

Toward an optimized strategy of using various airway mucus clearance techniques to treat critically ill COVID-19 patients

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Abstract: Coronavirus disease 2019 (COVID-19) caused by acute respiratory syndrome coronavirus 2 (SARS-Cov-2) is still threatening the human life and society throughout the world. For those critically ill patients, mechanical ventilation (MV) is essential to provide life support during treatment. However, both the virus infection and MV disrupt the balance between secretion and elimination of airway mucus and lead to mucus accumulation in the lung. Postmortem examination verified that the lungs in patients died of COVID-19 are indeed filled with sticky mucus, suggesting a great need to improve airway mucus clearance in critically ill COVID-19 patients. Therefore, it may be helpful to comprehensively review the current understanding regarding the changes of biochemical and rheological features of airway mucus associated with the disease, as well as the physiological principles and algorithm to decide airway clearance techniques suitable for the critically ill COVID-19 patients. Based on these considerations, optimized strategies may be developed to eliminate the airway mucus accumulated in the airways of critically ill COVID-19 patients.

Abbreviations

ACTs:	airway clearance techniques
ARDS:	acute respiratory distress syndrome
COVID-19:	coronavirus disease 2019
EPP:	equal pressure point
FRC:	functional residual capacity
HFCWO:	high frequency chest wall oscillation
Hi-PEP:	high pressure positive expiratory pressure
MCC:	mucociliary clearance
MHI:	manual hyperinflation
MI-E:	mechanical insufflation-exsufflation
MV:	mechanical ventilation
Osc-PEP:	oscillating positive expiratory pressure
PCL:	periciliary layer
PEF:	peak expiratory flow
PEP:	positive expiratory pressure
PIF:	peak inspiration flow
SARS-CoV-2:	severe acute respiratory syndrome coronavirus 2
VHI:	ventilator hyperinflation

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still threatening human health worldwide (Sohrabi *et al.*, 2020). COVID-19 patients usually start with common symptoms such as cough and fever, but within two weeks some of them will become either severely (15%) or critically ill (5%) suffering from acute respiratory distress syndrome (ARDS) and even respiratory failure, and most critically ill COVID-19 patients need mechanical ventilation (MV) to provide respiratory support (Brewster *et al.*, 2020; Cascella *et al.*, 2021; Huang *et al.*, 2020; Wu and McGoogan, 2020; Yam *et al.*, 2003; Yang *et al.*, 2020). Unfortunately, these mechanically ventilated COVID-19 patients are still risk an over 50% fatality rate (Baden and Rubin, 2020; Cao *et al.*, 2020; Murthy *et al.*, 2020; Wang *et al.*, 2020; Xie *et al.*, 2020).

One of the main reasons for this unacceptably high mortality may be the suffocating mucus accumulation in the airways (Chen *et al.*, 2020). Indeed, airway mucus accumulation or even mucus plug has been observed in both proximal bronchi and peripheral small airways in critically ill COVID-19 patients (Liu *et al.*, 2020a; Lu *et al.*, 2021; Xu *et al.*, 2020). In addition, airway mucus accumulation is thought to result in a common feature of lung opacity in severe COVID-19 patients (Dunican *et al.*, 2018) and severe

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hypoxemic respiratory failure in a significant proportion of COVID-19 patients (Brosnahan *et al.*, 2020; Wu *et al.*, 2020). It has also been suggested that airway mucus accumulation may lead to silent hypoxia, i.e., oxygen deprivation without breathing problems, which is presented as a unique life-threatening feature of COVID-19 (Khan *et al.*, 2021; Liu *et al.*, 2020b). Airway mucus accumulation is generally associated with respiratory viral infection, but is known to be deteriorated by MV, which may be responsible for airway obstruction and loss of pulmonary function in mechanically ventilated critically ill COVID-19 patients (Chen *et al.*, 2019b; Fahy and Dickey, 2010). Therefore, it is very important to properly manage airway mucus accumulation in COVID-19 patients, especially those critically ill and mechanically ventilated patients (Brosnahan *et al.*, 2020; Cook *et al.*, 2020; Saracoglu *et al.*, 2020; Szarpak *et al.*, 2020).

In clinical practice, airway mucus accumulation can be managed by either pharmacological intervention using mucus thinning and expectorant drugs or physical intervention using so-called airway clearance techniques (ACTs) (Balsamo *et al.*, 2010; Belli *et al.*, 2021; Castro *et al.*, 2013; Fan *et al.*, 2017; Gosselink *et al.*, 2008; Poole and Black, 2003; Rogers and Barnes, 2006). In fact, a spectrum of ACTs have been proved effective in helping clearing airway mucus accumulation such as postural drainage, breathing maneuvers, manual hyperinflation and mechanical assistive devices, and some of them have been recommended in several therapy guidelines for COVID-19 patients (Belli *et al.*, 2021; Murthy *et al.*, 2020; Thomas *et al.*, 2020). However, there have been few comprehensive and updated reviews focusing on different ACTs in regards of their working mechanisms and efficacies, which is not helpful to fully understand the pros and cons of different ACTs in treating different cases of airway mucus accumulation and thus prevent optimization of ACTs application in treating critically ill COVID-19 patients (Battaglini *et al.*, 2020; Denehy and Berney, 2006; Lazzeri *et al.*, 2020; Thomas *et al.*, 2020; Vitacca *et al.*, 2020).

Therefore, in this review article we first summarize the changes of biochemical and rheological properties of airway mucus and the correlated risk factors of airway mucus accumulation, especially in COVID-19 patients. Then, we focus on the basic rationales for airway mucus clearance and the associated ACTs that may be suitable for critically ill COVID-19 patients. Based on such deep understanding of airway mucus accumulation and ACTs, an optimized strategy may be established for application of ACTs in management of airway mucus accumulation in critically ill COVID-19 patients (Ambrosino and Clini, 2015; Stiller, 2000).

Airway Mucus Accumulation in COVID-19 Patients

In COVID-19, it has been reported that a large proportion of the patients present symptoms of cough (67.8%) and sputum production (33.7%), both of which are known to be associated with excessive airway mucus secretion (Guan *et al.*, 2020; Wang *et al.*, 2020). These data suggest that airway mucus accumulation may be a common phenomenon in COVID-19, due to a combination of dysfunction of mucociliary clearance (MCC), airway mucus hypersecretion, and changed rheology as described in the following.

Mucociliary clearance in healthy subjects and COVID-19 patients

Airway mucus in healthy subjects usually acts as an essential protective physical barrier by forming a thin layer on top of the airway lumen and then constructing MCC together with cilia (about 200 per cell, 6-7 μm long). Under the cyclic beatings of cilia including a fast effective stroke and a slow recovery stroke (10~15 Hz), the inhaled particles and pathogens can be trapped in the airway mucus and eliminated from the airway system (Fig. 1) (Loreng and Smith, 2017; McAuley *et al.*, 2019; Ridley and Thornton, 2018; Roy *et al.*, 2014; Sanderson and Sleight, 1981; Widdicombe, 1997; Xu and Jiang, 2019b).

According to its location to cilia, the airway mucus layer can be divided into an apical high viscosity layer (mucus gel, 10-50 μm depth) and a bottom low viscosity layer (periciliary layer, PCL, 7 μm depth) (Button *et al.*, 2012; Thornton and Sheehan, 2004). Since the height of cilia is similar to the depth of PCL layer, most of the movements of cilia occur in the PCL layer. Therefore, during the effective strokes the cilia intermittently sweep the subsurface of the mucus gel, which leads to the mucus cephalad transport (~100 $\mu\text{m}/\text{s}$) (Silberberg, 1990). This high turnover rate of airway mucus by MCC is a major feature of the respiratory system in healthy subjects. However, in COVID-19 patients, SARS-CoV-2 infection is known to seriously disrupt the function of MCC. For example, it has been reported that SARS-CoV-2 infection inadvertently leads to the shedding of ciliated cells from the airway epithelium (Fang *et al.*, 2020). This is partly because that a subpopulation of the ciliated cells highly express ACE2 and TMPRSS2 and thus mediate the initial infection and cellular entry of SARS-CoV-2 (Chilvers *et al.*, 2001; Lee *et al.*, 2020; Sungnak *et al.*, 2020; Ziegler *et al.*, 2020; Zou *et al.*, 2020). In addition, it has been demonstrated in a reconstituted human bronchial epithelial model that SARS-CoV-2 preferentially replicates in ciliated cells, which causes a rapid loss of the ciliary layer and thus compromises the motile function of the cilia (Brosnahan *et al.*, 2020). On the other hand, the intrinsic epithelial defense mechanisms likely occur too late to prevent such cilia loss due to SARS-CoV-2 infection (Robinot *et al.*, 2020).

Apart from the structural dysfunction of MCC, SARS-CoV-2 also disrupts the cilia beating through interference with the physiologically relevant signaling that modulates the cilia beating frequency, such as ATP, acetylcholine, cyclic adenosine monophosphate, cyclic guanosine monophosphate, nitric oxide, and inositol trisphosphate (Essaidi-Laziosi *et al.*, 2018; Kuek and Lee, 2020). Both the structural and signaling dysfunctions of the airway epithelium and the airway cilia would inevitably reduce the ability of MCC to remove airway mucus from the lung, which partially explains why airway mucus accumulation is so common and severe in critically ill COVID-19 patients.

Airway mucins in healthy subjects and COVID-19 patients

The principal components of airway mucus both structurally and functionally are mucins, and there are at least nine types of them in human airways (Chatterjee *et al.*, 2020; Lillehoj *et al.*, 2013). Among these mucins, MUC1, MUC4 and MUC16 mainly locate in the PCL layer tethered to the plasma

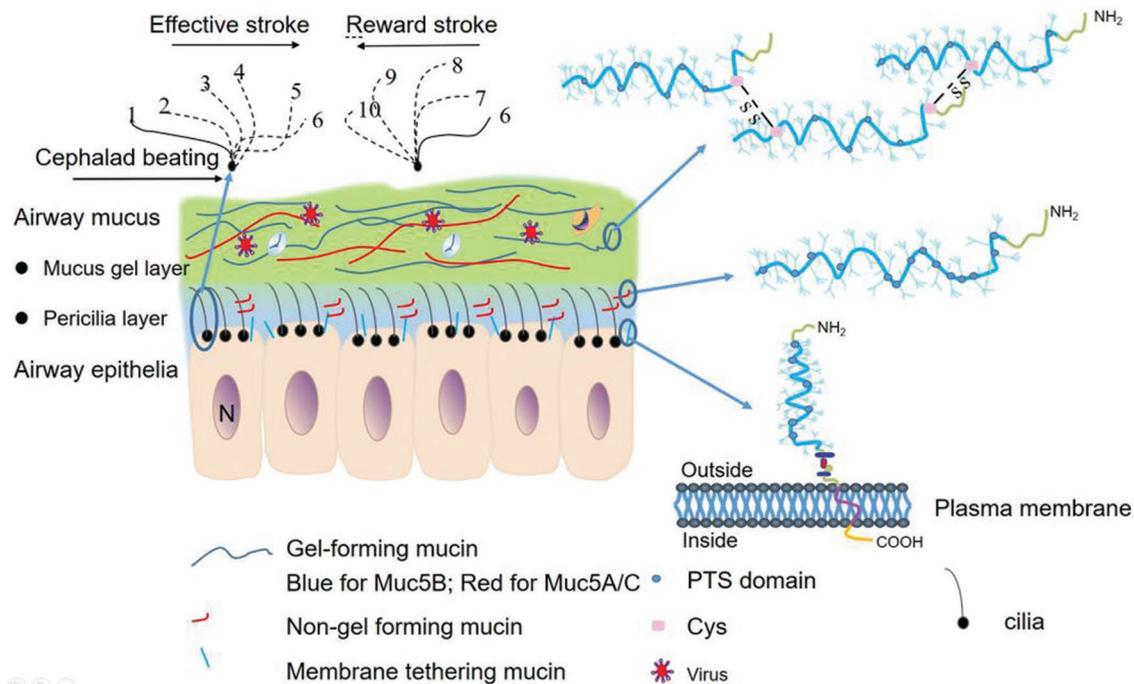


FIGURE 1. Mucociliary clearance (MCC) acts as a protective barrier in airways during SARS-CoV-2 infection, adapted from (Chatterjee *et al.*, 2020; Denny *et al.*, 2020; Hatrup and Gendler, 2008; Kuek and Lee, 2020; Xu and Jiang, 2019b). MCC consists of airway mucus and respiratory cilia. The major components of airway mucus are gel forming mucins (MUC5AC and MUC 5B, entrapping the virus and clearing them out driven by the continuous beating of cilia) and transmembrane mucins (MUC1, MUC4, and MUC16, binding to virus *via* the interaction of adhesion and sialic acid to prevent the entry of virus into cells), both of which are highly glycosylated proteins.

membrane of airway epithelium via a transmembrane domain, which attract water, thereby forming a lubricate fluid layer surrounding and protecting the cilia to promote movement of the mucus gel above. The gel-forming MUC2, MUC5A/C, MUC5B and MUC19, and the non-gel forming MUC7 and MUC8 mainly locate in the mucus gel layer after secretion by goblet cells (Fig. 1) (Denny *et al.*, 2020; Dhanisha *et al.*, 2018; Fahy and Dickey, 2010; Hatrup and Gendler, 2008; Ridley and Thornton, 2018; Thornton *et al.*, 2008).

Airway mucins always contain highly repeated domains of proline, threonine and serine. Since both threonine and serine mediate glycosylation via their oxygen, airway mucins are thus heavily glycosylated (20% protein core and 80% carbohydrates). In addition, the gel-forming mucins also contain abundant cysteine, which mediates the non-covalent and covalent crosslinks between the ends of different mucins due to their hydrophobic property and the disulfide formation, respectively. Consequently, the gel-forming mucins form polymers up to 5 μm long from only 0.2–0.6 μm long mucin monomers (Bansil *et al.*, 1995; Sheehan *et al.*, 1991). Once secreted, these heavily glycosylated long polymeric mucins interact with ions and absorb water and then increase the volume by several hundred times within seconds, forming a three dimensional lattice hydrostatic mucus gel (Quraishi *et al.*, 1998). Therefore, the airway mucus in the gel layer acquires both viscosity and elasticity, a physical property that is characteristic of complex fluids.

Besides their roles in determining airway mucus physical properties, airway mucins also have vital roles in protection of airways from infection by viruses such as SARS-CoV-2. For example, it has been shown that the terminal sialic acid glycans in gel-forming mucins including MUC5AC and

MUC5B can facilitate the interaction with coronavirus (Ganesan *et al.*, 2013). Consequently, the airway mucins may act as the primary site to entrap the viral particles and then remove them from the respiratory tract via MCC. The finding that COVID-19 patients have a higher level of MUC5B than their healthy counterparts seems to corroborate the protective role for MUC5B in this disease (Iyer *et al.*, 2020).

In addition, MUC1, MUC4 and MUC16 in the PCL layer (transmembrane mucins) can bind to coronavirus such as SARS-CoV-2 via the interaction of adhesion and sialic acid, which may prevent the entry of virus into the airway cells (Chatterjee *et al.*, 2020; Zou *et al.*, 2020). The transmembrane mucins are also known to shed their extracellular domains from the cell surface into the airway lumen, which is cleaved by endogenous protease that is activated due to mucin-virus interactions (McAuley *et al.*, 2017). Therefore, transmembrane mucins such as MUC1, MUC4, and MUC16 located on the surface of cilia can act as releasable decoy receptors and prevent the virus infection (Chatterjee *et al.*, 2020).

Hypersecretion of airway mucins in COVID-19 patients

In COVID-19 patients, the expression of airway mucins may be enhanced in response to SARS-CoV-2 infection via several signaling pathways, which leads to airway mucus accumulation (Bose *et al.*, 2021; Khan *et al.*, 2021; Plante *et al.*, 2020). For example, SARS-CoV-2 infection may stimulate differentiation and hyperplasia of the goblet cells in the airways, which is followed by hyperproduction and secretion of mucins. Up to date, enhanced expression of various mucins particularly MUC1, MUC2, and MUC4,

MUC5AC, MUC5B, and retention of highly viscous mucus in bronchial airways have been observed in COVID-19 patients (Chen *et al.*, 2020; Leng *et al.*, 2020; Lu *et al.*, 2021; Wang *et al.*, 2020; Ye *et al.*, 2020; Zhang *et al.*, 2021).

One of the causes of airway mucin hypersecretion in COVID-19 is the SARS-CoV-2 induced excessive inflammatory response known as cytokine storm, which is summarized by Castelli *et al.* (2020) in their review article. Briefly, numerous cytokines facilitate mucin expression via various signaling pathways. Among them, IL-2, IL-4, IL-8, IL-9, IL-13 and IL-19 facilitate mucin expression via activation of STATs signaling, IL-1 β , IL-5, IL-6, and IL-19 facilitate mucin expression via activation of MAPK signaling, while EGF, ATP and adenosine facilitate mucin expression via activation of NF- κ B signaling. In addition, it has been reported that SARS-CoV-2 increased IFN- β / γ expression, which enhanced expression of airway mucins such as MUC1, MUC2, MUC5AC, MUC5B, MUC13 and MUC16 via activation of IDO1-AhR axis (Hadjadj *et al.*, 2020; Liu *et al.*, 2020b). Since cytokine storm is a general feature in critically ill COVID-19 patients, these patients are also generally associated with airway mucin hypersecretion (Wang *et al.*, 2020).

The role of airway mucus rheology in COVID-19 patients

Airway mucus is a material that behaves like both a viscous fluid and a soft elastic solid, a rheological feature known as viscoelasticity. This airway mucus rheology can be impacted by virus infection and thus differently modulates the cilia beating and mucus transport.

Changes of airway mucus rheology in COVID-19 patients

In COVID-19 it has been reported that a proportion of the critically ill patients are often seen with highly viscous airway mucus that even forms colloidal mucus plugs in the bronchioles (Wang *et al.*, 2020). The abnormal rheological properties and mucus accumulation/plugging in the COVID-19 patients may result from various factors such as increased content of solids in the airway mucus (up to 15% as reported by (Button *et al.*, 2016) as well as changes in hydration, pH, and ion composition of the airway mucus (Ridley and Thornton, 2018).

The risk of mucus accumulation in critically ill COVID-19 patients

In healthy persons, mucus is continuously secreted into airways with totally about 10–100 mL/day. Under the function of MCC, mucus will move from peripheral airways to the glottis, and then be cleared by swallowing or coughing (Button *et al.*, 2012; Dickey, 2018). For critically ill COVID-19 patients, viral infection and the following inflammatory responses lead to airway mucus accumulation by changing mucus viscoelasticity, stimulating mucus hypersecretion, and disturbing mucus elimination as described above (Button *et al.*, 2016; Ridley and Thornton, 2018; Wang *et al.*, 2020).

Once the sticky airway mucus accumulates in the airways, it provides a favorable growth media for bacteria (Konrad *et al.*, 1994; Li Bassi *et al.*, 2008; Safdar *et al.*, 2005). Bacterial infection will cause airway inflammation,

which then induces goblet cells hyperplasia and submucosal gland hypertrophy, and leads to further mucus secretion and thus a vicious cycle of infection, inflammation, and hypersecretion. Additionally, the sticky airway mucus leads to the shedding of ciliated cells and impairs ciliary beating, and thus disrupts the function of MCC. Both the increased secretion of sticky airway mucus and the impaired MCC will further accelerate airway mucus accumulation (Anderson *et al.*, 2015; Zahm *et al.*, 1989).

Airway mucus accumulation can increase airway resistance and thus deteriorate lung function presenting as decreased forced expiratory volume in one second, which will lead to atelectasis, ARDS, ultimately the total obstruction of respiratory tracts and respiratory failure (Cerveri and Brusasco, 2010; Chen *et al.*, 2019b; Curran and Cohn, 2010; Darbee *et al.*, 2005; Dunican *et al.*, 2018; Fahy and Dickey, 2010; Hogg, 2004; Sprung *et al.*, 2010). Therefore, airway mucus accumulation may be one of the causatives for the high death rate of critically ill COVID-19 patients (Chen *et al.*, 2020; Lu *et al.*, 2021; Zhang *et al.*, 2021), and aggressive management of airway mucus accumulation by using appropriate ACTs is essential in clinical practice of treating respiratory conditions including critically ill COVID-19 patients (McKim *et al.*, 2011; Thomas *et al.*, 2020).

The direct impact of rheology on the transport of airway mucus

Airway mucus rheology determines the efficacy of MCC via modulating the mechanical coupling between the cilia and the mucus (Macchione *et al.*, 1995; Quraishi *et al.*, 1998; Rajendran and Banerjee, 2020; Silberberg, 1983). Therefore, a suitable viscoelasticity is required for the airway mucus to facilitate the conversion of energy from the cilia beating into mucus movement. Indeed, it has been widely reported that the airway mucus with an elastic modulus at 1–2 Pa and a zero shear rate viscosity (yield) 12 Pa·s (0.01 s⁻¹) is most suitable for the function of MCC (Majima *et al.*, 1991; Puchelle *et al.*, 1987; Rubin *et al.*, 1990; Shih *et al.*, 1977). Studies have also shown that the ratio of elasticity to viscosity and the overall impedance rather than viscosity and elasticity alone are more significantly associated with the function of MCC (Lorenzi *et al.*, 1992).

Interestingly, airway mucus can switch between viscous and elastic features depending on the magnitude of external deformation rates (Chen *et al.*, 2019a; Cone, 2009). For example, when it is sheared airway mucus undergoes a process of thinning due to a temporary realignment of mucins (Volsko, 2013). Because of these, several types of mechanical force including gravitational force, friction force, shearing force, and adhesion force may function together to determine the airway mucus rheological features and thus correlate the tendency of mucus movement (Fig. 2). This confers the ability of external mechanical forces due to tidal breathing (0.3 Hz), ciliary beating (10–15 Hz), forced expiratory maneuvers to regulate mucus rheology and the following mucus transport (Branson, 2007; Lai *et al.*, 2009).

Based on the rheological response of airway mucus to different external forces, it is possible to develop a variety of ACTs for clinical usage. For example, gravity, oscillation, and inspiration/expiration flow have been utilized to

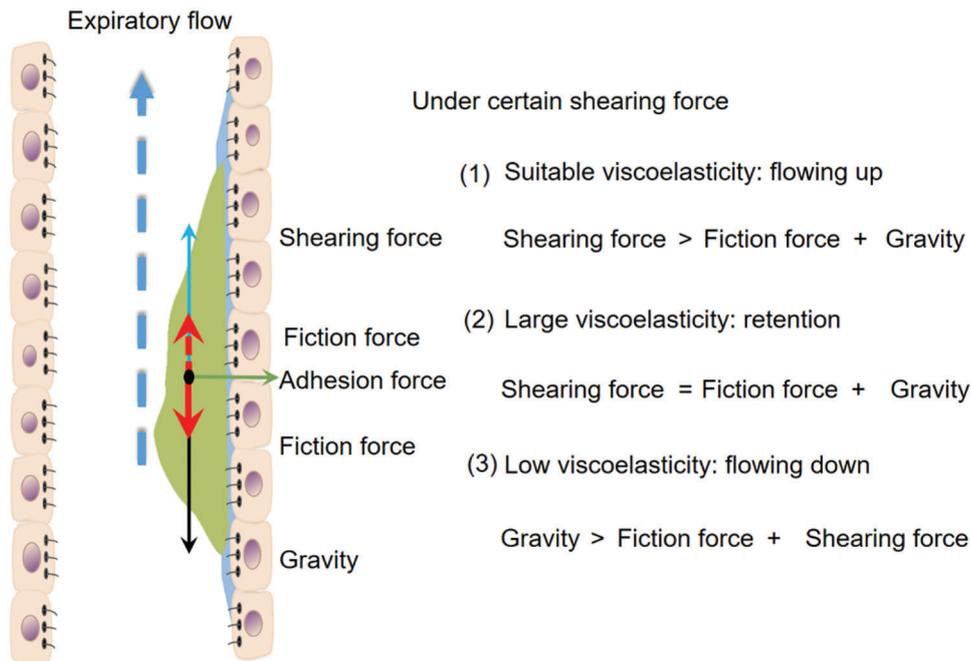


FIGURE 2. Mechanical forces and the rheological features of airway mucus that determine airway mucus movement.

modulate airway mucus transport. Indeed, using *in vitro* and *in vivo* models, it has been reported that oscillation of mucus can decrease its viscoelasticity in a time, frequency, and magnitude-dependent manner. Interestingly, the oscillation to reduce mucus viscoelasticity is found to be most potent at 12–15 Hz, which is similar to the cilia beating frequency (App *et al.*, 1998). Therefore, oscillation-based working mechanisms have been developed and integrated into different types of ACTs (Huang *et al.*, 2020; Rogers, 2007; Zhu *et al.*, 2020).

Basic Rationales for Clearance of Mucus from Obstructed Airways

Current methodologies for clearance of mucus-obstructed airways are all based on utilizing different physiological processes to effectively eliminate airway mucus, such as ventilating behind the obstructed airways/alveoli, increasing expiratory flow rate/flow bias, enhancing the beating of the cilia and decreasing the viscosity of airway mucus (Ambrosino and Clini, 2015; Denehy and Berney, 2006; Kim, 1997; Lester and Flume, 2009; McCarren and Alison, 2006; Pathmanathan *et al.*, 2015; Rogers and Doull, 2005). The basic rationales for these methods are discussed in detail as below.

Ventilating behind the obstructed airways/alveoli

If mucus obstructs the airways/alveoli, ventilating behind the obstructed regions is helpful to push the retained airway mucus out, which can be realized via interdependence of the adjacent alveoli and the collateral channels as shown in Fig. 3 (Cetti *et al.*, 2006; Terry and Traystman, 2016).

For obstructed alveoli that are adjacent and physically interconnected, the expansion of alveoli exerts traction forces and opens adjacent collapsed alveoli, thereby assisting ventilation to these regions, which is called interdependence of adjacent alveoli (McIlwaine *et al.*, 2017).

For obstructed airways especially the peripheral small airways, ventilation can be realized through some collateral channels that connect from either bronchioles to bronchioles (channels of Martin), bronchioles to alveoli (channels of Lambert) or alveoli to alveoli (pores of Kohn, ~50/alveolus). Once the airways are obstructed, these collateral channels can assist ventilation behind the obstructed regions to promote clearing the airway mucus since airflow prefers the path of least resistance (Slebos and Shah, 2017).

Ventilation through these accessory pathways can be generated by holding breath, increasing inspiratory flow rate/volume, and increasing positive pressure, which forms the basis of several ACTs such as breathing maneuvers, hyperinflation, and positive expiratory pressure (PEP) (Branson, 2007). In addition to ventilation behind the obstructed airways/alveoli, there are several other ways to physically enhance the fluidity and thus clearance of airway mucus that are discussed respectively in the following.

Increasing expiratory flow

Expiratory airflow especially peak expiratory flow (PEF) provides shearing forces on airway mucus surface to mobilize its cephalad movement via a two-phase gas-liquid flow mechanism (Fig. 4) (Kim *et al.*, 1986a; Kim *et al.*, 1987; Kim *et al.*, 1986b; Leith, 1968). Basically, once the shearing force exceeds the surface tension and the adhesive force of the mucus gel layer, the airway mucus starts to flow concurrently with the expiratory airflow in a pattern dependent on the magnitude of PEF, i.e., the expiratory air flows as either 1) fine bubbles in the airway mucus (bubble flow) for PEF <18 L/min; 2) large plugs in the airway mucus (plug flow) for PEF at 18–300 L/min; 3) a core inside an annulus of airway mucus (annular flow) for PEF at 300–750 L/min; or 4) fine drops combined with airway mucus (mist flow) for PEF >750 L/min. Among them, both the annular and mist flows can initiate cephalad movement of retained

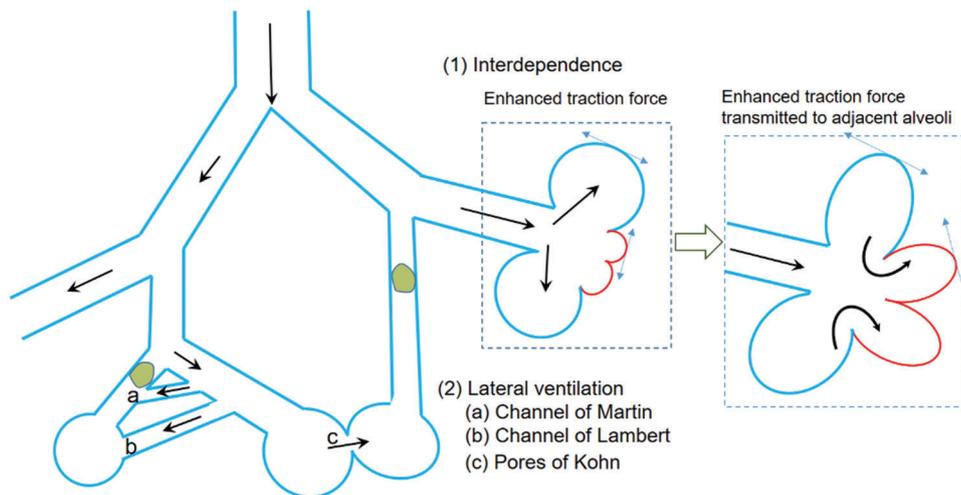


FIGURE 3. The accusatory pathways for ventilation in airways/alveoli, adapted from (Cetti *et al.*, 2006; Terry and Traystman, 2016).

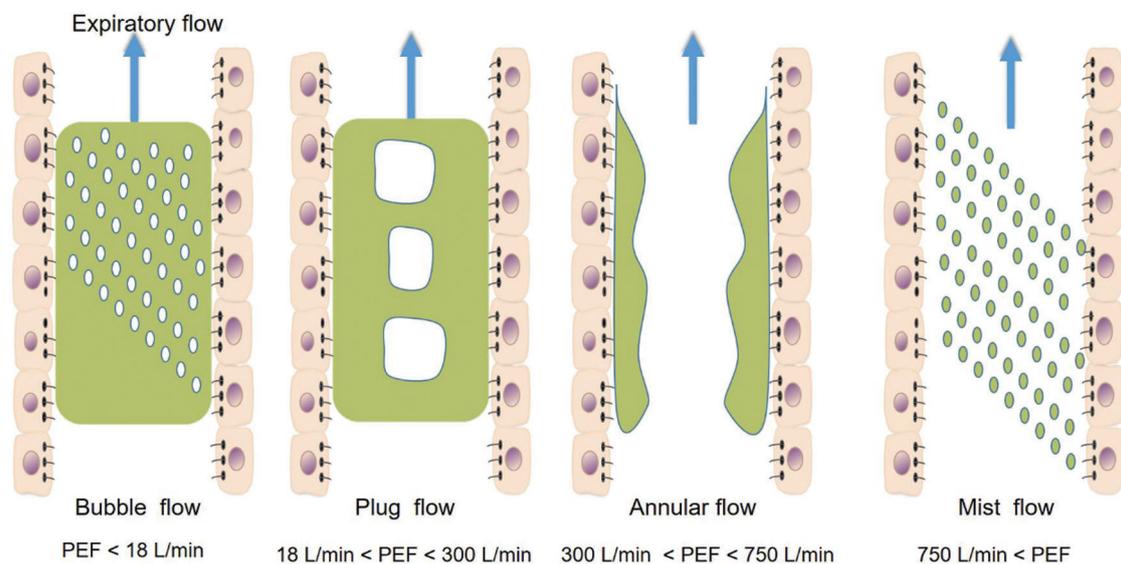


FIGURE 4. Four different flow patterns of expiratory airflow combined with concurrent airway mucus flow in the airway tract, adapted from Kim *et al.* (1986a); Kim *et al.* (1987); Kim *et al.* (1986b); Leith (1968).

airway mucus (Kim *et al.*, 1986a; Kim *et al.*, 1987; Kim *et al.*, 1986b; Leith, 1968).

It is generally known that PEF is proportional to the intrapulmonary pressure and inversely proportional to the airway resistance. Therefore, increasing the intrapulmonary pressure and decreasing the airway resistance have been used to increase PEF for airway mucus clearance in various ACTs (Maxwell and Ellis, 1998). For example, spontaneous forced expiration maneuvers such as cough and huff, mechanical device assistance such as mechanical insufflation-exsufflation (MI-E) can be used to generate increased intrapulmonary pressure. In addition, according to Poiseuille's law the airway resistance (R) is proportional to the length (l) and inversely proportional to the 4th power of radius (r) of the airways tract, as well as proportional to the viscosity (η) of air and the airway mucus ($R = 8\eta l/\pi r^4$). Therefore, the airway resistance can be decreased by either increasing airway radius via hyperinflation or decreasing the viscosity of the airway mucus via shearing-thinning by cough, huff, and MI-E (Kim *et al.*, 1987).

On the other hand, the intrapulmonary pressure during forced expiration will gradually decrease from the alveoli to

the mouth because of frictional pressure loss, and there will be a point where the intrapulmonary pressure equals to the surrounding pleural pressure (i.e., equal pressure point, EPP) (Oberwaldner, 2000). From the EPP toward the mouth in the downstream, the pleural pressure exceeds the intrapulmonary pressure and airway compression occurs, which narrows the airway transiently and thus increases PEF and favors a cephalad mucus flow according to Bernoulli's principle (Fig. 5) (Hollandl and Buttonl, 2006; Kaminsky and Chapman, 2020; van der Schans, 2007).

The location of EPP is mainly determined by the expiratory force and the elastic recoil of the lung, which usually occurs in the central airways. This is why cough and huff are usually effective for clearing airway mucus in the central airways especially above the sixth or seventh generations of airway branching (Button and Button, 2013; Oberwaldner, 2000). However, increasing expiratory force and elastic recoil can shift the site of EPP toward the alveoli, which provides the rationale for ACTs to clear airway mucus in small airways and alveoli by using enhanced force expiratory flow, PEP, and high frequency chest wall

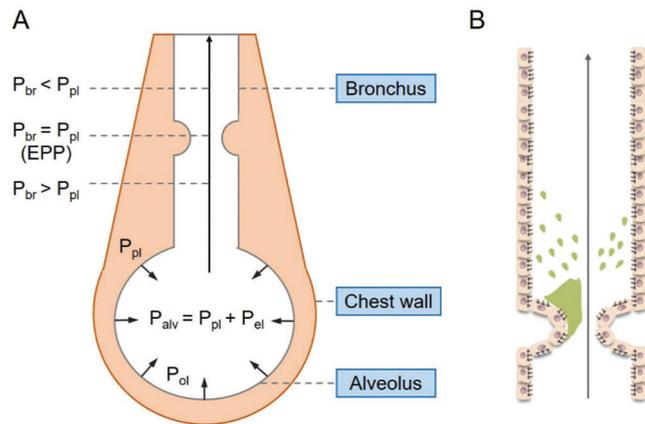


FIGURE 5. Mobilization of airway mucus via equal pressure point (EPP), adapted from Oberwaldner (2000); van der Schans (2007). (A) Expiratory flow with slight airway compression from EPP. P_{br} : pressure in bronchus; P_{pl} : pressure in pleural; P_{al} : pressure in alveolus; P_{el} : pressure induced by elastic recoil of lung. (B) The choke point catches the mucus and creates turbulent flow, which aerosolizes the mucus.

oscillation (HFCWO). Since the elastic recoil of the lung is also determined by the lung volume, it is another rationale for ACTs to remove mucus from the distal and central airways by using low and high lung volumes, respectively (Fink, 2007).

Increasing cephalad-bias flow

In addition to increasing expiratory flow, the flow bias between inspiratory and expiratory flows also influences the cephalad transport of airway mucus (Maxwell and Ellis, 1998; McIlwaine *et al.*, 2017; Volpe *et al.*, 2020). This is due to the fact that although the volume of air moving in and out of the airways during inspiration and expiration is equal, the peak flow during inspiration (PIF) is always different from that during expiration (PEF).

Early studies proposed the flow bias threshold for initiating a cephalad mucus movement in terms of PEF/PIF ratio > 1.1 (Kim *et al.*, 1987). Later study, however, demonstrated that it is the difference between PEF and PIF rather than the PEF/PIF ratio that determines the airway mucus mobilization (Volpe *et al.*, 2008). Although PEF-PIF > 17 L/min is the normal threshold for initiating cephalad mucus movement, PEF-PIF > 33 L/min is observed *in vivo* as a threshold to initiate mucus movement in mechanically ventilated patients and this threshold is probably more clinically relevant (Li Bassi *et al.*, 2012). Recently, it has been updated to include three criteria for an expiratory flow bias to mobilize airway mucus cephalad movement, i.e., PEF/PIF ratio > 1.1 , PEF-PIF > 17 L/min, PEF/PIF ratio > 4.3 and PEF-PIF > 33 L/min (Oliveira *et al.*, 2019b; Volpe *et al.*, 2008). It is worth emphasizing that this rationale does not depend on a high flow rate and can be applied even under tidal volume ventilation.

Oscillating intrapulmonary airflow

Oscillating intrapulmonary airflow can enhance mucus clearance by increasing the rate and bias of expiratory flow, ciliary beat frequency, or mucus hydration as well as by

altering the rheological properties of mucus, which can occur in both the central and the peripheral airways (Boucher, 2002; Dasgupta *et al.*, 1998; Hansen *et al.*, 1994; King *et al.*, 1983; Puchelle *et al.*, 1995; Winters *et al.*, 2007; Winters and Yeates, 1997).

The oscillation of intrapulmonary airflow can be induced by either directly oscillating the airflow at the mouth with an oscillatory positive expiratory pressure (Osc-PEP) or vibrating the thorax using HFCWO, both of which have been proved sufficient to overcome the mucus adhesion to the airway wall in the central and peripheral airways and consequently propel the mucus up and out of the airway (Button *et al.*, 2007; Dietl *et al.*, 2001; Tarran *et al.*, 2005).

Physically thinning airway mucus

As mentioned above, shear stress can physically thin airway mucus, which is also an essential pathway to promote mucus clearance (Shen *et al.*, 2018). Apart from shear stress, nanoparticles can also be used to physically thin airway mucus. For example, it has been reported that Fe_2O_3 nanoparticles (rod-shape, 8 nm diameter, 100 nm length) can experimentally induce physical thinning of simulated asthmatic airway mucus (Fig. 6), which may be mediated by the surface interaction between the Fe_2O_3 nanoparticles and the mucin polymers (Wang *et al.*, 2017). Our preliminary data also showed that magnetic field-driven movement of Fe_2O_3 can further decrease the viscoelasticity of the stimulated asthmatic airway mucus (data not shown). Since nanoparticles can be easily delivered into the central and peripheral airways, the phenomenon of mucus thinning induced by Fe_2O_3 nanoparticles provides another rationale for ACTs to physically thin the airway mucus and thus enhance the removal of mucus from the airways. These results suggest that novel nanotechnologies may provide alternative options for modulating airway mucus rheology for the purpose of promoting efficiency of ACTs.

Airway Clearance Techniques

Based on the above described rationales of mucus clearance, a variety of ACTs have been developed and applied in clinics including postural drainage, breathing maneuvers, conventional manual chest physiotherapy (percussion and vibration), airway oscillating PEP devices, external high-frequency chest oscillating devices, simulating cough devices such as manual hyperinflation (MHI), ventilator hyperinflation (VHI), and MI-E, as well as tracheal suctioning (Table S1).

Postural drainage

Postural drainage is an ACT that uses different body positions such as upright, side-lying, supine and prone to directly enhance airway mucus movement from the five lobes of the lung (Berney *et al.*, 2012; Button and Button, 2013; Li Bassi *et al.*, 2008; Safdar *et al.*, 2005). These precise postures comprehensively utilize the anatomy of the bronchial tree, and thus facilitate the sliding of airway mucus from the periphery toward the central airways and improve ventilation and perfusion via gravity-dependent strategies. Among them, the prone position is most effective and has been strongly recommended for mechanically ventilated

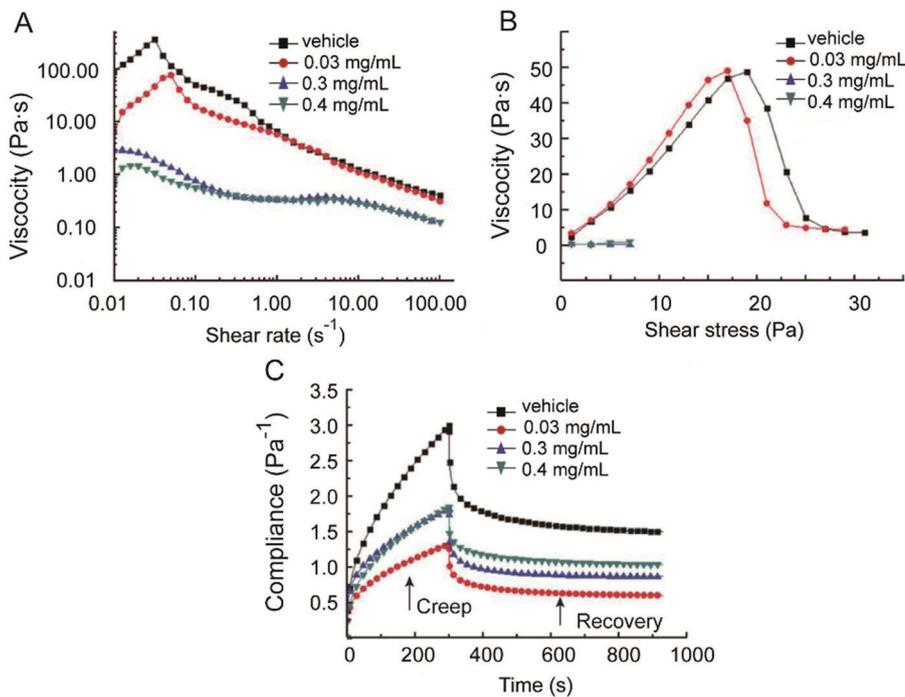


FIGURE 6. Effect of Fe₂O₃ nanoparticles on the viscoelasticity of simulated asthmatic airway mucus, adapted from (Wang *et al.*, 2017). (A) The dynamic viscosity as a function of the shearing rate. (B) The dynamic viscosity as a function of shearing stress curve. (C) The curve of creep and recovery.

COVID-19 patients, because it promotes drainage of airway secretion, homogenizes lung perfusion, and improves ventilation mismatch (Gattinoni *et al.*, 2020). It is also reported that although machine vibration or manual clapping does not affect severe-to-critical progression of COVID-19 patients, the prone position has been proved effective to avoid this progression via promoting mucus drainage (Wang *et al.*, 2020).

Breathing maneuvers

Various breathing maneuvers are known to be used as helpful ACTs to clear sticky mucus out of the obstructed regions, including 3-second breath holdings, thoracic expansion exercises, mobilization by increasing expiratory flow rate or volumes, forced expiration, active cycle of breathing, and autogenic drainage (Lewis *et al.*, 2012).

Each of these maneuvers works differently to promote mucus clearance. Specifically, the 3-second breath holdings can increase the constant time that allows the air to flow to the obstructed regions of the lung via accessory pathways as described before (Maxwell and Ellis, 1998; McIlwaine *et al.*, 2017). The thoracic expansion exercises use a deep, slow inspiration to increase the lung volume and thus promote ventilation to the obstructed airways via accessory pathways. Mobilization increases oxygen demand and thus increases the lung volume, which leads to ventilation behind the obstructed regions via accessory pathways (Amidei, 2012; Hodgson *et al.*, 2014; Thomas, 2013a, b). Forced expiration such as cough and huff relies on an adequate expiratory flow rate and volume to clear the airway mucus. The active cycle of breathing combines breath holding, deep inspiration and forced expiration (Bott *et al.*, 2009). This maneuver consists of one or two forced expirations followed by relaxed breathing. Autogenic drainage uses gently accelerated tidal volume breathing and gradually increased lung volumes to increase the expiratory flow rate and thus

improve mucus clearance from peripheral to central airways (Maxwell and Ellis, 1998; McIlwaine *et al.*, 2017). It needs to note that these breathing maneuvers may not be practical for critically ill COVID-19 patients because of their severe infirmity.

Percussion and vibration

Percussion is performed with cupped hands to clap on the thorax of the patients to generate a transient oscillation with a frequency of 3–6 Hz. Such oscillation transmits through the airways to dislodge the airway mucus from the airway wall so that the mucus can be removed out of the airways.

Similarly, the chest wall can be compressed either manually or using mechanical devices such as HFCWO to generate a transient oscillation and compression with a frequency of 3–17 Hz (McCarren *et al.*, 2006b). This vibration is proposed to remove mucus by increasing PEF (about 50%) and the expiratory flow bias or by decreasing the viscosity of mucus (Kim *et al.*, 1987; McCarren and Alison, 2006; McCarren *et al.*, 2006a). In addition, HFCWO utilizes an inflatable vest that is inflated with an oscillatory pressure below 25 cmH₂O at 5–25 Hz. This oscillation pressure can vibrate the chest wall and generate oscillatory airflow to clear airway mucus (Darbee *et al.*, 2005; Kendrick, 2007).

Positive expiratory pressure (PEP)

PEP helps to increase functional residual capacity and thus the tidal volume which can open obstructed airways via accessory pathways and support the airways during expiration to avoid small airway collapse (Branson, 2007; McIlwaine *et al.*, 2015). The patients need to exhale against an expiratory resistor for 8–10 cycles with moderately increased tidal breathing to finally achieve a positive pressure of 10–25 cmH₂O (Demchuk and Chatburn, 2021).

Apart from 10–25 cmH₂O, PEP can also function with either 40–140 cmH₂O high pressure (Hi-PEP), oscillation

(Osc-PEP), or ≤ 1 cmH₂O temporary PEP (T-PEP) (Figueiredo *et al.*, 2012). Evidence suggests that Hi-PEP helps to clear airway mucus by ventilating obstructed airways via collateral channels (Clini, 2009; Volsko *et al.*, 2003). It is especially suitable for patients who have an airway instability during forced expiration since it avoids the premature collapse of the airways and thus allows them to exhale a greater volume than the usual forced vital capacity. Osc-PEP is characterized by breathing against intermittent expiratory resistance to induce oscillation of variable frequency and can directly dislodge the sticky mucus and also decrease mucus viscoelasticity in a frequency- and amplitude-dependent manner. T-PEP applies an expiratory pressure at ≤ 1 cmH₂O only for a fraction of the expiratory phase. This increase in low pressure is created through a pulsatile flow at approximately 42 Hz in frequency. The vibration generated by the pulsatile flow is transmitted throughout the airways to detach the mucus. Analysis suggested that T-PEP provides greater benefit to patients with emphysema or on oxygen therapy, while PEP therapy would be of more benefit for patients on mechanical ventilation (D'Abrosca *et al.*, 2017; Nicolini *et al.*, 2018).

Intrapulmonary percussive ventilation

Intrapulmonary percussive ventilation (IPV) provides originally high-frequency mini bursts of air at a rate of 4–10 Hz, which creates an internal vibration in the lung and thus promotes mucus clearance. IPV is recommended for weak and fatigued patients with ineffective cough to loose secretions, which is feasible and safe in spontaneously breathing and non-intubated subjects in critical care (Berlinski, 2019; Hassan *et al.*, 2020). Additionally, IPV can be superimposed on conventional ventilation. The potential benefits of the concomitant use of IPV with ventilation include secretion clearance, improved oxygenation, and intermittent use (Dellamonica *et al.*, 2008).

Lung hyperinflation

Lung hyperinflation clears airway mucus by increasing inspiratory flow volume. This will recruit collapsed airways, reduce inspiratory resistance, and thus generate high expiratory flow rate bias and induce annular flow to remove airway mucus, which is predominantly used to move airway mucus from the peripheral airway to the tracheal for mechanically ventilated patients (Savian *et al.*, 2006).

It starts with a slow deep inspiration flow to deliver a high tidal volume up to a peak pressure of 40 cmH₂O, and follows by a 3-second breath holding, and then a fast uninterrupted expiratory flow that mimics a forced expiration (i.e., cough) (Berney and Denehy, 2002; Hodgson *et al.*, 2007). This deep inspiration will increase tidal volume by 50% and increase peak intrabronchial pressure to 40 cmH₂O (Haake *et al.*, 1987).

Such lung hyperinflation can be realized either manually, i.e., manual hyperinflation (MHI) or mechanically by a ventilator, i.e., ventilator hyperinflation (VHI). For mechanically ventilated COVID-19 patients, VHI is more suitable than MHI, since the patients are already connected to the ventilator and maintained with PEEP, and the outcome of VHI is more reproducible than that of MHI

(Haake *et al.*, 1987; Volpe *et al.*, 2020). Furthermore, it has been clarified that among different modes of VHI, the volume-controlled ventilation with inspiratory flow of 20 L/min and pressure support achieves the best effect to remove airway mucus (Ribeiro *et al.*, 2019).

Expiratory rib cage compression

Expiratory rib cage compression (ERCC), also known as manual chest compression, is one of the most commonly used ACTs in ventilated patients (Volpe *et al.*, 2020). ERCC is usually applied either to assist secretion clearance from peripheral to central airways, or to remove secretion from central airways. Unlike the previously described percussion and vibration, ERCC is applied with a steady force that gradually increases from gentle to strong intensity to prolong exhalation after the onset of the expiratory phase, which is used to remove the secretions from distal airways. To remove the secretions from proximal airways, ERCC is applied with hard compression to increase PEF and synchronized with the onset of expiration (Borges *et al.*, 2017; Guimaraes *et al.*, 2013; Martí *et al.*, 2013).

Positive end-expiratory pressure-zero end expiratory pressure

Positive end-expiratory pressure-zero end expiratory pressure (PEEP-ZEEP) consists of increasing PEEP to 15 cmH₂O during 5 cycles with peak inspiratory pressure limited to 40 cmH₂O, followed by an abrupt reduction of PEEP to 0 cmH₂O. By increasing the pressure change at the onset of the expiratory phase, this technique increases the PEF and, consequently, the expiratory flow bias, especially in the volume controlled mode (Amaral *et al.*, 2020; Oliveira *et al.*, 2019a; Volpe *et al.*, 2020). Although both ERCC and PEEP-ZEEP are responsible for increasing the expiratory flow bias, the latter is more potent in this aspect (7 L/min vs. 49 L/min) (Oliveira *et al.*, 2019a).

Mechanical insufflation-exsufflation

Mechanical insufflation-exsufflation (MI-E) is the most recently developed technique for mechanically ventilated patients, which delivers a positive inspiratory pressure to the patient to generate a deep inspiration (insufflation) and immediately (10 ms) followed by a negative expiratory pressure generating a deep expiration (exsufflation). In this way, a PEF greater than that induced by coughing can be achieved to remove airway mucus more effectively. In addition, the rapid switch from positive to negative pressure stimulates the bias flow. The advantage of MI-E is that it induces negative pressure throughout central and peripheral airways, suggesting that airway mucus in all generations of the airways can be removed (Ferreira de Camillis *et al.*, 2018; Terzi *et al.*, 2018).

Tracheal suctioning

Tracheal suctioning can clear sticky mucus from the trachea and the lower airways, which can be performed via an in-line closed system or an open system (Dodek *et al.*, 2004). Although tracheal suctioning should not be used as a routine ACT for non-intubated patients, it is recommended for the mechanically ventilated COVID-19 patients with

suction catheters of an in-line closed system, considering the risk of generating virus aerosol with an open system.

Airway Clearance Algorithm

Although various ACTs can effectively remove airway mucus, there is still no gold standard of clinical ACT practice for critically ill COVID-19 patients. One of the reasons is that one ACT may function differently to different patients depending on the specific pathological process and respiratory mechanics of each individual. Additionally, it may not be possible to compare all the ACTs and affirm the one with superiority. Lastly, each of the critically ill COVID-19 patients can present different respiratory conditions at a different stage of the disease, and thus may need different ACTs over the course of treatment (Battaglini *et al.*, 2020; Lazzeri *et al.*, 2020).

Nevertheless, for critically ill COVID-19 patients, passive ACTs such as postural drainage, percussion, vibration, oscillation, hyperinflation and MI-E may be more suitable than active ACTs such as breathing maneuvers and PEP, because of their physical frailty (Homnick, 2007; van der Schans *et al.*, 1999; Wilson *et al.*, 2019). In addition, the carers in close range of critically ill COVID-19 patients may be endangered by exposure to the virus aerosolized into the environment (Patel *et al.*, 2020; Zayas *et al.*, 2005). Considering this risk, mechanical devices such as HFCWO, VHI, and MI-E are more suitable than manual techniques to be used for the management of airway mucus of critically ill COVID-19 patients, despite that the manual techniques are also effective in mucus clearance. In practice, the treatment approach should be decided based on carefully evaluating the conditions of the patient and the features of available ACTs following the algorithm as shown in Fig. 7 (Chatburn, 2007; Konrad *et al.*, 1994; Sivasothy *et al.*, 2001).

Furthermore, it has been recommended to consider different internal rationales and application conditions of all the ACTs, and thus adapt a combinational therapeutic strategy of using several ACTs during the management of airway mucus (Ramos *et al.*, 2015). For example, although HFCWO is effective to mobilize secretions from the small airways, it does not provide ventilation behind obstructed airways. As a consequence, HFCWO can lead to 10%–50% reduction of the end-expiratory volume, which may close small airways and worsen pulmonary function (Dosman *et al.*, 2003; Jones *et al.*, 1995). However, this event can be avoided by 3-second breathing holds or deep inspiration prior to HFCWO. In addition, HFCWO can be used to mobilize mucus from the small airway to the proximal airways, and then tracheal suctioning can be further used to clear them out from the proximal airway. And when tracheal suctioning does not provide adequate mucus clearance in mechanically ventilated patients, it can be augmented by PEEP-DEEP and MI-E. Compared to tracheal suctioning that is routinely performed for invasive ventilation, MI-E may be more suitable for eliminating airway mucus in ventilated COVID-19 patients due to its advantages in convenience and comfortability in use (Garstang *et al.*, 2000; Sancho *et al.*, 2003). In fact, MI-E is recommended by the Canadian Thoracic Society to be added to or replace the tracheal suctioning (McKim *et al.*, 2011).

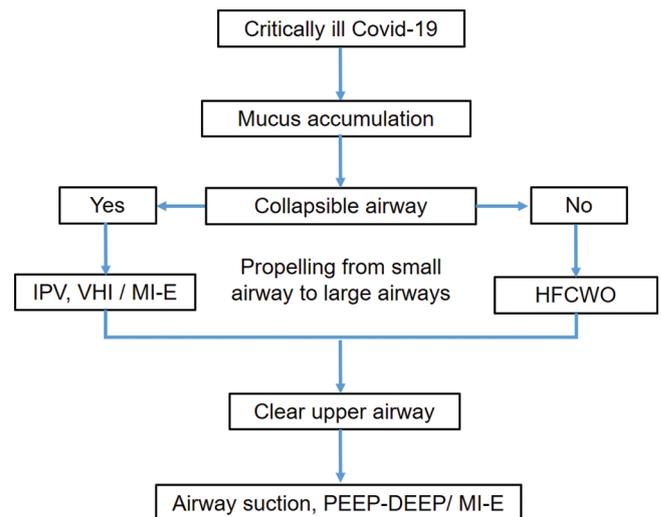


FIGURE 7. Airway clearance algorithm for critically ill COVID-19 patients, adapted from (Chatburn, 2007; Konrad *et al.*, 1994a; Sivasothy *et al.*, 2001).

Therefore, for critically ill COVID-19 patients, some ACTs such as HFCWO, MHI combined with 3-second breathing hold or IPV may be used together to dislodge the mucus in smaller airways and subsequently move the mucus to the larger airways. Then other ACTs such as tracheal suctioning and/or MI-E should be used to clear these mobilized secretions (Stiller, 2000, 2013; Volsko, 2013).

Limitations of Current ACTs and Future Directions

So far, the underlying mechanisms of airway mucus accumulation in COVID-19 are still unclear, which includes but is not limited to the unknown changes of hydration, biochemical constituents and correlated rheological features in the airway mucus of the patients. The other limitation is that although various ACTs have been used for critically ill COVID-19 patients, there are still not established criteria for determining what ACTs are the most suitable and when they should be used because there is not sufficient evidence to compare and evaluate the currently available ACTs for their efficacy and safety in clinical usage. Therefore, it is urgent for future studies to clarify the changes of specific types of mucins and the correlated rheological properties of airway mucus in COVID-19 patients. It is also necessary to explore new techniques such as abdominal electrical stimulation, nanotechnology, and artificial intelligence for their potential applications in airway mucus clearance, which may be key to optimize the use of existing ACTs as well as develop new ACTs for ultimate control of airway mucus clearance in COVID-19 (Spinou, 2018; Volpe *et al.*, 2020).

Conclusion

COVID-19 is an emerging infectious respiratory disease that has not been completely delineated, and clearance of excessive mucus accumulation in the airways remains one of the biggest challenges in the treatment of critically ill COVID-19 patients due to lack of knowledge of the mucus changes in the disease and corresponding techniques for optimal mucus clearance. In this review, we outline the

chemical and rheological features of airway mucus in physiopathological conditions especially in relation to COVID-19 and the currently available ACTs, as well as highlight the essential roles of mechanical forces and their interactions with airway mucus. A clear understanding of these aspects will provide novel insights for new therapeutic approaches or strategies for the airway mucus management of critically ill COVID-19 patients. Based on comprehensive considerations of all these principles and factors, an optimized strategy may be developed to meet the specific requirements for clearing airway mucus in critically ill COVID-19 patients.

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TABLE S1

Physiological basis of ACTs (França et al., 2012; McIlwaine et al., 2017)

ACTs	Ventilation to obstructed regions		Expiratory airflow		Oscillation	
	Interdependence	CV	PEF	Bias flow		
Postural drainage	Yes	Yes	Yes	Yes	No	
Breathing maneuvers	3-s breathing holds	Yes	Yes	No	Yes	No
	Thoracic expansion exercises	Yes	Yes	No	No	No
	Mobilization	Yes	Yes	No	No	No
	Forced expiratory techniques	No	No	Yes	Yes	No
	Autogenic drainage	No	Yes	Yes	Yes	No
PEP	Traditional	No	Yes	Yes	Yes	No
	Oscillating PEP with Flutter	Oscillation at >3 Hz	Yes	Yes	Yes	2–32 Hz
	Oscillating PEP with Acapella	No	Yes	Yes	Yes	10–18 Hz
Percussion	No	No	Yes	No	Yes	
HFCWO	Oscillation at >3 Hz;	No	Yes	Yes	Yes	5–25 Hz
Lung hyperinflation	MHI	Yes	Yes	Yes	Yes	No
	VHI	Yes	Yes	Yes	Yes	No
MI-E	Yes	Yes	Yes	Yes	Yes	No

Note: CV: collateral ventilation; HFCWO: high frequency chest wall oscillation; PEF: peak expiratory flow rate; PEP: positive expiratory pressure; PIF: peak inspiratory flow rate; MHI: manual hyperinflation; VHI: ventilator hyperinflation; MI-E: mechanical insufflation-exsufflation.