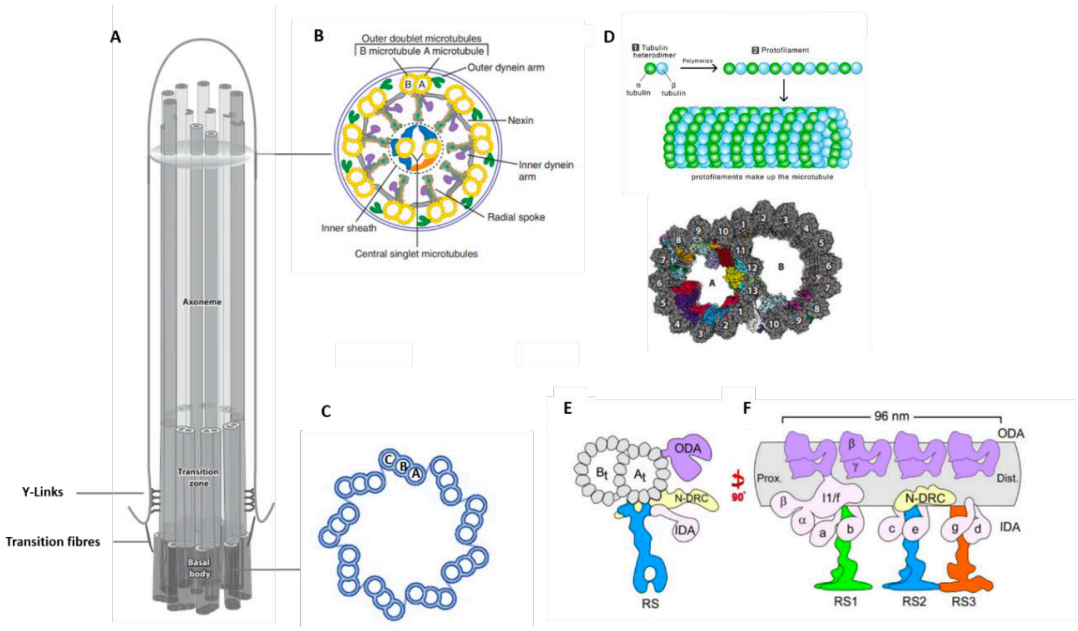

Figure 7: Structure of respiratory motile cilia. (A) Illustration showing the three main sections of the cilium: the basal body, transition zone, and axoneme. (B) Cross-sectional view depicting the axoneme's composition and (C) the structure of the basal body. (D) Diagram of a microtubule doublet formed by protofilaments made up of alpha and beta tubulin subunits. Microtubule A is comprised of 13 protofilaments, while the partially open microtubule B consists of 10 protofilaments. (E) and (F) Depiction of a 96-nm repeat unit of the axonemal multiprotein structure in human motile cilia, illustrating four outer dynein arms (ODAs, including dyneins β and γ), six single-headed inner dynein arms (IDAs a–e and g), one double-headed inner dynein arm (IDA I1/f), a nexin-dynein regulatory complex (N-DRC), and three radial spokes (RS1–RS3). IDA = inner dynein arm; N-DRC = nexin-dynein regulatory complex; ODA = outer dynein arm; RS = radial spoke. Lin J, Yin W, Smith MC, Song K, Leigh MW, Zariwala MA, et al. Cryo-electron tomography reveals ciliary defects underlying human RSPH1 primary ciliary dyskinesia. Nat Commun. 2014 Dec 4;5:5727. Reproduced with permission from Springer Nature.(65)

In addition to the axoneme and its associated structures, the cilium contains several sub-compartments that further contribute to its function. The **specialized ciliary membrane**, while continuous with the plasma membrane, is distinct in its composition and contains specific receptors and channels involved in sensory functions, growth control, and

epithelial homeostasis(12,72,75). The **ciliary matrix** is the soluble component of the cilium, housing the IFT machinery, which is essential for the transport of proteins synthesized in the cell body into the cilium. This bidirectional transport, facilitated by kinesin and dynein motor proteins, is critical for the maintenance and function of the cilium(67).

Finally, the **tip and basal body** of the cilium represent two additional sub-compartments. The tip contains specialized protein complexes whose functions are not fully understood, while the basal body, derived from mitotic centrioles, anchors the cilium to the cell and organizes ciliogenesis(53,75). The basal body is connected to the ciliary membrane by transition fibers, which act as docking sites for the IFT machinery, and the transition zone serves as a selective barrier at the base of the cilium, dynamically maintaining its composition and ensuring the proper entry of targeted ciliary proteins(67,72,75).

This detailed and intricately organized structure of the respiratory motile cilium highlights its crucial role in maintaining effective mucociliary clearance and overall respiratory health.

1.3.2 Intraflagellar transport

IFT is a crucial mechanism for the assembly, maintenance, and function of motile cilia in the respiratory epithelium, as these structures lack ribosomes and are unable to synthesize proteins within the cilium itself (76–78). All ciliary proteins, including those vital for the motility of ODAs and IDAs, must be synthesized in the cytoplasm and actively transported to their correct location within the cilium (67). This transport relies on IFT protein complexes, which function as structural units of the bidirectional transport system: **IFT-B** mediates **anterograde transport** (from the base to the tip of the cilium), assisted by kinesin-2 motor proteins, while **IFT-A** is mainly involved in **retrograde transport** (from the tip back to the base), in cooperation with dynein-2 motors (76–78). The transition zone at the base of the cilium functions as a selective barrier, regulating which proteins can

enter the cilium(76). While small molecules can diffuse freely, larger proteins must rely on the highly regulated IFT system to traverse this zone and reach their destination (Figure 8).

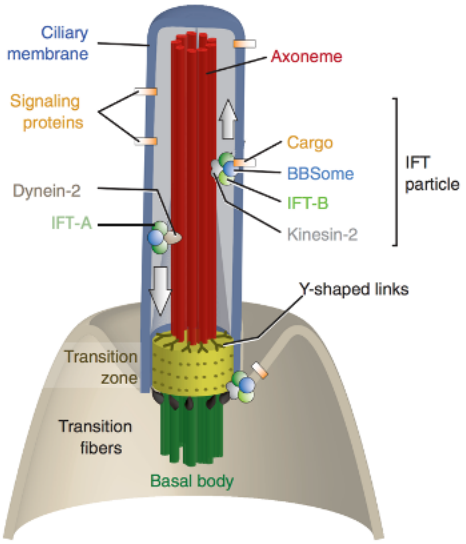

Figure 8: Mechanisms of protein transport in respiratory cilia: the role of intraflagellar transport in maintaining ciliary function. Adapted from vidor-Reiss T, Leroux MR. Shared and distinct mechanisms of compartmentalized and cytosolic ciliogenesis. Curr Biol. 2015 Dec 7;25 (23):R1143–50. Copyright © 2015 Elsevier Ltd. All rights reserved. (79)

IFT involves a bidirectional transport system, organized into train-like structures that move proteins either from the base to the distal tip of the cilium (anterograde transport) or from the tip back to the cell body (retrograde transport)(67,76). Anterograde transport is driven by the kinesin-2 motor complex, which moves along the B-tubule of the peripheral microtubule doublets (MTDs) at a speed of approximately 1.2 µm/sec (77,78,80). This process is essential for the delivery of proteins required for cilium assembly and elongation (71). Conversely, retrograde transport, mediated by the IFT dynein motor complex, occurs along the A-tubule of the MTDs at a speed of around 0.9 µm/sec. This process is critical for recycling proteins and maintaining the proper length and function of the cilia (71,77).

Unlike the dynein motors responsible for ciliary movement (ODA and IDA), the dynein complex involved in IFT, known as cytoplasmic dynein-2, is distinct (71,77). It functions alongside two key IFT protein complexes: IFT-A and IFT-B. IFT-A mediates retrograde transport by binding to the IFT dynein motor complex, while IFT-B is

responsible for anterograde transport, binding to the kinesin-2 motor complex (71). These complexes ensure the proper movement of proteins and other cargo molecules, which are carried along the ciliary axoneme (71). Cargo transport is facilitated by the BBSome, a protein complex that links specific cargo molecules to either IFT-A or IFT-B, depending on the direction of transport (72,79,81).

To better understand the molecular mechanisms underlying ciliary motility, it is crucial to distinguish between the dynein motors that drive intraflagellar transport (IFT) and those that generate the active beating of motile cilia.

Distinguishing between the dynein motors that drive intraflagellar transport (IFT) and those responsible for motile cilia beating is essential for understanding the molecular mechanisms of ciliary motility. The coordinated movement of motile cilia is driven by axonemal dynein arms**,** which are attached to the A-tubules of the outer doublet microtubules within the ciliary axoneme. ODAs generate the main force for ciliary beat frequency, while IDAs contribute to the regulation of the beat pattern and waveform (Figure 9) (82).

These dynein motors hydrolyze ATP to produce sliding forces between adjacent microtubule doublets. Because of the structural constraints imposed by nexin links and radial spokes, this sliding is converted into bending motions, allowing the cilium to generate coordinated, wave-like movements (83,84).

Defects in ODA or IDA structure, docking, or assembly are among the most well-documented ultrastructural abnormalities in PCD and are associated with specific genotypes (e.g., DNAH5, DNAH11 for ODAs; CCDC39, CCDC40 for IDAs) (85) .

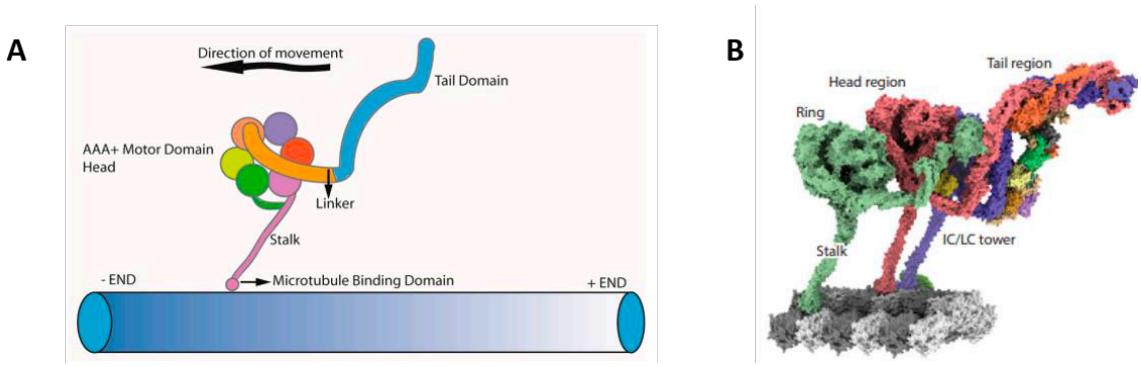

Figure 9: (A) Schematic or (B) three-dimensional illustration of the dynein heavy chain in the outer dynein arm, showing the tail (responsible for cargo binding), the stalk (attaching the outer dynein arm to the A-tubule of the peripheral microtubule doublets), the linker (which connects the tail to the head), and the head region (containing the site where ATP hydrolysis occurs). Antony D, Brunner HG, Schmidts M. Ciliary Dyneins and Dynein Related Ciliopathies. Cells. 2021 Jul 25;10 (8):1885.© 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/). (71)

This coordinated and highly regulated transport system is essential for the proper functioning and maintenance of the motile cilia in the respiratory epithelium, enabling the clearance of mucus and other debris from the airways. Errors or disruptions in IFT can lead to dysfunctional cilia, contributing to various respiratory conditions, including PCD.

1.3.3 Ciliary beating

Ciliary beating is a critical function of motile cilia in the respiratory system, essential for mucociliary clearance (MCC), which is the primary defense mechanism of the airways (1). The movement of cilia is coordinated in what are known as metachronal waves, where each cilium beats in a synchronized manner with a constant phase difference between adjacent cells(1,86). This wave-like beating pattern allows the effective clearance of mucus and entrapped particles from the respiratory tract. In healthy human airways,

motile cilia typically beat at a frequency of 10 to 20 Hz, generating a displacement of about 5 mm per minute(5,86).

The driving force behind ciliary beating lies in the interaction between the dynein arms and the microtubule doublets (MTDs), powered by ATP hydrolysis. This process involves structural changes in the dynein arms, which cause the microtubules to slide against one another, resulting in the movement of the cilia(7,34). The beating is divided into two key phases: the effective stroke and the recovery stroke. During the effective stroke, the cilium moves through an arc of approximately 110°, pushing mucus in the direction of clearance, perpendicular to the epithelium. This is followed by the recovery stroke, in which the cilium bends back through an arc of about 180°, returning to its initial position (Figure 10). In this phase, cilia deviate slightly, up to 5°, from the beating plane (7). Notably, the power stroke occurs two to three times faster than the recovery stroke, and both are separated by a brief pause. As the ciliary beat frequency (CBF) increases, the pause shortens, leading to an acceleration of both strokes (5,87,88).

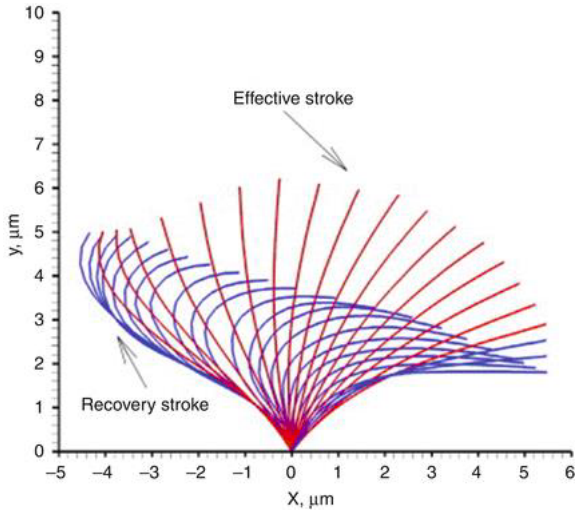

Figure 10: Typical motion of an airway cilium viewed from the side, illustrating the power stroke (in red) and the recovery stroke (in blue). M. Vanaki, S., Holmes, D., Jayathilake, P. G., & Brown, R. (2019). Three-Dimensional Numerical Analysis of Periciliary Liquid Layer: Ciliary Abnormalities in Respiratory Diseases. Applied Sciences, 9 (19), 4033. https://doi.org/10.3390/app9194033.

(http://creativecommons.org/licenses/by/4.0/). (89)

The structural division of the nine peripheral MTDs into two functional groups is key to ciliary beating: MTDs 1-4 are responsible for the effective stroke, while MTDs 5-9 control the recovery stroke. This precise division enables the efficient movement of the cilia and the metachronal wave formation. ODAs are responsible for controlling the frequency of the ciliary beat, while IDAs regulate the bending and waveform of the ciliary beat (1,74).

Ciliary beating is regulated by various physiological stimuli, including intracellular and extracellular pH, temperature, β2 agonists, NO, and ATP. Although the exact regulatory mechanisms are not fully understood, three key pathways have been identified: regulation through ions (Ca^{2+} and Cl^{-}), cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP). These pathways influence ciliary beating either independently or in combination (88,90–92).

The most studied regulatory pathway involves **Ca^{2+} ions**, which play a major role in controlling ciliary beat frequency. An increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) typically leads to an increase in CBF, while a decrease reduces it (88,91). The Ca^{2+} signaling pathways may act through G protein-coupled receptors (GPCRs), which release Ca^{2+} from intracellular stores like the endoplasmic reticulum (ER) via inositol trisphosphate (IP_3) (91). This release is triggered by the binding of ligands (such as ATP or acetylcholine) to GPCRs, activating the associated G-proteins and phospholipase C (PLC) (91). As Ca^{2+} is released into the cytosol, it binds to calmodulin, a regulatory protein that modulates the activity of proteins involved in ciliary beating. Additionally, Ca^{2+} voltage-dependent channels, such as TRPV4 and P2X receptors, contribute to the regulation of Ca^{2+} entry into the cell or cilium, further influencing ciliary function (91). Within the ionic regulation pathway, chloride ions (Cl^{-}) have also been shown to modulate ciliary beating, particularly through interactions with calcium-dependent mechanisms. The intracellular chloride concentration ($[Cl^{-}]i$) also plays a significant role in regulating ciliary beating. Yasuda et al. demonstrated that an increased $[Cl^{-}]i$ results in a reduction in both ciliary beat distance (CBD, similar to ciliary beat amplitude) and ciliary beat frequency (CBF). Conversely, reducing $[Cl^{-}]i$ led to an increase in CBD without affecting CBF at

37°C, indicating that the concentration range of $[Cl^-]i$ for CBF activation differs from that for CBD activation (92). Notably, at 25°C, a reduction in $[Cl^-]i$ was found to enhance both CBD and CBF (91). Since ODA is responsible for regulating CBF and IDA determines the ciliary waveform, changes in $[Cl^-]i$ can stimulate or inhibit the activity of both ODA and IDA, specifically at 25°C (92). This finding suggests the possibility that both ODAs and IDAs may contain a temperature-sensitive mechanism that influences chloride binding (92). Such changes in $[Cl^-]i$ can occur via activation of chloride or CFTR channels, or by inhibiting the $Na^+/K^+/2Cl^-$ cotransporter (92).

The second pathway involves **cAMP**, which is produced by adenylate cyclase (AC) in response to GPCRαs activation (88). cAMP activates protein kinase A (PKA), which phosphorylates proteins in the dynein arms, modulating both the CBF and ciliary beat pattern (88). β-adrenergic agonists and adenosine can increase cAMP levels, leading to enhanced ciliary activity (88).

Lastly, **cGMP**, produced by guanylate cyclase, plays a role in ciliary beating regulation (88). This molecule is activated by NO, which in turn activates protein kinase G (PKG), modulating CBF through phosphorylation of axonemal proteins (91). Although the precise mechanisms of cGMP regulation remain unclear, its role in the maintenance of efficient ciliary beating is supported by evidence of its interaction with other signaling molecules (88).

Furthermore, coordination between neighboring ciliated cells requires additional levels of regulation. Planar cell polarity signaling pathways, involving core proteins such as Vangl1/2 and Frizzled, establish the uniform orientation of cilia across the epithelial surface, ensuring that all cilia beat in the same direction (93,94). Furthermore, gap junctions composed of connexins facilitate intercellular communication by allowing the passage of ions and small signaling molecules such as Ca^{2+}, which synchronize CBF between adjacent cells (95). Intracellular calcium oscillations and cyclic nucleotides (e.g., cAMP, cGMP) also modulate CBF in response to mechanical or chemical stimuli, enabling dynamic adaptation to environmental conditions (96). Together, these mechanisms ensure that cilia across the epithelium beat in a metachronal wave, enhancing the efficiency of mucus transport and airway defense.

In summary, the regulation of ciliary beating is a complex and highly coordinated process involving multiple signaling pathways. The interaction between the dynein arms, microtubules, and signaling molecules such as Ca^{2+}, cAMP, and cGMP ensures that cilia can adapt to changes in the environment, maintaining effective clearance of mucus and pathogens from the respiratory tract

1.4 Primary ciliary dyskinesia

PCD is a rare, inherited ciliopathy resulting from genetic mutations that affect the formation, assembly, structure, and function of motile cilia, leading to impaired ciliary motility (97,98). Due to structural similarities, these mutations can also impact the function of non-motile cilia (99). PCD is predominantly inherited in an autosomal recessive manner, although autosomal dominant and X-linked inheritance patterns have been described.

The estimated prevalence is approximately 1 in 7,500 to 1 in 10,000 live births, though the true prevalence remains uncertain due to underdiagnosis and diagnostic delays, particularly in Europe (100–102). The median age at diagnosis is 9.8 years, and this delay can be attributed to multiple factors: insufficient awareness among healthcare professionals, low clinical suspicion due to symptom overlap with other respiratory conditions, and the absence of an accessible and straightforward diagnostic test (101,103–105). Early diagnosis is critical for reducing long-term pulmonary morbidity, avoiding unnecessary investigations, and ensuring appropriate treatment, which can significantly enhance quality of life for affected individuals (97,101).

PCD is characterized by impaired mucociliary clearance, a key mechanism responsible for removing mucus and secretions from the airways(97). This impairment leads to chronic respiratory symptoms, recurrent infections, and progressive disease affecting both the upper and lower respiratory tracts (97). The ineffective clearance of mucus results in chronic bacterial infections, contributing to bronchiectasis, which is marked by irreversible airway dilation and chronic inflammation (97,98). Respiratory

symptoms are prevalent in PCD, as ciliated epithelial cells line the upper and lower airways, as well as the Eustachian tubes (97). Chronic rhinosinusitis, persistent nasal congestion, and a wet cough are hallmark features that often present from infancy (97). The clinical phenotype of PCD varies substantially, even among individuals with the same genetic mutation, due to the genetic heterogeneity that influences ciliary morphology and function (97,98,102,105). Although clinical presentation can vary significantly among patients with PCD, the overall phenotype (characterized by early onset of symptoms, multi-organ involvement, and systemic manifestations) often differs from that of other causes of bronchiectasis. This distinct profile justifies a multidisciplinary approach to address the wide range of associated complications, including pulmonary, ENT, ophthalmologic, renal, cardiac, neurologic, and occasionally gastrointestinal manifestations.

Beyond respiratory symptoms, PCD also affects other organ systems due to the widespread presence of both motile and non-motile cilia. Motile cilia are found in several locations, including the reproductive tracts of both males and females, leading to infertility in some patients (72,74). In males, reduced sperm motility is a common cause of infertility (106). However, infertility is not universal among men with PCD, as some individuals with normal or reduced sperm motility have successfully fathered children (104,106,107). Women with PCD also face reduced fertility and a higher risk of ectopic pregnancy, which is linked to impaired ciliary function in the fallopian tubes and abnormal ovocyte transit.(108–112).

Furthermore, nodal cilia are crucial during embryonic development for establishing organ left-right asymmetry (113). Dysfunction of these cilia can lead to situs abnormalities, which are observed in over half of PCD patients (114). Situs inversus totalis, where the major visceral organs are mirrored from their usual positions, occurs in about 50% of cases, while other heterotaxy syndromes are found in an additional 10% (98,101,103). In rare instances, dysfunction of motile ependymal cilia, which help circulate cerebrospinal fluid, or non-motile sensory cilia, involved in signal transduction, may result in conditions such as hydrocephalus, retinitis pigmentosa, or polycystic kidney disease (29,115). These

additional manifestations underscore the multisystem complexity of PCD, requiring comprehensive management that extends beyond respiratory care.

The delay in diagnosis is partly due to limited awareness of PCD and its diverse clinical manifestations. In neonates, **respiratory distress is often the first clinical sign**—it may present at birth or develop in the first days of life (late-onset), and its severity can range from mild to severe cases requiring intensive care (97,101). Around 50% of PCD patients exhibit situs inversus or other laterality defects, which can serve as an important early indicator for clinicians (98,101,103). Some neonates also develop late-onset respiratory distress that necessitates intensive care, and approximately 5% are born with congenital heart defects, ranging from atrial or ventricular septal defects to more complex structural anomalies requiring urgent intervention (97,98,103,116). In contrast, young children with PCD often present with nonspecific symptoms, such as chronic rhinitis or a persistent wet cough, which are frequently overlooked or misattributed to more common pediatric conditions.

Nevertheless, many cases remain undiagnosed due to the subtlety of these early signs and their overlap with other conditions. These features highlight the importance of early and accurate diagnosis in reducing complications and improving long-term outcomes for affected individuals. Increased awareness among healthcare providers, coupled with the development of standardized diagnostic protocols, could facilitate earlier recognition and timely intervention, ultimately leading to improved prognosis and quality of life for PCD patients.

To date, approximately 50 genes have been identified as being associated with PCD (97,117). Despite this significant progress, these genes account for only about 70% of PCD cases (97,118). This genetic diversity contributes to variations in ciliary morphology and function, resulting in a wide range of phenotypic presentations among affected individuals (97).

1.4.1 Clinical features and manifestations of PCD

The clinical manifestations of PCD vary across different age groups and are linked to dysfunctional motile cilia in the conducting airways, middle ear, paranasal sinuses, reproductive organs, and embryonic node (97,105,118,119). At all ages, oto-sinopulmonary symptoms are the predominant clinical presentation of PCD (97,120). The main features include recurrent or chronic infections affecting both the upper and lower respiratory tracts, which can eventually lead to bronchiectasis (102,119). Patients often present with neonatal respiratory distress, a chronic wet cough, persistent nasal congestion, recurrent or chronic otitis media and sinusitis, as well as conductive hearing loss (97). A wet cough is characterized by the presence of airway secretions, leading to a moist, gurgling, or rattling sound during coughing. This contrasts with a dry (non-productive) cough, which lacks such secretions and sounds harsher or more paroxysmal. Clinically, a wet cough often suggests ongoing airway inflammation and mucus retention, which are hallmark features of PCD due to impaired mucociliary clearance. According to the European Respiratory Society guidelines and pediatric pulmonology literature, a cough is considered wet when it is described by the caregiver or clinician as sounding phlegmy, rattly, or when sputum is expectorated (if the child is old enough) (121,122). The clinical interpretation of wet cough can vary somewhat, especially in young children who cannot expectorate. In such cases, auditory characteristics (as perceived by caregivers or clinicians) and associated signs such as frequent throat clearing, vomiting of mucus, or moist crackles on auscultation are often used to identify a wet cough (123).

These symptoms generally emerge shortly after birth and continue throughout life on a year-round, daily basis (97,102). Unfortunately, most of these respiratory symptoms are also common among healthy children or those with other respiratory conditions, leading to frequent missed diagnoses during infancy and childhood, which can result in delayed intervention and inappropriate treatment. The key clinical features of PCD, along with several predictive diagnostic tools, have been identified, improving the recognition of patients affected by this rare condition (Table 1).

Stage of life	Symptoms highly suggestive of PCD
Newborns	- Situs inversus totalis with neonatal respiratory distress[a] - Situs ambiguus with neonatal respiratory distress[a] without cardiac defect - Situs ambiguus with neonatal respiratory distress[a] disproportionate to cardiac defect severity - Neonatal respiratory distress[a] at term birth requiring supplemental oxygen or positive pressure support for more than 24 hours, with lobar atelectasis on chest radiography - Neonatal respiratory distress[a] at term birth requiring supplemental oxygen or positive pressure support for more than 24 hours, along with a family history of PCD, chronic sino-oto-pulmonary disease, or unexplained bronchiectasis
Children	At least two of the following four key symptoms:

	- Year-round wet cough starting before 6 months of age - Year-round nasal congestion starting before 6 months of age - Neonatal respiratory distress[a] at term birth requiring supplemental oxygen or positive pressure support for more than 24 hours - Organ laterality defect - Unexplained bronchiectasis[b] with chronic sino-oto-pulmonary disease since early childhood or a family history of PCD
Adolescents and Young Adults	- Unexplained bronchiectasis[b] and at least one of the following key symptoms: - Chronic rhinosinusitis - Persistent otitis in adolescence or adulthood - Organ laterality defect - Infertility (in males or females) - Chronic sino-oto-pulmonary symptoms since early childhood

	- Family history of PCD
[a] In PCD, neonatal respiratory distress often develops 12 to 24 hours after birth and is accompanied by shifting and lobar atelectasis on chest radiography.	
[b] Bronchiectasis in PCD is often more prominent in the middle or lower lobes.	

Table 1: Overview of key clinical symptoms of PCD in newborns, children, and adults. Reproduced with permission from American Academy of Pediatrics: Wee WB, Kinghorn B, Davis SD, Ferkol TW, Shapiro AJ. Primary Ciliary Dyskinesia. Pediatrics. 2024 May 2;153 (6):e2023063064.
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To help clinicians, the "PrImary CiliAry DyskinesiA Rule" (PICARAR) is a tool that calculates a predictive score to assess the likelihood of PCD based on responses to seven specific questions (Table 2).

The total score provides an individualized estimate of PCD likelihood, as illustrated by the probability curve below (Figure 11).

PICADAR		
Does the patient have a daily wet cough that started in early childhood?	**Yes** – complete PICADAR **No** – **STOP**. PICADAR is not designed for patients without a wet cough	
1. Was the patient born pre-term or full term?	Term	2
2. Did the patient experience chest symptoms in the neonatal period (*e.g.* tachypnoea, cough, pneumonia)?	Yes	2
3. Was the patient admitted to a neonatal unit?	Yes	2
4. Does the patient have a situs abnormality (situs inversus or heterotaxy)?	Yes	4
5. Does the patient have a congenital heart defect?	Yes	2
6. Does the patient have persistent perennial rhinitis?	Yes	1
7. Does the patient experience chronic ear or hearing symptoms (*e.g.* glue ear, serous otitis media, hearing loss, ear perforation)?	Yes	1
	Total score =	

Table 2: PICADAR: clinical tool developed and validated in a **tertiary care setting** to help identify children likely to have PCD before undergoing diagnostic tests (126). It is based on key clinical features such as neonatal respiratory distress and laterality defects. A version adapted for **adult patients** has also been developed, reflecting differences in symptom presentation with age (127). Reproduced from Behan L, Dimitrov BD, Kuehni CE, et al. PICADAR: a diagnostic predictive tool for primary ciliary dyskinesia. Eur Respir J 2016; 47: 1103-1112 [DOI:10.1183/13993003.01551-2015].© ERS 2025 with permission from ERJ. (124)

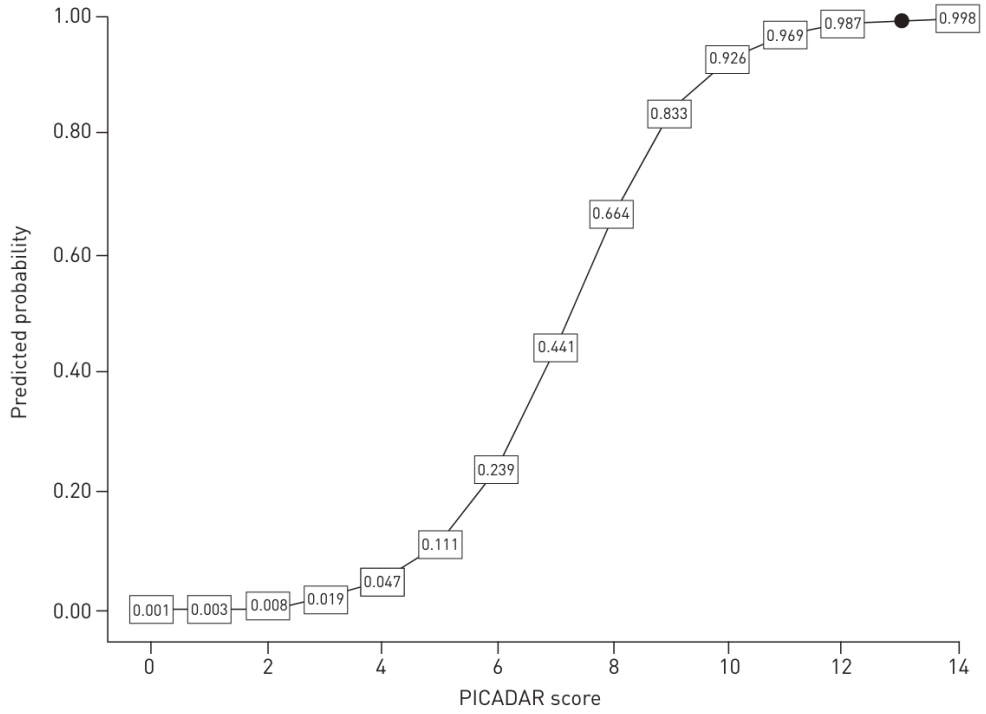

Figure 11: PICADAR probability curve: Estimating individual likelihood of PCD Diagnosis. After calculating the total PICADAR score from Table 2, the probability curve is used to estimate the individual risk of having PCD. Reproduced from: Behan L, Dimitrov BD, Kuehni CE, et al. PICADAR: a diagnostic predictive tool for primary ciliary dyskinesia. Eur Respir J 2016; 47: 1103-1112 [DOI:10.1183/13993003.01551-2015].© ERS 2025 with permission from ERJ. (124)

Prenatal stage

Prenatal ultrasounds cannot directly diagnose PCD, but they can detect anomalies suggestive of an underlying ciliopathy**,** which may raise suspicion. There have also been reported cases of identifying situs inversus during the second trimester ultrasound (129). Among individuals with situs inversus, approximately 20-25% are found to have PCD (114). The identification of situs inversus or other laterality abnormalities during prenatal imaging should prompt healthcare providers to consider PCD as a differential diagnosis (114,130–134). Furthermore, fetal cerebral ventriculomegaly has been reported in patients with PCD, particularly when it is associated with laterality abnormalities, cardiac malformations, or a family history of PCD (131). Such findings should heighten suspicion of PCD. The diagnostic value of prenatal ultrasound in this context depends on the skill of the operator and the quality of fetal imaging. While it does not serve as a standalone diagnostic tool for PCD, the prenatal identification of laterality anomalies such as situs inversus or heterotaxy may justify anticipating neonatal respiratory complications and planning delivery in a facility with access to neonatal intensive care, particularly in the presence of a positive family history (114,119).

Neonatal stage

Up to 85% of full-term neonates with PCD experience unexplained respiratory distress despite having a normal birth (107,130,133,135). The exact cause of respiratory distress in neonates with PCD is not fully understood, but it is hypothesized that respiratory cilia are involved in the rapid clearance of amniotic fluid from the lungs at birth, enabling effective air exchange (107,130,134,136). Unlike other causes of neonatal respiratory distress—such as transient tachypnea of the newborn (TTN) or neonatal pneumonia—which typically present immediately at birth, symptoms in neonates with PCD tend to appear later, most often between 12 and 24 hours of life (107,130,130). Initially, these infants may appear healthy but go on to develop respiratory distress within the first day of life, and some are discharged home only to be readmitted within a few days or weeks due to respiratory issues (107,130,130). Common clinical signs in neonates with PCD include

tachypnea, increased work of breathing, low oxygen saturation, and upper and middle lobe atelectasis, which is sometimes misdiagnosed as neonatal pneumonia.

This respiratory distress can progress to respiratory failure, requiring ventilatory support (107,119,133). While TTN generally resolves within five days, more than 75% of neonates with PCD require oxygen supplementation for an extended duration, ranging from days to weeks (119,130,134,137). Additionally, neonates with PCD frequently present with nasal congestion, which can lead to feeding difficulties, and an early cough—features that are uncommon in neonates but are highly indicative of PCD. From a clinical standpoint, the delayed onset of symptoms should raise suspicion for PCD in full-term neonates presenting with unexplained **r**espiratory distress beyond the immediate postnatal period, particularly when associated with prolonged oxygen dependency**,** bronchiectasis (or lobar collapse, which is rare in term neonates), and situs inversus (findings that are uncommon in typical neonatal respiratory conditions) (107,133).

Pulmonary disease

PCD presents with respiratory symptoms that typically emerge in early childhood and progressively worsen over time (97,107,119,136). A persistent, daily wet cough is one of the hallmark features of PCD, often accompanied by recurrent lower respiratory tract infections and chronic pulmonary manifestations (97,107,119,136). This wet cough, which usually begins in infancy typically before attending daycare, serves as a compensatory mechanism for impaired MCC (125,137,138). Despite its chronic nature, many patients, particularly children, perceive this cough as "normal," which often results in underreporting of symptoms (107,125,135,137,138). Although the severity of the cough may lessen temporarily with antibiotic treatment or seasonal changes, it rarely resolves completely(107,125). Notably, a seasonal or episodic cough, or a dry cough, is inconsistent with a diagnosis of PCD (107,125). Recurrent wheezing is also frequently reported, though it often shows minimal to no response to conventional treatment (107,125).

Recurrent lower respiratory tract infections, such as pneumonia and bronchitis, are common in young children with PCD (107,125). By preschool age, most patients have

experienced multiple episodes of lower respiratory infections; however, the frequent use of antibiotics for related conditions like otitis media and chronic rhinitis can obscure the diagnosis in some cases (107,139). The natural progression of PCD-associated lung disease is less well characterized compared to CF, but pulmonary involvement in PCD tends to be milder overall (137). Nevertheless, there is significant heterogeneity in disease progression among patients.

While some adults with PCD may progress to respiratory failure requiring lung transplantation, others experience only moderate respiratory symptoms throughout their lives (141,142). Estimates suggest that up to 25% of adults with PCD may develop respiratory failure (141), whereas other studies indicate a lower prevalence of around 4%, underscoring the variability in disease outcomes (142).

Lung function in PCD is frequently characterized by airflow obstruction and ventilation inhomogeneity, which can be assessed through lung clearance index (LCI) measurements (140,141,143,144). Many patients exhibit signs of abnormal lung function from an early age, with young children often displaying severe airflow obstruction comparable to the impairment seen in CF patients of similar age (140,143). However, in adolescents and adults with PCD, the decline in lung function is typically less severe compared to CF, likely due to preserved cough clearance that offers partial airway protection (125,138). Studies have shown that while lung function may remain stable in some treated patients, others may experience either improvement or deterioration despite receiving standard therapy (143,145). High resolution computed tomography (HRCT) is frequently used to detect early lung abnormalities in infants and young children with PCD, revealing changes such as consolidation, mucus plugging, bronchiectasis, atelectasis, air trapping, and peribronchial thickening (107,146). Although the development of bronchiectasis is age-dependent, it has been reported in toddlers as well (107,137,147). One study documented bronchiectasis in all adult patients and in over half of pediatric patients with PCD (146). Spirometry, which is traditionally used to monitor chronic lung disease progression, may be less sensitive than HRCT in detecting early changes (148). Recent studies have suggested that LCI may be more effective than forced expiratory volume in one second (FEV1) in identifying early lung disease and correlating with HRCT

findings, although correlations between LCI, spirometry, and radiographic changes in PCD remain inconsistent.

The progression of lung disease in PCD is intrinsically linked to chronic lower airway infection and inflammation (126,152). The spectrum of pathogens present in the lower respiratory tract evolves over time, with younger children frequently colonized by organisms such as Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, and Staphylococcus aureus (152). As patients age, bacterial diversity tends to increase, with pathogens such as Pseudomonas aeruginosa and Achromobacter xylosoxidans being more commonly isolated (152). Chronic infection with Pseudomonas aeruginosa becomes increasingly prevalent in teenagers and young adults, with reported rates ranging from 5% to 39%. Chronic Pseudomonas aeruginosa infection is associated with worse pulmonary function compared to PCD patients without chronic infection (142). Furthermore, approximately 10% to 15% of adults with PCD have been found to have non-tuberculous mycobacteria (NTM) infections (107,153). Unlike CF, where the Leeds criteria are widely used to define chronic Pseudomonas infection, there is no standardized definition of chronic infection in PCD, resulting in significant variability in clinical practice and patient management (154–156).

Otorhinolaryngologic disease

The ENT manifestations of PCD are very common and contribute significantly to the general morbidity of the disease. They are often recurrent during early childhood, despite a well-conducted antibiotic treatment (157).

The majority of PCD patients have chronic rhinosinusitis (CRS), which is defined by inflammation of the entire nasal mucosa and paranasal sinuses. CRS causes symptoms such as nasal congestion, facial pain, mucopurulent anterior or posterior rhinorrhea, hyposmia or anosmia. These symptoms are considered chronic if they persist for more than 12 weeks and if signs of rhinosinusitis are demonstrated by endoscopy or imaging.

Sinonasal polyposis may also be associated (157–159), usually occurring in older children, affecting 15-56% of adults (142,159). The nasal symptomatology associated with PCD is generally present throughout the year and is not influenced by the seasonal change, unlike allergic rhinitis (157). Chronic rhinorrhea and nasal congestion is often present since the neonatal period (107,138,160,161). Although it's a non-specific sign, clinical examination of the nasal cavities in patients with PCD frequently shows mucopurulent secretions in the nasal floor and swelling of the inferior turbinates (157,162). Hydrocephalus is uncommon in patients with PCD, but has been associated with ciliary aplasia, as it has been reported in 10 % with CCNO (Cyclin O) mutations (29). Hydrocephalus is the consequence of a dysfunction of the mobile cilia of the cerebral ventricles and of the ependymal duct, and can cause chronic headaches that can be mistakenly attributed to sinusitis (162). Pan-sinusitis is found on imaging (CT-scan) in most adult PCD patients and is often associated with sinus hypoplasia or agenesis (mainly frontal sinuses). While sinus opacification can occur as early as childhood, characteristic anatomical anomalies such as frontal and sphenoid sinus aplasia become more readily identifiable after sinus development is complete, usually from adolescence onward (162,163). These features reflect the long-term impact of PCD on sinus development during growth and represent a relevant diagnostic clue in clinical practice. Bilateral ethmoid sinus mucoceles have also been described in children with PCD (163,164). In terms of pathogens, H. influenzae, S. pneumoniae, and P. aeruginosa were the most frequently found bacteria in patients with PCD (165). Recently, Lam et al. analyzed data from the ENT prospective international cohort of patients with PCD (EPIC-PCD), which is the first cohort focused exclusively on examining upper airway manifestations in primary ciliary dyskinesia (166). According to recent findings from EPIC-PCD, rhinorrhea and nasal obstruction were reported in approximately 95% of the cohort (166). Additional clinical findings include recurrent sinus infections, hypertrophic nasal turbinates, deviated septum, and nasal polyps, with the latter occurring more frequently in adults compared to children, suggesting an age-related exacerbation of chronic sinonasal complications likely driven by ongoing inflammation (166). Furthermore, patients with PCD often experience sinonasal symptoms, which moderately affect their quality of life (166). However, they may underreport these impacts due to adaptation to chronic symptoms. Despite frequent sinonasal issues, many patients do not

receive appropriate treatment, which could be attributed to underreporting or the lack of standardized management protocols (166). This underscores the importance of regular sinonasal evaluations for PCD patients and the need for evidence-based treatments to improve care.

PCD is also expressed by very frequent involvement of the middle ear. Otologic manifestations of PCD include, but are not limited to, chronic otitis media with effusion (chOME), recurrent otitis media (ROM), and hearing loss (167). Patients frequently present with persistent otorrhea, especially after ventilation tubes insertion. Clinical examination of the ear may be normal or show chOME, acute otitis media, tympanosclerosis, as well as otorrhea in the external auditory canal if the tympanic membrane is ruptured spontaneously or by the ventilation tube insertion (167). Hearing loss caused by chOME is mild to moderate in severity, usually insidious in young children with PCD (157). However, even mild hearing loss in the first years of a child's life can affect language acquisition as well as the child's listening skills at school, which can affect children's performance at school (169). chOME is affecting at least 80% of children with PCD and is often persistent until the age of 12. Therefore, a special attention should be paid to hearing loss (157,160,168). Recent findings from the EPIC-PCD study indicated a high prevalence of ear-related symptoms in PCD patients, with over 50% of participants reporting ear pain and 46% reporting hearing problems (167). chOME was the most common otologic finding, predominantly affecting children, while tympanic sclerosis was more prevalent among adults, possibly due to chronic inflammation and recurrent ear infections. Audiometry revealed that over 40% of participants had some degree of hearing impairment, predominantly mild, with increasing age being a significant risk factor (167). Therefore, routine otologic assessments are essential for PCD patients of all ages to ensure effective monitoring and management of ear-related complications.

1.4.2 Management of PCD

General management

The limited evidence and lack of comprehensive data make the management PCD challenging (169). Due to the absence of randomized controlled trials, recommendations and consensus guidelines are predominantly drawn from small observational studies, extrapolated data from clinical trials for other diseases with impaired mucociliary clearance, such as CF and non-CF bronchiectasis, or the collective opinions and experience of experts in specialized PCD centers. Nevertheless, two randomized controlled trials have been carried out to evaluate the efficacy and safety of inhaled hypertonic saline and prophylactic azithromycin use in PCD patients (170,171). Findings from these trials offered limited evidence of quality-of-life improvement with hypertonic saline, whereas azithromycin prophylaxis was well-tolerated and significantly reduced respiratory exacerbation incidence by half (170,171). Furthermore, although not explicitly indicated in the inclusion criteria, some PCD patients were likely included in randomized controlled trials on non-CF bronchiectasis (172,173). Currently, no curative treatment exists for PCD, and available therapeutic approaches primarily focus on symptom management, prevention, and complication control (117,126). Consensus guidelines for monitoring and managing PCD were published by the ERS task force in 2009, and by the Genetic Disorder of Mucociliary Clearance Consortium (GDMCC) in 2016 (102,120). In 2021, an international consensus for infection prevention and control in PCD was also released (177).Patients with stable disease should receive follow-up every 3 to 6 months at an experienced PCD center with a multidisciplinary team comprising a respirologist, an ENT specialist, and a respiratory physiotherapist to assess growth, lung function, upper airway health, and hearing. Additional recommendations include regular physical activity, avoidance of known risk factors such as smoking (active or passive), and minimizing exposure to pathogens or air pollutants (97,139,175). Comprehensive management also involves heart monitoring, referral to a cardiologist if needed, especially for PCD patients with left-right laterality abnormalities, access to fertility clinics, and genetic counseling (97,176,177).Since the prevalence of infertility or subfertility in PCD remains uncertain,

patients should be informed about potential fertility concerns (111,178). Referrals to fertility clinics may be appropriate, particularly in the pre-conception phase (178). Given the age-related decline in sperm quality, young male PCD patients may be offered sperm cryopreservation (111). As PCD is a genetic disorder, genetic counseling is recommended both at diagnosis (to determine the need for family screening) and during pre-conception. In the following sections, we will discuss the detailed approaches to ENT management and pulmonary management in PCD.

Pulmonary management

Effective management of lower airway complications in PCD requires a multifaceted approach. Key components include routine airway clearance techniques, early detection and aggressive treatment of respiratory infections with antibiotics, preventive strategies such as regular pneumococcal and influenza vaccinations, patient segregation, and avoidance of inflammatory triggers like exposure to active and passive smoke (135,137,156,159).

Contrary to earlier perceptions, PCD is not a benign condition. Dependence on supplemental oxygen, severe pulmonary impairment (FEV1 < 40% predicted), or the necessity for lung transplantation is observed in 38% to 51% of adults with PCD (153,179). As a result, comprehensive long-term follow-up is essential and necessitates a multidisciplinary approach. To ensure optimal management of pulmonary disease in PCD, a comprehensive approach that includes consistent monitoring is essential to assess disease progression, initiate timely interventions, and optimize long-term outcomes. The following sections detail the specific methods and schedules for monitoring lung function, microbiology, and structural lung changes in patients with PCD.

- Lower Airways Monitoring: Regular monitoring is essential for tracking disease progression in PCD patients. It is recommended that individuals with stable

disease undergo outpatient follow-up visits with a specialist experienced in managing chronic suppurative lung disease every three to six months. (table 3)

Clinical visits
Pulmonology: 2–4 times/year
Otolaryngology: 1-2 time/year in children, as needed in adults
Audiology: at diagnosis and as needed per otolaryngology
Reproductive medicine: As clinically needed
Long-term surveillance
Chest radiography: every 2–4 years
Chest computed tomography: consider at least once after 5–7 years old (when sedation not required and images are of highest quality)[1]
Airway microbiology cultures: 2–4 times/year
Non-tuberculosis mycobacterial cultures: every 2 years (and with unexplained clinical decline)
Pulmonary function testing: 2–4 times/year
ABPA testing: IgE levels ± evidence of aspergillus specificity at diagnosis, with new onset wheezing, unexplained clinical decline
Preventative therapies
Airway clearance: daily
Nasal sinus lavage: daily (when pertinent)
Standard vaccinations: per local schedule
Influenza vaccine: annually[2]
13-valent pneumococcal vaccine: per ACIP guidelines[3]
23-valent pneumococcal vaccine: per ACIP guidelines[4]
RSV immunoprophylaxis: consider monthly in first winter[5]

[1]And as clinically indicated on a case by case basis.
[2]After 6 months old, including household members.
[3]ACIP guidelines.
[4]ACIP guidelines.
[5]Specifically consider in infants with complicated respiratory courses, including prematurity, prolonged mechanical ventilation, prolonged need for supplemental oxygen, need for home supplemental oxygen, or frequent respiratory illnesses.

Table 3: Recommended Monitoring, Investigations, and Clinical Care Schedule for Patients with Primary Ciliary Dyskinesia: Insights from the GDMCC Consensus Guidelines. Reproduced from Shapiro AJ, Zariwala MA, Ferkol T, Davis SD, Sagel SD, Dell SD, et al. Diagnosis, monitoring, and treatment of primary ciliary dyskinesia: PCD foundation consensus recommendations based on state of the art review. Pediatr Pulmonol. 2016 Feb;51 (2):115–32. © copyright 2016 with omission from John Wiley and Sons. (119)

- Microbiology Monitoring: Routine microbiological monitoring of the lower airways, using oropharyngeal cough swabs or expectorated sputum, is recommended every three to six months (119,175). Cultures should target common pathogens found in PCD, such as Pseudomonas aeruginosa and other Gram-negative bacteria (119,171). Screening for NTM is suggested every two years or when patients are on chronic macrolide therapy (119,171). In cases of unexplained clinical decline that do not respond to culture-directed antibiotics, additional testing (including bronchoalveolar lavage, NTM and fungal cultures, and assessments for allergic bronchopulmonary aspergillosis (ABPA) with IgE

levels and aspergillus-specific tests) should be conducted (118) (Table 3). Furthermore, by implementing a standardized eradication protocol (Tobramycin inhalation solution 300 mg/5 mL twice daily for 28 days, to be repeated for another 28 days if sputum cultures remained positive for Pseudomonas aeruginosa), lower airway colonization with Pseudomonas aeruginosa was successfully cleared in over 90% of children with PCD, with the majority maintaining negative airway cultures for Pseudomonas aeruginosa throughout the subsequent year. For the remaining patients with persistent Pseudomonas aeruginosa in sputum cultures despite two courses of tobramycin inhalation therapy, treatment was escalated to a 14-day course of intravenous ceftazidime and tobramycin, followed by an additional 28 days of tobramycin inhalation therapy (181).

- Lung Function Monitoring: Lung function monitoring using spirometry, particularly FEV1, is recommended every three to six months for stable PCD patients (130,156) (Table 3). Spirometry is the most accessible test in PCD centers but has limitations, including lack of sensitivity, inapplicability to children under five years of age, and variability even in stable patients (102,144,148,151). The LCI is a potential alternative for assessing lung function in PCD patients, though further validation is needed (144,150). Emerging evidence supports the use of hyperpolarized xenon gas and magnetic resonance imaging (MRI) for functional lung assessment, providing detailed anatomical imaging while measuring regional ventilation (182).

- Structural Lung Disease Monitoring: At diagnosis, initial lung structural assessment in PCD patients should begin with a chest radiograph as first-line imaging, particularly in children under five years of age, when a chest CT is not needed**,** as it generally requires sedation in this age group (119). A chest CT scan is recommended after the age of five, when it can usually be performed without sedation, to provide more detailed structural information if needed (119). To monitor disease progression in stable patients, chest radiographs are

recommended every two to four years and during exacerbations (119) (Table 3). HRCT should be performed at least once to confirm the presence of bronchiectasis, though routine serial CT scans are not recommended for monitoring disease progression (119). It is noteworthy that chest radiographs lack sensitivity for early detection of structural changes (119). In CF, quantitative scoring systems, such as Brody and Bhalla, are used for evaluating lung structural changes. In PCD, no specific scoring system has been developed, though the modified Brody score is commonly employed (182,183). MRI has shown comparable efficacy to HRCT in non-CF chronic lung disease, providing a non-radiative alternative, although its utility in PCD remains untested (184).

To provide effective long-term management of pulmonary disease in PCD patients, it is crucial to incorporate routine airway clearance and immunization strategies alongside individualized antibiotic use to address infections. Daily airway clearance techniques, including chest physiotherapy and cardiovascular exercise, form the foundation of therapy (119,185). Unlike cystic fibrosis, where MCC progressively worsens, PCD patients experience impaired MCC from birth, but their cough clearance remains relatively well-preserved (102,185). Available airway clearance techniques include forced coughing, breathing exercises, manual techniques, positioning, and positive expiratory pressure valves or oscillating devices (102,185). A study comparing high-frequency chest wall oscillation and conventional postural drainage found both techniques equally effective in improving lung function (186). Additionally, aerobic exercise is highly recommended, as reduced pulmonary function has been linked to poor exercise capacity (185,187,188). Notably, exercise provides a superior stimulus for airway clearance compared to inhaled beta2-agonists in children with PCD, suggesting that combining exercise with physiotherapy may enhance efficacy (119,188).

In parallel, PCD patients should adhere to standard immunization schedules, including annual influenza and pneumococcal vaccinations. Immunoprophylaxis against respiratory syncytial virus (RSV) may also be considered for infants under one year of age, especially those requiring prolonged oxygen supplementation during the neonatal period (119,139). COVID-19 vaccination is also strongly recommended for individuals with PCD (189,190).

Despite early concerns about increased susceptibility to severe COVID-19 due to chronic respiratory issues, studies have shown that people with PCD generally experience mild symptoms (189,190). However, vaccination remains crucial for this population (189,190). The COVID-PCD international cohort study reported high vaccination willingness and uptake among people with PCD, with 96% of adults being vaccinated or willing to be vaccinated. No severe side effects were reported, and half of the participants reported increased social interactions post-vaccination, indicating improved confidence and quality of life (189,190). On a case-by-case basis, chronic suppressive antibiotics may be employed for long-term management, focusing on eradicating pathogens such as Pseudomonas aeruginosa or Burkholderia cepacia, particularly following positive cultures (119,175,185). Chronic prophylactic antibiotics (e.g., macrolides or trimethoprim-sulfamethoxazole) are often recommended for patients experiencing frequent or severe exacerbations (119,175,185). While the evidence for chronic inhaled antibiotics in PCD is limited, therapies like inhaled aminoglycosides, colistin, or beta-lactam antibiotics may be considered for patients with severe bronchiectasis and chronic Pseudomonas colonization, similar to management strategies used in non-CF bronchiectasis (119,175,185,191).

To build on these approaches, additional therapeutic strategies, including inhaled agents and targeted treatments, aim to address the underlying challenges of mucus clearance and airway obstruction in PCD management.

Inhaled hypertonic saline is commonly utilized in PCD management, though evidence supporting its efficacy remains limited. The multicenter international "Clearing Lungs with ENaC Inhibition in PCD'' (CLEAN-PCD) trial demonstrated no significant benefit from hypertonic saline alone. However, when combined with idrevloride, a selective epithelial sodium channel inhibitor, a modest improvement in FEV1 was observed after four weeks of treatment.

Inhaled bronchodilators are not routinely recommended for PCD but may be used on a case-by-case basis with close monitoring of treatment response (102,119). While

short-acting bronchodilators have shown some improvement in FEV1, their long-term benefits remain uncertain (145).

N-acetylcysteine, known for its mucolytic and antioxidant properties, has demonstrated no significant benefits in PCD patients (185). As a result, its use is not currently recommended pending the outcomes of more robust and well-designed studies (193).

Azithromycin is increasingly used as a long-term maintenance therapy in patients with PCD, owing to its anti-inflammatory, immunomodulatory, and antibacterial properties (194). Several studies have demonstrated that chronic azithromycin therapy can reduce the frequency of pulmonary exacerbations and stabilize lung function in individuals with PCD (194). In a multicenter, double-blind, placebo-controlled trial (BESTCILIA study), azithromycin administered three times weekly over six months significantly reduced the number of respiratory exacerbations compared to placebo, without significant adverse effects (194). This aligns with observations in cystic fibrosis and non-CF bronchiectasis, where azithromycin has been shown to decrease neutrophilic airway inflammation and bacterial load (194). However, long-term use raises concerns about antibiotic resistance and requires careful patient selection and monitoring. Therefore, current guidelines suggest considering azithromycin in PCD patients with frequent exacerbations despite optimal airway clearance therapy (102).

Finally, **Management of exacerbations** involves aggressive antibiotic therapy guided by prior microbiology results. For mild cases, a 2–3-week course of broad-spectrum oral antibiotics, such as amoxicillin-clavulanic acid or a similar cephalosporin, is typically recommended(119,137). Severe exacerbations or those unresponsive to oral antibiotics may require parenteral antibiotics (102,119). Treatment strategies for PCD exacerbations are often extrapolated from CF protocols (156). However, as CF antibiotic doses are typically higher, there is an increased risk of toxicity if similar doses are applied to PCD patients (156).

ENT management

Management of nasal and sinus involvement in PCD consists of medical and surgical treatments. Nasal and sinus management primarily focuses on saline douching, physiotherapy, and antibiotics, which are key in reducing inflammation and preventing infections. Nasal and sinus irrigation with physiological saline is crucial for clearing mucus and maintaining sinus health. Respiratory drainage physiotherapy helps improve airway clearance, while antibiotics, either local (nebulized) or systemic, are essential for managing and preventing bacterial infections. Additional treatments, such as local corticosteroid therapy and, in some cases, anticholinergics, may be considered. However, polyps in PCD patients are mostly neutrophilic therefore they respond less to local intranasal corticosteroids.

In infectious exacerbations of rhinosinusitis, conservative treatment is not recommended, and antibiotics are preferred, the choice of antibiotic to be ideally guided by the nasosinus bacteriological analysis. Method of administration of antibiotics can be by oral, nebulized, or intravenous (120,157,158).

Long-term macrolide therapy may be useful for patients with frequent respiratory exacerbations, in whom azithromycin may reduce the morbidity of exacerbations, the need for additional antibiotic therapy and potentially prevent irreversible lung damage. In addition to their antibacterial effect, macrolides have beneficial anti-inflammatory effects and are increasingly used in various chronic respiratory pathologies, including PCD. Kobbernagel et al have shown that 6-month maintenance treatment with azithromycin halves respiratory exacerbations in patients with PCD (171).

In pediatric patients, CT scans are primarily used for preoperative planning to assess anatomy, extent of disease, and sites of nasal obstruction (196). Efforts should be made to reduce CT radiation exposure in patients with PCD. However, CT remains a valuable tool for diagnosing cases that require surgery. Cone Beam CT and MRI can evaluate paranasal sinus in children, respectively with minimal or no radiation exposure (197). MRI is particularly advantageous in follow-up settings where radiation sparing is a priority and when detailed assessment of soft tissue is sufficient (197). However, its routine use remains limited by several factors: lower spatial resolution for bone evaluation, longer acquisition

time (often requiring sedation in young children), and more limited availability in some clinical settings (197). Therefore, although systematic MRI for all follow-up imaging would reduce radiologic exposure, its practical limitations and lower utility in surgical planning currently restrict its widespread use. Imaging strategies in pediatric PCD should thus be individualized, taking into account the clinical indication, the child's age and cooperation, and the diagnostic question at hand (197).

Regarding surgical management, adenoidectomy and tonsillectomy can play a significant role in reducing bacterial load and alleviating symptoms associated with nasal and sinus congestion. The adenoids and tonsils are often reservoirs for chronic bacterial infections and their removal can lead to fewer respiratory infections and a decrease in the need for antibiotics. Furthermore, these surgeries can improve airflow, reduce nasal congestion, and relieve obstructive symptoms, such as breathing blockages and sleep-disordered breathing, which are common in PCD patients. However, careful patient selection is necessary to ensure that the benefits outweigh the risks, and these surgeries should be considered as part of a comprehensive management plan. Endoscopic sinus surgery (meatomy, polypectomy, ethmoidectomy, turbino-septal surgery) could be of benefit in the management of PCD. The goals of sinus surgery in patients with PCD are to treat nasal congestion, to restore nasal breathing, to improve olfaction, and to increase penetration of local treatments by improving mechanical sinus drainage (157). Some studies also suggest that sinus surgery may reduce the respiratory bacterial load (198). Bequignon et al. advise to orient the surgical procedure according to the predominant symptoms: if the facial pain is in the foreground an ethmoidectomy is performed, while in the event of predominant nasal obstruction a turbinectomy is performed. Endoscopic sinus surgery is not a curative treatment and persistant chronic rhinorrhea can be observed in post-operative consultations and follow up consultations. The choice of surgical technique is primarily guided by guidelines for CF patients (199). Therefore, endoscopic sinus surgery should be used in case of persistant rhino-sinus symptoms and/or no amelioration with a long-term macrolide therapy.

Biofilms of the upper, lower, and middle ear respiratory tracts are a complex association of bacteria, irreversibly attached to the mucous surface and enclosed in an adherent extracellular matrix, originating from both the host and the bacteria. The biofilm environment contains low level of oxygen and is not perfused by arterial blood, making it inaccessible to systemic antibiotics. These biofilms are likely to be present in patient presenting CRS, including those with PCD, and they are a reservoir of bacteria and mediators that contribute to systemic inflammation and recurrent respiratory tract infections. Therefore, some studies suggest that the sinuses can be considered as a bacterial reservoir that can infect the upper and lower respiratory tract, and that the incorporation of nasosinus bacteriological analyzes to guide the choice of medical and surgical treatments could improve the outcome of PCD patients. In these patients, the bacteria most commonly found in the sinuses are Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pneumoniae (157,165,200).

Management of otological complications of PCD aims to improve patients' hearing, aiming to avoid the possible sequelae of long-term hearing loss (cognitive disorders, language development disorders and academic delay), as well as to prevent the sequelae of chronic otitis in the tympanic membranes and middle ear (tympanosclerosis, retraction pockets or atelectasis of the eardrums, cholesteatoma, erosion of the ossicles). Chronic otitis media with effusion being very common in patients with PCD, especially in young children, hearing should therefore be monitored regularly using age-appropriate methods (160). Oral antibiotic treatment is recommended to treat acute otitis media (118). Use of trans-tympanic aerators for the treatment of OSM is controversial in the management of PCD, as these could be responsible for persistent and intractable otorrhea, causing discomfort and worsening hearing loss. European guidelines recommend to avoid the use of trans-tympanic ventilators in the management of patients with PCD, and treating hypoacusis by hearing aids and a regular follow-up in ENT consultations (102). Furthermore, no cases of cholesteatoma were found in PCD children with Chronic otitis media with effusion managed without trans-tympanic aerators (201). Antibiotics and local and / or general corticosteroids may be offered in the face of debilitating otorrhea, the choice of antibiotic having to be adapted according to the results of the bacteriological culture of the otorrhea.

Avoiding ototoxic antibiotics is important to prevent cumulative toxicity. PCD patients often experience recurrent infections from a very young age, requiring frequent antibiotic treatments. The repeated use of ototoxic antibiotics increases the risk of hearing loss over time. Since these patients are already predisposed to chronic respiratory issues, exposure to ototoxic drugs can exacerbate long-term complications. It is essential to carefully consider alternative antibiotics with a lower risk of ototoxicity and to closely monitor for any auditory changes during treatment. Early identification and prevention of ototoxicity can help preserve hearing function, which is vital for overall quality of life in PCD patients. Self-insufflation devices, also called nasal balloons, can also be used for the management of chronic otitis media with effusion, with limited evidence of benefit however (118,157,202–204).

Potential new therapies

The development of potential new therapies for PCD aims to restore ciliary function, ultimately leading to curative treatment options for patients. Given the heterogeneous nature of PCD, it is crucial to fully characterize both the genotype and phenotype of affected individuals, paving the way for personalized medicine (205). Despite advances in the characterization of PCD, research into new therapies remains limited, primarily due to the lack of effective models for studying and testing these therapies (206).
Emerging therapeutic approaches for PCD fall into four main categories:

I) **Gene Therapy:** Two studies on gene therapy for PCD have been published (205). The first study used a lentiviral vector containing DNAI1 cDNA to restore ciliary beating in DNAI1-deficient human airway epithelial cells (207). DNAI1, a component of the outer dynein arm (ODA), is involved in ciliary beating, and mutations in DNAI1 contribute to 10-14% of PCD cases (208). The study demonstrated partial restoration of ODA presence and ciliary motility. The second study by Ostrowski et al. involved a mouse model with an inducible deletion of the Dnaic1 gene (the murine homolog of DNAI1) to test gene transfer (209). By using a lentiviral vector, the authors partially restored Dnaic1 expression and ciliary activity in Dnaic1-/- mouse cells. Although CBF

was comparable to positive controls, the percentage of corrected cells remained limited (209). However, applying classical gene therapy in PCD presents significant challenges, including the large size of the PCD genes, differences in promoters, and the potential risks of host genome integration, such as off-target effects, immune responses, and safety issues (205).

II) **Gene Editing:** Gene editing represents a promising approach for PCD treatment, as it involves editing the specific defective portion of the gene without consideration of gene size. Lai et al. explored gene editing using transcription activator-like effector nucleases (TALENs) to target the DNAH11 gene (210). DNAH11 encodes a component of the dynein heavy chain in the ODA, and mutations in this gene contribute to approximately 6% of PCD cases associated with abnormal ciliary motility. The study demonstrated partial restoration of DNAH11 protein presence and ciliary activity in mutant epithelial cells, highlighting the potential of gene editing therapy (210). While both gene therapy and editing have shown feasibility in restoring ciliary function, challenges such as efficient transfection rates, immune responses, and achieving sustained long-term expression need to be addressed (205).

III) **Read-through therapy:** Read-through therapy is being investigated for PCD patients with nonsense mutations resulting in premature termination codons, which account for approximately 28% of cases (211). Eloxx Pharmaceuticals, in collaboration with Amsterdam University Medical Center and University Medical Center Utrecht, is conducting in vitro studies on read-through therapeutics for these patients (205).

IV) **mRNA-Based therapy:** mRNA-based therapies offer a reversible approach that does not directly alter genomic DNA. However, to be effective, the mRNA must be successfully delivered across the target cell membrane, requiring a specialized vector for safe transport. ETHRIS, a German biotechnology company, is developing an mRNA therapy for PCD involving the restoration of

the CCDC40 protein using a stabilized non-immunogenic mRNA (SNIM® RNA) platform (205,212). CCDC40 plays a role in the interaction between MTDs and RSs, and mutations in this gene are responsible for approximately 10% of PCD cases characterized by defective ciliary beating (205,213). Preliminary findings are encouraging, although the therapy remains in preclinical development (205,212). ReCode Therapeutics has developed a non-viral lipid nanoparticle (LNP) platform for the safe delivery of mRNA to target respiratory diseases. Their preclinical program targets PCD patients with biallelic DNAI1 variants. Recent results showed that LNP-derived DNAI1 mRNA aerosolized into cell culture successfully restored ciliary function in PCD cell models, even in the presence of mucus (205,212).

1.4.3 PCD diagnosis

Clinical features

The clinical presentation of PCD can overlap with other conditions, such as immunodeficiencies, CF, and recurrent respiratory infections, making the identification of potential PCD patients challenging (214). To address this, high-risk individuals (those with affected siblings or symptoms suggestive of PCD) should be prioritized for diagnostic testing (104). Various studies have proposed clinical criteria to guide referrals for PCD testing, leading to consensus recommendations by the ERS. According to these guidelines, PCD testing is advised in patients with an affected sibling or in those presenting with multiple clinical features**,** including:

- Persistent wet cough
- Situs abnormalities
- Congenital cardiac defects
- Persistent rhinitis
- Chronic middle ear disease, with or without hearing loss

- Neonatal respiratory symptoms in term infants
- Admission to a neonatal intensive care unit

In addition to these clinical indicators, predictive tools play a crucial role in identifying patients who warrant diagnostic testing. While the **PICADAR score** has been previously discussed as a valuable predictive model, other tools have also been developed to address limitations in certain patient populations and improve diagnostic accuracy.
The **modified PICADAR score (M-PICADAR)**, introduced to overcome challenges in recalling neonatal predictors during adulthood, offers a refined approach. In this version, neonatal respiratory symptoms and neonatal unit admission are consolidated into a single criterion (2 points), and gestational age is excluded. The M-PICADAR score ranges from 0 to 10 points, with a cut-off of ≥ 2 achieving 100% sensitivity and 89% specificity for identifying patients with PCD (215).

Another predictive model is the **North American Criteria Defined Clinical Features**, developed by Leigh et al. (125). This tool focuses on four key clinical features: laterality defects, unexplained neonatal respiratory distress, early-onset annual nasal congestion, and early-onset annual wet cough. A score of ≥ 2 effectively discriminates PCD cases, with sensitivity ranging from 80% to 92% and specificity between 46% and 72%, depending on the study. These criteria have been incorporated into the American Thoracic Society (ATS) Clinical Practice Guidelines (214).

The **Clinical Index**, designed by Martinu et al. (216), provides another option for assessing the probability of PCD. This seven-item questionnaire evaluates key early-life respiratory symptoms, such as neonatal respiratory distress, rhinitis, pneumonia, chronic bronchitis, and recurrent otitis. A score of ≥ 4 achieves a sensitivity of 96% and specificity of 72%, offering a reliable tool for identifying at-risk individuals (216).

In summary, while the PICADAR score remains a key predictive tool, other models, such as M-PICADAR, the North American Criteria, and the Clinical Index, provide complementary approaches to identifying high-risk patients. These tools, combined with

clinical criteria, facilitate early referral for diagnostic testing and ensure timely management of PCD.

Ciliary Ultrastructural Analysis via TEM

Ciliary ultrastructure defects characteristic of PCD are frequently visualized using TEM, which has historically been a cornerstone of PCD diagnostics since its introduction in 1976. Classification of PCD based on ultrastructural subtypes has advanced the understanding of its phenotypes, but it is important to note that approximately 30% of individuals with PCD exhibit normal axonemal ultrastructure, necessitating alternative diagnostic methods in these cases (104). TEM is performed on respiratory epithelial samples collected from the inferior nasal turbinate via brush or curette biopsy or from the lower respiratory tract using brush biopsy during bronchoscopy. These samples are chemically fixed, embedded, thinly sectioned using an ultramicrotome, stained, and then analyzed with TEM to assess transverse ciliary structures. Recently, international consensus guidelines for interpreting TEM results have been established (170).

Class 1 defects, which are classic for PCD, can confirm the diagnosis when combined with clinical features (170). These defects include outer dynein arm (ODA) defects, combined outer and inner dynein arm (ODA+IDA) defects, and inner dynein arm defects with microtubular disorganization (IDA+MTD) (170). ODA defects, associated with variants in genes encoding structural or docking proteins, are present in 26–59% of PCD patients (104,171–173). ODA+IDA defects, linked to genes involved in dynein assembly, are identified in 6–39% of patients evaluated via TEM. Meanwhile, IDA+MTD defects are typically associated with variants in CCDC39 or CCDC40 genes (168,174,187,217).

Class 2 defects, although not definitive, may support a PCD diagnosis if observed across multiple samples and corroborated by genetic testing (170). Central complex abnormalities, arising from mutations in radial spoke proteins (e.g., RSPH4A, RSPH1, RSPH9, DNAJB13), may manifest as the absence of the central microtubule pair, translocated outer microtubules, or misplaced outer doublets. However, these findings can also result from airway epithelial damage, leading to secondary anomalies such as compound cilia, axonemal blebs, and irregular microtubules (104,170,218).

Oligocilia, characterized by the displacement of basal bodies into the cytoplasm rather than their typical apical docking position, is associated with genetic mutations that impair the generation of multiple motile cilia (e.g., CCNO and MCIDAS) (43,170,219). In such cases, the few visible cilia generally exhibit normal ultrastructure, complicating differentiation from inadequate sampling. Additionally, isolated IDA abnormalities are not specific to PCD as they can also occur following recent respiratory infections or epithelial injuries in healthy individuals (104,177). TEM has high specificity for PCD when classic abnormalities are identified, capturing approximately 70% of cases (170). However, its sensitivity is limited, with 30% of PCD patients showing no ultrastructural defects. Consequently, normal TEM findings cannot definitively exclude PCD, and further diagnostic tests are necessary when clinical suspicion persists (103,104). Moreover, false positives can occur due to acute infections or environmental exposures (103). Cell culture techniques may mitigate secondary ciliary alterations and improve diagnostic accuracy (170,178).

Challenges associated with TEM include technical expertise, difficulty obtaining adequate samples, and interpreting results. Insufficient numbers of cilia in biopsies, especially in cases with subtle ciliary defects or oligocilia, may result in missed diagnoses (103). Up to 40% of biopsies may have inadequate ciliary content for TEM analysis (173,205,212). Furthermore, TEM evaluation requires specialized experience, though recent international guidelines have standardized interpretation practices. Emerging methods like cryotomography or advanced image processing may enhance structural visualization but remain experimental (206,207).

Genetic Testing for PCD-Related Genes

Primary ciliary dyskinesia (PCD) is a genetically diverse disorder, with most of the 54 known causative genes encoding components of ciliary ultrastructure or proteins involved in their assembly (220). While the majority of these genes follow autosomal recessive inheritance patterns, autosomal dominant (e.g., FOXJ1, TUBB4B) and X-linked inheritance patterns (e.g., OFD1, RPGR, DNAAF6) have also been identified. Genetic testing is highly specific when known pathogenic variants are detected, and its sensitivity has steadily improved as additional genes are incorporated into commercially available

panels (103). However, despite the expanding list of causative genes, 20–30% of patients with a confirmed PCD phenotype and other diagnostic findings lack identifiable pathogenic variants in currently known genes (50). As a result, negative genetic testing cannot definitively rule out PCD.

Efforts to expand the list of PCD-associated genes include investigating deep intronic and non-canonical splicing variants, which are often missed by standard genomic DNA analysis (124,214,221). Genetic testing is becoming more accessible due to advancements in sequencing technology and a significant reduction in associated costs (221). However, availability and affordability remain challenges in certain healthcare systems and resource-limited settings (222).

Multiple genetic testing strategies are currently employed. Commercially available multigene panels are widely used, offering a balance between comprehensiveness and cost-effectiveness (50). These panels typically target the most relevant genes and may include analyses for conditions with overlapping clinical features. Panels that incorporate both sequence analysis and assessments for large deletions or duplications are recommended for optimal diagnostic yield (50). Targeted testing is another option, particularly for families with known pathogenic variants or for patients whose ethnic or ancestral backgrounds suggest specific genetic predispositions (50,215). However, targeted approaches and multigene panels may not capture all genetic causes of PCD (125). When clinical suspicion remains high after negative panel results, more comprehensive methods such as whole-exome sequencing or whole-genome sequencing can be pursued (50,221). These broader approaches may not only confirm a PCD diagnosis but also uncover alternative diagnoses, such as inborn errors of immunity, which share overlapping clinical features with PCD (221,223).

Despite its advancements, genetic testing has limitations. For instance, the identification of two or more variants of uncertain significance within a single PCD-associated gene or across different genes is insufficient to confirm a diagnosis (50). Nevertheless, genetic testing has become a cornerstone in PCD diagnostics, often serving as a first-line investigation. Additionally, as genotype-phenotype correlations become better understood, genetic testing may provide valuable prognostic insights for patients and healthcare providers (12,34,224). In the future, certain genetic mutations may be amenable

to precision therapies aimed at restoring ciliary function, highlighting the potential for personalized treatment approaches.

Measurement of nNO

NO is a highly reactive gaseous molecule that plays numerous signaling roles in the respiratory tract (225). It is produced throughout the airways, with particularly high concentrations in the nasal sinuses (225). The biosynthesis of NO is typically upregulated during infection through increased transcription and activity of inducible nitric oxide synthase (iNOS) (226,227). Despite recurrent respiratory infections, nasal nitric oxide concentrations are significantly lower in the majority of patients with PCD compared to healthy individuals (227). For this reason, nasal NO is routinely used in clinical practice as a screening test for PCD (227). While the association between low nasal nitric oxide levels and PCD has been recognized for over 15 years, the underlying mechanisms of this phenomenon remain unclear (227).The nasal NO measurement is simple, non-invasive, and painless. No preparation is required, although children who can do so should clear their nasal passages before the test (227,228). The procedure typically takes only a few minutes (227,228). During the test, a small plastic nasal probe connected to an analyzer is placed at the entrance of one nostril, where it collects exhaled air for a few seconds (227,228). The collected air is then analyzed (227,228). The test is repeated at least twice, in each nostril, by a respiratory physiotherapist experienced with children who have chronic respiratory conditions. Depending on the child's age and abilities, they may be asked to either: Exhale slowly and steadily against resistance (children over 5 years old and adults), or breathe normally (children aged 2 to 5 years) (227,228). The test is generally not performed in children under 2 years of age (227,228).

In the literature, it is well established that some patients with PCD typically exhibit low nasal NO levels, generally below 250 ppb. However, there are instances of PCD patients with normal to high nasal NO levels, and other conditions can also result in reduced nasal NO levels, including cystic fibrosis, bronchiolitis, chronic sinusitis, nasal polyps, and both active and passive smoking (226). Therefore, relying solely on nasal NO levels is neither sufficiently sensitive nor specific for diagnosing PCD (226). The discovery that

individuals with PCD exhibit significantly lower nNO levels compared to healthy individuals and those with other diseases has established nNO as an adjunctive diagnostic tool for PCD. Nitric oxide (NO) is produced by nitric oxide synthases in epithelial cells near the basal bodies of cilia, provided appropriate cofactors and substrates, such as L-arginine and oxygen, are available (229). The majority of nNO in exhaled air originates from the sinuses and upper airways (225), and while NO is believed to play a role in ciliary motion, the precise mechanism behind reduced nNO production in PCD remains unclear (229).

The measurement of nNO utilizes chemiluminescence, where the emitted light is proportional to the NO concentration in a gas sample. Although electrochemical analyzers are available, they have yet to be fully validated as diagnostic tools for PCD. Testing in older, cooperative patients is performed during steady, low-flow exhalation with vellum closure to prevent contamination from lower airways, using a nasal catheter to collect gas samples. This method is generally applicable for children aged five years or older for ATS criteria, or six years or older according to European Respiratory Society (ERS) guidelines (227,230). For younger children who cannot perform vellum closure, tidal breathing measures of nNO are utilized (231). Normative nNO values are currently only available for individuals aged five years and older. To standardize reporting across devices with varying flow rates, nNO values are ideally expressed in nanoliters per minute (nL/min) rather than parts per billion (ppb) (232).

In chemiluminescence analyzer testing, an nNO cutoff of less than 77 nL/min has been shown to effectively differentiate PCD patients from healthy controls, as well as from individuals with asthma or chronic obstructive pulmonary disease (COPD) (232) . Sensitivity and specificity estimate for nNO testing range between 0.90–1.0 and 0.75–0.97, respectively (230). Despite these robust characteristics, nNO testing serves as an adjunctive tool rather than a definitive diagnostic measure, and individuals with reduced nNO levels require further confirmatory testing. Although nNO measurement is relatively inexpensive for centers equipped with the necessary technology, the acquisition of chemiluminescent analyzers remains costly, and these devices are not yet approved by the U.S. Food and Drug Administration for this specific use (214). Additionally, strict adherence to standardized testing protocols is essential to ensure accurate results (227). Non-diagnostic results, where

nNO levels exceed the diagnostic threshold of 77 nL/min, have been observed in patients with variants in PCD-related genes such as *RSPH1, FOXJ1, CCNO, GAS8, CCDC103, CFAP221, STK36, RPGR, DNAH9, GAS2L2, NEK10, SPEF2, HYDIN, TTC12, RSPH4A,* and *LRRC56* (214,228,233–235). Consequently, strong clinical suspicion of PCD warrants additional diagnostic investigations (102).

False-positive results with low nNO levels can occur in other conditions that share clinical features with PCD, such as diffuse panbronchiolitis (236) and CF (153,226,232). North American guidelines recommend excluding CF prior to evaluating for PCD. Furthermore, individuals with inborn errors of immunity may present with low nNO levels, and the overlapping features of chronic suppurative lung disease and recurrent infections can complicate the differentiation between PCD and immunodeficiency disorders (237,238). Temporary reductions in nNO can also arise during acute viral respiratory or bacterial sinus infections (239,240), highlighting the importance of repeat testing on separate occasions to confirm persistently low nNO levels (214,227).

Immunofluorescence (IF)

Immunofluorescent staining, initially a research tool for nearly two decades, has more recently been proposed as a diagnostic method for PCD (102,214,241,242). This technique allows for the localization of target proteins within the cilia of respiratory epithelial cells, enabling confirmation of the absence of proteins integral to ciliary ultrastructure. Various approaches have been developed, including the use of antibody panels to identify PCD ultrastructure subtypes based on the presence or absence of specific staining patterns (241,243). For instance, antibodies targeting DNAH5 identify ODA, DNALI1 targets IDA, radial spoke proteins such as RSPH1, RSPH4A, and RSPH9 are used for radial spoke analysis, and GAS8 identifies the nexin–dynein regulatory complex(213,241,242,244–246). These staining patterns guide targeted genetic analyses of specific genes associated with ultrastructural defects, further confirming the diagnosis (246).

Immunofluorescent staining has shown comparable accuracy and sensitivity to TEM (241,244). It can also detect certain loss-of-function and missense variants (246). Notably, this method requires fewer ciliated cells for analysis than TEM, making it a

suitable alternative for epithelial biopsy samples that would otherwise necessitate repetition (241). Additionally, immunofluorescence processing is faster and less costly than TEM (241). Costs can be further reduced by adopting a tiered approach, where a second panel of antibodies is applied only if no abnormalities are detected in the initial panel (241,244,247). This cost-effectiveness, combined with its diagnostic potential, makes immunofluorescent staining particularly valuable in lower-resourced settings where advanced diagnostic tools or expertise may be inaccessible (241,244).

With the ongoing identification of additional antibodies targeting ciliary structural proteins, antibody panels could be expanded in the future, potentially capturing a larger proportion of PCD patients (241,247,248). However, similar to TEM, immunofluorescent staining has limitations in diagnosing PCD cases with normal ciliary ultrastructure. Therefore, it cannot be used as a standalone diagnostic tool (241,244).

High-Speed Video Microscopy (HSVM)

High-speed video microscopy analysis (HSVMA) is a diagnostic technique that utilizes a high-speed digital video camera attached to a microscope to evaluate the movement of cilia on epithelial cells. These cells are obtained through brush or curettage biopsies of the inferior nasal turbinate or bronchus (7,102). The recorded frames are played back at slower rates to assess ciliary motion patterns and beat frequency, allowing for a direct evaluation of ciliary function and movement (7,102). Visualization can occur directly or after cells are cultured under air-liquid interface conditions to minimize the impact of secondary ciliary defects (7,102,249). Key results from this method include qualitative descriptions of the CBP, CBF, and assessments of particle clearance (102,250–252). Some groups have also developed quantitative measures and computer programs to analyze ciliary movement to reduce subjectivity (251,253).

HSVMA provides a functional assay for diagnosing PCD, particularly in individuals without identified genetic or ultrastructural defects (102,254). Additionally, recordings can be reviewed by experts for diagnostic confirmation or utilized in research (102). Findings from HSVMA correlate with specific ultrastructure defects and genotypes (250–252). Common findings in PCD include immotile cilia with slow, short, stiff

flickering patterns, and dyskinetic patterns characterized by minimal but highly disorganized beating, often associated with ODA or ODA+IDA defects (250–252). IDA and IDA+MTD defects typically exhibit a stiff forward power stroke with reduced amplitude, although immotile cilia are also observed (251,252). Rotational beat patterns have been linked to central complex and radial spoke defects (250). Importantly, CBF analysis should always be combined with CBP assessments, as patients with PCD may exhibit increased, decreased, or normal beat frequencies depending on their genotype (250).

Despite its utility, HSVMA is not a gold standard for PCD diagnosis. It may fail to identify individuals with genotypes exhibiting nondiagnostic, normal, or subtle changes in HSVMA findings (e.g., HYDIN variants) (70,250). Similarly, genotypes that impair ciliogenesis, such as CCNO or MCIDAS variants, are challenging to detect using this method (255). The technique also requires considerable expertise and training, which limits its availability (102,214). While sensitivity and specificity are high when performed by experts, interobserver agreement decreases for inconclusive or atypical findings (102,254,256). Additionally, genetic confirmation has not been consistently incorporated into studies evaluating HSVMA's diagnostic performance (254,256).

Challenges to the widespread adoption of HSVMA include the lack of standardization in result interpretation and variability in cell processing and culture techniques across centers (102). Inadequate samples and inconclusive results that necessitate repeat sampling are not uncommon, and cell culture success rates vary significantly (249,256). The high cost of the required equipment further limits its routine use, particularly in resource-constrained settings (256). As such, while HSVMA has been integrated into some European and Canadian diagnostic guidelines, it is not universally available and has not been standardized or validated to the extent required for broader implementation. Consequently, the ATS diagnostic guidelines for PCD do not recommend HSVMA as a routine diagnostic tool (195,214).

Furthermore, To date, there is no universally accepted standardization of reference values for ciliary beat frequency and pattern as assessed by DHSV. While several studies have proposed normative data, these are typically based on small cohorts and vary considerably in methodology, including sample collection, temperature control, and

analysis protocols. As a result, reported reference values for normal ciliary function differ substantially from one publication to another, limiting their reproducibility and clinical applicability across diagnostic centers. This heterogeneity has been consistently highlighted in the literature, which emphasizes the lack of large-scale studies establishing robust normative benchmarks for DHSV assessment (257,258). Recent international guidelines also underline the absence of harmonized standards and the need for further efforts to unify interpretation criteria (259). This diagnostic variability remains a significant challenge in the accurate and consistent identification of primary ciliary dyskinesia.

Additional Diagnostic Methods to Enhance PCD Detection

Cell culture

The culture of airway epithelial cells can enhance the diagnostic accuracy of PCD, particularly by helping to differentiate true PCD cases from false positives. Factors such as acute or chronic respiratory infections, inflammation, and environmental or demographic influences can transiently induce secondary ultrastructural or functional abnormalities in cilia (260). Unlike genetic causes, these secondary abnormalities typically resolve after cell culture, reinforcing its value in confirming a PCD diagnosis (249,261,262).

There are two primary techniques for culturing ciliated cells: suspension cell culture (notably spheroid cell culture) and air-liquid interface (ALI) cell culture (249,263–265). While both methods aim to regenerate cilia by promoting ciliogenesis on the apical surface of cultured cells, they differ in their approaches and applications. Spheroid cell culture is particularly suited for assessing CBF and coordination, as it enables evaluation through spheroid rotation and the migration of fluids or debris. In contrast, ALI cell culture allows for a comprehensive functional assessment of cilia, including both CBF and CBP, similar to the evaluations performed on freshly brushed samples. This is achieved by mechanically detaching cells from the newly formed airway epithelium (249,263).

Despite their diagnostic advantages, both methods have limitations. They are resource-intensive, requiring significant expertise and time. Spheroid cell culture is generally faster than ALI cell culture but does not provide the same depth of functional

assessment (227,263). These challenges underscore the need for careful implementation in clinical practice.

To ensure the reliability of ciliary videomicroscopy for PCD diagnosis, the ERS consensus recommends repeating both functional and structural evaluations of cilia (via ciliary videomicroscopy and TEM, respectively) after cell culture (102,260). This approach minimizes the risk of misdiagnosis due to transient secondary ciliary abnormalities and reinforces the accuracy of the diagnostic process.

Genotype–Phenotype Relationships in PCD

Efforts to characterize genotype-phenotype relationships in PCD have gained momentum with the recognition of an increasing number of genes impacting ciliary structure and function, as well as the variability in clinical presentations. Historically, PCD classifications relied on groupings based on ultrastructural changes; however, not all genetic mutations associated with similar ultrastructural defects produce identical clinical manifestations. For instance, variants within the same gene can result in differing phenotypes depending on whether they cause a complete loss of protein function or only a reduction in its activity (266,267) (Table 4).

Gene	Reference	Ciliary component affected	Ultrastructural defect	Ciliary beat pattern
DNAH5	[3]	ODA: Heavy-chain dynein	Absent or shortened ODA	Mostly immotile with some residual movement
DNAH11	[74]	ODA: Heavy-chain dynein in proximal region of the axoneme	Normal Absent heavy chain in proximal portion in ET	Hyperkinetic, reduced amplitude with stasis in the proximal portion and/or completely immotile
DNAI1	[82]	ODA: Intermediate-chain dynein	Absent ODA	Immotile
DNAI2	[83]			
DNAL1	[84]	ODA: Light-chain dynein	Absent ODA	Impaired motility
RSPH1 RSPH4A RSPH9	[86]	Radial spoke head proteins	Intermittent central-pair loss and transposition	Circling on top view
RSPH3	[85]	Radial spoke component (unknown)	Reduction in number of radial spokes, intermittent central MT pair abnormalities	Some immotile cilia Motile cilia with reduction in beat amplitude
CCDC39	[87]	Nexin–Dynein Regulatory Complex assembly	IDA and microtubule disorganization	Stiff cilia with reduced amplitude
CCDC40	[88]			
CCDC65	[89]	Nexin–Dynein Regulatory Complex	Subtle microtubule disorganization	Hyperkinetic, subtle reduction in beat amplitude
CCDC164	[73]			
GAS8	[90]	Nexin–Dynein Regulatory Complex	Normal/Subtle microtubule disorganization	Subtle reduction in beat amplitude
HYDIN	[10]	Central microtubular pair projection protein	Majority of cilia normal, some central pair abnormalities. Absent C2b projection in ET	Discoordinated
CCDC103	[75]	Outer dynein arm assembly factor	Variable: Absent IDA + ODA Missense mutation Partial absence IDA + ODA Normal ultrastructure	Variable: Immotile cilia Missense mutation reduced amplitude, static patches, and normal beat pattern
CCDC114	[9]	ODA docking complex	Absent ODA	Immotile
CCDC151	[91]			
ARMC4	[92]			
ZMYND10	[93]	Proteins involved in cytoplasmic assembly of dynein arm complexes ('preassembly' proteins)	Absence or partial absence of IDA + ODA	Immotile
LRRC6	[94,95]			
DNAAF5 (HEATR2)	[96]			
C21ORF59	[97]			
DNAAF1 (LRRC50)	[98]			
DNAAF2 (KTU)	[73]			
DNAAF3	[99]			
DYX1C1 (DNAAF4)	[100]			
SPAG1	[101]			
TXND3 (NME8)	[102]	Component associated with ODA	Heterogeneous ultrastructure: normal, absent, or shortened ODA	Immotile
CCNO	[103]	Factors involved in human multiciliated cell differentiation	Ciliary depletion (1 or 2 per cell) CCNO: mislocalized basal bodies MCIDAS: mislocalized basal bodies additional lack of axonemal components	Reduced cilia numbers. May be misinterpreted as secondary ciliary absence
MCIDAS	[104]			
OFD1	[105]	PCD co-segregation with other syndromes: RPGR – associated with X-linked retinitis pigmentosa: OFD1: centrosome and basal body protein – associated with X-linked orofacialdigital syndrome	Normal ultrastructure, RPGR: disturbed cilia orientation	OFD1: disorganized ciliary beat
RPGR	[106]			

ODA: Outer dynein arms, IDA: inner dynein arms, MT: microtubules, ET: electron tomography; RPGR: retinitis pigmentosa GTPase regulator.

Table 4: Association Between Genetic Mutations, Ciliary Ultrastructural Defects, and Abnormal Ciliary Beating Patterns. Dehlink E, Hogg C, Carr SB, Bush A. Clinical phenotype and current diagnostic criteria for primary ciliary dyskinesia. Expert Rev Respir Med. 2016 Nov;10 (11):1163–75.© copyright 2016, reprinted by permission of Informa UK Limited, trading as Taylor & Francis Group, https://www.tandfonline.com (268).

Patients with variants in *CCDC39* and *CCDC40*, genes encoding molecular rulers essential for ciliary assembly, show more pronounced lung function decline compared to other genotypes. These variants, which lead to IDA and MTDs defects, are associated with more severe reductions in spirometry and LCI and are often linked to extensive bronchiectasis and mucus plugging observed on CT scans (143,269,270). In contrast, individuals with *DNAH11* variants exhibit relatively preserved lung function over time, possibly due to a less severe impact on ciliary function, despite normal ultrastructure on TEM (269,270).

Laterality defects, including situs inversus totalis (SIT) and situs ambiguus (SA), are typically observed in PCD patients with genes affecting nodal ciliary structure and assembly. Genes like *CCNO* and *MCIDAS*, which do not play roles in nodal cilia, are not associated with laterality defects (55,56,235). Conversely, mutations in genes such as *DNAH5* and *CCDC103*, which encode components of ODA, and *CCDC39* and *CCDC40* (IDA+MTD defects), frequently result in laterality abnormalities (271). Interestingly, some variants, like those in *FOXJ1*, contribute to both motile and nodal ciliary dysfunction, underscoring their involvement in laterality determination (271).

Subfertility is another area where genotype-phenotype relationships are evident. Genes such as *CCDC39*, *CCDC40*, and *DNAH5* are expressed in both respiratory epithelial cells and reproductive tissues, contributing to male and female infertility in PCD patients (111,178). In contrast, mutations in genes like *RSPH4A*, which are expressed at lower levels in testicular tissues, are associated with reduced male fertility but less severe reproductive consequences overall (111).

Certain PCD-related genotypes are linked to syndromic presentations or overlapping features with other ciliopathies. For instance, *OFD1* mutations cause oral-facial-digital syndrome type I, characterized by dysmorphic features and intellectual disabilities, alongside classic PCD manifestations such as recurrent respiratory infections and laterality defects (268). Similarly, mutations in *RPGR*, primarily associated with retinitis pigmentosa, may also lead to recurrent pulmonary infections and bronchiectasis, though without laterality abnormalities (273).

International Consensus and Guidelines for PCD Diagnosis

The ERS Task Force (2017) and the ATS Task Force (2018) have published evidence-based guidelines for the diagnosis of PCD, each with distinct diagnostic algorithms and criteria (102,195,214).

ERS Guidelines

The ERS guidelines classify PCD diagnosis into three categories: "confirmed," "highly likely," and "highly unlikely." The diagnostic pathway involves three sequential steps (101):

1. Step One: nNO and DHSV Testing
    - Normal results for both nasal nitric oxide (nNO) and DHSV suggest PCD is "highly unlikely."
    - Abnormal results in either test necessitate repeating the tests or proceeding to Step Two.
2. Step Two: TEM
    - Hallmark ultrastructural ciliary defects identified by TEM confirm a PCD diagnosis.
    - If TEM results are normal, clinicians are advised to repeat DHSV testing after cell culture or consider genetic testing in Step Three.
    - If all post-culture results are normal, PCD is "highly unlikely." Conversely, low nNO combined with suggestive DHSV findings after cell culture indicates PCD is "highly likely."

3. Step Three: Genetic Testing
    - Pathogenic bi-allelic mutations in PCD-associated genes confirm the diagnosis.
    - Normal or inconclusive results warrant follow-up testing as diagnostic technologies advance.

ATS Guidelines

The ATS guidelines use a binary diagnostic framework: PCD is classified as either "positive" or "unlikely." The diagnostic process includes four steps (214):

1. Step One: Clinical History
    - Strong clinical suspicion (assessed using the North American Criteria Defined Clinical Features) leads to Step Two.
    - Patients with low/moderate suspicion are deemed PCD-negative.
2. Step Two: nNO Testing
    - Repeated low nNO values are considered diagnostic for PCD, provided cystic fibrosis (CF) is excluded and nNO is measured during velum closure using a chemiluminescence analyzer in cooperative patients aged ≥5 years.
    - Normal nNO values make PCD "unlikely," though genetic testing may be recommended for patients with strong clinical features.
    - If nNO testing is unavailable, proceed to Step Three.
3. Step Three: Genetic Testing
    - Pathogenic bi-allelic mutations confirm PCD.
    - Single or absent pathogenic variants require further evaluation in Step Four.
4. Step Four: TEM
    - Hallmark ultrastructural defects confirm PCD.
    - Normal TEM results do not exclude PCD; repeated testing is advised if samples are inadequate or results inconclusive.

Comparison of Guidelines

Both the ERS and ATS guidelines recognize hallmark ultrastructural defects on TEM and/or non-ambiguous bi-allelic mutations in PCD-associated genes as definitive for diagnosis (102,214). However, the ATS guidelines also accept repeated low nNO levels as diagnostic, provided CF is excluded and specific testing protocols are followed. Unlike the ERS guidelines, the ATS does not include ciliary videomicroscopy in its diagnostic algorithm (214).

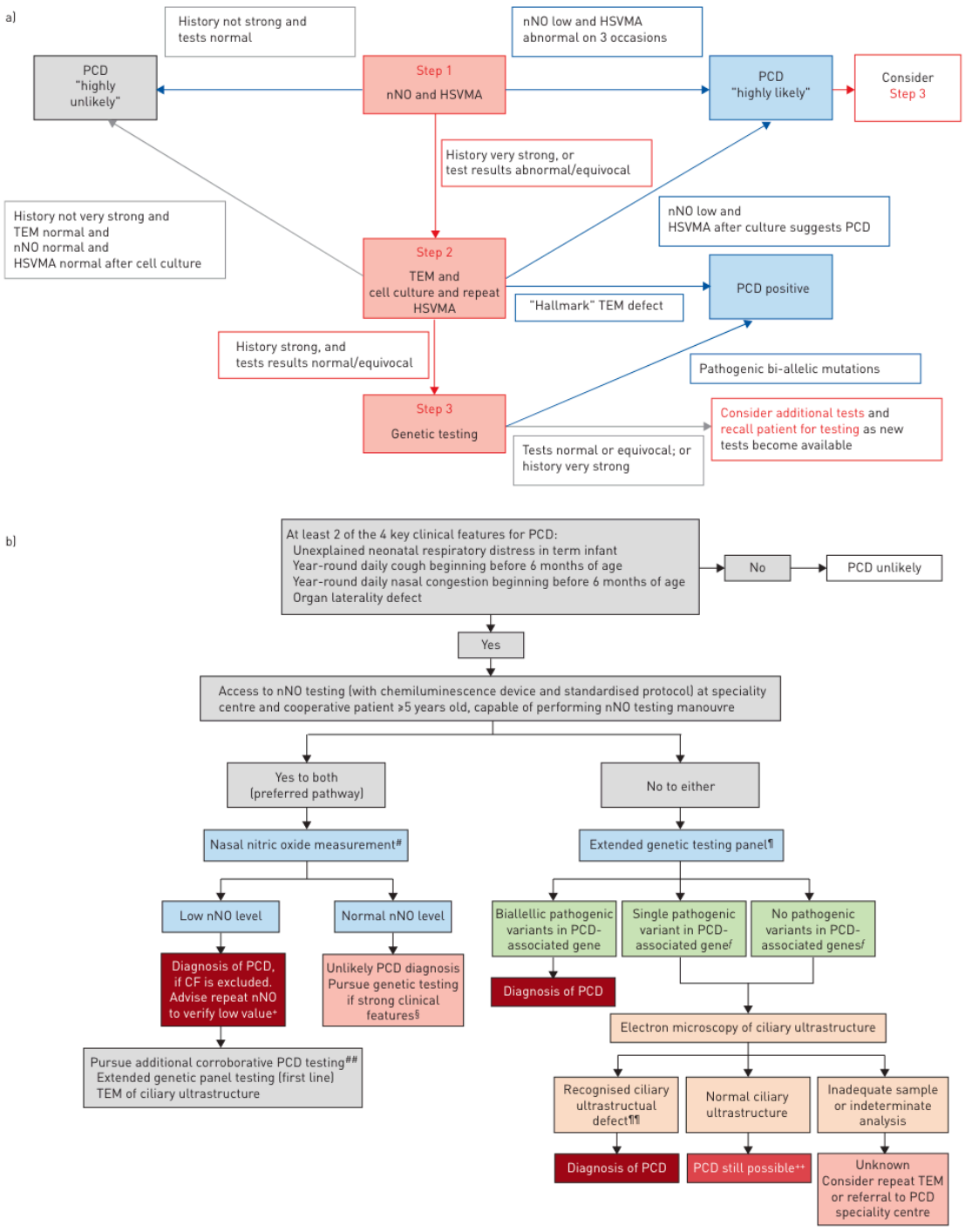

Figure 12: Diagnostic algorithm of primary ciliary dyskinesia (A) according to the European Respiratory Society guidelines and (B) according to the American Thoracic Society guidelines. Shoemark A, Dell S, Shapiro A, et al. ERS and ATS diagnostic guidelines for primary ciliary dyskinesia: similarities and differences in approach to

diagnosis. Eur Respir J 2019; 54: 1901066 [https://doi.org/10.1183/13993003.01066-2019].Reproduced with permission of European respiratory journal © ERS 2025 (195)

Limitations

Both task forces acknowledge that no single test or combination of tests can definitively rule out PCD in all cases, especially for patients with strong clinical suspicion (based on tools like PICADAR for ERS and the North American Criteria for ATS) (195). These findings underscore the need for comprehensive diagnostic approaches and ongoing refinement of tools and protocols to enhance diagnostic accuracy.

1.5 Ciliary functional analysis (CFA)

DHSV employs a high-speed digital video camera (120–500 frames per second) attached to a microscope, enabling real-time recording of ciliary beating. This system provides the capability for detailed analysis through slow-motion replay, creating recordings that can be used for diagnostic evaluation, expert consultation, and research purposes (7,102). The ciliary motion is observed from three planes: a sideways profile, a frontal view, and a top-down perspective (274). Using DHSV, a comprehensive CFA can be conducted, encompassing evaluations of CBF and CBP, either manually or with computer-assisted methods (275,276).

Traditionally, manual evaluation of CBF involves measuring the time for a cilium or group of cilia to complete 10 beat cycles. Kempeneers et al. demonstrated that evaluating five cycles instead of ten yields comparable accuracy, reducing the time required for manual analysis (274). CBP evaluation, integral to DHSV, characterizes motion patterns across the ciliated epithelium and requires high-speed recording at a minimum of 400 frames per second. Three abnormal CBPs have been described: immotile cilia (static), stiff cilia with reduced amplitude and bending, and circular motion where the tip of the cilia

moves in a gyratory manner (252) (Figure 13).

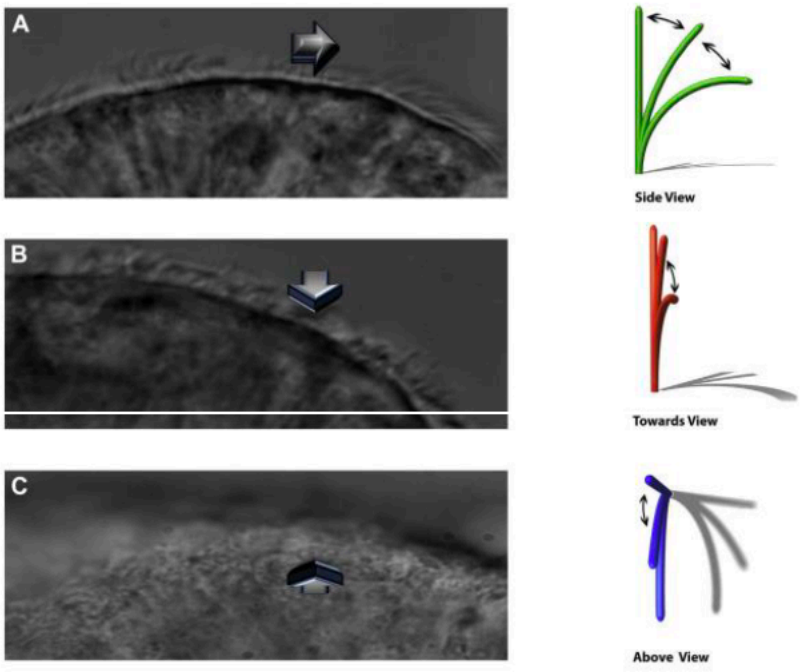

Figure 13: Illustrated Depictions of Ciliary Beating in Three Distinct Planes: (A) Sideways Profile, (B) Beating Toward the Observer, and (C) Top-Down View. Reproduced from : Kempeneers C, Seaton C, Chilvers MA. Variation of Ciliary Beat Pattern in Three Different Beating Planes in Healthy Subjects. Chest. 2017 May;151 (5):993–1001. Copyright © 2017 with permission from Elsevier. (274)

Additionally, specific markers of ciliary dyskinesia have been proposed, including the immotility index (percentage of immotile cilia), dyskinesia score (proportion of abnormal CBP across edges), and percentage of dyskinetic edges within the sample. Other observations, such as efficiency in particle removal and the presence of ciliated conical

protrusions, further inform the diagnosis of PCD (277,278) (Figure 14).

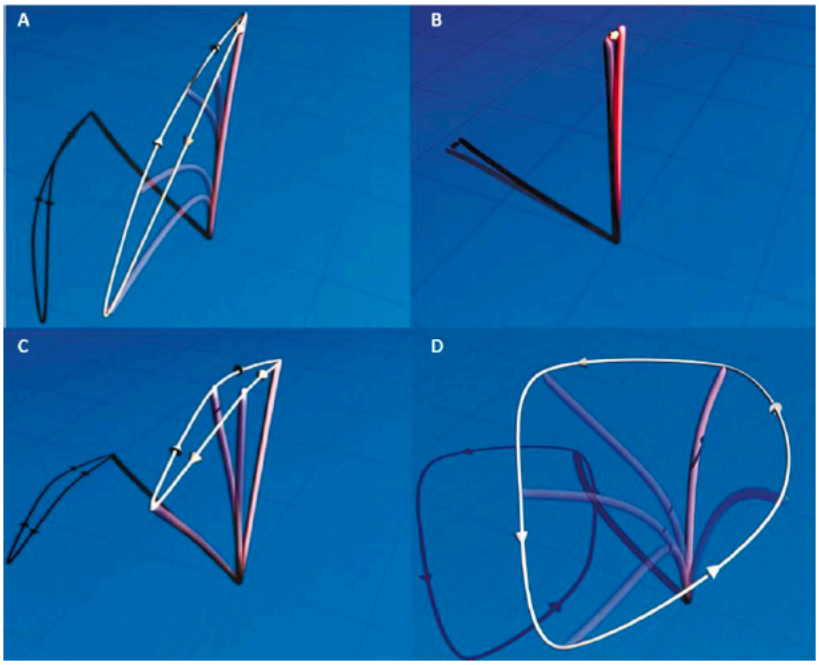

Figure 14: Visualization of Various Ciliary Beat Patterns Using DHSV: (A) Normal Pattern, (B) Immotile Pattern, (C) Stiff Pattern with Reduced Amplitude, and (D) Circular Pattern. Reproduced from Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. J Allergy Clin Immunol. 2003 Sep;112 (3):518–24. Copyright © 2003 Mosby, Inc. All rights reserved. (252)

Given the time-intensive and subjective nature of manual CBF and CBP evaluations, various software programs have been developed for automated or semi-automated analysis. These programs use algorithms to assess light intensity changes in recorded videos, allowing for CBF calculation. However, differences in CBF measurements between manual and automated methods, particularly in PCD cases, underscore the need for standardized exclusion criteria and processing techniques (275,279).

DHSV is a highly sensitive and specific diagnostic tool for PCD in expert laboratories, with sensitivity ranging from 0.95 to 1.00 and specificity from 0.91 to 0.96 (254). Despite its promise, international guidelines (ERS and ATS) do not currently recommend DHSV as a standalone confirmatory test. The ERS guidelines allow its use as part of a diagnostic approach but stress that hallmark ultrastructural defects on TEM or definitive bi-allelic mutations in PCD-associated genes are required for confirmation. Limitations of DHSV

include variability in laboratory protocols, environmental factors influencing ciliary function, and insufficient standardization of operating procedures (102,260,276).

Furthermore, previous assessments of DHSV's diagnostic efficacy have often relied on incomplete reference standards (e.g., TEM alone or in combination with nNO) or evaluations incorporating DHSV itself, which may inflate diagnostic accuracy estimates (251,256). These challenges, coupled with the cost and expertise required, limit its broader adoption and recognition as a definitive diagnostic tool for PCD. Standardization of methodologies and additional validation studies are necessary to enhance its utility in clinical practice.

One of the main challenges in evaluating ciliary function using DHSV lies in distinguishing PCD from secondary ciliary dyskinesia (SCD). While DHSV is a powerful tool for visualizing ciliary motion in real time, it may detect abnormal beat patterns that are not necessarily indicative of a primary disorder, but rather reflect secondary, inflammation-induced dysfunction.

SCD refers to an acquired, non-genetic alteration in ciliary beat pattern or frequency, resulting from environmental or inflammatory insults to the airway epithelium. Unlike PCD, which is due to ultrastructural or functional abnormalities of motile cilia caused by gene mutations, SCD arises secondarily in the context of infections, chronic inflammation, exposure to pollutants, or surgical trauma. Inflammatory cytokines, oxidative stress, and epithelial remodeling can lead to reversible or persistent disruptions in ciliary coordination, resulting in ineffective mucociliary clearance (7,280). In diseases such as CRSwNP, secondary dyskinesia is frequently observed and is believed to contribute to disease persistence and recurrence. Importantly, while primary dyskinesia typically shows consistent and repeatable ciliary motion abnormalities, secondary dyskinesia often presents with heterogeneous patterns , and may improve after resolution of inflammation or appropriate treatment (280,281). HSVM enables detailed assessment of ciliary motility and, when combined with TEM, immunofluorescence, and genetic testing, can help differentiate between primary and secondary forms of dysfunction(282,283).

2. Purpose of the Study

A major challenge in the diagnostic pathway for PCD lies in the need for repeated nasal ciliary sample collections. This necessity arises due to variability in sample quality, potential contamination from concurrent infections, and occasional technical failures, leading to multiple procedures often spaced 4 to 6 weeks apart. DHSV, a key tool for PCD diagnosis, relies heavily on high-quality ciliary samples to ensure accurate results. Such repeated collections are particularly distressing for pediatric patients, who may experience pain, fear, and trauma during the sampling process. This not only causes immediate discomfort but also risks eroding the trust and cooperation essential for a long-term relationship between patients and healthcare providers. Trust and cooperation are critical for managing a chronic, lifelong condition like PCD. Negative experiences during the diagnostic process may lead to reluctance to return for follow-up testing, delaying diagnosis and complicating overall care.

The integration of anesthesia in the diagnostic protocol for PCD, particularly during ciliary sampling for DHSV, holds the potential to transform this experience. By alleviating the pain and anxiety associated with sample collection, anesthesia can significantly improve patient comfort while enhancing diagnostic reliability. Improved procedural comfort may also result in higher-quality samples, reducing the need for repeat collections and minimizing issues such as suboptimal results. This can streamline the diagnostic process and contribute to faster, more accurate diagnoses.

Local anesthesia emerges as a particularly promising option. It offers a balance between patient comfort and procedural efficiency, allowing nasal ciliary samples to be collected with minimal invasiveness while maintaining patient cooperation and safety. In cases where local anesthesia proves insufficient, general anesthesia is not routinely used solely for ciliary sampling due to its invasive nature. However, it may be considered when the patient is already undergoing general anesthesia for another procedure. This approach is especially useful for highly sensitive or uncooperative children. By ensuring reliable and high-quality sample collection on the first attempt, anesthesia could facilitate a smoother diagnostic process and improve patient compliance.

To achieve these outcomes, further research is needed to develop and validate standardized protocols for the use of both local and general anesthesia in PCD diagnostics. Importantly, this includes investigating whether anesthetic agents alter CBF and CBP, as

previous studies have suggested that certain anesthetics can affect ciliary function. The two studies presented in this thesis address this gap by providing essential data on the feasibility, safety, and efficacy of anesthetic approaches during ciliary sampling for DHSV, while also evaluating their impact on ciliary function. By enhancing both diagnostic accuracy and patient experience, this research seeks to reduce distress, strengthen trust between healthcare providers and families, and ensure more effective long-term management. Ultimately, these advancements could play a pivotal role in improving care, outcomes, and quality of life for children with PCD.

3. Clinical Manifestations and Management of Primary Ciliary Dyskinesia in ENT Practice

Lionel Benchimol, Simon Dubois, Noemie Bricmont, Romane Bonhiver, Marie-Christine Seghaye, Philippe P. Lefebvre, Jean-François Papon, Céline Kempeneers, Anne-Lise Poirrier (**2024**).

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Clinical Manifestations and Management of Primary Ciliary Dyskinesia in ENT Practice

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ABSTRACT

Primary ciliary dyskinesia (PCD) comprises a wide range of phenotypes related to the impaired function of epithelial cilia. Histologically altered or absent cilia lead to multiple and variable consequences at the clinical level. Several research and clinical interest have surrounded respiratory ciliated epithelium because of its key role in clearing mucus from the ear, nose, and respiratory tract. Our aim was to provide a current state of the art on the ENT signs and symptoms of primary ciliary dyskinesia and their practical management. We systematically searched the following databases from 2011 until 2021: Cochrane Central Register of Controlled Trials, PubMed, and ScienceDirect. The searches were performed by 2 independent investigators. After removing duplications, articles were selected after the evaluation of the publications by reading their titles and abstracts. Eventually, full-text reading took place. Early onset of upper and lower respiratory symptoms in a full-term born child is suggestive of ciliary dyskinesia, especially in the absence of a usual triggering factor (passive smoking, allergy). The cornerstone of care is improving mucociliary clearance, using nasal and sinus irrigations, autoinflaction devices for middle ear effusion, physiotherapy and/or physical exercise for upper airway recovery. Decongestants, mucolytics, steroids, and antihistamines are part of the therapeutic arsenal with a low level of evidence. Early eradication of airway infections should be based on bacteriological analysis. Surgical interventions are common and mainly aim at restoring drainage. in summary, PCD is associated with ENT manifestations from the first days of life. The key to management is restoring adequate drainage of the upper airway and ENT cavities, using medical and surgical interventions.

Keywords: Chronic rhinitis, chronic rhinosinusitis, management, otitis media with effusion, primary ciliary dyskinesia

Introduction

Primary ciliary dyskinesia (PCD) is an inherited motor ciliopathy, in which the beating of the respiratory cilia is absent, slow, or abnormal, leading to a deficit in mucociliary clearance and significant respiratory and ENT pathologies.[1]

Clinically, PCD is primarily characterized by recurrent or chronic infections of the upper and lower respiratory tract, the development of bronchiectasis, chronic cough, chronic nasal congestion, recurrent or chronic otitis media, and chronic sinusitis beginning from childhood.[2]

Primary ciliary dyskinesia is rare, and its prevalence is difficult to establish, ranging from 1 : 10000 to 1 : 20000. However, the actual prevalence of PCD is probably higher because the diagnosis is difficult and often missed or delayed due to lack of clinical suspicion and difficulties in confirming this diagnosis. This results in a significant delay in diagnosis or inadequate treatments.[3]

Diagnosis of primary ciliary dyskinesia is based on a combination of tools such as genetic analysis, measurement of nasal NO, transmission electron microscopy, high-speed video microscopy analysis after cell culture, and

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immunofluorescence. Guidelines of the American Thoracic Society and the European Respiratory Society agree that a positive genetic analysis or an ultrastructural defect observed by transmission electron microscopy confirms the diagnosis of PCD.[4]

The objective of this article is to carry out a current state of the art on the clinical manifestations and management of primary ciliary dyskinesia in the practice of otorhinolaryngology.

Methods

The Cochrane Central Register of Controlled Trials, PubMed, and ScienceDirect databases were searched using keywords, subject headings, and Medical Subject Headings. The search was completed on February 15, 2022. We used keywords, including Primary ciliary dyskinesia AND ENT OR ear OR sinus OR ventilation tube OR middle ear ventilation OR sinonasal OR nasal AND treatment OR manifestations, to identify all previously published studies that investigated the clinical manifestations and management of primary ciliary dyskinesia. The search was adapted to each database, and we included clinical trials, meta-analyses, randomized controlled trials, reviews, and systematic reviews. The reference lists of the identified studies were searched manually for additional related papers. A repeated search using the same strategy was performed on February 15, 2022, and revealed the same results. Titles and abstracts of all articles extracted from the primary search were screened after removing duplicates. The full texts of relevant papers were reviewed for eligibility by 2 authors independently. Finally, 32 articles were selected.

ENT Clinical Manifestation of PCD

Primary ciliary dyskinesia is a disease affecting the structure and/or function of the mobile cilia, thereby causing a disorder of mucociliary clearance with an accumulation of mucus and bacteria in the airways. The upper respiratory tract and the middle ear are covered with ciliated epithelium. One of the characteristics of PCD is the retention, from the neonatal period, of secretions and chronic infections in the middle ear, nose, and facial sinuses (Table 1).[3,5]

The clinical presentation of PCD is heterogeneous, and the symptoms of this disease are nonspecific. Therefore, although most patients with PCD have symptoms of preschool onset, diagnosis is often delayed.[6] Sommer et al[7] found that the majority of patients had seen a doctor more than 50 times before being diagnosed with PCD, with the average age of diagnosis for PCD being 10.9 years.

Main Points

- Primary ciliary dyskinesia is underdiagnosed, and mean time to diagnosis is 11 years.
- Recent guidelines for management of primary ciliary dyskinesia in ENT practice are summarized.
- ENT care is paramount for the quality of life of primary ciliary dyskinesia patients.
- Coordination of medical and surgical treatments is key for primary ciliary dyskinesia patients.

Table 1. The Early Onset (Before Attending Daycare) in a Full-term Born Child is Suggestive of Ciliary Dyskinesia, Especially with a History of Neonatal Respiratory Distress and/or Neonatal Cough, and in the Absence of a Usual Triggering Factor (Passive Smoking, Allergy)

ENT Manifestations of PCD	
Nose / Sinus	Early onset (preschool)
	Perennial
	Despite medical treatment
	Recurrence after surgery
Ears	Early onset (preschool)
	Perennial
	Hearing loss
	Despite medical / surgical treatment
Upper airway	Chronic cough
	Sleep apnea

The ENT manifestations of PCD are very common and contribute significantly to the general morbidity of the disease. They are often recurrent during early childhood, despite well-conducted antibiotic treatment.[8]

The majority of PCD patients have chronic rhinosinusitis (CRS), which is defined by inflammation of the entire nasal mucosa and paranasal sinuses. Chronic rhinosinusitis causes symptoms such as nasal congestion, facial pain, mucopurulent anterior or posterior rhinorrhea, hyposmia, or anosmia. These symptoms are considered chronic if they persist for more than 12 weeks and if signs of rhinosinusitis are demonstrated by endoscopy or imaging. Sinonasal polyposis may also be associated,[5,8] usually occurring in older children and affecting 15-56% of adults.[6,9] The nasal symptomatology associated with PCD is generally present throughout the year and is not influenced by seasonal change, unlike allergic rhinitis.[8] Chronic rhinorrhea and nasal congestion are often present since the neonatal period.[7,10-12] Although it is a non-specific sign, clinical examination of the nasal cavities in patients with PCD frequently shows mucopurulent secretions in the nasal floor and swelling of the inferior turbinates.[8] Hydrocephalus is uncommon in patients with PCD, but has been associated with ciliary aplasia, as it has been reported in 10% of patients with CCNO mutations.[14] Hydrocephalus is the consequence of a dysfunction of the mobile cilia of the cerebral ventricles and of the ependymal duct, and can cause chronic headaches that can be mistakenly attributed to sinusitis.[13]

Pan-sinusitis is found on imaging (CT scan) in most adult PCD patients, and is often associated with sinus hypoplasia or agenesis (mainly frontal sinuses). Bilateral ethmoid sinus mucoceles have also been described in children with PCD.[15,16] In terms of pathogens, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* were the most frequently found bacteria in patients with PCD.[17]

Primary ciliary dyskinesia is also expressed by very frequent involvement of the middle ear. Otologic manifestations of PCD include but are not limited to, chronic otitis media with

effusion, recurrent otitis media, and hearing loss. Patients frequently present with persistent otorrhea, especially after ventilation tube insertion. Clinical examination of the ear may be normal or show chronic otitis media with effusion, acute otitis media, tympanosclerosis, as well as otorrhea in the external auditory canal if the tympanic membrane is ruptured spontaneously or by the ventilation tube insertion. Hearing loss caused by chronic otitis media with effusion is mild to moderate in severity and is usually insidious in young children with PCD.[8] However, even mild hearing loss in the first years of a child's life can affect language acquisition as well as the child's listening skills at school, which can affect children's performance at school. Chronic otitis media with effusion affects at least 80% of children with PCD and is often persistent until the age of 12. Therefore, special attention should be paid to hearing loss.[7,8,18]

Upper respiratory tract and middle ear infections are common in the general pediatric population, and they can be difficult to distinguish from the nasal and otologic manifestations of PCD. This type of frequent infections may delay the diagnosis of PCD in children. In addition, the chronic ENT and respiratory symptoms associated with PCD are common to other diseases that should also be considered, such as cystic fibrosis, immunodeficiency, asthma, allergic rhinitis, prolonged bacterial bronchitis, gastroesophageal reflux, and secondary ciliary dyskinesia.[2,19] The combination of several signs suggestive of PCD makes it possible to distinguish PCD from these conditions: unexplained neonatal respiratory distress, persistent daily productive cough of early onset, daily nasal congestion of early onset, and abnormal lateralization of the viscera. About 50% of PCD patients present with situs inversus and 12% with situs ambiguous.[20]

Symptoms affecting the ENT sphere in patients with PCD may progress with age. Chronic rhinosinusitis is the main manifestation in PCD adult patients, while otological manifestations are more common in children.[7,21]

Management and Treatment of ENT Complications

The current recommendations concerning the management of PCD are extrapolated from guidelines for the management of cystic fibrosis and personal experiences of various medical centers, without real proof of benefit. The management of PCD should be multidisciplinary and carried out in centers with expertise in the disease. Consultations with a respirologist and ENT specialist are usually done every 3-6 months, depending on the severity of the disease and the patient's age (Table 2).

Currently, as there is no treatment that corrects the genetic dysfunction of mobile cilia, the goal of PCD management is to treat the symptoms, to prevent infectious exacerbations and their complications, and to delay as much as possible the functional decline of the respiratory system, while preserving as much as possible the quality of life and the socio-psychological well-being of the patients.[5]

Management of nasal and sinus involvement in PCD consists of medical and surgical treatments. The medical treatment of CRS is based on nasal and sinus irrigation with physiological liquid, respiratory drainage physiotherapy, local corticosteroid therapy, local antibiotic therapy (nebulization) or general, and sometimes anticholinergics. Nasal and sinus irrigation with isotonic or hypertonic saline is the basis of treatment, allowing to drain the secretions as well as the bacteria and the biofilm contained in the sinuses. Local intranasal corticosteroids may help decrease mucosal inflammation, especially in the presence of polyps. However, polyps in PCD patients are mostly neutrophilic; therefore, they respond less to local intranasal corticosteroids.[4,22] In infectious exacerbations of rhinosinusitis, conservative treatment is not recommended, and antibiotics are preferred, with the choice of antibiotic to be ideally guided by the nasosinus bacteriological analysis. Methods of administration of antibiotics can be oral, nebulized, or intravenous.[5,8,21]

Long-term macrolide therapy may be useful for patients with frequent respiratory exacerbations, in whom azithromycin may reduce the morbidity of exacerbations, the need for additional antibiotic therapy, and potentially prevent irreversible lung damage. In addition to their antibacterial effect, macrolides have beneficial anti-inflammatory effects and are increasingly used in various chronic respiratory pathologies, including PCD. Kobbernagel et al[23] have shown that 6-month maintenance treatment with azithromycin halves respiratory exacerbations in patients with PCD.

Endoscopic sinus surgery (meatomy, polypectomy, ethmoidectomy, turbino-septal surgery) could be of benefit in the management of PCD. The goals of sinus surgery in patients

Table 2. The Cornerstone of Care is Restoring Adequate Drainage of the Upper Airway and ENT Cavities, Using Nasal and Sinus Irrigations, Autoinflaction Devices for Middle Ear Effusion, Physiotherapy and/or Physical Exercice for Upper Airway Recovery. Decongestants, Mucolytics, Steroids, and Antihistamines are Part of the Therapeutic Arsenal with a Low Level of Evidence. Antibiotics Should be Based on bacteriological Analysis. Surgical Interventions are Common and Mainly Aim at Restoring Drainage

ENT Management of PCD	
Nose / Sinus	Saline douching (hypertonic)
	Antibiotics (nebulization, systemic)
	Functional endoscopic sinus surgery
	Endoscopic sinus surgery
Ears	Autoinflation devices
	Antibiotics (local, systemic)
	Transtympanic drainage (controversial)
	Hearing aid
	Speech therapy
	Surgery of middle ear complications
Upper airway	Respiratory drainage physiotherapy
	Antibiotics (nebulization, systemic)
	Macrolide maintenance therapy (azithromycin)
	Adenotonsillectomy
	Continuous positive airway pressure

with PCD are to treat nasal congestion, to restore nasal breathing, to improve olfaction, and to increase penetration of local treatments by improving mechanical sinus drainage.[8] Some studies also suggest that sinus surgery may reduce the respiratory bacterial load.[24] Bequignon et al[21] advise orienting the surgical procedure according to the predominant symptoms: if facial pain is in the foreground, an ethmoidectomy is performed, while in the event of predominant nasal obstruction, a turbinectomy is performed. Endoscopic sinus surgery is not a curative treatment, and persistent chronic rhinorrhea can be observed in post-operative consultations and follow-up consultations. Therefore, endoscopic sinus surgery should be used in cases of persistent rhino-sinus symptoms and/or no amelioration with long-term macrolide therapy.

Biofilms of the upper, lower, and middle ear respiratory tracts are a complex association of bacteria, irreversibly attached to the mucous surface and enclosed in an adherent extracellular matrix, originating from both the host and the bacteria. The biofilm environment contains low levels of oxygen and is not perfused by arterial blood, making it inaccessible to systemic antibiotics. These biofilms are likely to be present in patients presenting CRS, including those with PCD, and they are a reservoir of bacteria and mediators that contribute to systemic inflammation and recurrent respiratory tract infections. Therefore, some studies suggest that the sinuses can be considered as a bacterial reservoir that can infect the upper and lower respiratory tracts, and that the incorporation of naso-sinus bacteriological analyzes to guide the choice of medical and surgical treatments could improve the outcome of PCD patients. In these patients, the bacteria most commonly found in the sinuses are *H. influenzae*, *P. aeruginosa*, *Staphylococcus aureus*, and *S. pneumonia*.[8,17,25]

Management of otological complications of PCD aims to improve patients' hearing, aiming to avoid the possible sequelae of long-term hearing loss (cognitive disorders, language development disorders, and academic delay), as well as to prevent the sequelae of chronic otitis in the tympanic membranes and middle ear (tympanosclerosis, retraction pockets or atelectasis of the eardrums, cholesteatoma, erosion of the ossicles).[8,15] Chronic otitis media with effusion being very common in patients with PCD, especially in young children; therefore, hearing should therefore be monitored regularly using age-appropriate methods.[7] Oral antibiotic treatment is recommended to treat acute otitis media.[26] Use of trans-tympanic aerators for the treatment of chronic otitis media with effusion is controversial in the management of PCD, as these could be responsible for persistent and intractable otorrhea, causing discomfort and worsening hearing loss. European guidelines recommend to avoid the use of trans-tympanic ventilators in the management of patients with PCD, and treating hypoacusis by hearing aids and regular follow-up in ENT consultations.[27] Furthermore, no cases of cholesteatoma were found in PCD children with chronic otitis media with effusion managed without trans-tympanic aerators.[28] Antibiotics and local and/or general corticosteroids may be offered in the face of debilitating otorrhea, with the choice of antibiotic having to be adapt according to the results of the bacteriological culture of the otorrhea. It is important to avoid ototoxic antibiotics. Self-insufflation devices, also called nasal balloons, can also be used for the management of chronic otitis media with effusion, with limited evidence of benefit.[8,26,29-31]

Vaccination against influenza and pneumococcus is important for patients with chronic respiratory diseases, including PCD.[8] Concerning the severe acute respiratory syndrome coronavirus 2 pandemic, PCD patients were considered to be at high risk. Therefore vaccination is also recommended.[32]

Perspectives

The cornerstone of care has been to restore adequate drainage to the upper airway and ENT cavities. To collect standardized data focusing on ENT disease in PCD patients, the ENT prospective international cohort of patients with PCD (EPIC-PCD) was born. This international prospective study aims to longitudinally characterize ENT disease in PCD patients and its association with lung disease, and to identify determinants of its prognosis.[33] While observational, this multicenter study was not designed to develop new treatments. However, it helps to better identify the ENT manifestations of PCD, better define the exacerbations, better understand their evolution, the possible need for surgery, and their prognosis.

Future treatments may target ciliary function itself. PCD is genetically heterogeneous and currently includes more than 50 known genes. To date, three studies in the preclinical stage have been published, describing the partial restoration of ciliary function in ciliopathies using classical gene therapy and one study using gene editing. Gene therapy aims to replace or repair the DNAI1 gene, which encodes a component of the outer arm of dynein, associated with approximately 10-14% of PCD cases. Lentiviral and adenoviral vectors of the DNAI1 gene were delivered directly into the airways of mouse models of PCD by aerosol, showing promising outcomes. Aerosolization of DNAI1 gene through viral vectors was able to normalize ciliary beat frequency. However, before it can be applied to patients, gene therapy has to address safety concerns following alterations in genomic DNA and the resistance of differentiated airway epithelium to transduction by viral vectors.

More recently, RNA therapy or transcript therapy has been studied with the aim of restoring DNAI1 or CCDC40 protein expression in knockout mice.[34] By acting downstream of the genome, these options avoid DNA alteration and are preferably reversible. The first results of the preclinical stage in transcript therapy for CCDC40 protein are encouraging. There is no current data for RNA therapy in PCD humans.

Conclusion

Primary ciliary dyskinesia is associated with ENT manifestations from the first days of life. The current management of PCD is based mainly on expert opinions and extrapolations from cystic fibrosis patients. The key management is restoring adequate drainage of the upper airway and ENT cavities, using medical and surgical interventions. Unfortunately, there is no curative management for PCD patients. Hence, these patients must have regular follow-ups in ENT and pneumology to reduce as possible complications that could affect lung function. Management in a specialized center with a multidisciplinary approach should be prioritized for PCD patients.

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

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ORIGINAL ARTICLE

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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 (PCD) 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 PCD). 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 videomicrocopy, 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 single-blind 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 100× 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 µL 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 1 h and 3 h after sample collection.

For ciliary functional analysis (CFA), only normal edges or edges with minor projections,[18] measuring at least 50 µm 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 H0 (for saline and local anesthesia condition), H1 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-Withney *U*-test compared lidocaine-naphazoline administration to saline for CBP and CBF. The evolution of CBF and CBP was analyzed immediately (T0), 1 h (T1) and 3 h (T3) after lidocaine-naphazoline 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 non-PCD-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 volonteers 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 lidocaine-naphazoline 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 lidocaine-naphazoline administration ($p = 0.906$ and 0.271, respectively).

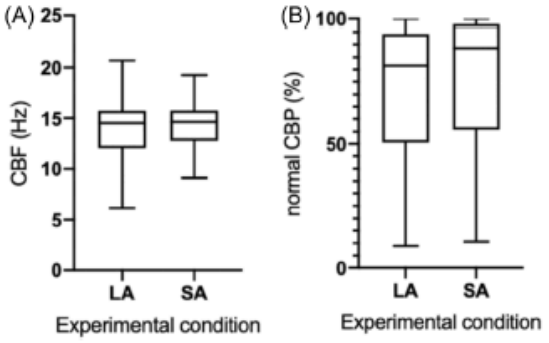

FIGURE 1 Effects of Local Anesthesia on CBF (A) and CBP (B) in the total population, including referred patients (non-PCD referred patients and PCD-referred patients) and healthy volunteers. No significant difference was observed following nasal administration of Lidocaine 2% and Naphazoline 0.5% in 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.

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).

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

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-Withney *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.

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.

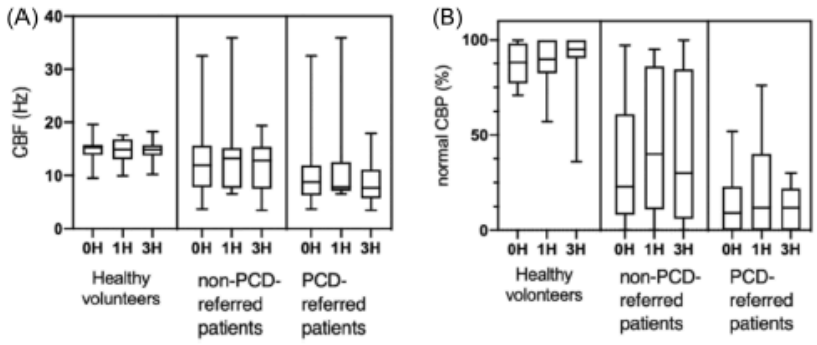

FIGURE 2 Temporal Evolution of CBF (A) and CBP (B) Following Nasal Administration of Lidocaine 2% and Naphazoline 0.5% in Healthy Volunteers and Referred Patients (Non-PCD and PCD). No significant difference observed among the three groups in terms of CBF and CBP. CBF (Hz), ciliary beat frequency in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

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 semi-automatic 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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5. Impact of General Anesthesia on Ciliary Functional Analysis by Digital High-Speed Videomicroscopy in Suspected Primary Ciliary Dyskinesia

Lionel Benchimol, Noemie Bricmont, Romane Bonhiver, Gregory Hans, Philippe P. Lefebvre, Céline Kempeneers, Anne-Lise Poirrier (**2024**).

Diagnostics

diagnostics

MDPI

Case Report

Impact of General Anesthesia on Ciliary Functional Analysis by Digital High-Speed Videomicroscopy in Suspected Primary Ciliary Dyskinesia

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Abstract: Digital high-speed videomicroscopy (DHSV) is a crucial tool for evaluating ciliary function in children suspected of primary ciliary dyskinesia (PCD). However, until now, samples are taken without anesthesia due to uncertainty about its effect on ciliary function and DHSV interpretation. This study aimed to investigate the impact of general anesthesia on ciliary functional analysis by DHSV in a series of three patients listed for ENT surgeries, which could improve diagnostic procedures for pediatric patients. Patient 1 (7-year-old girl) underwent adenotonsillectomy and tympanostomy placement tube, while patients 2 (17-month-old boy) and 3 (15-month-old girl) underwent adenoidectomy and tympanostomy placement tube. All patients underwent nasal brushing before general anesthesia (control sample). Experimental samples were taken in the contralateral nostril at the time of equilibration of the anesthetic agents (sevoflurane, propofol, sufentanil). Ciliary beat frequency and pattern were measured using digital high-speed videomicroscopy. Our findings highlighted the variability of respiratory ciliary function under general anesthesia among individuals. Our results emphasize the need for caution when interpreting ciliary function data obtained during general anesthesia. Further research with larger cohorts is warranted for validation.

Keywords: primary ciliary dyskinesia; digital high-speed videomicroscopy; general anesthesia; ENT surgeries

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1. Introduction

Maintaining effective airway clearance and defense mechanisms relies on the production, drainage, and regulation of periciliary fluid and mucus, alongside the coordinated function of the ciliated epithelium. Impaired mucociliary clearance can stem from primary conditions such as cystic fibrosis or primary ciliary dyskinesia, or it may develop as a secondary consequence of toxic exposures, infections, or chronic diseases such as chronic rhinosinusitis [1,2].

Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by disrupted ciliary function, leading to recurrent ENT and respiratory infections [2]. Its prevalence is difficult to determine, estimated between 1 in 10,000 and 1 in 20,000 people. However, the true prevalence of PCD is likely higher due to the complexity of diagnosis, often resulting in underdiagnosis or delayed diagnosis attributed to insufficient clinical suspicion and diagnostic difficulties [3]. Therefore, there is a significant delay in diagnosing PCD or providing appropriate treatments. Diagnosis of PCD relies on a combination of diagnostic tools

including genetic analysis, measurement of nasal nitric oxide (NO), transmission electron microscopy, high-speed videomicroscopy analysis after cell culture, and immunofluorescence [2,4]. The American Thoracic Society and European Respiratory Society guidelines agree that a positive genetic analysis or identification of ultrastructural defects by transmission electron microscopy confirms the diagnosis of PCD [5,6]. Ongoing research and international patient registries aim to improve understanding and treatment options for PCD [2].

Digital high-speed videomicroscopy (DHSV) is a sensitive and specific tool for assessing ciliary function in PCD [7–11], examining ciliary beating frequency (CBF) and ciliary beating pattern (CBP) [12,13]. In young patients suspected of having PCD, frequent ENT procedures necessitate general anesthesia. Despite the need for ciliary samples to confirm PCD diagnosis, concerns persist regarding the potential impact of general anesthesia on ciliary function assessment. Currently, ciliary samples for diagnostic purposes are not obtained under general anesthesia due to the perceived risk of anesthesia-induced alterations in ciliary beat frequency potentially leading to inaccurate diagnoses [14,15].

Currently, there are no studies regarding the assessment of ciliary function by DHSV when sampling is performed under general anesthesia. Our study addressed this critical gap in clinical practice by investigating whether general anesthesia truly influences ciliary function analysis using DHSV. Answering this question is essential for optimizing diagnostic protocols in pediatric patients, potentially allowing for ciliary sample collection during routine interventions under general anesthesia. We sought to provide empirical evidence from real clinical settings to guide future diagnostic practices in suspected PCD cases. Proving that general anesthesia does not affect ciliary function could streamline diagnostics and improve the clinical experience of young ENT patients. Conversely, if anesthesia does alter ciliary function, clinicians should avoid using it to collect samples to avoid inaccurate results. Our aim was to compare the performance of DHSV to analyze ciliary function with and without general anesthesia for sample collection in a small case series. The following three cases present a rare analysis of ciliary function in pediatric patients, both under and outside of general anesthesia. Ciliary function over time was characterized by CBF and CBP using DHSV as co-primary endpoints.

2. Case Presentation

2.1. Material and Methods

Three pediatric patients referred to the PCD diagnosis center and listed for adenotonsillectomy, adenoidectomy, or tympanostomy tube placement under general anesthesia were included in this case series. Middle turbinate brushing for DHSV analysis was first performed before the induction of general anesthesia as a control sample. Controlateral middle turbinate brushing was then performed under general anesthesia.

- Anesthesia was induced by the inhalation of sevoflurane at an inspired concentration of 6% in a 50% air/oxygen mixture. A bolus of 1–2 mg/kg of propofol and 0.1–0.2 mcg/kg sufentanil was then administered after intravenous access was secured and before endotracheal intubation. After controlling the airway, anesthesia was maintained with sevoflurane at an end-tidal concentration equivalent to 1–1.2 minimal alveolar concentration corrected for age in an air-oxygen mixture. The inspired oxygen fraction was adjusted to maintain the oxygen saturation of the arterial blood above 95%. In addition, throughout the intervention, mean arterial pressure (MAP) was carefully monitored as a reliable indicator of cerebral perfusion. Importantly, MAP was consistently maintained above 33 mmHg to ensure safety and adequate cerebral perfusion. The samples were taken 10 min after airway control so that the concentration of sevoflurane had reached an equilibrium between the different compartments.
- Nasal brushing samples were placed in 2 mL of medium 199 (Thermo Fisher, Waltham, MA, USA) containing an antibiotic solution (1% penicillin/streptomycin (Thermo Fisher, Waltham, MA, USA)) and an antifungal, 1% amphotericin B (Thermo Fisher, Waltham, MA, USA). Ciliary function was assessed immediately (T0) as well as

at 1 h (T1) and 3 h (T3) following brushing to study the evolution of a possible anesthetic washout.

Video sequences of ciliated beat edges were recorded using an inverted microscope with a 100× oil immersion interference contrast objective (Axio Vert.A1, Zeiss, Oberkochen, Germany) and a video camera at high speed (CrashCam Mini 1510, IDT Innovation in motion, Pasadena, CA, USA), at a frame rate of 500 hertz (Hz) at a controlled temperature of 37 °C. To record video sequences of cilia beating, 60 µL of the respiratory ciliated edges in medium 199 were placed under the microscope and heated to 37 °C using a heated box (Ibidi, Gräfelfing, Germany) and a microscope lens heater (Tokai Hit, Fujinomiya, Japan), and the temperature was strictly controlled and stable at 37 °C [16].

Only the edges considered normal or having minor projections, measuring at least 50 µm in length, were documented and utilized for ciliary functional analysis (CFA) [17]. Among these edges, only cilia free of mucus and displaying sideways beating profiles were examined. CFA was assessed based on a minimum of three high-quality edges meeting the specified criteria for each condition. For the manual assessment of CBF, cilia, or groups of cilia beating in the sideways profile were identified. The process included counting the number of frames needed to complete five beat cycles, which was then converted to CBF through a simple calculation [12]. A maximum of 10 CBF measurements were obtained from each ciliated beating edge. Ciliated edges that did not allow at least four CBF measurements to be performed were excluded from the analysis. If immobile cilia were present, a CBF of 0 Hz was recorded [18]. The average CBF for each sample was calculated for each treatment condition.

The specific movement of a cilium or group of cilia throughout a complete beating cycle was compared to the normal CBP observed with DHSV [1,2]. Each cilia or ciliary group evaluated was classified as having a distinct normal or abnormal CBP. The percentage of normal CBP in the sample was calculated for each condition [18]. This study received approval from the ethics committee of the University Hospital of Liège 2021-393. Informed written consent was obtained from all patients and their guardians prior to their involvement.

Transmission electron microscopy (TEM) was used to investigate ciliary ultrastructural defects. For TEM analysis, the ciliated cells were initially fixed with 2.5% glutaraldehyde in Sorensen's phosphate buffer (pH 7.4), then treated with 1.3% osmium tetroxide, dehydrated in graded ethanol, and dried using hexamethyldisilazane. Samples were embedded overnight in a 1:1 1,2-epoxypropan-epon mixture at 4 °C. After polymerization, sections were placed on copper grids and stained with Reynold's lead citrate.

In addition to TEM, molecular genetic testing was performed to screen for mutations associated with primary ciliary dyskinesia (PCD). PCD is a genetically heterogeneous disorder, typically inherited in an autosomal recessive manner, except for mutations in FOXJ1 (autosomal dominant) and PIHID3, OFD1, and RPGR (X-linked recessive). To date, mutations in more than 50 genes have been implicated in PCD, with the most common being DNAH5, DNAH11, DNAI1, CCDC39, and CCDC40. In this study, a custom gene panel (Gent) was used to screen 125 genes associated with isolated or syndromic congenital heart disease, heterotaxy, and both motile and non-motile ciliopathies.

2.2. Case Series

Case #1 was a 7-year-old North African female listed for adenotonsillectomy and tympanostomy tube placement for recurring upper airway obstruction and chronic otitis media with effusion. The patient had a past medical history of congenital heart disease, characterized by a large atrial septal defect and a large interventricular septal defect. These cardiac anomalies were surgically corrected at the age of 3 months. Despite thorough genetic testing, no underlying genetic etiology for the congenital heart defects was identified. She had a previous ENT history of adenoidectomy and tympanostomy tube placement at the age of 2 years. Although she initially experienced relief, symptoms of snoring, mouth

breathing, and chronic otitis media with effusion recurred 4 years later, accompanied by auditory impairment.

The patient was not exposed to passive smoking. Extensive diagnostic workup including a sweat test was conducted to rule out cystic fibrosis, which returned negative. Additionally, immunological and allergic assessments were unremarkable. The patient did not respond to conservative treatments including intensive saline nasal irrigation, intranasal glucocorticosteroids, and myofunctional therapy. The ENT examination revealed nasal congestion, chronic otitis media with effusion, and hypertrophy of both the adenoids and tonsils. Tympanometry results were type B curves. Pure tone audiometry showed an average hearing threshold of 25 dB in the right ear and 30 dB in the left ear, indicating mild hearing loss. The PICADAR score was not applicable due to the absence of a persistent wet cough. Given the persistence of symptoms, the patient was listed for adenotonsillectomy and tympanostomy tube placement, with concurrent ciliary sampling, in our ENT department.

The postoperative recovery was uneventful. The patient experienced significant clinical improvement, with the restoration of normal nasal breathing, resolution of snoring, and normalization of hearing. She remains symptom-free with a follow-up period of 5 months.

Case# 2 was a Caucasian male aged 1 year and 5 months listed for adenoidectomy and tympanostomy tube placement for adenoid hypertrophy and chronic otitis media with effusion. The patient had a history of recurrent respiratory issues including multiple episodes of bronchitis, recurrent acute otitis media, and asthma.

There was no history of exposure to passive smoking. Comprehensive immunological and allergic evaluations were unremarkable, ruling out underlying immunodeficiencies or allergic conditions. The ENT evaluation demonstrated bilateral glue ear, adenoid hypertrophy, and nasal congestion. Tympanometry indicated type B curves. Pediatric visual reinforcement audiometry, conducted using sound field speakers, revealed an average hearing threshold of 45 dB. Due to the lack of a chronic wet cough, the PICADAR score could not be assessed. Prior to surgery, the patient received respiratory physiotherapy, selective ß2-adrenergic receptor agonists for asthma management, saline nasal douching, and intranasal glucocorticosteroids. Despite these treatments, the symptoms persisted, and the patient was listed for adenoidectomy and tympanostomy tube placement, with concurrent ciliary sampling. Due to his asthma, he was kept under observation overnight following the surgery to ensure respiratory stability.

The postoperative recovery was uncomplicated. The patient showed significant improvement, with a marked reduction in the frequency of respiratory and ear infections. Hearing returned to normal up to 11 months post-surgery.

Case #3 was an African girl aged 1 year and 3 months listed for adenoidectomy and tympanostomy tube placement for adenoid hypertrophy and chronic otitis media with effusion. The patient had a medical history of situs inversus totalis, a rare congenital condition in which the major visceral organs are positioned as mirror images of their typical locations.

The genetic analysis did not identify any homozygous mutations in genes commonly associated with PCD and situs inversus including *DNAH5*, DNAI1, CCDC39, CCDC40, DNAH11, LRRC6, RPGR, RSPH4A, RSPH9, CCNO, DNAAF1, and *HYDIN*. Nose congestion and ear infections started at 2 months of age. Additional workup successfully ruled out other cardiac diseases, cystic fibrosis, allergies, and immunodeficiencies. Initial treatment included saline nasal irrigation and intranasal glucocorticosteroids. However, these conservative measures did not resolve the patient's symptoms. The ENT examination showed signs of mouth breathing, chronic otitis media with effusion, and hypertrophy of both the tonsils and adenoids. Tympanometry yielded type B results. Pediatric visual reinforcement audiometry using sound field speakers demonstrated an average hearing threshold of 30 dB, with a notable elevation to 50 dB at 500 Hz. The PICADAR score was deemed inapplicable as the patient did not exhibit a chronic cough. The patient was listed

for adenoidectomy and tympanostomy tube placement, with concurrent ciliary sampling. Postoperatively, the patient experienced an improvement in nasal breathing. However, she presented recurrent episodes of otorrhea, particularly on the right side, necessitating ongoing local care. The hearing is currently normal, with a follow-up period of 8 months. Although the patient exhibits organ transposition, PCD diagnosis remains uncertain. Genetic testing including a panel of 125 PCD-related genes and electron microscopy failed to identify any definitive mutations or structural abnormalities typically seen in PCD.

All patients underwent a complete evaluation for suspected PCD including nasal NO measures (only for case #1), genetic analysis (using the PCD heterotaxy gene panel, which covers 125 genes linked to PCD; see Supplementary File S1), electron microscopy analysis, and samples for DHSV taken in the outpatient clinic. Genetic analysis of the PCD heterotaxy panel did not detect any pathogenic mutations, and electron microscopy revealed no structural abnormalities in any of the cases.

In the three cases, no clear underlying cause was identified for the recurrent respiratory infections, aside from adenoid and/or tonsil hypertrophy in Case 1 and Case 2. The diagnosis of PCD in the third case remains uncertain, and she is currently being managed with respiratory physiotherapy and saline nasal irrigation.

2.3. Ciliary Beat Frequency

Analysis of CBF in brushing when exposed to general anesthesia (T0, T1, and T3), compared with control pre-anesthesia (control), revealed notable variations compared to the control side (Table 1). In patient #1, there was a consistent increase in CBF under general anesthesia at all time points. Conversely, patient #2 showed a mixed response. While there was an initial increase in CBF observed at 1 h under general anesthesia, a subsequent decrease was noted at 3 h compared to the control side. Patient #3 demonstrated a relatively stable response under general anesthesia. There was a slight increase in CBF at T1 followed by stable CBF levels at T3 compared to the control condition (SA). The CBF for healthy pediatric volunteers in our laboratory was 15.74 Hz ± 1.64 Hertz, measured in a sideway view and pre-culture conditions.

Table 1. Temporal evolution of ciliary beat frequency (CBF) after exposure to general anesthesia compared to control side pre-operatively. Results are expressed as the median [P25–P75].

CBF, Median [P25–P75]	Control	General Anesthesia		
		T0	T1	T3
Case #1	17.1 [16.0–17.7]	16.5 [14.5–17.7]	17.6 [16.7–18.9]	19.1 [17.7–20.0]
Case #2	14.6 [12.7–16.8]	15.4 [14.1–17.0]	16.6 [14.4–17.1]	13.5 [11.7–13.8]
Case #3	18.3 [15.1–19.8]	17.2 [14.9–18.5]	18.7 [17.2–19.0]	18.1 [14.2–18.5]

2.4. Ciliary Beat Pattern

Analysis of the pattern of ciliary beats as a percentage of normal beats after exposure to general anesthesia at different times (T0, T1, T3) compared to the control side revealed variable effects depending on the patients (Table 2). Patient #1 demonstrated relatively stable ciliary function under general anesthesia, with a non-significant decrease in normal CBP observed at 3 h compared to the control side. Conversely, patient #2 showed a notable decrease over time in normal CBP under general anesthesia compared to saline exposure. Patient #3 showed a relative stable ciliary beat pattern over time with minor variations. The CBP for healthy pediatric volunteers in our laboratory was 86.89% ± 6.83 Hertz, measured in a sideway view and pre-culture conditions.

Table 2. Temporal evolution of ciliary beat pattern (CBP) expressed as a percentage of normal CBP after exposure to general anesthesia compared to saline pre-operatively (control). Results are expressed as a percentage of normal CBP.

CBP, Percentage %	Control	General Anesthesia		
		T0	T1	T3
Case #1	100	100	100	96.5
Case #2	90.5	100	87.0	75.0
Case #3	84.0	96.0	100	91.4

3. Discussion

Our findings highlighted the variability of respiratory ciliary function under general anesthesia among individuals. Our results emphasize the need for caution when interpreting ciliary function data obtained during general anesthesia. General anesthesia is frequently used in the pediatric population with recurrent respiratory infections. However, taking a sample of ciliary epithelium for ciliary function analysis under general anesthesia is not advisable, despite the temptation to improve patient comfort. The impact of general anesthesia on CBF and CBP is essential to understand the dynamics of mucociliary clearance, particularly in pediatric patients undergoing surgery. Patient #2 showed pronounced changes in CBP and CBF under general anesthesia, indicating disturbances in ciliary function (Figure 1). These results question the impact of general anesthesia on ciliary function.

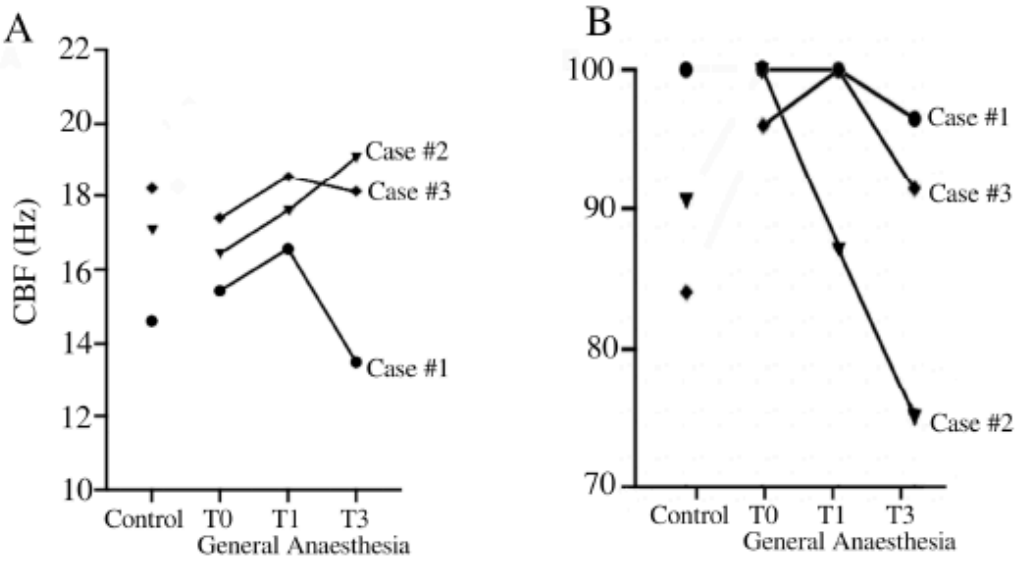

Figure 1. Temporal evolution of CBF (**A**) and CBP (**B**) in Case #1 (●), Case #2 (▼), and Case #3 (♦). CBF (Hz) = ciliary beat frequency in Hertz. CBP = ciliary beat pattern.

Concerning general anesthesia, in vivo studies in humans have demonstrated a decrease in CBF after exposure to isoflurane, and interestingly, despite this decrease in CBF, isoflurane did not change the CBP or the proportion of immobile cilia [19,20]. Furthermore, investigations in guinea pigs revealed no significant effects of individual anesthetics such as propofol, midazolam, and sufentanil on tracheal CBF [15,21]. However, when administered in combination, propofol and midazolam exhibited a synergistic interaction, slowing CBF [15]. This finding is consistent with their common target of the $GABA_A$ receptor site [15]. On the other hand, the non-significant effect of the propofol-sufentanil combination in the absence of midazolam on CBF suggests a unique pharmacodynamic

profile. Notably, similar observations were made in human respiratory cilia samples, where a propofol-alfentanil combination was used [22]. Another study on human ciliated nasal epithelium demonstrated the absence of the effect of halothane on CBF on 24 healthy volunteers and over 3 h [23]. While Marusiakova et al. demonstrated that children with adenoid hypertrophy had significantly lower median CBF than healthy controls [24], we were not able to repeat this finding. All of our patients had normal CBP and CBF without anesthesia compared to our normal laboratory values for children (15.74 Hz ± 1.64 for CBF and 86.89% ± 6.83 for normal CBP) (Figure 1). In fact, Case #3 presented a higher value compared to our normal laboratory values for CBF. The elevated CBF values observed in Table 1 can mostly be explained by the effect of general anesthesia. However, it is important to note that Case #3 exhibited a high CBF prior to anesthesia. It is possible that this patient had a baseline CBF that is higher than our laboratory's standard. Additionally, this patient's diagnosis remains uncertain, which could further explain this atypical finding. In addition, for Cases #1 and 3, it is hypothesized that general anesthesia at T0 caused an initial slowing of CBF, followed by a return to pre-anesthesia patterns after the washout period, explaining the higher CBF observed at T1 and T2. In contrast, Case 2 exhibited an initial increase in CBF at T0 and T1, with a return to pre-anesthesia levels later on. While these findings suggest that general anesthesia may influence ciliary function, it is difficult to draw definitive conclusions based on only three cases.

Nonetheless, it is important to acknowledge that patients included in this study have a history of recurrent upper respiratory infections, which raises the possibility of secondary ciliary dyskinesia (SCD). SCD, which can be caused by chronic inflammation or infection, may temporarily impair ciliary function and affect the baseline CBF. This factor could influence our findings, particularly the response of ciliary function under general anesthesia. While our study aimed to assess the impact of anesthesia on ciliary movement, it is essential to recognize that pre-existing conditions like SCD could complicate the interpretation of the observed changes in CBF. Future studies with larger sample sizes and detailed analyses of both primary and secondary dyskinesia are needed to better differentiate these effects.

To date, no similar cases have been published showing the evolution across time of ciliary function using CBF and CBP by DHSV in nasal brushing samples obtained from pediatric patients during general anesthesia for ENT surgery. These cases highlight the importance of carefully interpreting ciliary function data obtained in this context, as anesthesia may influence ciliary activity. The primary objective of this work was to present specific clinical observations in patients suspected of primary ciliary dyskinesia (PCD) who underwent anesthesia. These case reports, while limited in number, provide important insights that contribute to the understanding of this rare condition.

In conclusion, this study reports observations from three individual cases and therefore cannot provide definitive conclusions on the effects of general anesthesia on ciliary beat frequency (CBF) in patients with or without primary ciliary dyskinesia (PCD). The variability observed in CBF across the cases suggests that general anesthesia may influence ciliary function, but further investigation with a larger cohort is necessary to fully understand this relationship. Until more robust data are available, we recommend caution in interpreting ciliary function from samples taken under anesthesia.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/diagnostics14212436/s1, File S1: H9.1-OP2-B11: Genpanel Heterotaxie PCD, v2, in voege op 27/06/2022.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of CHU of Liège (2021-393). Approved date 7 February 2022.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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6. Ciliary Functional Analysis in Chronic Rhinosinusitis with Polyps after Multimodal Intervention: Oral Corticosteroid, Functional Endoscopic Sinus Surgery, and Omalizumab Injection

Lionel Benchimol, Olivier Bouchain, Noemie Bricmont, Romane Bonhiver, Céline Kempeneers, Philippe Lefebvre, Anne-Lise Poirrier (**2024**).

Case Reports in otolaryngology

Background

CRS is a highly prevalent inflammatory condition, affecting approximately 10.9% of the adult population in Europe, with the CRSwNP subtype accounting for around 2–4% (280,284). CRSwNP is frequently encountered in otolaryngology practice and is typically associated in Europe with a Th2 inflammatory response, often characterized by tissue eosinophilia and elevated serum IgE levels. In contrast, PCD is generally associated with a Th1 or neutrophilic inflammation, even when nasal polyps are present (285). In this case report, we applied our standardized ciliary functional analysis protocols established for PCD to a patient with CRSwNP exhibiting a Th2-dominant endotype. The patient underwent a multimodal therapeutic approach, including systemic corticosteroids, functional endoscopic sinus surgery and anti-IgE biotherapy (omalizumab). Our objective was to explore how DHSV might provide clinically relevant insights into secondary, inflammation-driven ciliary dysfunction, and to assess the potential of targeted interventions to partially restore ciliary function in a reversible context.

Perspective

This case highlights the potential utility of dynamic high-speed video microscopy (DHSV) not only in the diagnostic workup of primary ciliary dyskinesia, but also as a tool to monitor ciliary function in patients with CRSwNP undergoing biologic therapy. Our findings suggest that ciliary dysfunction in Th2-dominant CRSwNP may, in some cases, be reversible with targeted anti-inflammatory interventions. In future clinical practice, assessing ciliary function in patients receiving biologics could offer valuable insights into treatment response and serve as a useful adjunct in evaluating functional changes in response to treatments. Future studies could explore the role of ciliary function assessment in tracking therapeutic response and disease progression, and potentially in identifying cases of secondary ciliary dyskinesia amenable to reversal. This approach could contribute to more personalized management strategies in chronic airway inflammatory diseases.

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Case Report

Ciliary Functional Analysis in Chronic Rhinosinusitis with Polyps after Multimodal Intervention: Oral Corticosteroid, Functional Endoscopic Sinus Surgery, and Omalizumab Injection

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In her late 50 s, a woman with a medical history of endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps (CRSwNP) experienced a relapse of nasal polyps, significantly impacting her breathing and sense of smell. She underwent a multifaceted treatment approach, including oral corticosteroids, functional endoscopic sinus surgery, and omalizumab injections. Digital high-speed videomicroscopy (DHSV) revealed only partial improvement in ciliary beat pattern and ciliary beat frequency with oral corticosteroid treatment, while significant improvement in these ciliary parameters was observed with omalizumab injections. Furthermore, administration of omalizumab resulted in a decrease in her SNOT-22 (Sinonasal Outcome Test 22) score. Notably, this case report represents the first study investigating ciliary function using DHSV in a patient treated with omalizumab.

1. Introduction

Clearance and protection of the respiratory tract involve periciliary fluid and mucus production and drainage, as well as ciliated epithelium function. Disruption of mucociliary clearance may be caused by primary diseases such as cystic fibrosis or primary ciliary dyskinesia, or may be secondary to toxic exposure, infections, or chronic disease such as chronic rhinosinusitis [1]. In case of ciliary dyskinesia secondary to chronic rhinosinusitis, the ideal treatment would target the causative disease in order to restore the physiological function and the capacity to respond to future illness. Compromised cilia motility accompanies epithelial hyperplasia in individuals with nasal polyps (NP) [2]. This impairment in ciliary function is thought to likely contribute to persistent mucosal inflammation or infections, such as biofilm formation, commonly seen in people with chronic rhinosinusitis [2]. In addition to epithelial cell dysfunction, there is a prevalent type 2 inflammatory pattern in chronic rhinosinusitis with nasal polyps (CRSwNP) in Western countries, characterized by the expression of interleukins (ILs) IL-4, -5, and -13, as well as elevated IgE concentrations [3]. This trend was observed in 85% of patients with CRSwNP [3].

Chronic rhinosinusitis with nasal polyps (CRSwNP) is associated with elevated IgE production and eosinophilic inflammation [4]. Omalizumab, an anti-IgE antibody, has been shown to be effective in patients with both CRSwNP and coexisting asthma [4]. In fact, omalizumab has demonstrated its efficacy in dampening the type 2 inflammatory response in CRSwNP [4]. This reduction in inflammation may contribute to improved ciliary function by relieving the chronic irritation and inflammation [2].

Unfortunately, to date, no studies have specifically investigated the ciliary function both before and after

administration of omalizumab treatment in a patient presenting with CRSwNP. Assessment of ciliary function is achievable through digital high-speed videomicroscopy (DHSV) [5–7]. DHSV facilitated the examination of ciliary beat frequency (CBF) and beat pattern (CBP) [8].

This case report investigates ciliary epithelium function throughout chronic rhinosinusitis management by corticosteroids, surgery, and the administration of subcutaneous omalizumab.

2. Case Presentation

A Caucasian female in her late 50s with a history of chronic rhinosinusitis with nasal polyps presented to the ENT outpatient clinic after an unsuccessful course of oral and local corticosteroid. She had a history of functional endoscopic sinus surgery for obstructive nasal polyps 5 years before. The ENT examination with nasal endoscopy showed grade 3 polyposis in both nasal cavities. The SNOT-22 score was 45/110. The blood eosinophils count was normal (120/mm^3, 1.6%) and the total serum IgE was increased. At this time, the patient was listed to undergo functional sinus surgery and omalizumab injection afterwards. Pathological evaluation confirmed the eosinophilic nature of the polyps. Starting on the 5th month following surgery, a regimen of omalizumab treatment was administered monthly. At baseline, total IgE levels stood at 464kU/l. Clinical and endoscopic follow-ups were conducted up to 1 year post-surgery and 7 months post-omalizumab initiation.

In our case, four samples of ciliated epithelium were obtained from nasal brushing without local anaesthesia of the middle turbinate under endoscopic view, and ciliary function was analyzed using DHSV.

Nasal brushing samples were placed in 2 ml of medium 199 (Thermo Fisher, Waltham, MA, USA) containing an antibiotic solution (1% penicillin/streptomycin (Thermo Fisher, Waltham, MA, USA)) and an antifungal (1% amphotericin B (Thermo Fisher, Waltham, MA, USA)). Video sequences of ciliated beat edges were recorded using an inverted microscope with a 100x oil immersion interference contrast objective (Axio Vert.A1, Zeiss, Oberkochen, Germany) and a video camera at high speed (CrashCam Mini 1510, IDT Innovation in motion, Pasadena, CA, USA), at a frame rate of 500 hertz (Hz) and at a controlled temperature of 37°C. To record video sequences of cilia beating, 60 μL of respiratory ciliated edges in medium 199 was placed under the microscope and heated to 37°C using a heated box (Ibidi, Gräfelfing, Germany) and a microscope lens heater (Tokai Hit, Fujinomiya, Japan), and the temperature was strictly controlled and stable at 37°C [9].

Solely the edges considered normal or having minor projections, measuring at least 50 μm in length, were documented and utilized for ciliary functional analysis (CFA) [10]. Among these edges, only cilia free of mucus and displaying sideways beating profiles were examined. CFA was assessed based on a minimum of 3 high-quality edges meeting the specified criteria for each condition.

To perform manual assessment of ciliary beat frequency (CBF), the assessment involved identifying cilia or groups of cilia that beat in the sideways profile. The process included counting the number of frames needed to complete 5 beat cycles, which was then converted to CBF through a simple calculation [8]. A maximum of 10 CBF measurements were obtained from each ciliated beating edge. Ciliated edges that did not allow at least 4 CBF measurements to be performed were excluded from the analysis. If immobile cilia were present, a CBF of 0 Hz was recorded [9]. The average CBF for each sample was calculated for each treatment condition.

The specific movement of a cilium or group of cilia throughout a complete beating cycle was compared to the normal ciliary beating pattern (CBP) observed with DHSV [8, 11]. Each cilia or ciliary group evaluated was classified as having a distinct normal or abnormal CBP. The percentage of normal CBP in the sample was calculated for each condition [9].

The first sample was before functional endoscopic sinus surgery; the patient was under a treatment of oral corticosteroids. The second sample was three months after surgery, and the patient used only nasal corticosteroids and did not use it the day of sampling collection. The third sample was on the day of the third omalizumab injection, approximately 1 month after the first injection. Finally, the fourth sample was the day of the ninth omalizumab injection, approximately 3 months after the first injection. The results of the samples are presented in Table 1.

The patient showed a notable improvement in her condition, marked by a significant reduction of symptoms. Following treatment, the patient reported a remarkable improvement in her sense of smell, accompanied by improved sleep quality and a notable increase in her overall energy levels daily. Additionally, as a precautionary measure, the patient now undergoes regular ENT check-ups every three months, involving nasal endoscopy to monitor any signs of recurrence or polyp formation.

Patients with comorbid nasal polyps and asthma present a better reduction in asthma exacerbation rate, asthma control tests, rhinosinusitis outcome, and related quality of life under omalizumab compared to control patients [12]. However, the exact mechanism is still under investigation [13]. Our case report suggested that the mechanism may involve airway remodelling, including increase of cilia presence, allowing better local ciliary function [14]. Domingo Ribas et al. showed that baseline membrane thickness and intercellular spaces were reduced, and epithelial damage was improved [14]. Effective interaction between the mucus layer and coordinated ciliary beating resulted in better mucociliary clearance and symptom improvement.

3. Discussion

A remarkable observation in this case study is the similarity in ciliary function (CBF and CBP) between sample #1 (before FESS, with treatment of oral corticosteroids) and sample #4 (after three months of omalizumab injections). This similarity implies that treatment with oral corticosteroids may have played a crucial role in maintaining ciliary function by reducing inflammation even before the start of omalizumab treatment.

Table 1: Results of ciliary function and SNOT-22 score across different treatment conditions in a patient presenting with CRSwNP.

	CBF (Hz)	CBP (percentage of normal beating)	SNOT-22
Sample #1 (preoperative)	16.78 ± 1.51	95.65	45
Sample #2 (postoperative)	14.25 ± 4.25	41.18	45
Sample #3 (1 month omalizumab)	13.60 ± 2.75	90	Not evaluated
Sample #4 (3 months omalizumab)	16.03 ± 1.72	100	13

The first sample was taken preoperatively, under oral corticosteroids. The second sample was taken postoperatively, under intranasal glucocorticosteroid. The third sample was taken after one month of omalizumab injections. The fourth sample was taken after 3 months of omalizumab injections. CBF (Hz) = ciliary beat frequency in hertz; CBP = ciliary beat pattern; SNOT-22 = 22-Item Sinonasal Outcome Test. Numerical values in CBF column are expressed as mean ± SD (standard deviation).

Sample #1 demonstrated a CBF of 16.78 ± 1.51 Hz, which remains consistent with sample #4 (16.03 ± 1.72 Hz). Similarly, the CBP in sample #1 was 95.65% and sample #4 had a CBP of 100%. This suggests that treatment with oral corticosteroids effectively preserved ciliary function, possibly by attenuating inflammation of the nasal mucosa. In fact, previous study demonstrated the direct influence of cytokines on ciliary function in the respiratory epithelium, contributing to impaired mucociliary clearance in a cell culture model of ciliated human respiratory epithelial cells [15]. Specifically, interleukin-4 (IL-4) and IL-13, which are associated with the T helper (TH) 2 cytokine profile, have been shown to decrease CBF, while IL-5 and l IL-9, also TH2 cytokines, lead to an increase in CBF [15]. However, another hypothesis suggests a potential link between ciliary dysfunction and chronic mucosal inflammation or infection observed in patients with CRSwNP [2]. In fact, nasal polyps can lead to abnormal cilia architecture, causing alterations in motile cilia and ciliogenesis-associated markers, which subsequently affect ciliary beat frequency (CBF) [16–18]. This abnormal architecture, characterized by untidy, overly dense, and lengthened cilia, has been observed in patients with nasal polyps both in vivo and in vitro [16, 17]. Furthermore, studies have demonstrated that the overexpression of ciliogenesis-associated markers in patients with nasal polyps is associated with abnormal cilia architecture, potentially contributing to the observed CBF slowdown [16, 18, 19].

The initial decrease in ciliary function observed in sample #2 three months after FESS may be attributed to the switch from oral to nasal-only corticosteroids. This transition could have temporarily impacted inflammatory control, leading to a transient reduction in CBF and CBP. However, as shown in the following samples (sample #3 and sample #4), ciliary function gradually improved, reaching or even exceeding baseline levels.

Furthermore, SNOT-22 scores aligned with the ciliary function results, showing a notable reduction from 45 in sample #1 to 13 in sample #4. This improvement corresponds to the switch to omalizumab treatment and restoration of ciliary function, strengthening the potential synergistic effect of functional sinus surgery and omalizumab in the management of CRSwNP.

In summary, despite attempts with oral corticosteroids and sinus surgery, CBF, CBP, and SNOT-22 remained altered in this patient. However, following omalizumab therapy, CBF and CBP were successfully normalized. SNOT-22 showed a notable improvement by 32 points. A direct relationship between improved CBP and CBF values and lower SNOT-22 scores was observed (i.e., 13/110 post-omalizumab), while poorer CBP and CBF values corresponded to higher SNOT-22 scores (i.e., 45/110 pre-omalizumab). Omalizumab achieved better control of ciliary function (CBF and CBP) and symptoms, while avoiding the known side effects of oral corticosteroids.

To date, no similar cases have been published showing ciliary function by DHSV across different treatment lines. A large-scale study could be interesting concerning the introduction of biotherapy such as omalizumab, in the monitoring of ciliary function. Previous studies carried out by DHSV mainly studied CBF after cell culture [1, 3, 14, 15]. The objective of this case was to analyze ciliary function on the same day of sampling while maintaining the conditions of inflammation to which the respiratory mucosa was chronically affected.

Data Availability

The data supporting the findings of this case report are available upon request. Interested parties may obtain access to the data by contacting the authors via e-mail at Lioneljbenchimol@gmail.com. We are committed to promoting transparency and facilitating the sharing of research materials to contribute to the scientific community's understanding.

Consent

Informed consent has been obtained from the patient.

Disclosure

The authors conducted this work independently.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Anne-Lise Poirrier, Celine Kempeneers, Philippe Lefebvre, and Olivier Bouchain were responsible for study conception and design. Lionel Benchimol, Romane Bonhiver, and Noemie Bricmont were responsible for data collection. Lionel Benchimol and Anne-Lise Poirrier were responsible

for analysis and interpretation of results. Lionel Benchimol, Anne-Lise Poirrier, and Olivier Bouchain were responsible for manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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7. Repeating ciliary videomicroscopy improves the specificity for PCD diagnosis

7.1 Abstract

Background: Primary ciliary dyskinesia (PCD) is an inherited motor ciliopathy in which respiratory cilia are dyskinetic. High speed videomicroscopy analysis (HSVA) allows to evaluate beat frequency (CBF) and pattern (CBP). HSVA is highly sensitive and specific for PCD when combining CBF and CBP evaluation, as CBF alone lacks sensitivity and specificity. Currently, only electron microscopy (TEM) and genetics are recognized as confirmatory diagnostic tests for PCD. ERS guidelines state that HSVA should be repeated on 3 separate occasions to suggest a PCD diagnosis. However, the 3 visits imposed on the patient constitute a heavy burden and a significant cost.

Aims: To compare the sensitivity and specificity for PCD diagnosis of 1 HSVA evaluation versus HSVA repeated on 3 separate occasions.

Methods: We defined PCD positive if TEM and/or genetics were positive, and PCD negative if TEM and genetics were negative. We selected patients who had successful HSVA on 3 separate occasions. HSVA was considered as abnormal if the percentage of abnormal CBP was higher than our laboratory normal values. We compared the sensitivity and specificity between the first HSVA and the 3 repeated HSVA. We considered that the results of 3 HSVA was positive if the patient had 3 abnormal HSVA, and negative if the patient had ≥ 1 normal HSVA.

Results: 10 patients (4 PCD positive and 6 PCD negative) had 3 successful HSVA. The sensitivity of 1 HSVA versus 3 repeated HSVA to diagnose PCD were similar (75%). However, the specificity of 3 repeated HSVA was higher than a single HSVA to diagnose PCD (75% vs 33%, respectively).

Conclusion: This pilot study suggests that repeating HSVA on 3 separate occasions improves the specificity of the test.

Repeating ciliary videomicroscopy improves the specificity for PCD diagnosis

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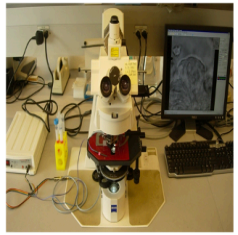
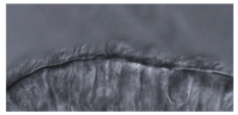
Background

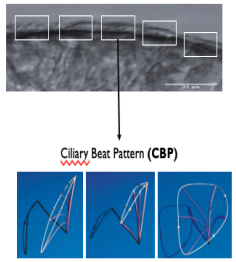
Primary ciliary dyskinesia(PCD) is an inherited motor ciliopathy in which respiratory cilia are dyskinetic. High speed videomicroscopy analysis (HSVA) allows to evaluate beat frequency(CBF) and pattern(CBP). HSVA is highly sensitive and specific for PCD when combining CBF and CBP evaluation, as CBF alone lacks sensitivity and specificity. Currently, only electron microscopy(TEM) and genetics are recognized as confirmatory diagnostic tests for PCD. ERS guidelines state that HSVA should be repeated on 3 separate occasions to suggest a PCD diagnosis. However, the 3 visits imposed on the patient constitute a heavy burden and a significant cost.





Aims

To compare the sensitivity and specificity for PCD diagnosis of 1 HSVA evaluation versus HSVA repeated on 3 separate occasions.



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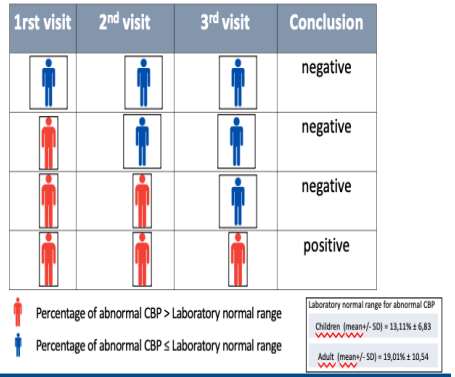
Results

10 patients(4 PCD positive and 6 PCD negative)had 3 successful HSVA

	sensitivity	specificity
1 HSVA	75%	33%
3 HSVA	75%	75%



Conclusion

This pilot study suggests that repeating HSVA on 3 separate occasions improves the specificity of the test.

8. Discussion and perspective

8.1 General discussion

DHSV is a key tool in the diagnostic workup of PCD, provided that sampling conditions are carefully controlled. Topical nasal anesthesia is effective in reducing patient discomfort without compromising ciliary analysis, whereas general anesthesia may alter ciliary beat analysis. Additionally, a case study of CRSwNP with a Th2-dominant inflammatory profile highlights the complexity of secondary ciliary dysfunction and the potential of integrating ciliary functional assessment into therapeutic monitoring.

PCD is a rare inherited motile ciliopathy caused by genetic mutations that disrupt the formation, assembly, structure, and function of cilia, leading to impaired motility. The condition has an estimated prevalence of 1:7,500 live births but is often underdiagnosed (100,103). In Europe, the median age at diagnosis is 9.8 years (100,103). The diagnostic delay in PCD is largely due to limited awareness among healthcare providers and the absence of a simple, standardized diagnostic pathway. Currently, no single test or combination of tests offers 100% sensitivity and specificity for PCD diagnosis (101,105,214). International guidelines specify that hallmark ciliary ultrastructural defects observed via TEM and/or non-ambiguous bi-allelic mutations in PCD-associated genes are required to confirm a diagnosis, yet these methods fail to identify approximately 30% of cases (102,260).

DHSV is a diagnostic technique that allows for the visualization and assessment of respiratory ciliary beating, including CBF and CBP. Studies have shown that DHSV has a high sensitivity (0.95–1.00) and specificity (0.91–0.95) for diagnosing PCD (102,251,254,256,286). However, its standalone use is not recognized by the ERS or ATS guidelines. The ERS does not accept DHSV in isolation for diagnosis, and the ATS excludes it entirely from the diagnostic algorithm (102,260).

This lack of recognition stems from two main challenges. First, there are no standardized protocols for DHSV, leading to variability in how the technique is performed across diagnostic centers (275). Differences in sample collection, in sample processing, environmental conditions, and analysis methods result in inconsistent reference values for ciliary function (275). Second, the diagnostic accuracy of DHSV is difficult to determine. Past evaluations have used incomplete reference standards, such as TEM alone or a combination of TEM and nNO measurements or included DHSV itself as part of the diagnostic criteria, which may skew sensitivity and specificity estimates (251,254,256). These limitations underscore the need for standardized protocols and further validation of DHSV to establish its role in the PCD diagnostic pathway.

This thesis sought to address critical gaps in the diagnosis and management of PCD through a multidisciplinary and innovative approach. **The first part of the thesis** was to examine and improve ENT clinical management strategies for PCD patients by analyzing diagnostic pathways, treatment efficacy, and the role of tailored interventions. By addressing challenges in early diagnosis and evaluating current treatment protocols, this study aimed to optimize patient outcomes while highlighting the importance of multidisciplinary care in managing this lifelong condition.

The second part of the thesis investigated the potential impact of a commonly used local anesthetic in ENT practice on diagnostic accuracy during DHSV assessments, focusing on its effect on CBF and CBP. Given the routine use of local anesthetics to enhance patient comfort, understanding their potential influence on diagnostic integrity is essential. This research not only assessed whether such anesthetics compromised diagnostic reliability but also explored whether their judicious use could improve procedural outcomes by ensuring higher-quality samples. In clinical practice, local anesthesia can be implemented routinely to facilitate repeated sample collections for diagnosing PCD. Our study showed that it is well tolerated and enhances patient comfort without altering CBF or CBP. Since ciliary function remains unaffected, the reliability of high-speed video microscopy for diagnostic assessment is preserved.

Expanding on the use of anesthesia in diagnostics, **the third part of the thesis** evaluated the feasibility and reliability of conducting ciliary sampling under general

anesthesia, particularly in pediatric patients or individuals unable to cooperate with standard sampling techniques. This research is especially relevant in pediatric ENT settings, where general anesthesia is often required for routine interventions (ventilation tubes, adenoidectomy, tonsillectomy, adenotonsillectomy). Considering our findings on the impact of general anesthesia on ciliary function, we do not recommend performing high-speed videomicroscopy analysis immediately after sampling under general anesthesia. While ciliary function assessment remains feasible following general anesthesia, it should be conducted on epithelial cells obtained from per operative sampling only after cell culture, in order to ensure reliable and interpretable results. In our prior study, transient alterations in CBF and CBP were observed within the first three hours following anesthesia, particularly in one of the three patients assessed. If general anesthesia is required for other medical reasons, particularly in children who often undergo multiple surgeries, it may provide an opportunity to collect samples. However, in such cases, it is crucial that ciliated samples are cultured before analysis using DHSV. This step allows for the removal of any residual effects of the anesthesia, ensuring more reliable results. While routine culturing is not typically practical due to the time-consuming nature of the process and despite a low risk of contamination, it remains a valuable approach when applied selectively. Cell culture plays a critical role in differentiating primary from secondary ciliary dyskinesia, primarily by excluding reversible dyskinesia associated with infection or inflammation. Additionally, it allows the assessment of ciliary function independent of transient alterations potentially induced by anesthetic agents.

Finally, the fourth part of this thesis assessed ciliary function in patients with CRSwNP, a condition frequently observed in PCD but also prevalent in the general population. Omalizumab treatment led to normalization of ciliary function (CBF and CBP) and marked clinical improvement (SNOT-22: 45 → 13) after partial response to oral corticosteroids and sinus surgery. The beneficial effect on ciliary function appears linked to improved control of nasal inflammation. A larger study is warranted to assess the impact of biologics on ciliary function. While not directly related to PCD diagnosis, this investigation illustrated a potential clinical application of standardized high-speed videomicroscopy protocols in evaluating ciliary impairment in inflammatory sinonasal diseases. By analyzing the effect of treatments, including biotherapies, on ciliary function

in CRSwNP patients, this work highlights how the standardized assessment technique developed for PCD can be extended to other clinical contexts. Although anti-IgE biotherapy was used in this study, future work could investigate the effect of IL-4/IL-13 inhibitors, given their known association with ciliary dysregulation. In this perspective, CRSwNP could serve as an inflammatory model to further explore secondary dyskinesia mechanisms and the broader clinical utility of ciliary function analysis.

The overarching goal of this thesis was to improve the diagnostic and therapeutic landscape for PCD while enhancing the patient experience. By investigating the integration of anesthetic protocols, this work highlights the potential to reduce procedural distress, ensure accurate diagnostics, and strengthen trust between patients and healthcare providers. Furthermore, the findings underscore the importance of a standardized approach to DHSV, and anesthesia use in diagnostics, paving the way for evidence-based protocols that prioritize both accuracy and patient comfort. Consequently, we advocate for the systematic incorporation of anesthetic administration into the DHSV protocol to enhance diagnostic accuracy while simultaneously improving patient comfort and procedural tolerance. Ultimately, the outcomes of this thesis are poised to contribute to more effective long-term management and improved quality of life for individuals living with PCD.

8.2 Limitations and futures perspectives

This thesis advances our understanding of PCD diagnostics and management; however, several limitations must be addressed in future research. A significant limitation lies in the limited scope of subject cohorts used in some methodological studies, with investigations relying primarily on healthy subjects rather than on patients with confirmed or suspected PCD. While these studies provide essential baseline data, their findings need validation in larger, more diverse cohorts, particularly among individuals referred to PCD diagnostic centers. As PCD is a rare and heterogeneous disease characterized by various phenotypes and genotypes (102,195), international multicenter collaborations will be vital for gathering sufficient patient data to explore variations across subtypes effectively. Such

efforts will enhance the applicability of findings and may reveal unique characteristics linked to specific phenotypes or genotypes.

Additionally, respiratory ciliary function in this thesis was predominantly evaluated using nasal airway epithelium samples, given their accessibility and the minimally invasive nature of nasal brushing. While studies have demonstrated that CBF measured from nasal samples correlates well with bronchial samples, subtle differences in the CBP or ultrastructure across different respiratory regions cannot be excluded (287). Nevertheless, nasal brushing remains the preferred technique for routine sampling due to its speed, safety, and low risk of complications, particularly when compared to invasive biopsy methods (288,289). Further studies examining bronchial samples may provide complementary data for general anesthesia CFA.

The variability in CBF and CBP within samples from the same individual also poses challenges for diagnostic standardization (7,274). While multiple high-quality edges were analyzed for each subject to mitigate this issue, intra-subject variability was not systematically assessed. This limitation is particularly relevant in the context of our findings showing that local anesthesia does not alter CBF or CBP. Future research should aim to quantify this variability and refine evaluation protocols to ensure diagnostic precision.

Furthermore, we explore the methodological aspects and diagnostic potential of ciliary videomicroscopy for identifying PCD using fresh nasal brushing samples. However, evidence suggests that evaluating CBF and CBP and ultrastructure through cultured airway epithelial cells significantly enhances diagnostic accuracy, particularly in differentiating true PCD cases from false positives (249,263). Acute or chronic respiratory infections, inflammation, and various environmental can induce transient secondary abnormalities in ciliary ultrastructure and function (260). Unlike genetic defects, these secondary abnormalities typically resolve following cell culture, further underscoring the value of this approach in refining diagnostic reliability (261–263).

Another key limitation is the lack of a universally standardized protocol for ciliary videomicroscopy across diagnostic centers. Variability in sample collection, processing, and analysis techniques may lead to inconsistencies in reference values and diagnostic outcomes, complicating inter-center comparisons (290–299). Establishing an international

protocol would enable more reliable comparisons of CFA across laboratories, fostering global advancements in PCD diagnostics.

The absence of fully automated tools for evaluating CBF and CBP is also a limitation in my studies. Manual assessments are subjective, time-intensive, and prone to interobserver variability. Even though interobserver variability in our laboratory was minimal, this difference could still be a source of variability that may impact the diagnosis of PCD. Developing artificial intelligence (AI)-driven systems for automated analysis could significantly enhance diagnostic accuracy and efficiency. However, training these algorithms requires large datasets comprising well-characterized PCD and non-PCD samples, which can be challenging to obtain due to the rarity and variability of the condition. One limitation is that not all cases of PCD are the same. There is considerable heterogeneity in the clinical presentation, pattern of ciliary beating and underlying genetic mutations. This diversity means that AI systems trained on more common patterns of PCD may fail to recognize rare or atypical cases, leading to missed diagnoses or misinterpretations. Additionally, AI algorithms are highly dependent on the quality and representativeness of the data they are trained on. If the dataset is biased or lacks sufficient representation of rarer phenotypes, the AI may be less accurate in diagnosing those patterns. Furthermore, AI systems are typically designed to recognize patterns within the data they are trained on, and their ability to adapt to new, unseen variations may be limited. This could result in a reduced ability to detect novel or less common forms of PCD. The reliance on large, high-quality datasets also presents challenges in ensuring data privacy and standardization, especially when data is collected across multiple centers with varying protocols and patient populations. Thus, while AI holds great potential for improving PCD diagnosis, these limitations must be carefully considered to ensure its effectiveness and safety in clinical practice. Given the rarity of PCD, achieving this will necessitate extensive collaboration among international diagnostic centers. The adoption of standardized videomicroscopy protocols will be critical to ensure consistency in training data and subsequent diagnostic performance.

Finally, the findings related to the impact of local and general anesthesia on diagnostic reliability are noteworthy. While local anesthesia shows promise for routine sample collection by improving patient comfort and reducing procedural variability, the

use of general anesthesia remains complex. General anesthesia is not routinely recommended due to its potential to interfere with ciliary function during videomicroscopy. However, when general anesthesia is required for other medical procedures, such as in pediatric ENT settings, it provides an opportunity for sample collection, with cell culturing serving as a critical step to ensure diagnostic accuracy. Future studies should focus on refining anesthesia protocols and evaluating their effects on diagnostic outcomes in larger patient populations.

A future avenue of research lies in investigating ciliary function in PCD patients with CRSwNP undergoing biotherapy. While it is unlikely that ciliary function would return to normal, biotherapy could potentially mitigate secondary dyskinesia caused by chronic inflammation, leading to relative improvements in ciliary function. Such studies could provide novel insights into managing both PCD and its nasal manifestations, ultimately contributing to more personalized and effective therapeutic strategies.

9. Conclusion

PCD is a rare and complex genetic disorder, characterized by impaired ciliary function leading to significant respiratory, otologic, and sinonasal manifestations. Despite advancements in our understanding of its pathophysiology and diagnostic tools, PCD remains challenging to diagnose due to its rarity, heterogeneity, and the absence of a single gold standard diagnostic test. International guidelines emphasize the importance of combining clinical assessments with a range of diagnostic tools, including ciliary ultrastructure analysis, genetic testing, nasal nitric oxide measurement, and ciliary videomicroscopy, to enhance diagnostic accuracy.

Efforts to standardize diagnostic protocols have highlighted the need for further refinement in methodologies, such as ensuring consistency in sample handling and optimizing the assessment of ciliary function. Collaborative international efforts are critical to establish uniform protocols and reference standards, enabling reliable comparisons across diagnostic centers worldwide. Additionally, the integration of automated systems and artificial intelligence in ciliary function analysis holds promise for improving the efficiency and objectivity of diagnostic procedures. In this context, our results provide important insights regarding the impact of anesthetic methods on ciliary functional analysis. We found that local anesthesia does not affect CBF or CBP and improves patient comfort, supporting its implementation in the standardization of DHSV protocols for PCD diagnosis. In contrast, general anesthesia can alter ciliary parameters, and we therefore recommend avoiding direct DHSV analysis on fresh samples obtained under general anesthesia. Instead, cellular culture prior to analysis should be favored to ensure diagnostic reliability.

Beyond diagnostics, the management of PCD underscores the importance of a multidisciplinary approach, tailored to address the chronic and progressive nature of the disease. Early diagnosis, combined with personalized interventions and close monitoring, plays a pivotal role in preventing complications and improving long-term outcomes. Enhancing patient comfort during diagnostic and therapeutic procedures, particularly in

pediatric settings, is essential to fostering trust and cooperation in managing this lifelong condition. In this context, the use of local anesthesia within a standardized DHSV protocol for PCD diagnosis appears particularly valuable in pediatric populations, as it facilitates better tolerance of nasal sampling without compromising ciliary function assessment.

As knowledge of PCD continues to grow, future research must focus on unraveling genotype-phenotype correlations, developing innovative treatments, and exploring how interventions can mitigate disease progression. International collaboration will remain central to advancing diagnostic and therapeutic strategies, ultimately improving care and quality of life for individuals living with this challenging condition. For SCD, future research intefrating ciliary functional assessment into therapeutic monitoring could refine our understanding of MCC and support the development of more tailored treatment strategies for selected patients.

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