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 'Atemwegpflege' (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 eindämmern") [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 CIVID-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.[13] 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, pathophysiological 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-spreading 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]-,[36],[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 s 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]-,[47],[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]-,[62],[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, this 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_x channel, over-stimulating ENaC and leading to electrolyte movements stressing the mitochondria (unlikely to explain the mechanism, as the threshold for Na_x activation in vitro is 150 mM of extracellular Na^+ [[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 TMPRRSS2, 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_2O_2 [[113]]. Alternatively, as

Cl^- 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 ≥0.5 log10/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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Tables

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

Report/Study of	Procedure	Results & (Proposed) mechanism	Ref.
Virus containing bio-aerosol or aliquots *in vitro* – without saline			
Bio-aerosol in culture medium	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]
Bio-aerosol review	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	Intubation, extubation and related procedures, prone positioning, disconnecting patient from ventilator, tracheotomy/ tracheostomy manipulation, manual ventilation, open suctioning, bronchoscopy or non-invasive ventilation	Viral spread by invasive procedures causing basal/airway damage and spread of surfactant-containing ALF is being referred to	WHO [12]
Nebulised salbutamol	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]
Bio-aerosol-generating procedures	Systematic review of transmission of acute respiratory infections to healthcare workers	Nebuliser treatment found not to be significant	Tran et al. 2012 [25]
Bio-aerosol-generating procedures	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]
Bio-aerosol-generating procedures	Assessment of various aerosol-generating procedures	No enhanced transmission risks of saline nebuliser treatment identified	Jespers at al. KCE, Belgium, 2020 [28]
Bio-aerosol-generating procedures	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]
Bio-aerosol-generating procedures	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			
Saline aerosol	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	Edwards et al. 2004 [31] Fiegel et al. 2006 [32]
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	Hendley and Gwaltney 2004 [35]
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 ≥0.5 log10/day and transmission within household contacts by 35% (P = 0.006) as compared to controls	Ramalingam et al. 2015 [36]
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	Edward et al. 2020 [37]
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	Delgado-Enciso et al. 2020 [34]
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.	Pfeifer et al. 2020 [13]
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	Watanabe et al. 2006 [38]
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	Patel et al. 2019 [39]

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 Superspreading events in closed and non-ventilated areas	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 Ciliary beat is inhibited by dry mucosa	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 Stasis of thick, dehydrated mucus within the nasal cavities and nasopharynx	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	Strong Cl^--dependency of ACE2-receptor From a certain concentration onwards, NaCl induces steric hindrance of ACE2 receptor for its substrates	ACE2 is the entry receptor for SARS-COV-2 -> *in vitro* replication of the virus is inhibited by pure saline	NaCl causing steric hindrance of ACE2 receptor *in vitro*: in HEPES · MIC : 0.5% * · IC_{50}: 1.4% * NaCl inhibiting SARS-CoV-2 replication in vitro in Vero-cells: pure saline: · MIC : 0.6% * · IC_{50}: 0.9% - $IC_{100:}$ 1.5% *
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_x	Shared proteases are hijacked by SARS-CoV-2, leading to less availability for ENaC and so to less fluid absorption in the lungs	Na_x sensing of NaCl (at 0.9%), stimulating ENaC and so sodium (re)absorption, contributing to the volume control and Na+-homeostasis, the control of ALF height and MCC
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	Apical Cl^- secretion is mediated mainly by CFTR, relevant to dehydrated ALF (as in CF).	Not reviewed - 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.]

ALF = Alveolar lining fluid; CFTR cystic fibrosis transmembrane conductance regulator; Cl^-: Chloride; HOCl = hypochlorous acid; IC_{50}: inhibitory concentration to inhibit 50%; IC_{100}: inhibitory concentration to inhibit 100% (*MIC and IC deduced from graphs);MCC = Mucociliary clearance; MIC: Minimum Inhibitory Concentration; MPO = myeloperoxidase; Na_x = sodium channel x;.

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

· **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[a]: 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.

Figures

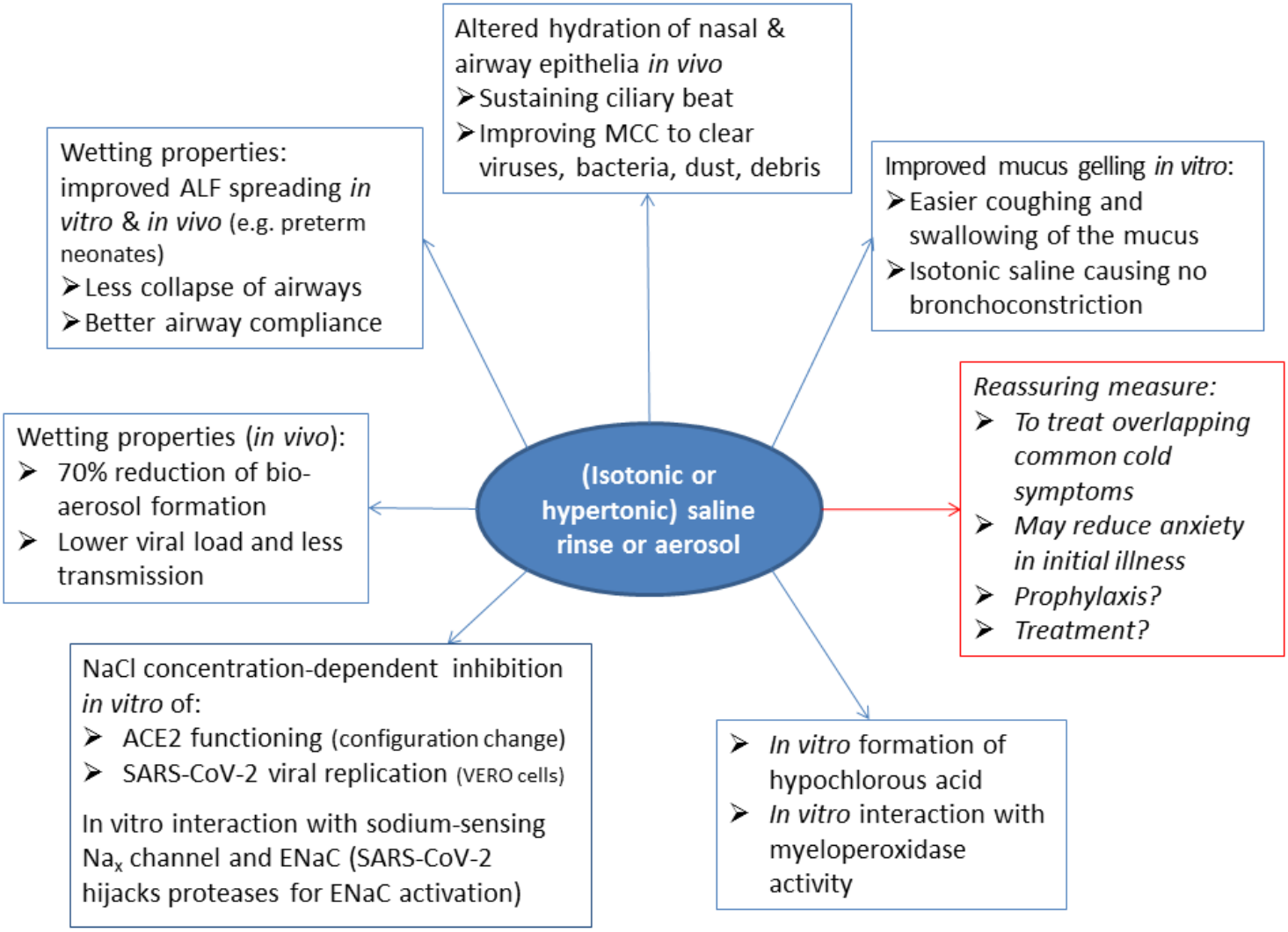
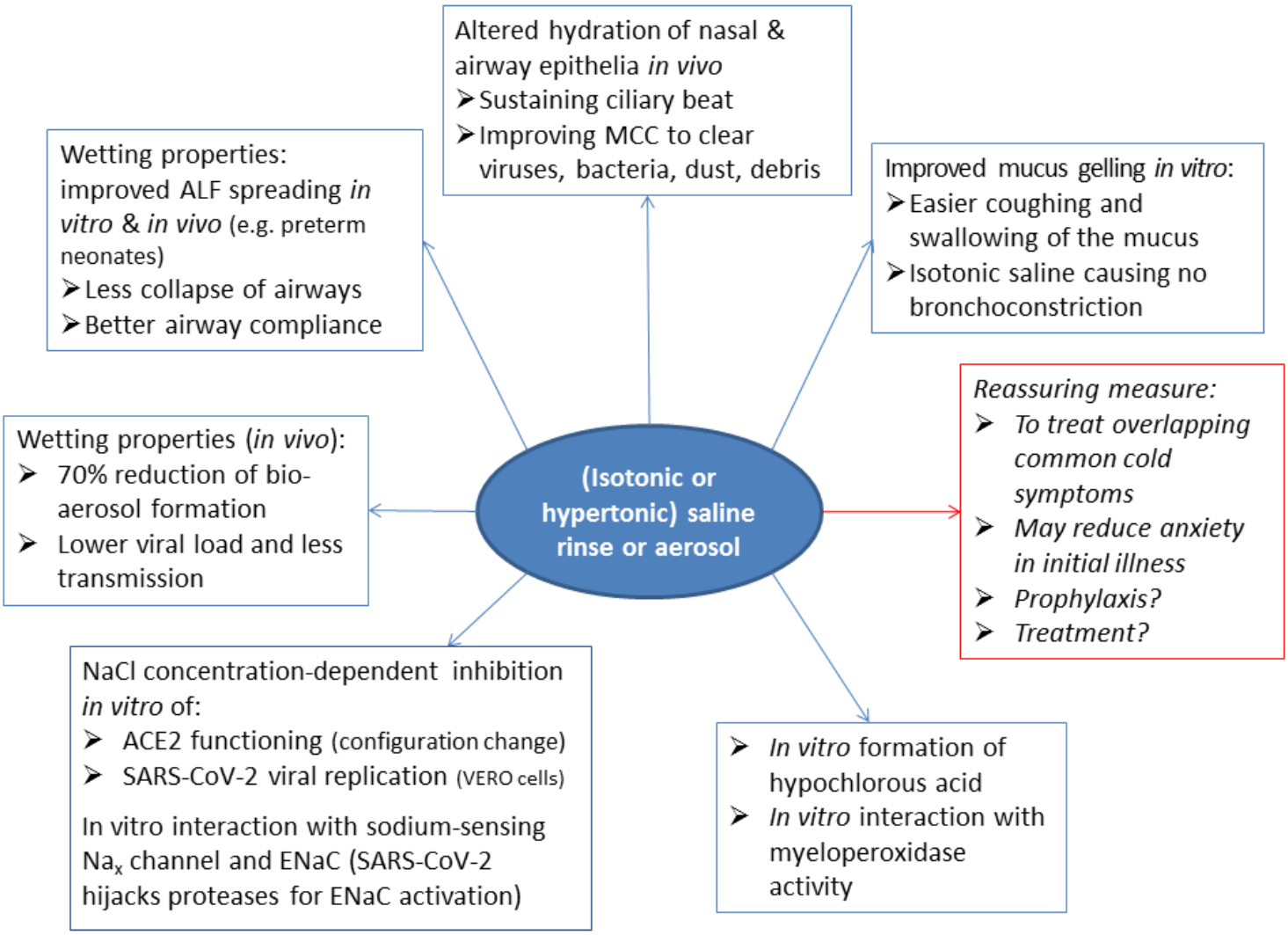


Figure 1

Effects of isotonic and/or hypertonic saline on hydration & mucociliary clearance, alveolar liquid spreading, bio-aerosol/droplet transmission, ACE2, sodium channel ENaC and viral replication, hypochlorous acid formation and myeloperoxidase activity. Bio-aerosol refers to micro-droplets, spontaneously produced during exhalation, such as during speaking, singing and coughing; aerosol refers to nebulizing and inhaling saline for inhalation in the respiratory airways, while saline rinse refers to nasal spraying/oral gargling of saline. ALF= alveolar lining fluid; isotonic = 0.9% NaCl (or 9 g/L) and hypertonic = varying concentrations above 0.9% NaCl (2%-7%, often 3%). ACE2=Angiotensin Converting Enzyme 2; MCC=mucociliary clearance.



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