Essentials in saline pharmacology for nasal or respiratory hygiene in times of COVID-19

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Research Article

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Abstract

**Purpose:** Nasal irrigation or nebulizing aerosol of isotonic or hypertonic saline is a traditional method for respiratory or nasal care. A recent small study in outpatients with COVID-19 without acute respiratory distress syndrome suggests substantial symptom resolution. We therefore analysed pharmacological/pharmacodynamic effects of isotonic or hypertonic saline, relevant to SARS-CoV-2 infection and respiratory care.

**Methods:** Progressive and systematic searches.

**Results:** Due to its wetting properties, saline achieves an improved spreading of alveolar lining fluid and has been shown to reduce bio-aerosols and viral load. Saline provides moisture to respiratory epithelia and gels mucus, promotes ciliary beating and improves mucociliary clearance. Coronaviruses and SARS-CoV-2 damage ciliated epithelium in the nose and airways. Saline inhibits SARS-CoV-2 replication in Vero cells; possible interactions involve the viral ACE2-entry mechanism (chloride-dependent ACE2 configuration) and sodium channel ENaC. Saline shifts myeloperoxidase activity in epithelial or phagocytic cells to produce hypochlorous acid. Clinically, nasal or respiratory airway care with saline reduces symptoms of seasonal coronaviruses and other common cold viruses. Its use as aerosol reduces hospitalisation rates for respiratory syncytial virus infection in children. Preliminary data suggest symptom reduction in symptomatic COVID-19 patients if saline is initiated within 48 hours of symptom onset.

**Conclusions:** Saline interacts at various levels relevant to nasal or respiratory hygiene (nasal irrigation or aerosol). If used from the onset of common cold symptoms, it may represent a useful add-on to first-line interventions for COVID-19. Formal evaluation in mild COVID-19 is desirable as to establish efficacy and optimal treatment regimens.

**Introduction**

The use of ‘Atemwegspflege’ (care of the airways) or nasal care is a traditional German practice, as to provide moist to the airways and can be achieved by nasal sprays or inhalation of nebulized isotonic or hypertonic saline (Kochsalzlösung) [[1],[2],[3],[4],[5],[6],[7],[8]]. This practice is being promoted by lung specialists, health care and consumer organizations during COVID-19, whereby the use of nebulizing/aerosols with saline is recommended, either stating that, while it may not change the risk of infection, it helps to mitigate the first symptoms, or claiming that it effectively can ‘dam’ (thus reduce) virus infection (“Einfaches Inhalieren kann Tröpfcheninfektion effektiv eindämmen”) [2-6].

Whether nebulising isotonic or hypertonic saline may help to alleviate shortage of breath is not known. Respiratory secretions due to COVID-19 infection may behave similarly as those of a severe bronchitis or bronchiolitis; cough can be dry, but secretions can be clear to mucopurulent, and thus need to be mobilised to be removed from the airways. Aerosol use was actively discouraged in Belgium, as it is believed to create more risks for viral transmission, according to the APB (official pharmacist organisation in Belgium) and Sciensano reports [9],[10],[11]. Also the World Health Organisation (WHO) discourages the use of aerosolising procedures in general [[12]]. This risk was also raised at some stage in Germany, because of the fear that this technique would generate bio-aerosol drops, which could promote virus spread. This concept however was contradicted by a positioning statement of the German pneumologists.[13] A recent small study in outpatients with COVID-19 without acute respiratory distress syndrome (ARDS) suggests substantial symptom resolution with hypertonic saline [[14],[15]].
We therefore investigated the potential roles that saline nasal spray/irrigation or aerosol may play in reducing COVID-19 infectivity. In particular, we discuss the evidence from the literature about the effects of saline on surface tension, mucus and alveolar lining fluid (ALF), ciliary beat and mucociliary clearance (MCC), the Angiotensin Converting Enzyme 2 (ACE2) activity and the sodium channel (ENaC) and their interaction with SARS-CoV-2 in causing COVID-19. Additionally, we review relevant clinical data supporting the use of isotonic or hypertonic saline as a simple rinse or aerosol as an early reassuring intervention for upper respiratory infection and COVID-19. This assessment is not meant to propose isotonic or hypertonic saline as the solution of severe COVID-19 ARDS. We rather aim to evaluate the mechanisms by which nasal or respiratory hygiene with saline may serve to limit viral infectivity and spread. In this paper “aerosol” thus refers to simple nasal spraying of isotonic saline (0.9% NaCl; also called “physiologic serum”) or hypertonic saline and/or to nebulizing iso- or hypertonic saline, using a mist-forming device for inhalation and humidification to clear the airways and to remove phlegm in viral respiratory infections. We use the term “bio-aerosol”, when referring to the micro-droplets, spontaneously produced during exhalation, such as during speaking, singing and coughing.

**Methods**

In an attempt to summarize differences in first-line treatment approaches for COVID-19 between Germany [low excess mortality on EUROMOMO (www.euromomo.eu), low hospitalization and case-fatality ratio (www.RKI.de)] and Belgium [high excess mortality on EUROMOMO (www.euromomo.eu) and high hospitalization and case-fatality rate (Epidemiological updates on www.Sciensano.be)], we initially searched for guidance articles concerning COVID-19 treatment, as are accessible to German and Belgian consumers on the internet. We combined general key words such as ‘treatment’ and ‘corona/Covid-19’, in Dutch/French and German on online search engines (Google.be, Google.de, Yahoo.de). In addition, the recommendations of Sciensano (Belgium) and the positioning statement of the German lung specialists (pneumologists) were taken into account. The German sources retrieved were related to ‘AtemwegPflege’ (respiratory care), aerosol and Kochsalz (kitchen salt)/saline [1-7]. However, such information was not found on the Belgian internet. Subsequently, progressive and systematic qualitative literature searches were undertaken, adding multidisciplinary searches on key words relevant to iso- or hypertonic saline (keywords in the title or abstract), as well as to relevant aspects related to respiratory viruses and COVID-19 in particular, thereby searching PubMed and Internet in general. These included evidence for: (1) effects on, and risk-benefit evaluation of saline (aerosol) in the formation of bio-aerosol and viral spreading; for the effects of saline on bio-aerosols and viral decay, ample sources were found related to pollution; only the most relevant sources relevant to the dynamics on viral stability in droplets or bio-aerosols were retained; (2) the role of saline in mucosal hydration; (3) its effects on the MCC; (4) the localization and interactions between sodium chloride (NaCl or salt) and SARS-CoV-2 with regard to ACE2 and ENaC; (5) the role of NaCl in generation of hypochlorous acid and the involvement of myeloperoxidase (MPO); (6) relevant clinical results with saline to common colds/upper respiratory symptoms/bronchiolitis or ARDS. As the literature related to saline for respiratory care is very extensive, comprehensive sources were retained for this analysis, if available (e.g. meta-analyses and Cochrane for the clinical effects). Sources that rather focus on cystic fibrosis or chronic respiratory conditions were not retained, unless if relevant to discriminate from a pharmacodynamic, pathophysiologica or safety point of view; recently published articles on saline in relation to COVID-19 by companies developing devices for nasal irrigation of saline were rejected, as containing incomplete, if any, information on the pharmacological/pharmadynamic effects of saline.
Results

The effects of isotonic and/or hypertonic saline are summarized in Figure 1. They include the wetting/gelling properties, effect on hydration and MCC, SARS-CoV-2 viral replication and underlying mechanisms, as well as effect on formation of hypochlorous acid (HOCl).

1. Wetting properties of saline and effect on viral spreading/transmission

Saline changes the physicochemical properties of the ALF and mucus, including the molecular behaviour of ionic and non-ionic surfactants and substrates, proteins in particular. Collectively, these effects are referred to as “wetting properties” \([16],[17]\). Surface tension is an important factor in alveolar wetting, the MCC and the phenomenon of capillarity. From a pathophysiological perspective, the role of lung surfactants in wetting, re-spreadening and compressing the ALF to ultra-low surface tensions is a well-known mechanism in preterm children suffering from infant respiratory distress syndrome: isotonic saline aerosol has been proven to remediate this problem and be lifesaving by improving airway compliance \([18],[19]\). Alveolar type II epithelial cells (AT2) in the ALF promote the biosynthesis of lung surfactant. SARS-CoV-2 attacks these cells causing defective functionality of AT2 cells, possibly leading to exhaustion of pulmonary surfactant, so raising alveolar surface tension \([20],[21]\). Hence, the wetting properties of NaCl may provide a benefit in reducing surface tensions, thus improving airway compliance.

The role of nebulizing saline aerosol in reducing viral spreading of SARS-CoV-2 so far has not been formally assessed in clinical studies. Saliva droplets carrying the virus are believed to convert into a bio-aerosol infecting the environment and bystanders, and therefore the use of aerosolising procedures has been discouraged by several authorities \([9-12]\). Yet, one should not confound saline aerosol with viral bio-aerosols \([22],[23]\), or bio-aerosol-generating procedures, such as listed by the WHO \([12]\). Differences are illustrated in Table 1. Concern about viral spreading by aerosol was mainly based on a Hong Kong hospital case of SARS-CoV, associated with contamination in a ward following 7 days of nebulizing salbutamol \([24]\). Several subsequent independent evaluations have not found a significant effect of nebulizing treatment on transmission \([13-],[25],[26],[27],[28]\). Although the KCE (Belgian Health Care Knowledge Centre) identified no enhanced transmission risks of saline aerosol nebulizer treatment \([28]\), saline aerosol use was discouraged in Belgium, unless in an isolated home situation or in the open air; users should then ventilate the room for a minimum of 30 minutes after the atomization \([11]\). This contrasts to the German situation, as an early German position paper by pneumologists on COVID-19 refers to two retrospective analyses regarding the procedure-related risk of nebulizer applications that were carried out during the SARS epidemic in Canada in 2003 that could not establish an increased risk of infection for the medical personnel \([29],[30]\). The German paper refers to the study by Edwards \([31]\), showing that simple isotonic saline inhalation reduces the release of exhaled bio-aerosols from the lungs by an average of 72% for up to 6 hours. The highest effect was obtained in high emitters as illustrated by Fiegel \([32]\). The conclusion is further summarized under point 3.3 of their statement: “Although nebulizers with nozzles increase the amount of aerosol in room air, they do not increase the risk of infection for medical staff. The inhalation of isotonic saline solution significantly reduces (bio) aerosol release from the lungs”\([13]\). Only recently, it has been shown that saline inhibits replication of SARS-CoV-2 in vitro in Vero cells (see further point 3) \([33]\), while a study with nebulized
neutral electrolyzed saline was found to reduce the qPCR positivity of the virus in 6 out of 10 subjects by day 4, in 80% by day 6 and in all of them by day 9 (for clinical results, see point 6) [34]. Other studies of the effect of saline on bio-aerosols, listed in Table 1 [35]-[37], have generated similar results with other viruses or lung particles, confirming a reduction bio-aerosols.

From a mechanistic focus, it is proposed that isotonic saline inhalation changes surface tension of the liquid film on the airway epithelium, leading to less droplet formation and, as such, to less release of exhaled bio-aerosols [13]. In vitro, saline induces droplet aggregation and stronger gel formation leading to faster deposition, while surfactant in contrast breaks up the droplets to smaller sizes [38],[39]. Salinity in evaporated respiratory droplets also affects the structure of virus particles and viral decay, whereas processes at the air-liquid interface drive the inactivation of viruses in droplets [40],[41]. Such bio-aerosols of enveloped viruses would fail to undergo rapid rehydration upon entry of the nearly saturated humidity of the respiratory tract, as surfactant in the droplets would inhibit fast reabsorption of water [40]. This underlines the role of the MCC in clearing inhaled particles from the air.

2. **Role of saline in mucosal hydration**

Mucosal hydration is essential to the MCC, the major primary innate defence mechanism of the lung, continuously clearing the airways from dust, infectious and other particles. These ciliary movements, clearing the airways are temperature, pH and moisture-dependent [42],[43]. Passage of cold air current or chilling depresses the mucous membrane temperature and ciliary movement, which manifests to a greater extent in the nasopharynx than in postnasal spaces [43]. Drying of the respiratory mucus or excessive dryness in the nose reduce ciliary function [43]. The speed of warming up of inhaled air depends on the respiratory frequency and volume of air inhaled, but hyperventilation leads to faster drying of the mucosa. These findings also point to the important role for ambient temperature and humidity in the efficiency of ciliary beating and so in the MCC. Altered cilia, slower ciliary beating, changes in the properties of mucus and lower MCC, are specifically found in elderly people and more polluted environments, and older patients more frequently suffer from a dehydrated mucosa [44],[45]. Patients with acute nasal involvement and increased metabolic activity also appear to have higher body temperatures, the temperature of the nose fluctuating directly with the core body temperature [43], so that also during high fever, there may be faster drying of the respiratory mucosa, making elderly patients with fever in particular susceptible to viral aggression.

As a consequence, humidification with saline aerosol may be beneficial, especially in these circumstances. It has been shown that ‘a fringe of ciliary activity persists’ as long as there is sufficient moisture [43]. However, if dryness lasts longer than 15-18 minutes, air humidification or water flushing can no longer restore the ciliary movement, and only the use of physiological sodium chloride solutions or Ringer’s solutions can do so [43]. Using different techniques, it has been shown that isotonic saline induces a positive effect on the ciliary beat frequency, can reverse ciliostasis and promotes MCC, both under physiological and damage-induced conditions [46]-[48],[49],[50]. The osmolality of saline plays an important role. Pure water severely damages the normal human nasal epithelial cells, while isotonic saline (in contrast to hypo- and hypertonic saline) does not affect their morphology [51],[52]. Hypertonic saline has been shown to decrease the ciliary movement in human nasal epithelium [49],[53], while others report faster MCC in healthy subjects, after-single dose nasal irrigation, or in the airways at 30 min, but not 4 h after inhalation (attributed to depletion of airway mucin) [47]-[54],[55],[56]. Hypertonic saline induces osmotic pressure, but has also been found to decrease the potential difference in nasal epithelia - a rapid, reversible and dose-related effect indicating a direct effect of NaCl on ion transport across the human airway epithelium (not just attributable to a simultaneous change in osmolarity) [57]. Hypertonic saline has also been
found to affect the nasal epithelial permeability \[58],[59]\, and to cause nasal burning/irritation \[60]\, while both hypo- and hypertonic saline aerosols may induce bronchoconstriction or cough, as has been shown in patients with asthma or with moderate to severe chronic obstructive pulmonary disease \[61]-[63],[64]. In chronic bronchopulmonary disease, which is associated with dehydrated airway surface liquid (ASL) and concentrated mucus, hypertonic saline has been shown to increase the MCC, drawing additional water onto the ASL \[65],[66]\.

The mechanism of hydration is further explained under the MMC.

3. **Role of saline in the MCC**

Nasal and respiratory mucin forms a gel layer, serving as a liquid reservoir for the periciliary layer \[67]\.

Basically, the MCC clearance not only depends on the mucin properties, but also on the properties of the ASL and requires coordination between the periciliary liquid near the cell surface and the overlaying transported mucus layer \[68],[69]\.

These layers need to be appropriately hydrated in the lungs and airways, allowing the cilia to beat properly and move the mucus and transporting trapped pathogens and particles. The height of the ALF and hydration of the periciliary liquid layer depend on opposing mechanisms in water transport: the outward chloride (Cl\(^-\)) secretory transport through apical chloride channels (CFTR and CACC mediated), and the inward movement of water following active (re)absorption of Na\(^+\) through apical sodium channels ENaC, in concertation with the basolateral Na\(^+\)/K\(^+\)-ATPase, located in the ciliated cells \[42],[70],[71]\.

Whereas many more ion transport processes are involved, it is to note that the upper layer of ASL likely contains high concentrations of ions above 100mM, while the periciliary liquid layer normally contains NaCl in an amount below 50mM (< 0.29%), which normally ensures and maintains effective transepithelial transport of ions and water, as allowing effective ciliary beating \[71]\.

Saline affects several MCC-related processes, relevant to nasal or respiratory hygiene. Firstly, saline will affect the hydration of the mucus, thereby dramatically affecting its viscous and elastic properties and transportability, and so define how effectively it is cleared by ciliary action and cough \[67]\.

While healthy mucus is a gel with low viscosity and elasticity, easily transported by ciliary action, pathologic mucus has higher viscosity and elasticity, so being less easily cleared. The mucus composition changes during ARDS, containing viral, bacterial and lysed cell material. Adding 0.5% (90 mM) saline to sputum samples has been shown to increase their ciliary transportability, while dehydrated sputum such as in cystic fibrosis may need more NaCl as to equilibrate the sputum for maximal transport \[72]\.

For influenza A infection, it was shown that the viral yield in porcine ciliated airway epithelium was about two- to threefold higher 24-48 h post-infection in the case of ciliary stasis, as compared to normal ciliary activity \[48]\.

Saline (2%) was found to reverse the ciliostasis, altering the MCC and impeding the viral infection: the 2% concentration was chosen in that experiment because full recovery of the ciliary activity was seen up to this concentration, while above 2% NaCl, recovery decreased the more the saline concentrations increased up to 11% \[48]\.

Secondly, impaired mucus clearance can induce cough and dyspnoea \[67]\.

In elderly patients in particular, there is stasis of thick, dehydrated mucus within the nasal cavities and nasopharynx leading to postnasal drip, cough, and globus (pharyngeus) sensation \[45]\.

Saline aerosol will equilibrate in the mucus while gelling it \[observed at 100 mM (0.6%)\] \[73]\ and reducing its adhesion \[0.9% saline\] \[74]\, so helping to relieve chronic cough \[75]\.

Thirdly, decreased MCC and persistent accumulation of mucus can lead to infection and inflammation by providing an environment for microbial growth: mucin affects bacterial adhesion, and fluidizing the mucin by saline in the deeper airway layers may help to remove mucin-attached pathogens involved in secondary pulmonary infections \[76]\.
Apart from reversing ciliostasis [47], nebulising isotonic saline provides hydration of the airway epithelial surface and easier removal of the mucus by its wetting effect, thereby also increasing the diffusivity of particles such as viruses within the mucin [77]. It improves the mucus’ gelling properties, leading to better entrapment of the viruses and other pathogens, while this also alters the viscoelasticity properties at the ALF surface [38]. These mucin-related phenomena lead to easier, more efficient coughing-up and swallowing of the mucus. Consequently, also the physical properties of ALF itself are changed by saline, leading to a better spread of ALF and a reduced tendency of ALF to disintegrate into small droplets or bio-aerosols, as already discussed [38]. The latter phenomena are relevant, as virus infected bio-aerosols are thought to be formed during coughing, or due to vibration when the bronchial mucoid secretions move over the vocal cords during speaking or singing [78],[79]. SARS-CoV-2 targets ciliated cells in the nose and airways releasing virus or abundant secretory vesicles, and impairs the MCC [80],[81],[82]. SARS-COV-2 spike protein also binds sialic acids (the main constituent of mucus) [83], but apparently not if the mucus is buffered at pH 7.0 [84]. So, pure saline (pH 5.5) may assist to contain the infection by gelling and clearing the mucus and impring the MMC.

Remains to be noted that adding bronchodilators to saline aerosols, such as salbutamol or terbutaline, improves the MCC and dilates the airways. In contrast, many preservatives, antimicrobial agents, lidocaine/anaesthetics, opioids, and mucolytics such as acetyl cysteine, decrease the MCC [85].

4. **Interactions of saline with SARS-CoV 2**

NaCl can directly affect the SARS-CoV-2 virus by interacting with its ionic or electrostatic charges. NaCl is listed as an antimicrobial against coronaviruses MHV-2, MHV-N (mouse hepatitis viruses) and CCV (canine coronavirus), as these viruses lose infectivity after exposure to NaCl 0.23% [86]. Moreover, a recent in vitro assessment of SARS-CoV-2 with saline (0.8-1.7% NaCl) showed a dose-dependent inhibition of viral replication in Vero CL-81 cells [33]. Inhibition of viral replication started from 0.6% onwards, increasing to 50% at 0.9% (isotonic) saline and reaching 100% at 1.5% hypertonic saline. NaCl, however, had no direct effect on SARS-CoV-2, as saline-pretreatment of the virus was unable to induce an inhibition of subsequent viral replication in the Vero cells. The authors proposed as mechanisms: (1) NaCl-induced hyperosmotic stress leading to the SARS-CoV-2 inhibition (yet, no direct effect on the virus was shown), (2) decreased expression of the PKC signalling pathway (yet, this would require time for down-regulation) and (3) depolarization via ENaC and its sodium sensor, the Na<sub>x</sub> channel, over-stimulating ENaC and leading to electrolyte movements stressing the mitochondria (unlikely to explain the mechanism, as the threshold for Na<sub>x</sub> activation in vitro is 150 mM of extracellular Na<sup>+</sup> [87]: at this isotonic (0.9% NaCl) condition already 50% inhibition of the replication was observed.)

We identified two other interactions of saline relevant to viral tropism and its interactions during respiratory infection. The first interaction concerns ACE2, the entry receptor of the virus, which is present in the nose, oropharynx and airways (particularly in ciliated cells) [88],[89],[90], and which has a (sodium) chloride-sensitive conformation: increasing saline concentrations dose-dependently induce immediate steric hindrance in the ACE-2 receptor configuration for binding of Angiotensin (Ang) II: saline starts to block the cleavage of Ang II by ACE2 onwards 0.1 M (0.5%) which is close in concentration at which also the blocking effect of saline on SARS-CoV-2 replication became observed [33,[91],[92]. The ACE2-virus interaction is possibly also subjected to a pH-effect: pure saline has a pH of 5.5 yet is not buffered. NH4Cl (pH range 4.6-6.0) but not phosphate buffered saline (PBS pH 7.4) has been shown to block viral replication in vitro [93]. A direct pH-dependent effect may also involve
hampered unfolding of the SARS-CoV-2 spike [[94]]. The role of pH may be relevant because the nasal cavity and airways, as well as sputum have a slight acidic pH (pH 5.5-6.5), while this pH changes, for instance, during common colds or some chronic respiratory conditions to more alkaline pH 7.2-8.3 [[95]]. This suggests that pure saline may change the configuration of the ACE2 receptors in the nose thereby blocking the viral receptor-binding and impeding viral entry for replication. The second interaction involves ENaC. As discussed, ENaC is the main mechanism for maintaining the necessary hydration of the ASL and ALF [42]. Based on the protein sequencing, Anand [[96]] identified a unique S1/S2 cleavage site in the SARS-CoV-2 virus that can mimic the proteolytic activation of human ENaC. In fact, the virus can hijack several proteases for its replication, which are also involved in the activation and regulation of ENaC activity, such as TMPRSS2, furin, prostasin and matriptase [[97],[98],[99]]. By hijacking these proteases, the virus may lead to dysregulation of ENaC and fluid absorption. Sodium is actively absorbed by ENaC [42,70,71] while the fluid homeostasis is also regulated by the cooperation of ENaC and the sodium sensing Na$_x$ channel [[100]]: Na$_x$ can activate ENaC following the sensing of extracellular Na$^+$ at 150 mM (0.9% NaCl) and higher salt concentrations [87]. These mechanisms involving saline require further study as to elucidate their relevance for preventing nasal infection and (as aerosol) for limiting alveolar flooding following SARS-CoV-2 infection.

5. **Formation of HOCl**

Inhibition of viral replication in presence of chloride and halide salts was first reported in the 1960s: viral inactivation was observed at NaCl concentrations between 15-300 mM (corresponding to 0.09%-1.7% of saline) and appeared to be related to the presence of Cl$^-$ or similar anions, rather than Na$^+$ [[101]]. This observation was subsequently expanded to a number of DNA, RNA, enveloped and non-enveloped viruses, including the human coronavirus 229E (HCoV-229E) [[102]]. Viral inhibition was dose-dependent and measurable from 10 mM NaCl (0.058%) onwards, as well as dependent on the virus tested. The *in vitro* effect was not due to a direct effect of NaCl on the host cells, but happened during viral replication [102]. It is suggested that this mechanism is part of the innate antiviral immune mechanism to clear viral infections.102 As to support this hypothesis, a small randomised controlled trial using nasal irrigation versus no irrigation was performed [36] (for results see section 6), its results leading to a recent recommendation of saline irrigation for COVID-19 by these investigators [[103]].

HOCl has the well-known effect of bleach, being effective against all virus types for house-hold purposes. Yet, HOCl is also cytotoxic and may injure airway epithelial cells *in vitro* [[104]]. So, tight regulation of this metabolic route is needed. Human MPO activity is involved in phagocytosis by neutrophils and macrophages and in oxidative processes, as well as their feedback mechanisms, in the bronchi and lungs [[105],[106],[107],[108]]. Four-fold enhanced MPO activity in airway fluid is associated with infections in the airways of children with cystic fibrosis compared to those without respiratory infection [[109]]. Also in sera of COVID-19 patients, particularly if mechanically ventilated, MPO is increased (no data on the lungs) [[110]]. Nebulised saline (0.9%) may provide an additional benefit by influencing MPO in lung disease. More precisely, neutrophil MPO activity increases with increasing NaCl concentrations from 0.025 to 0.14 M (0.14%-0.82%) [[111]], while the HOCl generation in the phagosomes requires a continuous supply of chloride: the local chloride disposition will drive chloride redistribution into the neutrophil phagosomes by various mechanisms and sustain HOCl production [[112]]. So adding NaCl to cell culture medium *in vitro* shifts the MPO activity to production of HOCl, the chlorination dominating over peroxidation, producing HOCl with higher antimicrobial action than H$_2$O$_2$ [[113]]. Alternatively, as
also competes with thiocyanate as a natural substrate for MPO activity, saline may shift the substrate thiocyanate towards alternative signalling pathways, thereby exerting host defence and antioxidant properties, in addition to the effects of HOCl \([114]\). To note, isothiocyanate is used in TRIzol to inactivate SARS-CoV-2 for extraction and qPCR analysis \([115]\).

6. **Saline use in a clinical context**

**Studies in non-COVID-19 ARDS or bronchiolitis.** Based on a meta-analysis of studies in non-COVID-19-associated ARDS, isotonic saline aerosol (0.9%, also called physiologic serum) has been proposed as an active treatment for acute viral bronchiolitis, rather than an inert placebo \([116],[117]\): patients with viral bronchiolitis treated with nebulized normal saline showed significant improvement in the respiratory rate, clinical scores after therapy and reduced hospital length of stay by 24h. Tolerance in infants was excellent. When comparing “saline placebo” with other (non-drug containing) placebos, patients treated with nebulized isotonic saline showed greater improvements in posttreatment scores \([116,117]\). In a Cochrane analysis of studies with nebulised hypertonic saline and a meta-analysis \([118],[119]\), it was concluded that hypertonic saline use may modestly reduce length of stay among infants hospitalised with acute bronchiolitis and improve clinical severity score. Furthermore, treatment with nebulised hypertonic saline was found to reduce the risk of hospitalisation among outpatients and emergency department patients. Yet, in a large direct comparative study in infants, nebulized hypertonic saline did less well than isotonic saline, while worsening of cough occurred more frequently among children in the hypertonic saline group \([120]\). Also other studies failed to confirm a clinical benefit on length of stay or readiness for discharge when compared to normal saline/care \([121],[122]\).

**Studies with nasal saline irrigation for common cold.** Regarding nasal irrigation with saline, the WHO has only recently acknowledged that its use may promote recovery from common cold; they further state that to date, there is no evidence that it can protect people from infectious respiratory diseases or COVID-19 \([123],[124]\). A 2014 Cochrane analysis found limited data from five randomised controlled trials (RCTs) suggesting that saline nasal irrigation may have some benefit in patients with acute upper respiratory tract infections, but the included trials were generally small and were found to carry a high risk of bias \([125]\). A recent meta-analysis of saline nasal irrigation for acute upper respiratory tract infections in infants and children showed that saline significantly improved rhinological symptoms, but not respiratory symptoms \([126]\). Its use, however, appeared to reduce the use of other treatments, whether local or systemic, and particularly antibiotics. Long-term use led to a decrease in the incidence of acute rhinosinusitis and its complications. Finally, with regard to common colds, studies of isotonic saline have shown a reduction in number of illness days and infectious episodes in adults and children, including substantially reduced absence from school (17% vs 35%) and secondary medical complications (8% vs 32%) in children \([127],[128]\).

**Studies in upper respiratory infections of SARS-CoV-2 and other human coronaviruses.** Regarding COVID-19, a recent publication called for the use of hypertonic saline nasal irrigation and gargling as a treatment option, based on prior data generated from a randomised pilot study in 68 patients with an upper respiratory tract infection, of whom 56% were infected by rhinovirus and 31% by “common” (non-COVID-19) coronaviruses \([36,103]\). Patients were enrolled within 48 hours of onset of symptoms and the parameters were rated for a maximum of 14 days or until patients felt well for two consecutive days. In this study, the rinse procedure (used maximum 12 times/day), which was proposed to act through formation of hypochlorous acid (see Point 5), reduced the duration of upper
respiratory tract infection by an average of 1.9 days (P = 0.01), the transmission within household contacts by 35% (P = 0.006) and viral shedding by $\geq 0.5 \log_{10}/d$ as compared to controls. The relevance of these data in the context of COVID-19 needs confirmation. A study in non-morbid COVID-19 outpatients is ongoing including the use of hypertonic saline nasal irrigation up to 12 times daily in addition to standard hygiene and social distancing recommendations [[129],[130]].

A recent interim analysis of a small open-labelled study in 45 non-hospitalised COVID-19 patients suggests substantial symptom resolution, with nasal congestion and headache resolving a median of 7-9 days earlier in the nasal saline and saline plus detergent irrigation groups: the viral load data and results in the planned 90 patients are awaited [14]. A more recent publication proposes the use of isotonic alkaline saline solution containing 0.6% non-iodide salt and 0.3% sodium bicarbonate (NaHCO3) (pH 8.3), attributing a potential benefit of such nasal rinse to the formation of hypochlorous acid, while the bicarbonate ions would reduce the viscosity of the mucus [[131]]. So far there is no clinical assessment available.

Another prospective study in 45 COVID-19 patients receiving usual medical care plus intravenous or nebulised electrolysed saline compared with 39 patients in the control group (usual medical care alone) [34]. The process of electrolysis would form HOCl. The intervention led to a decrease of hospitalisation by 92% (p=0.02), a faster acceptable symptom status after 4.6 days on average (instead of 11.0 days in the control group, p=0.015) and reduced mortality (0 versus 12.8% (p=0.019), but nebulization was less efficacious than its combination with its intravenous administration. Simple saline nebulisation was not assessed.

**Discussion**

Our analysis suggests that, although not claiming to cure COVID-19 infection, the timely use of isotonic or hypertonic saline irrigation or aerosol may help to contain COVID-19 infection, if started early and so possibly prevent the evolution to severe disease, as also proposed to the German consumers on the internet [1-7]. Our analysis shows that this effect may take place by different mechanisms.

Firstly, saline irrigation or aerosol may provide moisture, and as such, protect the airways. Saline may wet proteins, altering their functioning, contributing to a better quality of the ALF, its improved spreading, less bio-aerosol production, and – by its isotonic nature – it may also reverse bronchoconstriction, in case of hyper-secreted, potentially hypotonic ALF [16-19,31,37,38,61] Through these mechanisms, isotonic saline may aid to improve the oxygen passage and ventilation of the lungs during COVID-19.

Secondly, isotonic saline promotes ciliary beating and reverses ciliostasis [43,46,47], while the mucus properties change, allowing a better MCC with more efficient trapping and removal of viruses, pathogens and debris, and resulting in better gelling properties, allowing more efficient mucus coughing and swallowing [42,43,46,47,67,68,77]. Improvement of MCC takes place upon the usage of saline nasal irrigation at room temperature (no heating for inhalation required) [[132]].

Thirdly, saline may interfere with the SARS-CoV-2 infectivity, possibly by interacting with the viral ACE2 entry mechanism, as well as with ENaC [33].

Finally, saline may interact with SARS-CoV-2 infectivity by shifting the MPO activity in epithelial or phagocytic cells to produce HOCl [113-114].
The pharmacological/pharmacodynamic effects of saline are effectuated at concentrations already reached by isotonic saline. *In vivo*, the limited action of isotonic saline on the viral ACE2-receptor binding and SARS-CoV-2 replication [33,91,92] will be complemented by its effect on ciliary beating and the MCC for clearing virus [46,47,69-71]. Overall, the complex interactions with NaCl, particularly those impacting with ACE2 and ENaC, suggest that a therapeutic strategy for recovering the balance between alveolar fluid formation and reabsorption may contribute to the treatment for alveolar lung injury. The beneficial effect on the MCC is also relevant in view of the recent findings of an infection gradient for SARS-CoV-2 along the respiratory tract: this finding made the investigators propose that micro-aspiration from the nose would seed the lower airways, while the tracheal-produced virus would lead to further aspiration into the deep lung; aspiration of SARS-CoV-2 into the lung seems consistent with the patchy, bibasilar infiltrates observed by chest CT in COVID-19 [133]. So patients may benefit from early controlled removal of virus or viral loaded mucus by the rinse effect and/or improved MCC with saline as to avoid the progression of COVID-19 to ARDS.

The administration of saline as orinasal rinse or nebulized aerosol should not be confounded with the viral transmission-prone aerosolising procedures – a common misconception in respiratory care. Whereas the use of oxygen flow or invasive procedures may cause the formation of virus-loaded bio-aerosols from the infected surfactant-containing environment of the respiratory tract, saline in contrast leads to re-spreading and compressing the ALF to ultra-low surface tensions and to a reduction in exhaled bio-aerosol [18,19,31,36-38]. The resulting improved airway compliance has been well-established with isotonic saline in preterm children suffering from the infant respiratory distress syndrome [18,19]. Obviously, saline irrigation should be combined with the basic hygiene measures for COVID-19 (see Table 3), with use of disposable or washable tissues to collect superfluous rinse and mucus, as well as hand washing, and adequate room ventilation while or after nebulizing.

Whether (ori)nasal irrigation or rather inhalation of nebulised iso- or hypertonic saline is the best approach early during COVID-19 infection and whether it adds to other treatment strategies to limit the damage of SARS-CoV-2 to the lungs, deserves further evaluation. While the measure is simple and at low cost, it may moreover work reassuring in early stages of the disease when COVID-19 symptoms are indistinguishable from other common colds. Table 3 summarizes a number of recommendations, based on the results from this analysis, for use of saline for nasal and/or respiratory hygiene at initial onset of common cold symptoms, which clinically overlap with COVID-19 upper respiratory symptoms. While isotonic seawater has a rinsing effect improving the MCC [134], the use of pure isotonic saline is rather proposed, because nasal spray compositions with more (buffering) ions such as seawater, or with surfactants, emulsifiers, and/or active substances may not necessarily lead to the desired effects, such as observed with pure saline on SARS-CoV-2 replication *in vitro* [33,93]; moreover, the addition of surfactants/emulsifiers may enhance bio-aerosol formation [31,39], while these products or active substances may inhibit the ciliary beating [85] or be ciliotoxic (e.g. polyvidone-iodine [134,135]). Trypsin–containing sprays, claiming to protect against the virus based on a viral trypsin digest in a laboratory test tube (using trypsin inhibitor before applying it to host cells) [136] are to be avoided because trypsin, if incubated in presence of host cells, does not stop, but enhances SARS-CoV-2 invasion and syncytia formation, as well as potentiates the viral multiplication and cellular inflammation of other viruses such as influenza [93,137]; such sprays sold OTC as medical devices in the European Union do not undergo regulatory assessment like for medicinal drugs, and are prohibited for sale in Germany because of lack of proof of efficacy.

We propose isotonic saline rather than hypertonic saline as it is devoid of the side effects that have been associated with hypertonic saline (changes in cell morphology [51,52], increased nasal epithelial permeability
[58,59], nasal burning/irritation [60] and when nebulizing: induction of bronchoconstriction or cough [61-64]. Until more clinical documentation becomes available, isotonic saline might thus be preferred to hypertonic saline for nasal hydration/hygiene, either as nasal spray or nasal irrigation - to be used from the first onset of common cold symptoms. The use of nebulizing isotonic saline may demand more precautions, such as a well aerated room. Hypertonic aerosol best includes a bronchodilator, as it can induce bronchoconstriction [62-64]. Home-made saline (preparing saline by using simple kitchen salt) is often proposed in the internet. Sterile sprays and uni-doses may be safer for use for nebulizing aerosol. Usual applications for airway care are 2 to 3 times a day, while the ongoing clinical trial in symptomatic COVID-19 patients proposes nasal irrigation up to 12 times per day [36].

At last, common colds are frequent at young age, whereas dehydration of the nose and airways increases with age. Improving the hydration and ciliary beat function of dry nasal mucosa may be of particular interest in the elderly. Whether children and adolescents benefit preventively from saline irrigation upon the first common cold symptoms for nasal hygiene during COVID-19, best needs further evaluation as to establish the optimal daily dosing regimen. The same applies to the elderly, as to find out whether elderly persons with dry mucosa benefit from prophylactic applications of saline for hydration during COVID-19 outbreaks.

**Abbreviations**

air-liquid interface (ALI), Acute Respiratory Distress Syndrome (ARDS), alveolar lining fluid (ALF), mucociliary clearance (MCC), Angiotensin Converting Enzyme (ACE), Angiotensin (Ang), Alveolar type 2 cells (AT2).

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**References**

[1] HNO-Ärzte im Netz (2020) Herausgegeben vom Deutschen Berufsverband der Hals-Nasen-Ohrenärzte e.V.) Tipps zur richtigen Nasenpflege [Tipps for adequate nasal care]. https://www.hno-aerzte-im-netz.de/unsere-sinne/hno-hygiene/tipps-zur-richtigen-nasenpflege.html. Accessed 19 June 2020

[2] Lungenartze im Netz (Lung doctors in the Net) (2020) Einfaches Inhalieren kann Tröpfcheninfektion effektiv eindämmern. [Simple inhalation can limit efficiently droplet infection] https://www.lungenaerzte-im-netz.de/news-archiv/meldung/article/einfaches-inhalieren-kann-troepfcheninfektion-effektiv-eindaemmern/ . Accessed 19 June 2020

[3] Praxisvita (das Portal für Gesundheit & Medizin) (2020) Inhalieren bei Corona: Wie wirksam ist das Hausmittel? [Inhalation during Corona; How effective is this home remedy?]
https://www.praxisvita.de/coronavirus-dieses-hausmittel-hilft-bei-leichten-symptomen-18411.html. Accessed 19 June 2020

[4] Leichter Atmen bei Lungen- und bronchialerkrankungen (2020) Corona: Pflege der Atemwege vermindert Infektionsrisiko [Corona: Care of the airways reduces the risk of infection]. [24.03.2020] https://www.leichter-atmen.de/copd-news/atemwegspflege. Accessed 19 June 2020

[5] PARI-Blog (2020) Treatment and nebuliser therapy for COVID-19 in hospital. Interview with the Prof. Dr Kamin, Medical Director of the Hamm Lutheran Hospital. https://www.pari.com/int/blog/treatment-and-nebuliser-therapy-for-covid-19-in-hospital-interview-with-the-prof-dr-kamin-medical-director-of-the-hamm-lutheran-hospital/. Accessed in English 27 July 2020. - Firstly accessed in German: Accessed 19 June 2020

[6] Betreut.de (2020) Coronavirus: Was Senioren & ihre Betreuer wissen müssen. [Coronavirus: What seniors and care givers need to know] www.betreut.be. Accessed 14 July 2020

[7] ETH Zurich (2020) Mit Atemwegspflege das Infektionsrisiko senken. [With airway care decrease the risk of infection.] https://ethz.ch/de/news-und-veranstaltungen/eth-news/news/2020/03/zukunftsblog-viola-vogel-mitatemwegspflege-das-infektionsrisiko-senken.html. Accessed 14 July 2020

[8] Bronchiectasis Toolbox (2020) Hydration and humidification https://bronchiectasis.com.au/physiotherapy/principles-of-airway-clearance/hydration-and-humidification. Accessed 13 July 2020

[9] Sciensano (2020) Consensus over het rationeel en correct gebruik van mondmaskers tijdens de COVID-19-pandemie [Consensus on the rational and correct use of mouth masks during the COVID-19 pandemic]. https://covid-19.sciensano.be/sites/default/files/Covid19/consensus%20on%20the%20use%20of%20masks_RMG_NL.pdf. Accessed 13 July 2020

[10] Sciensano (2020) Procedure voor huisartsen in geval van een mogelijk geval van COVID-19. Versie 08 juli 2020. [Procedure for doctors in the event of a possible case of COVID-19]. https://covid-19.sciensano.be/sites/default/files/Covid19/COVID-19_procedure_GP_NL.pdf. Accessed 13 July 2020

[11] APB (2020) Aerosoltoestellen [Aerosol devices]. Information Update 20 March 2020. https://www.apb.be/APB%20Documents/NL/All%20partners/Coronavirus_Aerosol_Verhuur_20_03_20.pdf. Accessed 19 June 2020

[12] World Health Organization (2020) Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations. Scientific brief, 29 March 2020. https://www.who.int/publications-detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations. Accessed June 19, 2020.

[13] Pfeifer M, Ewig S, Voshaar T, Randerath WJ, Bauer T, Geiseler J. et al (2020) Position paper for the state-of-the-art application of respiratory support in patients with COVID-19. Respiration 99:521–541. https://doi.org/10.1159/000509104
[14] Kimura KS, Freeman MH, Wessinger BC, Gupta V, Sheng Q, Huang LC, et al (2020) Interim analysis of an open-label randomized controlled trial evaluating nasal irrigations in non-hospitalized patients with COVID-19. Int Forum Allergy Rhinol Sep 11 [Epub ahead of print]. https://doi.org/10.1002/alr.22703

[15] ClinicalTrials.gov Identifier: NCT04347538. Impact of nasal saline irrigations on viral load in patients with COVID-19. https://clinicaltrials.gov/ct2/show/record/NCT04347538?term=saline&cond=covid-19&draw=2&rank=1

[16] Santos FKG, Barros Neto EL, Moura TMCPA, Castro Dantas TN, Dantas Neto AA (2009) Molecular behavior of ionic and nonionic surfactants in saline medium. Colloids and Surfaces A: Physicochemical and Engineering Aspects 333:156-162. https://doi.org/10.1016/j.colsurfa.2008.09.040

[17] Staszak K, WieczorekD, Michocka K (2015) Effect of sodium chloride on the surface and wetting properties of aqueous solutions of cocamidopropyl betaine. J Surfact Deterg 18;321–328. doi.org/10.1007/s11743-014-1644-8

[18] Avery ME, Mead J (1959) Surface properties in relation to atelectasis and hyaline membrane disease. AMA J Di Child 97(5_Part_I):517–523. https://doi.org/10.1001/archpedi.1959.02070010519001

[19] Ghadiali SN, Gaver DP (2008) Biomechanics of liquid-epithelium interactions in pulmonary airways. Respir Physiol Neurobiol 163(1-3):232-243. https://doi.org/10.1016/j.resp.2008.04.008

[20] Huang J, Hume AJ, Abo KM, Werder RB, Villacorta-Martin C, Alysandratos KD, (2020) SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. bioRxiv [Preprint]. Jun 30:2020.06.30. 175695. https://doi.org/10.1101/2020.06.30.175695

[21] Takano H (2020) Pulmonary surfactant itself must be a strong defender against SARS-CoV-2. Medical Hypotheses 144:110020. doi.org/10.1016/j.mehy.2020.110020.

[22] van Doremalen N, Morris DH, Holbrook MG, Holbrook MG, Gamble A, Williamson BN (2020) Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 382:1564-1567. https://doi.org/10.1056/NEJMmc2004973

[23] Scheuch G (2020) Breathing is enough: for the spread of influenza virus and SARS-CoV-2 by breathing only. J Aerosol Med Pulm Drug Delivery 33:230-234. doi.org/10.1089/jamp.2020.1616

[24] Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, (2003) A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 348:1986e94. https://doi.org/10.1056/NEJMoa030685

[25] Tran K, Cimon K, Severn M, Pessoa-Silva CL, Conly J (2012) Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: a systematic review. PLOS ONE 7:e35797. https://doi.org/10.1371/journal.pone.0035797

[26] Harding H, Broom A, Broom J (2020) Aerosol-generating procedures and infective risk to healthcare workers from SARS-CoV-2: the limits of the evidence. J Hospital Infection 105:717-725. https://doi.org/https://doi.org/10.1016/j.jhin.2020.05.037

[27] Simonds A, Hanak A, Chatwin M, Morrell M, Hall A, Parker K (2010) Evaluation of droplet dispersion during non-invasive ventilation, oxygen therapy, nebuliser treatment and chest physiotherapy in clinical practice:
implications for management of pandemic influenza and other airborne infections. Health Technol Assess 14:131-172. https://doi.org/10.3310/hta14460-02

[28] Jespers V, Roberfroid D (2020). COVID-19 – KCE Contributions. Aerosol-generating procedures. https://kce.fgov.be/sites/default/files/atoms/files/2020-51_COVID_Aerosol%20KCE_FINAL_19052020_3.pdf

[29] Raboud J, Shigayeva A, McGeer A, Bontovics E, Chapman M, Gravel D (2010) Risk factors for SARS transmission from patients requiring intubation: a multicentre investigation in Toronto, Canada. PLOS ONE 5(5):e10717. https://doi.org/10.1371/journal.pone.0010717

[30] Loeb M, McGeer A, Henry B, Ofner M, Rose D, Hlywka T (2004) SARS among critical care nurses, Toronto. Emerg Infect Dis 10(2):251–255. https://doi.org/10.3201/eid1002.030838

[31] Edwards DA, Man JC, Brand P, Katstra JP, Sommerer K, Stone HA (2004) Inhaling to mitigate exhaled bioaerosols. Proc Natl Acad Sci USA 101(50):17383–17388. https://doi.org/10.1073/pnas.0408159101

[32] Fiegel J, Clarke R, Edwards DA (2006) Airborne infectious disease and the suppression of pulmonary bioaerosols. Drug Discov Today 11(1-2):51-7. https://doi.org/10.1016/S1359-6446(05)03687-1

[33] Machado RRG, Glaser T, Araujo DB, Petiz LL, Oliveira DBL, Durigon GS (2020) Hypertonic saline solution inhibits SARS-CoV-2 in vitro assay. BiorXiv https://doi.org/10.1101/2020.08.04.235549

[34] Delgado-Enciso I, Paz-Garcia J, Barajas-Saucedo CE, Mokay-Ramírez KA, Meza-Robles C, Lopez-Flores R (2020) Patient-reported health outcomes after treatment of COVID-19 with nebulized and/or intravenous neutral electrolyzed saline combined with usual medical care versus usual medical care alone: a randomized, open-label, controlled trial. Res Sq [Preprint] 10:rs.3.rs-68403. https://doi.org/10.21203/rs.3.rs-68403/v1.

[35] Hendley JO, Gwaltney JM. Viral titers in nasal lining fluid compared to viral titers in nasal washes during experimental rhinovirus infection. J Clin Virol 2004 30(4):326-328. https://doi.org/10.1016/j.jcv.2004.02.011

[36] Ramalingam S, Graham C, Dove J, Morrice L, Sheikh A (2019). A pilot, open labelled, randomised controlled trial of hypertonic saline nasal irrigation and gargling for the common cold. Sci Rep 9:1015. https://doi.org/10.1038/s41598-018-37703

[37] Edwards D, Hickey A, Batycky R, Griel L, Lipp M, et al. (2020). A new natural defense against airborne pathogens. QRB Discovery 1:e5. https://doi.org/ 10.1017/qrd.2020.9.

[38] Watanabe W, Thomas M, Clarke R, Klibanov AM, Langer R, et al. (2007) Why inhaling salt water changes what we exhale. J Colloid Interface Sci 307:71–8. https://doi.org/ 10.1016/j.jcis.2006.11.017

[39] Patel A, Longmore N, Mohanan A, Ghosh S (2019) Salt and pH-induced attractive interactions on the rheology of food protein-stabilized nanoemulsions. CS Omega 4 (7):11791–11800. https://doi.org/10.1021/acsomega.8b03360

[40] Vejerano EP, Marr LC (2018) Physico-chemical characteristics of evaporating respiratory fluid droplets. J R Soc Interface 15: 20170939. doi.org/10.1098/rsif.2017.0939
[41] Yang W, Elankumaran S, Marr LC (2012) Relationship between humidity and Influenza A viability in droplets and implications for influenza's seasonality. PLoS ONE 7(10): e46789. https://doi.org/10.1371/journal.pone.0046789

[42] Bustamante-Marin XM, Ostrowski LE (2017) Cilia and mucociliary clearance. Cold Spring Harb Perspect Biol 9(4):a028241. https://doi.org/10.1101/cshperspect.a028241

[43] Rivera JA(1962) Cilia, ciliated epithelium, and ciliary activity. International Series of Monographs and Applied Biology. 1st edn. Pergamon Press Ltd, Oxford-London-NewYork-Paris pp.50-58. ISBN 978008009623

[44] Paul P, Johnson P, Ramaswamy P, Ramadoss S, Geetha B, & Subhashini AS (2013) The effect of ageing on nasal mucociliary clearance in women: a pilot study. Pulmonol Article ID 598589:5 pages. https://doi.org/10.1155/2013/598589

[45] Pinto JM, Jeswani S (2010) Rhinitis in the geriatric population. Allergy Asthma Clin Immunol 6(1):10. https://doi.org/10.1186/1710-1492-6-10

[46] Wolf G, Koidl B, Pelzmann B (1991) [Zur Regeneration des Zilienschlages humaner Flimmerzellen] Regeneration of the ciliary beat of human ciliated cells. Laryngorhinootologie 70(10): 552-555. https://doi.org/10.1055/s-2007-998095

[47] Daviskas E, Anderson SD, Gonda I, Eberl S, Meikle S, Seale JP, Bautovich G (1996) Inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects. Eur Respir J 9(4):725-32. https://doi.org/10.1183/09031936.96.09040725

[48] Fu Y, Tong J, Meng F, Hoeltig D, Liu G, Yin X, Herrler G (2018) Ciliostasis of airway epithelial cells facilitates influenza A virus infection. Vet Res 49(1):65. https://doi.org/10.1186/s13567-018-0568-0

[49] Keojampa BK, Nguyen MH, Ryan MW (2004) Effects of buffered saline solution on nasal mucociliary clearance and nasal airway patency. Otolaryngol Head Neck Surg 131(5):679-82. https://doi.org/10.1016/j.otohns.2004.05.026

[50] Sood N, Bennett WD, Zeman K, Brown J, Foy C, Boucher RC, Knowles MR (2003) Increasing concentration of inhaled saline with or without amiloride: effect on mucociliary clearance in normal subjects. Am J Respir Crit Care Med. 167(2):158-63. https://doi.org/10.1164/rccm.200204-293OC

[51] Kim C-H, Song MH, Ahn YE, Lee G-G, Yoon YH (2005) Effect of hypo-, iso- and hypertonic saline irrigation on secretory mucins and morphology of cultured human nasal epithelial cells. Acta Oto-Laryngologica 125:1296-1300. https://doi.org/10.1080/00016480510012381

[52] Sumaily I, Alarifi I, Alsuwaian R, Alsiwat L, Alsaleh S (2020) Impact of nasal irrigation with iodized table salt solution on mucociliary clearance: proof-of-concept randomized control trial. Am J Rhinol Allergy 34(2):276-279. https://doi.org/10.1177/1945892419892172

[53] Min YG, Lee KS, Yun JB, Rhee C S, Rhyoo C, Koh YY et al. (2001) Hypertonic saline decreases ciliary movement in human nasal epithelium in vitro. Otolaryngol Head Neck Surg 124(3):313-316. https://doi.org/10.1067/mhn.2001.113145
[54] Bencova A, Vidan J, Rozborilova E, Kocan I (2012) The impact of hypertonic saline inhalation on mucociliary clearance and nasal nitric oxide. J Physiol Pharmacol 63(3):309-13. PMID: 22791646.

[55] Talbot AR, Herr TM, Parsons DS (1997) Mucociliary clearance and buffered hypertonic saline solution. Laryngoscope 1997;107(4):500-3. https://doi.org/10.1097/00005537-199704000-00013

[56] Bennett WD, Wu J, Fuller F, Balcazar JR, Zeman KL, et al. (2015) Duration of action of hypertonic saline on mucociliary clearance in the normal lung. J Appl Physiol 118(12):1483-90. https://doi.org/10.1152/japplphysiol.00404.2014

[57] Middleton PG, Pollard KA, Wheatley JR (2001) Hypertonic saline alters ion transport across the human airway epithelium. Eur Resp J 17: 195-199. https://erj.ersjournals.com/content/17/2/195

[58] Jiao J, Yang J, Li J, Li Y, Zhang L (2020) Hypertonic saline and seawater solutions damage sinonasal epithelial cell air-liquid interface cultures. Int Forum Allergy Rhinol 10(1):59-68. https://doi.org/10.1002/alr.22459

[59] Miwa M, Matsunaga M, Nakajima N, Yamaguchi S, Watanabe K (2007) Hypertonic saline alters electrical barrier of the airway epithelium. Otolaryngol Head Neck Surg 136(1):62-6. https://doi.org/10.1016/j.otohns.2006.08.013

[60] Hauptman G, Ryan MW (2007) The effect of saline solutions on nasal patency and mucociliary clearance in rhinosinusitis patients. Otolaryngol Head Neck Surg 137(5):815-21. https://doi.org/10.1016/j.otohns

[61] Balmes JR, Fine JM, Christian D, Gordon T, Sheppard D (1988) Acidity potentiates bronchoconstriction induced by hypoosmolar aerosols. Am Rev Respir Dis 138(1):35-39. https://doi.org/10.1164/ajrccm/138.1.35

[62] Makker HK, Holgate ST (1993) The contribution of neurogenic reflexes to hypertonic saline-induced bronchoconstriction in asthma. J Allergy Clin Immunol 92:82-88. https://doi.org/10.1016/0091-6749(93)90041-d

[63] Taube C, Holz O, Mücke M, Jörres RA, Magnussen H (2001) Airway response to inhaled hypertonic saline in patients with moderate to severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med 164:1810-1815. https://doi.org/10.1164/ajrccm.164.10.2104024

[64] Lowry RH, Wood AM, Higenbottam TW (1988) Effects of pH and osmolarity on aerosol-induced cough in normal volunteers. Clin Sci (Lond) 74 (4): 373–376. https://doi.org/10.1042/cs0740373

[65] Elkins MR, Bye PT (2011) Mechanisms and applications of hypertonic saline. J R Soc Med 104 (Suppl 1):S2-S5. https://doi.org/10.1258/jrsm.2011.s11101

[66] Goralski JL, Wu D, Thelin WR, Boucher RC, Button B (2018) The in vitro effect of nebulised hypertonic saline on human bronchial epithelium. Eur Respir J 51(5):1702652. https://doi.org/10.1183/13993003.02652-2017

[67] Fahy JV, Dickey BF (010) Airway mucus function and dysfunction. N Engl J Med 2363(23):2233-2247. https://doi.org/10.1056/NEJMra0910061

[68] Mandelberg A, Amirav I (2010) Hypertonic saline or high volume normal saline for viral bronchiolitis: mechanisms and rationale. Paed pulmonol 45:36-40. https://doi.org/10.1002/ppul.21185
[69] Bartoszewski R, Matalon S, Collawn JF (2017) Ion channels of the lung and their role in disease pathogenesis. Am J Physiol Lung Cell Mol Physiol 313(5):L859-L872. https://doi.org/10.1152/ajplung.00285.2017

[70] Hollenhorst MI, Richter K, Fronius M (2011) Ion transport by pulmonary epithelia. J Biomed Biotechnol Article ID 174306, 16 pages. https://doi.org/10.1155/2011/174306

[71] Iwan IH, Dziembowska I & Słonina DA (2019) Airways surface liquid and ion Transport - The mechanism maintained patency. Biomedical Journal of Scientific & Technical Research 14(3):1-7. (doi:10.26717/BJSTR.2019.14.002543) https://biomedres.us/fulltexts/BJSTR.MS.ID.002543.php

[72] Wills PJ, Hall RL, Chan Wm, Cole PJ (1997) Sodium chloride increases the ciliary transportability of cystic fibrosis and bronchiectasis sputum on the mucus-depleted bovine trachea. J Clin Inv 99(1):9-13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC507760/pdf/990009.pdf

[73] McCullagh CM, Jamieson AM, Blackwell J, Gupta R (1995) Viscoelastic properties of human tracheobronchial mucin in aqueous solution. Biopolymers 35(2):149-159. https://doi.org/10.1002/bip.360350203

[74] Button B, Goodell HP, Atieh E, Chen Y-C, Williams R, et al. (2018) Roles of mucus adhesion and cohesion in cough clearance. PNAS 115 (49):12501-12506. https://doi.org/10.1073/pnas.1811787115

[75] Lin L, Chen Z, Cao Y, Sun G (2017) Normal saline solution nasal-pharyngeal irrigation improves chronic cough associated with allergic rhinitis. Am J Rhinol Allergy 31(2):96-104. https://doi.org/10.2500/ajra.2017.31.4418

[76] Lillehoj EP, Kato K, Lu W, Kim KC (2013) Cellular and molecular biology of airway mucins. Int Rev Cell Mol Biol 303:139-202. https://doi.org/10.1016/B978-0-12-407697-6.00004-0

[77] Lieleg O, Vladescu I, Ribbeck K (2010) Characterization of particle translocation through mucin hydrogels. Biophys J 98:1782-1789. https://doi.org/10.1016/j.bpj.2010.01.012

[78] Jayaweera M, Perera H, Gunawardana B, Manatunge J (2020) Transmission of COVID-19 virus by droplets and aerosols: A critical review on the unresolved dichotomy. Environ Res 188:109819. https://doi.org/10.1016/j.envres.2020.109819

[79] WHO (2020) Transmission of SARS-CoV-2: implications for infection prevention precautions. https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions

[80] Ehre C (2020) SARS-CoV-2 infection of airway cells. N Engl J Med 383:969. https://doi.org/10.1056/NEJMicm2023328

[81] Zhu N, Wang W, Liu Z, Liang C, Wang W, Ye F (2020) Morphogenesis and cytopathic effect of SARS-CoV-2 infection in human airway epithelial cells. Nat Commun 11:3910. https://doi.org/10.1038/s41467-020-17796-z

[82] Robinot R, Hubert M, Dias de Mehlo G, Lazarini F, Bruel T, et al. (2020) SARS-CoV-2 infection damages airway motile cilia and impairs mucociliary clearance. bioRxiv https://doi.org/10.1101/2020.10.06.328369
[83] Baker AN, Richards SJ, Guy CS, Congdon TR, Hasan M, et al. (2020) The SARS-COV-2 spike protein binds sialic acids and enables rapid detection in a lateral flow point of care diagnostic device. ACS Cent Sci https://doi.org/10.1021/acscentsci.0c00855

[84] Hao W, Ma B, Li Z, Wang X, Gao X, Li Y (2020) Binding of the SARS-CoV-2 spike protein to glycans. bioRxiv https://doi.org/10.1101/2020.05.17.100537

[85] Rusznak C, Devalia JL, Lozewicz S, Davies RJ (1994) The assessment of nasal mucociliary clearance and the effect of drugs. Respir Med 88(2):89-101. https://doi.org/10.1016/0954-6111(94)90020-5

[86] Newster (2020) Eco-sustainable technology for the processing of healthcare waste (HCW), on-site or in centralized treatment centers. Coronaviruses: SARS, MERS and Covid19. 28/02/2020 http://www.newstergroup.com/news/coronaviruses__sars_mers_and_covid19

[87] Noda M, Hiyama TY (2015) The Nax Channel: What it is and what it does. The neuroscientist 21(4): 399-412. https://doi.org/10.1177/1073858414541009

[88] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, et al. (2020) SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181(2):271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052

[89] Hou Y, Zhao J, Martin W, Kallianpur A, Chung MK, et al. (2020) New insights into genetic susceptibility of COVID-19: an ACE2 and TMPRSS2 polymorphism analysis. BMC Med 18: art. No. 216. https://doi.org/10.1186/s12916-020-01673-z

[90] Sungnak W, Huang N, Bécavin C, Berg M, Queen R, et al. (2020) SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med 26:681–687. https://doi.org/10.1038/s41591-020-0868-6

[91] Rushworth CA, Guy JL, Turner AJ (2008) Residues affecting the chloride regulation and substrate selectivity of the angiotensin-converting enzymes (ACE and ACE2) identified by site-directed mutagenesis. FEBS J 275(23):6033-6042. https://doi.org/10.1111/j.1742-4658.2008.06733

[92] Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ (2003) Angiotensin-converting enzyme-2 (ACE2): comparative modeling of the active site, specificity requirements, and chloride dependence. Biochemistry 42(45):13185-13192. https://doi.org/10.1021/bi035268s

[93] Ou X, Liu Y, Lei X, Li P, Mi D, et al. (2020) Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11:1620. https://doi.org/10.1038/s41467-020-15562-9

[94] Zhou T, Tsybovsky Y, Olia AS, Gorman J, Rapp MA, et al. (2020) A pH-dependent switch mediates conformational masking of SARS-CoV-2 spike. bioRxiv https://doi.org/10.1101/2020.07.04.187989

[95] Fischer H, Widdicombe JH (2006) Mechanisms of acid and base secretion by the airway epithelium. J Membr Biol 211(3):139-50. https://doi.org/10.1007/s00232-006-0861-0
[96] Anand P, Puranik A, Aravamudan M, Venkatakrishnan AJ, Soundararajan V (2020) SARS-CoV-2 strategically mimics proteolytic activation of human ENaC. eLife 9:e58603. https://doi.org/10.7554/eLife.58603

[97] Jaimes JA, Millet JK, Whittaker GR (2020) Proteolytic cleavage of the SARS-CoV-2 spike protein and the role of the novel S1/S2 site. iScience 23:101212. https://doi.org/10.1016/j.isci.2020.101212

[98] Ji HL, Zhao R, Matalon S, Matthay MA (2020) Elevated plasmin(ogen) as a common risk factor for COVID-19 susceptibility. Physiol Rev 100(3):1065-1075. https://doi.org/10.1152/physrev.00013.2020.

[99] Kleyman TR, Carattino MD, Hughey RP (2009) ENaC at the cutting edge: regulation of epithelial sodium channels by proteases. J Biol Chem 284(31):20447-51. https://doi.org/10.1074/jbc.R800083200.

[100] Marunaka Y, Marunaka R, Sun H, Yamamoto T, Kanamura N, Taruno A (2016) Na+ homeostasis by epithelial Na+ channel (ENaC) and Nax channel (Nax): cooperation of ENaC and Nax. ATM 4(Suppl 1):S11. https://doi.org/10.21037/atm.2016.10.42

[101] Speir RW (1961) Effect of several inorganic salts on the infectivity of Mengo virus. Proc Soc Exp Biol Med 106:402–404. https://doi.org/10.3181/00379727-106-26352.

[102] Ramalingam S, Cai B, Wong J, Twomey M, Chen R, et al. (2018) Antiviral innate immune response in non-myeloid cells is augmented by chloride ions via an increase in intracellular hypochlorous acid levels. Sci Rep 8:13630. https://doi.org/10.1038/s41598-018-31936-y

[103] Ramalingam S, Graham C, Dove J, Morrice L, Sheikh A (2020) Hypertonic saline nasal irrigation and gargling should be considered as a treatment option for COVID-19. J Glob Health 10(1):010332. https://doi.org/10.7189/jogh.10.010332

[104] Regelmann WE, Schneider LA, Fahrenkrug SC, Gray BH, Johnson S, et al. (1997) Proteinase-free myeloperoxidase increases airway epithelial permeability in a whole trachea model. Pediatr Pulmonol 24(1):29-34. https://doi.org/10.1002/(sici)1099-0496(199707)24:1<29::aid-ppul5>3.0.co 2-e

[105] Klebanoff SJ, Kettle AJ, Rosen H, Winterbourn CC, Nauseef WM (2013) Myeloperoxidase: a front-line defender against phagocytosed microorganisms. J Leukoc Biol 93(2):185-198. https://doi.org/10.1189/jlb.0712349

[106] Haegens A, Vernooy JHJ, Heeringa P, Mossman BT, Wouters EFM (2008) Myeloperoxidase modulates lung epithelial responses to pro-inflammatory agents. Eur Respiratory J 31:252-260. https://doi.org/10.1183/09031936.00029307

[107] Casciaro M, Di Salvo E, Pace E, Ventura-Spagnolo E, Navarra M, Gangemi S (2017) Chlorinative stress in age-related diseases: a literature review. Immun Ageing 14:21. https://doi.org/10.1186/s12979-017-0104-5

[108] Khan AA, Alsahli MA, Rahmani AH (2018) Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives. Med Sci (Basel) 6(2):33. https://doi.org/10.3390/medsci6020033

[109] Kettle AJ, Chan T, Osberg I, Senthilmohan R, Chapman AL, et al. (2004) Myeloperoxidase and protein oxidation in the airways of young children with cystic fibrosis. Am J Respir Crit Care Med 170(12):1317-1323. https://doi.org/10.1164/rccm.200311-1516OC
[110] Zuo Y, Zuo M, Yalavarthi S, Gockman K, Madison JA, et al. (2020) Neutrophil extracellular traps in COVID-19. JCI Insight 5(11):e138999. https://doi.org/10.1172/jci.insight.138999.

[111] Suzuki K, Yamada M, Akashi K, Fujikura T (1986) Similarity of kinetics of three types of myeloperoxidase from human leukocytes and four types from HL-60. Arch Biochem Biophysics 245(1):167-173. https://doi.org/10.1016/0003-9861(86)90201-8

[112] Wang G, Nauseef WM (2015) Salt, chloride, bleach, and innate host defense. J Leukocyte Biol 98(2): 163–172. https://doi.org/10.1189/jlb.4RU0315-109R

[113] Zhang N, Francis KP, Prakash A, Ansaldi D (2013) Enhanced detection of myeloperoxidase activity in deep tissues through luminescent excitation of near-infrared nanoparticles. Nat Med 19(4):500-505. https://doi.org/10.1038/nm.3110

[114] Chandler JD, Day BJ (2012) Thiocyanate: a potentially useful therapeutic agent with host defense and antioxidant properties. Biochem Pharmacol 84(11):1381-1387. https://doi.org/10.1016/j.bcp.2012.07.029

[115] Darnell ME, Subbarao K, Feinstone SM, Taylor DR (2004) Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J Virol Methods 121(1):85-91. https://doi.org/10.1016/j.jviromet.2004.06.006

[116] House SA, Gadomski AM, Ralston SL (2020) Evaluating the placebo status of nebulized normal saline in patients with acute viral bronchiolitis. A systematic review and meta-analysis. JAMA Pediatr 174(3):250-259. https://doi.org/10.1001/jamapediatrics.2019.5195.

[117] Sauvaget E, David M, Bresson V, Retornaz K, Bosdure E, Dubus JC (2012) Sérum salé hypertonique nébulisé et bronchiolite aiguë du nourrisson : données actuelles [Nebulized hypertonic saline and acute viral bronchiolitis in infants: current aspects]. Arch Pediatr 19(6):635-41. https://doi.org/10.1016/j.arcped.2012.03.018

[118] Zhang L, Mendoza-Sassi RA, Wainwright C, Klassen TP (2017) Nebulised hypertonic saline solution for acute bronchiolitis in infants. Cochrane Database Syst Rev12(12):CD006458. https://doi.org/10.1002/14651858.CD006458.pub4

[119] Hsieh CW, Chen C, Su HC, Chen KH (2020) Exploring the efficacy of using hypertonic saline for nebulizing treatment in children with bronchiolitis: a meta-analysis of randomized controlled trials. BMC Pediatr 20(1):434. https://doi.org/ 10.1186/s12887-020-02314-3

[120] Angoulvant F, Bellêttre X, Milcent K, Teglas JP, Claudet I, et al. (2017) Effect of nebulized hypertonic saline treatment in emergency departments on the hospitalization rate for acute bronchiolitis: a randomized clinical trial. JAMA Pediatr 171(8):e171333. https://doi.org/10.1001/jamapediatrics.2017.1333.

[121] Everard ML, Hind D, Ugonna K, Freeman J, Bradburn M, et al. (2015) Saline in acute bronchiolitis RCT and economic evaluation: hypertonic saline in acute bronchiolitis - randomised controlled trial and systematic review. Health Technol Assess 19(66):1-130. https://doi.org/10.3310/hta19660.

[122] Morikawa Y, Miura M, Furuhata MY, Morino S, Omori T, et al. (2018) Tokyo Pediatric Clinical Research Network. Nebulized hypertonic saline in infants hospitalized with moderately severe bronchiolitis due to RSV
infection: A multicenter randomized controlled trial. Pediatr Pulmonol 53(3):358-365. https://doi.org/10.1002/ppul.23945

[123] WHO (2020) Saline. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/myth-busters#saline

[124] WHO (2020) Can rinsing your nose regularly with saline solution prevent Covid-19?. https://www.who.int/docs/default-source/searo/thailand/12myths-final099bfbf976c54d5fa3407a65b6d9fa9d.pdf

[125] King D, Mitchell B, Williams CP, Spurling GKP (2015) Saline nasal irrigation for acute upper respiratory tract infections. Cochrane Database of Systematic Reviews Issue 4. Art. No.: CD006821. HTTPS://DOI.ORG/10.1002/14651858.CD006821.pub3

[126] Cabaillot A, Vorilhon P, Roca M, Boussageon R, Eschalier B, Pereirad B (2020) Saline nasal irrigation for acute upper respiratory tract infections in infants and children: A systematic review and meta-analysis. Paediatr Respir Rev S1526-0542(20)30016-6. https://doi.org/10.1016/j.prrv.2019.11.003

[127] Slapak I, Skoupa J, Strnad P, Hornik P (2008) Efficacy of isotonic nasal wash (seawater) in the treatment and prevention of rhinitis in children. Arch Otolaryngol Head Neck Surg 134:67–74. https://doi.org/10.1001/archoto.2007.19

[128] Tano L, Tano KA (2004) daily nasal spray with saline prevents symptoms of rhinitis. Acta Otolaryngol 124(9) 1059- 1062. https://doi.org/10.1080/00016480410017657

[129] The University of Edinburgh (2020) ELVIS-COVID-19. https://www.ed.ac.uk/usher/elvis-covid-19/contact-us

[130] ClinicalTrials.gov Identifier: NCT04382131. Hypertonic saline nasal irrigation and gargling in suspected or confirmed COVID-19 (ELVIS COVID-19). https://clinicaltrials.gov/ct2/show/NCT04382131?term=saline&cond=covid-19&draw=2&rank=6

[131] Borah H, Goswami A (2020) Nasal irrigation in Covid-19 pandemic: is it justified? IOSR-JDMS 19:19-21. https://doi.org/10.9790/0853-1906071921

[132] Nimsakul S, Ruxrungham S, Chusakul S, Kanjanaumporn J, Aeumjaturapat S, Snidvongs K (2018) Does heating up saline for nasal irrigation improve mucociliary function in chronic rhinosinusitis? Am J Rhinol Allergy 32(2):106-111. https://doi.org/10.1177/1945892418762872.

[133] Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, et al. (2020) SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. Cell 182(2):429-446.e14. https://doi.org/10.1016/j.cell.2020.05.042

[134] Bastier PL, Lechot A, Bordenave L, Durand M, de Gabory L (2015) Nasal irrigation: From empiricism to evidence-based medicine. A review. Eur Ann Otorhinolaryngol Head Neck Dis 132(5):281-5. https://doi.org/10.1016/j.anorl.2015.08.001.

[135] Niedner R (1997) Cytotoxicity and sensitization of povidone-iodine and other frequently used anti-infective agents. Dermatology 195 Suppl 2:89-92. https://doi.org/10.1159/000246038
Tables

Table 1. Discriminatory results and their respective mechanisms on bio-aerosols, bio-aerosol generating procedures and nebulised saline.

[136] Gudmundsdottir Á, Scheving R, Lindberg F, Stefansson B (2020) Inactivation of SARS-CoV-2 and HCoV-229E in vitro by ColdZyme® a medical device mouth spray against the common cold. J Med Virol https://doi.org/10.1002/jmv.26554

[137] Kido H (2015) Influenza virus pathogenicity regulated by host cellular proteases, cytokines and metabolites, and its therapeutic options. Proc Jpn Acad Ser B Phys Biol Sci 91(8):351-68. https://doi.org/10.2183/pjab.91.351
## Virus containing bio-aerosol or aliquots *in vitro* – without saline

| Procedure                                                                 | Results & (Proposed) mechanism                                                                 | Ref.                      |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|
| Nebulizing viable viral culture on various surfaces                       | Survival of SARS-CoV-2 virus in bio-aerosol, yet originating from a nebulised virus-growing culture medium as carrier | van Doremalen et al. 2020 [22] |
| Hypothesis built on studies with various types of aerosols               | - Bio-aerosols are generated in the deep lung through reopening of collapsed small airways during inspiration  
- Deposition of inhaled 0.1–0.5 µm particles is only 30% -70% of inhaled particles are exhaled again | Scheuch et al. 2020 [23] |

## Bio-aerosol-generating procedures in hospital care

| Bio-aerosol-generating procedures | Results & (Proposed) mechanism                                                                 | Ref.                      |
|----------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|
| Intubation, extubation and related procedures, prone positioning, disconnecting patient from ventilator, tracheotomy/tracheostomy manipulation, manual ventilation, open suctioning, bronchoscopy or noninvasive ventilation | Viral spread by invasive procedures causing basal/airway damage and spread of surfactant-containing ALF is being referred to | WHO [12] |
| Hong Kong hospital case report with SARS-CoV - Aerosol use with salbutamol – 0.5 mg through jet nebuliser, delivered by oxygen at a flow rate of 6 L/min, 4/day, 7 days. | Association with contamination in a ward following 7 days of nebulizing salbutamol | Lee et al. 2003 [24] |
| Systematic review of transmission of acute respiratory infections to healthcare workers | Nebuliser treatment found not to be significant | Tran et al. 2012 [25] |
| Evaluation of infective risk to healthcare workers for SARS-CoV-2 | “Currently there is very little evidence detailing the transmission of SARS-CoV-2 associated with any specific procedures.” | Harding et al. 2020 [26] |
| Assessment of various aerosol-generating procedures | No enhanced transmission risks of saline nebuliser treatment identified | Jespers et al. KCE, Belgium, 2020 [28] |
| Evaluation of nebuliser applications during SARS epidemic 2003 in Canada | No increased risk of infection for medical staff with use of nebulisers | Raboud et al. 2010 [29] |
| Evaluation of nebuliser applications during SARS epidemic 2003 in Canada | No increased risk of infection for medical staff with nebulisers | Loeb et al. 2004 [30] |

## Nebulised saline/saline aerosol studies in human

| Procedure                                                                 | Results & (Proposed) mechanism                                                                 | Ref.                      |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|
| Nebuliser treatment and various aerosol-generating procedures:            | - Small- and medium-size aerosol/droplet generation, no increase in large-size droplet count | Simonds et al. 2010 [27] |
| Nebulised saline droplets dispersion | - Systematic error possible: not investigated whether particles originated from patient or nebuliser, and whether viruses could be isolated from aerosol |
|-----------------------------------|--------------------------------------------------------------------------------------------------|
| Saline aerosol (nebulised) (0.9%) | Assessment of exhaled bio-aerosol particles after saline or a surfactant formulation |
|                                   | - Number of exhaled bio-aerosol particles was reduced by 70% with nebulised saline compared with surfactant, particularly in “high-producers” |
|                                   | - Median droplet size containing surfactant was smaller compared with saline |
| Saline nasal lavage (0.9%)        | Viral concentrations after nasal lavage of infected volunteers |
|                                   | Lower viral titres after saline nasal lavage in rhinovirus infections; after a single rinse, titres only returned to initial values after day 5 |
| Saline irrigation & gargling (3%) | Open-label, controlled trial in patients with common cold (coronaviruses other than SARS-CoV-2) |
|                                   | Hypertonic saline reduced viral shedding of coronavirus by $\geq 0.5$ log10/day and transmission within household contacts by 35% ($P = 0.006$) as compared to controls |
| Mixed saline/calcium aerosol (nebulised) | Open-label trial in volunteers |
|                                   | Fast production (within 15min) of less and finer bio-aerosol lasting (up to at least 6 h). Suppression was most pronounced (99%) among those who exhale large numbers of particles |
| Electrolysed saline aerosol (nebulised) | Open-label, controlled trial in COVID-19 patients |
|                                   | Nebulised neutral electrolyzed saline reduced qPCR positivity in 6/10 subjects by day 4, 80% by day 6 and in all of them by day 9 |
| Saline aerosol (nebulised) (0.9%) | German position paper of pneumologists on COVID-19 |
|                                   | Isotonic saline inhalation changed surface tension of the liquid film on the airway epithelium, leading to less droplet formation and, as such, to less release of exhaled bio-aerosols. |
| **Saline (bio)aerosol studies in vitro** | |
| Saline bio-aerosol (0.9%) + Mucin | Mechanistic study/biophysical characterization in presence of mucins (confirmed in bull calves) |
|                                   | Charge shielding of mucin or mucin-like macromolecules that consequently undergo gelation, stabilizing ALF/air interface and reducing its breakup, resulting in a reduced tendency of the ALF to disintegrate into very small droplets |
| Saline spray / droplets | Mechanistic study of NaCl droplets +/- surfactant |
|                                   | Added to nanoemulsions, NaCl makes finer micellar droplets “aggregate”, making the droplet size distribution to move to a bigger size range (so will lead to faster deposition), while surfactant in contrast breaks up the droplets to smaller sizes |
| Saline +/- surfactant bio-aerosol ( +/- influenza virus particles) | Study of viral decay in droplets evaporated at different RH and concentrations of saline and protein | - Viability depends on the RH (higher at RH <50% and at 100%)  
- Viability decreased in saline solutions, the extent dependent on the salt concentrations and presence of protein | Yang et al. 2012 [41] |
| --- | --- | --- | --- |
| Saline +/- surfactant bio-aerosol ( +/- influenza virus particles) | Mechanistic study in evaporated droplets | - Salinity affects the structure of viral particles, whereas processes at the air-liquid interface drive virus inactivation in droplets, also depending on droplet composition and RH  
- Bio-aerosols of enveloped viruses would fail to undergo rapid rehydration upon entry of the nearly saturated humidity of the respiratory tract, as surfactant arrangement in the droplets would inhibit the reabsorption of water | Vejerano et al. 2018 [40] |

ALF=alveolar lining fluid; NaCl = saline, sodium chloride; RH = relative humidity

**Table 2. Pharmacological effects of saline in the setting of COVID-19 (for references, see text)**
| Parameter                      | Physiology/pathophysiology relevant to common cold / respiratory infection | Pathophysiology with regard to SARS-CoV-2                                                                 | Pharmacological effects of saline                                                                 |
|-------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Bio-aerosol generation        | Airborne transmission of COVID-19 by aerosol                              | Infection of lower airways and lungs thought to occur via micro-aspiration of ultrafine droplets           | NaCl (0.9%) leads to:                                                                               |
|                               |                                                                          |                                                                                                           | - Wetting and fluid aggregation, leading to:                                                       |
|                               |                                                                          |                                                                                                           | - Easier deposition of heavier /larger drops                                                       |
|                               |                                                                          |                                                                                                           | - 70% reduction of bio-aerosol formation                                                           |
|                               |                                                                          |                                                                                                           | - Reduction of viral shedding (shown for other viruses), improved ALF spreading and airway compliance |
| Viral shedding of rhinovirus  | Transmission of common cold viruses and COVID-19 by apical shedding of viral particles and/or exosomes (secretion of virus containing vesicles) | Viral shedding of SARS-CoV-2 may take place during up to 10-14 days                                    | NaCl (0.9%-3%) provides (shown for viruses other than SARS-CoV-2):                                 |
|                               |                                                                          |                                                                                                           | - *In vivo* rinse effect, causing lower viral titres (5 days until back to initial values)         |
|                               |                                                                          |                                                                                                           | - *In vivo* reduces viral shedding as demonstrated for other viruses (rhino- and other coronaviruses) |
| Mucosal dehydration           | Periciliary fluid normally contains <50 mM NaCl (0.29%) as to maintain ciliary movement | More severe SARS-CoV-2 is frequently associated with conditions of dry mucosa, such as in the elderly | *In vitro & in vivo:*                                                                               |
|                               |                                                                          |                                                                                                           | - Isotonic (0.9% NaCl): hydrating effect                                                          |
|                               |                                                                          |                                                                                                           | - Hypertonic (usually 3% NaCl): rinsing effect (osmosis); in vitro various effects on the epithelial cell membrane (altered electrical conductance, permeability, cell deformation) |
| Mucins, containing sialic acids | Captures pathogens, viral particles and debris to remove these by MCC or upon coughing | SARS-CoV-2 spike protein binds sialic acid (if not buffered at pH>7) and is found in sputum             | NaCl gels the mucus *in vitro* [shown at >90 mM ~ 0.6%], so altering the MCC and cough clearance [≥ 0.9%] |
| Mucociliary clearance (MCC)   | Primary defense mechanism to expel pathogens, viral particles and debris | SARS-CoV-2 preferentially targets ciliated cells in nose, nasopharynx, airways, olfactory bulb and reduces the MCC | NaCl (0.9%) promotes ciliary beat and MCC *in vitro* and *in vivo*                                 |
|                               |                                                                          |                                                                                                           | Variable effect with hypertonic saline                                                            |
| Cough clearance               | Associated with MCC                                                     | SARS-CoV-2 is frequently associated with cough, while present in sputum                                | NaCl (0.6%) gels the sputum and (0.9%) reduces its adhesion, so promoting cough clearance          |
lead to postnasal drip and cough

### (Na)Cl concentration at ACE2-expressing cells

| (Na)Cl concentration at ACE2-expressing cells | Strong Cl-dependency of ACE2-receptor | ACE2 is the entry receptor for SARS-CoV-2 |
|-----------------------------------------------|---------------------------------------|-----------------------------------------|
| From a certain concentration onwards, NaCl induces steric hindrance of ACE2 receptor for its substrates | ACE2 is the entry receptor for SARS-CoV-2 | NaCl causing steric hindrance of ACE2 receptor in *vitro* in HEPES |
| - NaCl inhibiting SARS-CoV-2 replication in *vitro* in Vero-cells: pure saline: |
| - NaCl inhibiting SARS-CoV-2 replication in *vitro* in HEPES |
| - MIC: 0.5% * |
| - IC<sub>50</sub>: 1.4% * |

### ENaC

| ENaC determines hydration and height of ALF and MCC, as well as drives reabsorption of diluted ALF hypersecretion | ENaC activity is regulated by various proteases and by the sodium sensor Na<sub>x</sub> | Shared proteases are hijacked by SARS-CoV-2, leading to less availability for ENaC and so to less fluid absorption in the lungs |
|-------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Na<sub>x</sub> sensing of NaCl (at 0.9%), stimulating ENaC and so sodium (re)absorption, contributing to the volume control and Na<sup>+</sup>-homeostasis, the control of ALF height and MCC |

### Hypochlorous acid (HOCl), myeloperoxidase (MPO)

| Hypochlorous acid (HOCl), myeloperoxidase (MPO) | Antiviral effects of NaCl are attributed to the production of HOCl from Cl- ions |
|-----------------------------|-----------------------------------------------|
| - HOCl is mainly produced by MPO: HOCl generation in the phagosomes requires a continuous supply of chloride |
| SARS-CoV-2 is sensitive *in vitro* to HOCl |
| NaCl 15-300 mM (= 0.09% to 1.7%) results in HOCl production |
| MPO activity in neutrophil phagosomes increases with increasing NaCl concentrations from 25 mM (0.14%) NaCl onwards |

### CFTR

| CFTR | Apical Cl<sup>-</sup> secretion is mediated mainly by CFTR, relevant to dehydrated ALF (as in CF). |
|-----------------|------------------------------------------------|
| - No specific interactions relevant to viral infection known so far |
| (?) Rationale remains unclear besides the known effect of hypertonic saline in osmosis creation [so far not found to be relevant to SARS-CoV-2; relevant to conditions characterized by dehydrated ALF, thick mucus and impaired MCC.] |

**Table 3. Pharmacy practice recommendations for saline use, based on this literature analysis.**

ALF = Alveolar lining fluid; CFTR cystic fibrosis transmembrane conductance regulator; Cl<sup>-</sup>: Chloride; HOCl = hypochlorous acid; IC<sub>50</sub>: inhibitory concentration to inhibit 50%; IC<sub>100</sub>: inhibitory concentration to inhibit 100% (*MIC and IC deduced from graphs); MCC = Mucociliary clearance; MIC: Minimum Inhibitory Concentration; MPO = myeloperoxidase; Na<sub>x</sub> = sodium channel x;
- **Saline does not destroy SARS-CoV-2** and is thus only to be used as an add-on to basic hygiene measures.

- **In case of acute** common cold or upper respiratory symptoms in times of COVID-19

### Nasal rinse:

| From first symptoms of common cold or upper respiratory symptoms |
|---------------------------------------------------------------|
| Rinse with pure “isotonic” saline (0.9%) 2-3 times/day*       |
| - This concentration combines a reliable positive effect on the MCC with desirable partial receptor block of the entry receptor and is well established for treatment or prevention of common cold and as nebulisation/aerosol for treatment of bronchiolitis. |
| - Isotonic saline is devoid of the side effects that have been reported for hypertonic saline (effect on cell morphology, increased nasal epithelial permeability, nasal burning/irritation, and when nebulizing: induction of bronchoconstriction or cough) |
| - [*Unless hypertonic saline is already used in the frame of other indications, in which case it is continued*] |
| - Heating the saline is not needed; concentrations of salt reached upon inhaling sea salt solution are unknown. |
| - No special devices are needed. |

**Preferably do not use:**

- Seawater and other nasal sprays: these may have a rinsing effect, yet not all promote the MCC. If they contain other (e.g. buffering ions), the desired block of the entry receptor for viral replication, as observed in vitro in host cells, may possibly not be obtained. Some sprays may contain emulsifying ingredients (such as carrageenan or polyvidone) possibly leading to enhanced bio-aerosol formation.

- Trypsin-containing sprays: trypsin can digest viruses in a laboratory test tube, but in presence of host cells trypsin has been found to enhance SARS-CoV-2 invasion and syncytia formation, as well as potentiate influenza virus replication en cellular inflammation; such sprays sold as medical devices in the European Union are prohibited for sale in Germany, because of lack of proof of efficacy.

**+ Gargling**

In case of common cold symptoms with throat involvement:

*Or in case of COVID-19 positive testing*

-> Gargling can be done with self-made hypertonic saline*: up to 12 times per day

-> Don't swallow; discard in sink

**Respiratory care**

When using a saline aerosol with a nebulizing apparatus to remove phlegm, hydrate the airways and/or reduce cough:

-> Continue with habitual strengths of sterile saline concentration (0.9%), unless otherwise indicated, or ask pharmacist

-> Follow the cleaning instructions of the manufacturer, to end with hand hygiene

-> Preferentially in well-aerated place or outside

**See your doctor if not getting better and/or if feeling short of breath or/and very sick with high temperature**

**Preventive use**

Awaiting further studies, nasal and oral gargling is not systematically recommended as a preventive measure unless:
- As nasal saline for hydration of the nasal mucosa (e.g. when feeling dehydrated due to carrying a mouth mask)
- Before seeing a frail or immune compromised person, or after visiting an unexpectedly crowded location, and thus if risk of contamination is believed to be higher

Distancing and hygiene measures will prevail at any time

| Protective measures: always combine with protective measures |
|-------------------------------------------------------------|
| **Nose & Mouth:**                                           |
| · Collect superfluous liquid with tissue paper and discard  |
| · Wash and/or safely collect the saline recipients, to end with hand hygiene |
| **Nebulisation/Aerosol:**                                  |
| · If there is no way to isolate or to aerate the room, use cotton sheets to cover your lap (plus head) to prevent aerosol dispersion |
| · Ventilate the room and wash hands                        |

a Awaiting further studies from clinical trials: 1 table spoon or 20 mg kitchen salt per litre.