4 Physiology of the Nose and Paranasal Sinuses: Mucociliary Clearance

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Introduction

The conducting airways in the nose and sinuses are lined with a pseudostratified epithelium consisting of ciliated cells, secretory cells and goblet cells.

The epithelium in the sinonasal cavity is a main entry port for respiratory pathogens, allergens and pollutants, and it also plays an important role in the initial host responses against infection. Normal mucociliary clearance (MCC) is essential for the maintenance of an effective primary defence mechanism and healthy sinonasal cavities. Effective MCC necessitates appropriate mucus, and effective and synchronized ciliary beating accompanied with a proper periciliary fluid layer.

Cilia propel respiratory mucus. After inhalation of a pathogen, allergen, debris or a pollutant, the foreign material is trapped in the mucus and then phagocytised or removed by the process of MCC. The coordinated and continuous unidirectional beating of the cilia transports the mucus to the oropharynx, where it is cleared by ingestion, coughing and expectoration.

If MCC is compromised, the airways become vulnerable to infection and inflammation. This phenomenon is evident in patients with chronic rhinosinusitis who experience persistent cycles of infection and inflammation resulting in ciliary loss and a hyper-viscous mucus. Damage or disruption of mucociliary function due to viral infection is probably a major cause of secondary bacterial infection.

This chapter will review the essential components of the mucociliary apparatus and discusses important clinical examples of compromised MCC. Factors that can improve MCC will also be discussed.

Mucus

The normal mucosal lining of the nasal cavity is coated by a mucus layer up to 70 μm thick [1]. The periciliary fluid layer is approximately 5 μm thick [2].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-28690-2_4.

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A. C. Swift et al. (eds.), *Contemporary Rhinology: Science and Practice*,
https://doi.org/10.1007/978-3-031-28690-2_4

Mucus is produced by goblet cells and by submucous glands in the mucosa [3]. In the nose, 100–200 mL of mucus is produced per day [2]. Mucus is a barrier to prevent water loss by diffusion and to remove inhaled foreign substances such as viruses, bacteria, allergens, inflammatory cells and pollutants. Upon infection of the nasal epithelium, secretory cells release anti-microbial surfactants and mucus to delay pathogen transmission in the airway [2]. Mucus is characterized by its volume, viscosity, elasticity and thread-forming capacity [4].

Mucus is a gel consisting of predominantly water (approximately 95%). The other key components are ions, proteins and macromolecules. The major macromolecular components of mucus are the mucin glycoproteins. These can be subdivided into:

1. Secreted mucins
2. Cell-associated mucins that are anchored at cell surfaces
3. Gel-forming mucins

This arrangement of cell-associated and gel-forming secreted mucins creates a two-layered airway surface mucus barrier with a periciliary liquid layer next to the cell surface and a gel-forming mucin layer. The most important cell-associated mucins are MUC1, MUC4, MUC16 and MUC20. The periciliary fluid, both in composition and volume, appears to be critical for proper mucociliary transport [1]. The cell-associated mucins attached to airway epithelial microvilli and cilia generate an osmotic barrier that preserves the periciliary layer [3].

The most important gel-forming secreted mucins are MUC5AC and MUC5B. These are responsible for the characteristic viscoelastic properties of the mucus gel layer. MUC5AC production from goblet cells increases following viral infection. It has been shown that rhinovirus infection induces temporary mucus hypersecretion which is evident during the common cold.

Mucins can also mediate inflammatory cascade pathways, and they contain innate immune proteins such as lactoferrin, lysozyme and s-IgA which aid in the local immune defences [5].

All of these rheologic and physical properties are influenced by the degree of hydration and the glycoprotein composition, factors that are host-regulated.

Mucus hyperproduction is also an important hallmark of type 2 inflammation via activation of Il-13 and Il-5; please see below.

Cilia

Ciliated cells are the major component of the pseudostratified epithelium. Cilia are hair-like organelles that are organized with microtubule, Fig. 4.1. There are 50–200 cilia per epithelial cell. The length of cilia is typically 6 μm, and they reach through the periciliary liquid layer and just into the mucus layer.

Cilia are coated with cell-associated mucins that exclude mucus from the periciliary space and promote the formation of a distinct mucus layer and a periciliary liquid layer as mentioned above [3].

Cilia are composed of structural proteins and motor proteins that drive their coordinated unidirectional beating of the cilia which are critical for MCC. Under normal conditions, the cilia beat at a frequency of 6–17 Hz [6, 7].

The normal ultrastructure of motile cilia contains nine outer doublets of microtubules and a central pair called the “9+2 axonemal appearance.” Outer and inner dynein arms are attached and contain enzymes for ATP hydrolysis producing power. Nexin links connect the doublets and stabilize the structure, whereas radial spokes interlock the outer doublet to the central pair. When activated, the dynein arms slide one microtubule-duplet relative to another, and since these are connected by the nexin links, the whole axoneme bends (Figs. 4.1 and 4.2).

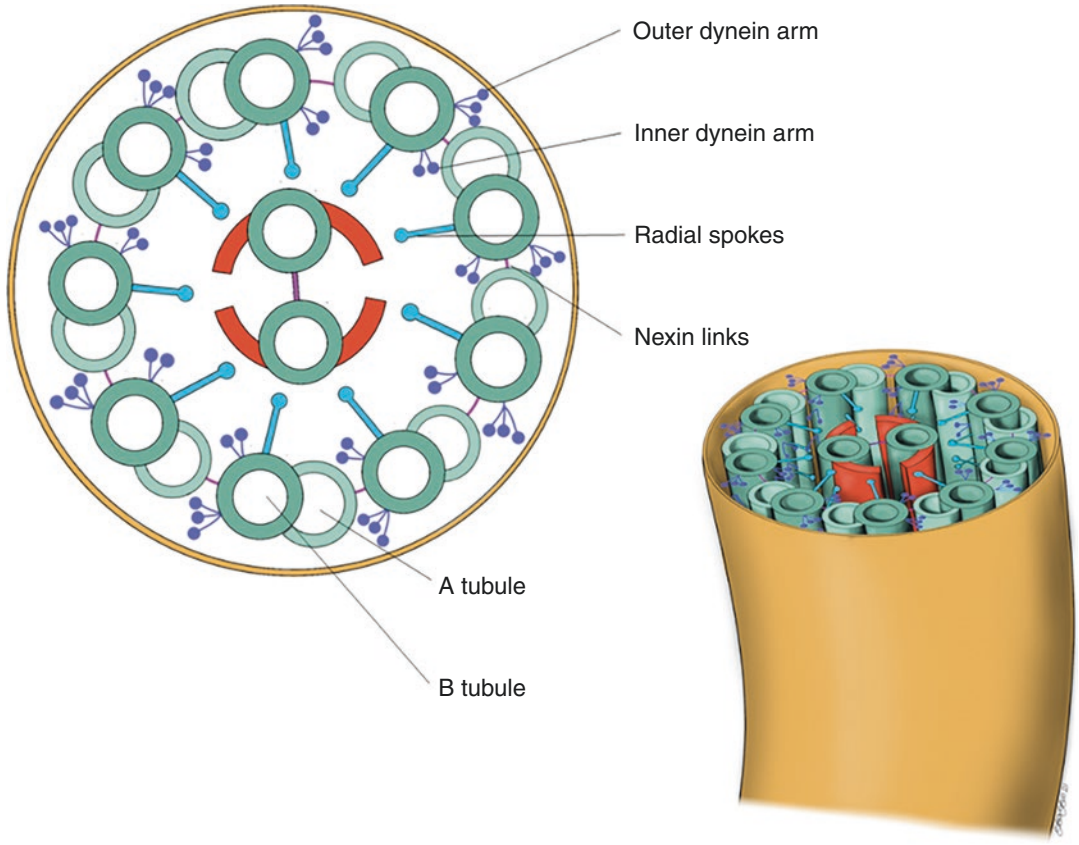

Fig. 4.1 Normal ultrastructure of cilia. Normal cilia have nine outer doublet (A + B tubule) and a central pair "9 + 2 appearance." Dynein arms are attached to the outer doublets. Nexin links connect the outer doublets, and radial spokes connect the outer doublets with the central pair

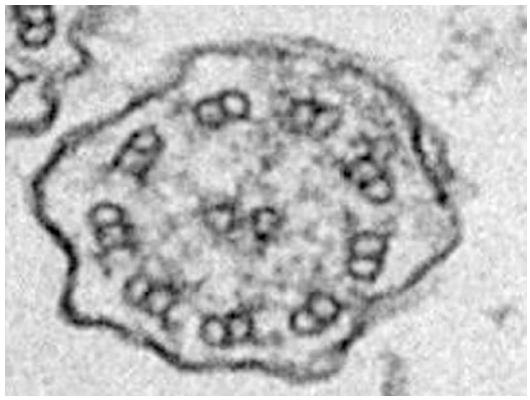

Fig. 4.2 Normal ultrastructure by TEM. The "9 + 2" axonemal appearance is evident

Assessment of Ciliary Ultrastructure and Ciliary Beat Function

Ciliary ultrastructure can be assessed by transmission electron microscopy (TEM). TEM is used for research purposes and in the clinical setting to aid in the diagnosis of primary ciliary dyskinesia (PCD); please see below. The required ciliated epithelial specimen can be obtained using a cytology brush on the inferior nasal turbinate [8].

Precise ciliary beat frequency and ciliary beat pattern can also be assessed from brush biopsies of the inferior turbinate using high-resolution, high-speed video microscopy with slow-motion

replay. Like TEM, it is used clinically to establish a diagnosis of PCD (Video 4.1).

Nasal Mucociliary Clearance Testing

The MCC time is the time taken for a molecule inserted into the nares to reach the oropharynx. Mucus moves at a speed of approximately 10 mm per minute in vivo under normal conditions, and normal values in adults are approximately 10–15 min. It can be assessed using different methods [6, 9].

Saccharin Test

A 5 mg particle of saccharin is placed on the inferior turbinate, 1.5 cm from the nares under direct visualization. A timer is started, and the transit time is reported as the elapsed time from the placement of the particle until the patient reports a sweet taste. Normal values reported for this assay are between 11 and 15 min and it has been recommended that further investigations are necessary in patients with a transit time of 60 min or more [9]. The saccharin particle can be dissolved with methylene blue. Thus, when the patient reports the taste sensation, the objective finding of blue dye in the oropharynx confirms the subjective taste report [10].

Scintigraphy with Technetium-99

A droplet of a suspension of colloid particles labelled with technetium-99 (usually 50 [mu]Ci diluted in 0.05 mL of saline) is placed 1 cm posterior to the mucocutaneous junction of the nasal cavity on the inferior turbinate or along the lateral floor. Movement of the radioactivity is recorded with a gamma camera with images obtained every 30 s during a 10-min period [7]. Most studies report an average velocity of 10.9 mm/min for control populations. To determine MCC in the lower airways, a turboinhaler may be used with labelled particles of different sizes. Larger particles typically deposit in the nose and pharynx, while smaller particles are deposited in the trachea, and minute particles remain suspended in inhaled air [4] (Video 4.2).

Examples of Compromised MCC

Impaired MCC leads to stagnant mucus in the respiratory tract, which predisposes to infection and inflammation.

Primary Ciliary Dyskinesia

PCD is an autosomal recessive genetic disease. Well-described mutations in more than 30 genes involved in ciliary structure and function are characterized, and genetic testing can identify approximately 60% of the phenotypically identified PCD patients. In PCD, MCC is impaired by genetic mutations resulting in non- or hypofunctional cilia.

The commonest ultrastructural defect in PCD is defects in one or both dynein arms. This is observed in >80% of patients with recognized structural defects (Fig. 4.3 [11]).

Initially, the composition of the mucus is presumably normal in the PCD airway; however, during prolonged or chronic infection and inflammation, DNA and actin released from neutrophils may increase the viscosity of the mucus.

PCD manifests primarily as an oto-sino-pulmonary disease comprising chronic otitis media with effusion, chronic rhinosinusitis with or without nasal polyps and recurrent or chronic lung infections leading to structural lung damage

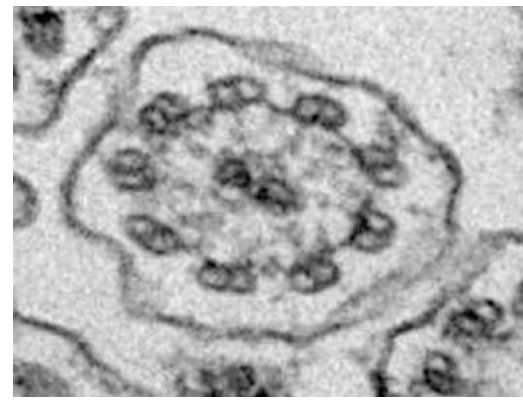

Fig. 4.3 Abnormal TEM in a patient with PCD. Transition electron microscopy displaying missing outer dynein arm, representing one of the most common findings in patients with PCD

such as bronchiectasis and declining lung function.

CRS and bacterial sinusitis are ubiquitous in patients with PCD affecting more than 70% of the patients. Sinus surgery can improve QoL in patients with PCD and may also be effective in eradicating Gram-negative bacteria from the global airways [12] (Video 4.3).

Cystic Fibrosis (CF)

Cystic fibrosis (CF) is a life-shortening genetic disease caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene on chromosome 7. The gene encodes chloride channels, and the defect leads to abnormal transport of chloride and sodium across the cell. Loss of CFTR function results in deficient chloride and bicarbonate secretion and dysregulation of the epithelial sodium channel with excessive sodium absorption at the apical cell membrane. The resultant decrease in salt concentration in the airway secretion more than doubles the viscosity. This leads to a dehydrated and sticky mucus which reduces MCC by preventing normal ciliary movement and predisposes to infection. Recurrent or chronic lung infection with especially CF-pathogenic Gram-negative bacteria (GNB) including *Pseudomonas aeruginosa*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* causes structural lung damage, declining lung function, premature death or lung transplantation. In CF, the cilia are apparently normal. However, a recent study demonstrated abnormal accumulation of an intracellular transport protein (IFT88) and disrupted intra-ciliary trafficking which suggest that disrupted ciliary function is also a feature of the CF phenotype, which might contribute to defective airway MCC [13].

Cough clearance is weakened in CF due to the depletion of the airway surface liquid which is not the case in PCD. Airway inflammation in both PCD and CF are dominated by neutrophilic infiltration compared to eosinophilic inflammation in patients with CRS with nasal polyps and asthma.

CRS with or without nasal polyposis is common in patients with CF, and radiographic evidence of CRS in CF is almost 100%. Nevertheless, <50% report symptoms, but they can have a substantial negative impact on QoL. Sinus surgery with adjuvant medical therapy can reduce pulmonary infections with CF-pathogenic GNB and improve QoL [14].

CFTR modulators serve as correctors or potentiators of the chloride channel, and there is substantial evidence that they can improve lung function, quality of life and slow the progression of lung disease. Emerging evidence support that CFTR modulators also may improve sinonasal symptoms, i.e. SNOT 22 in CF [15].

In contrast to PCD patient, OME is very rare in CF.

Secondary Ciliary Dyskinesia

Ciliary abnormalities detected after infection and inflammation are referred to as secondary ciliary dyskinesia. Mucostasis, hypoxia, microbial products and toxic inflammatory mediators can induce secondary ciliary changes, and ciliary impairment is a feature of both viral and bacterial rhinosinusitis.

Impairment of nasal MCC including a fall in the number of ciliated cells and a moderate and short-lasting change in beating frequency and synchrony has been observed in patients during the common cold. Other studies have further confirmed that impaired ciliogenesis is prominent following viral infections consistently leading to loss of cilia and ciliated cell ultrastructural abnormalities. Characteristically, influenza virus infection can be followed by apoptotic and necrotic cell death causing the loss of epithelium including ciliated cells, impacting ciliary function. During sinusitis, a study found a prolonged nasal MCC time of 18 min versus 10 min for matched controls [6]. Impairment of MCC following viral infection is probably a major cause of secondary bacterial infections.

Smoking

Ciliary impairment is associated with cigarette smoking. Smoking significantly prolongs nasal

MCC probably due to a reduced beat frequency, a reduction in number of cilia and changes in viscoelastic properties of mucus as a result of significantly increased goblet cell density and mucin volume density [3, 16]. It is also well known that smoking can contribute to the development of CRS [17].

Drugs

Several studies on the effect of nasal steroids have found no change on MCC in healthy subjects, but they may be effective in patients with perennial rhinitis; see below.

Studies on the imidazoline derivatives oxymetazoline and xylometazoline which are alpha adrenergic receptor agonists have found that they exhibit ciliotoxic effects and inhibit ciliary function and thus MCC [18]. Long-term use may also lead to rhinitis medicamentosa. It is believed that when the imidazoline derivatives are withdrawn, increased parasympathetic activity leads to rebound congestion as a consequence of vasodilation and mucosal swelling. Long-term use may also lead to goblet cell hyperplasia and destruction of nasal cilia which compromise MCC [19].

Gastroesophageal Reflux Disease (GERD)

Reflux of gastric acid into the pharynx and nasopharynx is thought to cause mucosal inflammation which may impair MCC.

Type 2 Inflammation

The immune system covers a wide variety of inflammatory cells with different functions and features. Inflammation is generally defined as a response to an invading pathogen or endogenous signals from, e.g. damaged cells. Toxic, non-allergic or allergen-induced inflammation of the nasal mucosa causes swelling resulting in reduced MCC.

Patients with asthma and CRS with nasal polyps usually present with type 2 helper T-cell (Th2) cytokine-mediated inflammation in the mucosa, which has similarities to allergic inflammation/hay fever. Controversially, neutrophilic Th1-dominated inflammation is seen in patients with COPD. Key Th1 cell cytokines are interferon (INF)-γ and tumour necrosis factor that trigger macrophages while inhibiting mast cells, eosinophils and IgE production.

Th2 cell-mediated production of interleukins is dominated by Il-4, Il-5 and Il-13. Il-5 production increases tissue eosinophilia. Il-13 hyperproduction leads to bronchial hyperreactivity, goblet cell metaplasia and vessel wall priming that allows eosinophils to extravasate, and they inhibit macrophages. Especially, mucus hyperproduction and bronchial smooth muscle proliferation are hallmarks of type 2 inflammation.

Mucus plugging of bronchi is seen in severe asthmatics and associated with airway eosinophilia. Similarly, mucus plugging in the sinus cavities is evident in severe Th2 cell-mediated inflammation. Activated eosinophils will release galectin-10 that will undergo a transition to a crystalline form as Charcot-Leyden crystals (Fig. 4.4 [20]). These crystals are sharp and act as a barbed wire infiltrating the mucus making it increasingly sticky—comparable to dried glue [21]. Mucus plugging in the nose and sinus cavities compromise MCC.

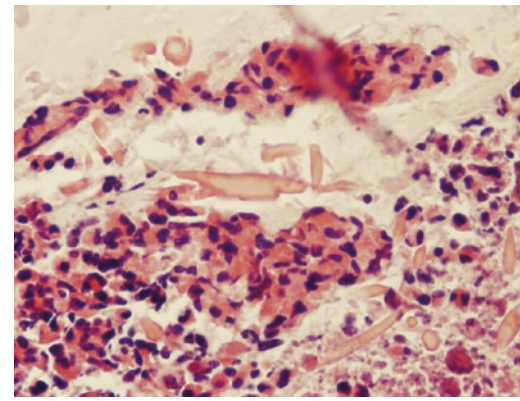

Fig. 4.4 Charcot-Leyden crystals formed in severe Th2 cell-mediated inflammation. Source: Original image kindly supplied by Andrew C. Swift

Improving Mucociliary Clearance (MCC)

Nasal Irrigation with Saline

Nasal irrigation with isotonic and hypertonic saline can improve the mucociliary transport function of the nasal mucosa [22]. Different kinds of nasal irrigation solutions, such as normal saline as well as various concentrations of hypertonic saline, have been used clinically. Saline solutions have been widely used in nasal irrigations for many years and are recommended for the treatment of various nasal diseases by several international expert groups including the EPOS 2020 [23]. Besides stimulating MCC, nasal irrigation may also be effective in reducing nasal congestion and secretions and moisturize the mucosa.

Drugs

Intranasally administered drugs can speed up or slow down MCC, which may be used in the clinical setting. For instance, a drug that increases MCC may lead to a faster clearance of pathogens or allergens from the mucosa. In contrast, drugs that prolong MCC may increase the bioavailability of topically administered drugs. However, many studies are conflicting, but it is an interesting area of future research [18].

Mucoactive drugs are regularly used as a therapeutic option for mucus alteration, including hypersecretion. The drugs can be divided into expectorants (e.g. hypertonic saline), mucoregulators that regulate mucous secretion (e.g. carbocisteine), mucolytics that decrease mucous viscosity (e.g. N-acetylcysteine and DNase) and mucokinetics that increase MCC by acting on the cilia (e.g. bronchodilators and surfactants). Long-term treatment of patients with perennial rhinitis with fluticasone propionate can increase nasal MCC, whereas treatment with xylometazoline may prolong it [9].

Endoscopic Sinus Surgery (ESS)

ESS can improve MCC by addressing the natural drainage pathways from the sinuses or by clearing polyps from the nasal cavity. ESS has also been found to significantly improve the number of cilia and can reduce the number of goblet cells in the mucosa which may facilitate MCC. In addition, ESS can facilitate nasal irrigation and subsequent topical treatment with steroids and antibiotics of the nose and sinuses [24].

Key Learning Points

- Effective mucociliary clearance necessitates proper mucus composition.
- Effective mucociliary clearance necessitates normal respiratory cilia.
- Mucociliary clearance can be tested but is primarily used for research purposes.
- Genetic diseases such as primary ciliary dyskinesia and cystic fibrosis lead to compromised mucociliary clearance.

Infection, inflammation, gastroesophageal reflux disease, smoking and various drugs can affect mucociliary clearance.

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