Results of clinical trials of nasal or oropharyngeal decontamination procedures for prophylaxis of COVID-19 infection, for treatment of COVID-19 patients and for reduction of their infectivity – a living review.

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Vorbemerkung für deutsche Plagiatsjäger:

Ja, dies ist ein Plagiat und keine eigene Studie oder Abhandlung. Es handelt sich lediglich um eine Datensammlung und zum Teil auch eine Zitatsammlung, die auf den Ergebnissen von Hunderten von Studien anderer beruht. Dabei kann es auch vorkommen, dass einige "Kernsätze" wörtlich zitiert werden, insbesondere dann, wenn eine Umformulierung zu einer unnötigen Verlängerung des Textes oder zu einem Verlust an Präzision and Prägnanz geführt hätte. Es wird daher **ausdrücklich nicht** der Anspruch erhoben, dass es sich hier in irgendeiner Weise um eine eigene wissenschaftliche Leistung handele.

Abstract

Decontamination of the nose, nasopharynx, oropharynx by nose drops, nose spray, nasal irrigation, oropharyngeal gargle or oropharyngeal spray, or inhalation/nebulization of antiseptic/decontaminating agents, are supposed to reduce the viral load in the nasal, nasopharyngeal or oropharyngeal areas at least for a short and maybe for a longer time. These locations are considered as the main ports of entry for SARS-CoV-2. Such methods may also be effective as a sort of chemoprophylaxis (pre- or postexposure) and are subject of a few ongoing trials. In infected patients, decontaminating procedures may attenuate the risk of progression as long as the infection is still limited to the upper respiratory tract, its primary port of entry. It is suggested that infection of the lungs occurs by microaspiration. In

optimistic scenarios, local decontamination may contribute to the eradication of the infection and quick viral clearance. However, this will be impossible as soon as the infection disseminated to the lungs or other organs, or in cases when the lungs were affected by the infection already at the very start of the disease.

Moreover, such decontaminating procedures can only kill and inactivate free virus particles that were already released from infected cells. It cannot be expected that oral or nasal antiseptics are able to inhibit viral replication within the infected cells, i.e. they cannot act in a *curative* manner. After the decontaminating procedure, new alive viral particles will continue to be shedded from infected cells. At best, they can be inactivated as long as remnants of the antiseptic are retained on the mucosal membranes. However, mucosal secretions will dilute these remnants continuously so that they fall below the limit of their effectiveness.

Anyway, as long as the patient is in an infectious stage of the disease, oral decontamination by oral gargle and spray and nasal decontamination may reduce the infectivity of the patient for a while as long as there is less viable virus after such a procedure in speech droplets or exhaled aerosols, in the case of sneezing or coughing. Local decontamination procedures may thus play an important role in ring prophylaxis e.g. in household contacts of infected people.

Whereas there are meanwhile a lot of *in silico* and *in vitro* results about agents which seem to be suitable for nasal or oropharyngeal decontamination in the case of COVID-19, and also some trial results of such procedures in the context of *other* upper respiratory tract infections, including common coronaviruses, and a wealth of literature about the theoretical background and the supposed mechanisms behind these local strategies, results from clinical trials with humans are very sparse. As of September 11th, only six clinical trials were published in peer-reviewed journals or as unreviewed preprints, and all of them involved only a small number of participants (2 – 31), and only two had a control group.

One trial showed rather good effectiveness of 1 % povidone-iodine oral rinse for oral decontamination for at least three hours in patients with high pre-rinse viral load in saliva. In contrast, 1 % hydrogen peroxide was found ineffective for the same purpose in another trial. 3 % hydrogen peroxide for nasal irrigation (once), followed by daily hypertonic saline nasal irrigation, was able to suppress pharyngeal PCR positivity in patients with long-lasting or reactivated infection for several days until reappearance, but may cause viral clearance in some of these cases (as far as the time frame of 14 days of that study is concerned).

Gargling with 0.12 % CHX reduced viral load in saliva at 1 and 2 hours following gargling in 75 – 100 % of cases, but the effect became smaller after 4 hours.

Gargling with 1 % povidone-iodine (PVP-I) or, alternatively, Listerine “Original” was able to accelerate viral clearance (indicated by negative oropharyngeal and nasopharyngeal swab) in

recently diagnosed asymptomatic COVID-19 cases, and the effect was found to be significant compared to the control group (no gargle or gargle with tap water).

Mouthwash with 1 % PVP-I or 1.5 % hydrogen peroxide abolished viral load in respiratory droplets for at least 20 minutes, and reduced viral load a lot (but not completely) at 60 minutes.

In a large RCT with 606 outpatients from Bangladesh, the combination of mouthwash, gargle, nose drops and eye drops with 1 % PVP-I, four times every day for 4 weeks, was able to reduce the risk of hospitalization, hospitalization with the need for oxygen support, and death by 84 %, 84 % and 88 %, and accelerated viral clearance so that most of the participants in the PVP-I group were PCR- already at day 3.

Finally, inhalation of diluted acetic acid accelerated the recovery from symptoms and viral clearance in patients with mild disease compared to a control group who instead took lopinavir/ritonavir tablets.

There are so far no results from prophylactic trials except for one trial with interferon nose drops (and thymosin alpha injections in a subgroup of participants), but in the absence of a control group, the meaning of the results is unclear. Moreover, interferon is not a decontaminant.

Much more data are needed until the chances and limitations of local decontamination procedures are well understood. This living review was initiated in order to collect the results from such trials. It will be confined to clinical trials with humans or non-human primates, either (i) for prophylaxis, (ii) treatment at any stage of the disease, or (iii) reduction of infectivity. It is not about *in vitro* studies*, in silico* studies or theoretical papers about that subject.

So far, and highly provisional (!), it can be recommended that people with suspected or confirmed COVID-19 infection and their contacts should gargle thrice a day with ~ 1 % PVP-iodine or Listerine Cool Mint (Listerine especially for those for whom PVP-I is contraindicated or unwanted). Based only on *in vitro* results (and thus outside the scope of this review), Dequonal may be another alternative. Combination of gargle and throat spray may enhance effectiveness, since throat spray is more effective to reach the oropharynx compared to gargling. In case of infection or symptoms suspective of COVID-19, the procedure from the Bangladesh PCR seems to be extremely promising as a simple home-based treatment.

Based on theoretical assumptions (and thus also outside the scope of this review), it would be probably advantageous to combine this procedure with nasal spray or irrigation of 0.5 – 1.0 % PVP-I diluted in isotonic saline solution, or iota-carrageenan-based nasal spray, in both the infected or suspected index patient and his contacts (e.g., family members). It is doubtful whether nasal irrigation is superior to nasal spray since nasal irrigations may clean mucosal

surfaces from protective natural substances like interferons, lysozyme, defensins or immunoglobulins (IgA).

Introduction

Nasal/nasopharyngeal and oropharyngeal decontamination from viable COVID-19 virus is a matter of great importance since it may have the potential

● to possibly accelerate viral clearance in the upper respiratory tract in infected people

● to reduce infectivity of infected people with regard to contacts or surfaces (fomites) (by reducing the viral load that is associated with aerosol or droplet production during exhaling, speaking, coughing, sneezing)

● to kill very recently acquired virus (viral contamination) for the purpose of postexposure prophylaxis (PEP) in exposed healthy people (if the decontaminating agent has a longer lasting effect on the mucosa, it may also act as preexposure prophylaxis = PREP) as long as there is no manifest infection of the mucosal cells

● to reduce the risk of progression of the infection of the upper respiratory in people with suspected or confirmed COVID 19 infection, and of dissemination down to the lungs and other organs:

It is generally suggested meanwhile that the mucosal lining of the nasal/nasopharyngeal or oropharyngeal area (and also the eyes which are connected to that area by the nasolacrimal duct) are the primary ports of entry for SARS-CoV-2 in all or nearly all of the cases. Only after replication of the virus in that area and a massive rise of the local viral load, the infection may expand downward in the airways and into the lung, eventually causing pneumonia, and, distributed by the blood stream, to more distant organs like heart, kidney, liver and others (e.g. LIANG et al., MADAS et al.).

Moreover, there is some evidence for a vascular route of transfer of SARS-CoV-2 from the oral cavity to the lungs: *“Saliva is a reservoir of SARS-CoV-2, thus any breach in the immune defenses of the mouth may facilitate entrance of the virus to the vasculature through the gingival sulcus or periodontal pocket. From the oral vasculature, the virus would pass through veins of the neck and chest, and reach the heart, being pumped into pulmonary arteries, and to the small vessels in the lung periphery”* (LLOYD-JONES et al.). LLOYD-JONES et al. based these assumptions on radiological and oral cavity findings. They also proposed that dental plaque accumulation and periodontal inflammation intensify this pathways. Besides daily oral hygiene, use of specific mouthwashes is thus assumed to decrease the salivary viral

load, so that less virus is transferred from the oral cavity to the lungs and other organs by the vascular route.

Following these theories, nasal and oropharyngeal decontamination are suggested to have a central role in (i) early treatment (to avoid progression), (ii) reduction of infectivity with regard to contacts, (iii) viral clearance in the upper respiratory tract, and (iv) chemoprophylaxis.

Moreover, MOOSAVI et al. point out that patients with respiratory infections have an altered oral microflora with an increase of pathogenic microorganisms (besides the causal virus), and mouthwash may reduce the pathogenetic flora. This is regarded as the mechanism how chlorhexidine mouthwash or gel reduces the risk of ventilator-associated pneumonias (independent of COVID-19) and contributes to the improvement of symptoms in people with respiratory infections (MOOSAVI et al.).

While there are a lot of agents which are suggested to be effective for nasal or oropharyngeal decontamination based on theoretical assumptions, *in vitro* data based on experiments with COVID-19 (e.g., on Vero cells), or clinical experiences and studies in the context of other or unspecified infections of the upper respiratory tract (e.g., influenza, rhinovirus, RSV, common coronaviruses), clinical studies in humans are sparse, both with COVID-19-infected people and for prophylactic use of decontaminating methods. There is a need for a living review which will collect the available and new evidence, but restricted to *in vivo* trials in humans (and non-human primates) involving only SARS-CoV-2 (*not* MERS, SARS-CoV-1, common human coronaviruses, other upper respiratory tract infections), in order to highlight this sparse and thus very valuable evidence from the wealth of promising laboratory or *in silico* data and theoretical papers.

As TELLES-ARAUJO et al. pointed out, *in vitro* studies have the limitation that they don't take into account the impact of host immunity, thus the response to the agent can be different *in vivo.*

Based on theoretical assumptions, experiences with other URT infections or laboratory data, the following agents have already been proposed for nasal and/or oropharyngeal decontamination procedures:

- N-chlorotaurine,
- lactoferrin,
- liposomal Lactoferrin (see SERRANO et al.),
- interferon alpha, beta or kappa (IFN kappa combined with TFF2: FU et al.),
- PVP-iodine,

● essential iodine drops (Iodine-V: KONTOS Z),

● alcohol-based nasal antiseptics,

● quarternary ammonium compounds (ammonium chloride, cetylpyridinium chloride, miramistin) (all proposed by CEGOLON et al.),

● hydrogen peroxide,

● beta-chitosan, amphiphilic chitosan (PYRC et al.),

● iota-carrageenan,

● lambda-carrageenan,

● nasal spray with a combination of lambda-carrageenan and gellan with high mucoadhesive properties (MOAKES et al.; *"a mechanism for both prophylaxis and prevention is proposed; where entrapment within a polymeric coating sterically blocks virus uptake into the cells, inactivating the virus, and allowing clearance within the viscous medium");*

● hypertonic saline solution (e.g., 2 %, see KHAN et al.),

● dequalinium chloride,

● essential oils (like Listerine),

● octenidine (very favorable *in vitro* results for Octenisept: STEINHAUER et al.),

● polyhexanide,

● xylitol,

● astrodimer,

● chlorhexidine,

● Neem extract *(Azardirachti indica)* (KHAN et al.),

● ArtemiC (artemisinin, curcumin, frankincense and vitamin C),

● Citrox (a bioflavonoid) (CARROUEL et al., CARROUEL et al. 2),

● cyclodextrin (CARROUEL et al., CARROUEL et al. 2),

● chlorine dioxide,

● Kerecis spray (omega 3 viruxide - containing neem oil and St John's wort),

● nitric oxide releasing solution,

● saline with baby shampoo,

● GLS-1200 oral spray (BURTON et al., see below),

● C31 G (amphoteric surface-active agents; see MOOSAVI et al.),

● ethanol/ethyl lauroyl arginate (STATKUTE et al., in vitro data),

● Xlear nasal spray (xylitol + grapefruit seed extract) (FERRER et al. *in vitro*, GO et al. *in vivo*) and grapefruit seed extract alone (FERRER et al., *in vitro*),

● black and green tea (OHGITANI et al., rapid inactivation of SARS-CoV-2 in saliva by green and black tea). However, black tea inactivated alpha, gamma, delta and kappa variants *in vitro* only in the absence of milk/milk caseins (while sugar or lemon juice hat no deleterious effect on the inactivation capacity at all). *"These results suggest the possibility that intake of black tea without milk by infected persons may result in inactivation of the virus in saliva and attenuation of spread of SARS-CoV-2 to nearby persons through droplets"* (OHGITANI et al. 2).

● nasal administration of an Indian herbal ayush oil formulation (Anu oil) reduced viral load in lungs and severity of the disease in Syrian hamsters (RIZVI et al.)

● intranasal application (twice daily) of nafamostat (*but not:* camostat) was highly effective for prophylaxis in a Syrian hamster model (NEARY et al.)

● simvastatin 1 % as mouthwash (ABDULRAB et al.),

● inhalation of diluted acetic acid,

● aerosolisation of the secretome of oral stem cells (DIOMEDE et al.; *pre-experimental*),

● oil-in-water nanoemulsion (nanodroplet) formulation containing the antiseptic 0.13% benzalkonium chloride (PANNU J et al).,

● extract from the common dandelion (TRAN et al.),

● niclosamide-lysozyme particles (BRUNAUGH et al., *efficacious in animal models),*

● nebulized heparin (VAN HAREN et al.),

● inhalation of EDTA solutions (CASHMAN et al.),

● inhalation of biguanides (buformin) (LEHRER),

● nebulized hypertonic ibuprofen solution (GARCIA et al.),

● hypothiocyanite or hypothiocyanite/lactoferrin combination as aerosol (CEGOLON et al. 2),

● neutral electrolyzed saline solution (a solution that contains reactive species of chlorine and oxygen (ROS)) (DELGADO-ENCISO et al.),

● Drug-free nasal spray AM-301 (Bentrio TM) (based on clay: bentonite); (99 % efficient in an *in vitro* study of 3 D human primary nasal epithelial model) (FAIS et al.)

● cationic amphiphilic peptide (CAP) surfactant inhalation (MANDAL and PANDA),

● inhaled optate (DAVIS et al.),

● ethacridine (LI X et al.),

● ivermectin nasal spray (ERRECABLE et al.),

● human defensin 5 nasal spray (recombinant HDEF5) (NIV et al.),

● intranasally delivered chlorpheniramine maleate (an antihistamine with antiviral activity) (WESTOVER et al.),

● intranasal application of monoclonal antibodies that binds the RBD (HALWE et al., experiments with mice)

● oral rinsing with black chokeberry *(Aronia melanocarpa)* juice or pomegranate *(Punica granatum)* juice or green tea *(Camellia sinensis)* (virucidal activity according to *in vitro* data; CONZELMANN et al.),

● toothpaste integrients like sodium tetradecene sulfonate, sodium N-lauroyl-N-methyltaurate, sodium N-lauroylsarcosinate, sodium dodecyl sulfate, copper gluconate, tranexamic acid and 6-aminohexanoic acid thanks to their function as serine protease inhibitors (inhibiting TMPRSS2 protease activity) or interaction between spike protein and ACE2 (TATEYAMA-MAKINO et al.),

● chloroquine nasal drops (THAKAR et al.),

● Covispray (nasal spray; experimental; not available; no antiviral, but osmotic polymeric film (SHRIVASTAVA et al.),

● *Lactococcus lactis* W136 nasal irrigation (in saline solution (MFUNA ENDAM et al.; small RCT already published),

● ColdZyme mouth spray (glycerol, water, buffer, $CaCl_2$, menthol, and trypsin from the Atlantic cod) (POSCH et al.),

● glyzyrrhycin (topical use on nasal and ocular surfaces; *see* PASALI et al.; VAN DE SAND et al.),

● ARGOVIT (silver nanoparticles for mouthwash and nasal rinse; special formulation from Russia, including also hydrolyzed collagen and thus different from "common water with colloidal silver" on the market; **RCT** from ALMANZA-REYES et al.),

● VIRUXAL nasal spray and oral spray (natural fatty acids: oleic acid, palmitic acid, linoleic acid, stearic acid, linolenic triglycerides and free fatty acids), in vitro results see KRISTJANSSON and ROLFSSON; virucidal activity > 90 % on Vero cells

● Ni-Dihydrogalactochitosan (nasal administration), a novel mucoadhesive immunostimulatory polymer (see WEISS et al. for results in a mouse model), regarded as a soluble immunoadjuvans, e.g. for prevention of severe disease

● niclosamide (NEEDHAM:) *"low dose (20uM) prophylactic solution of niclosamide at a nasally safe pH of 7.9 and a (up to 300uM) throat spray at pH 9.1 would be one of the simplest and potentially most effective formulations from both an efficacy standpoint as well as manufacturing and distribution, with no cold chain"*

● RD-X19, a handheld medical device to emit blue light through the oral cavity to target the oropharynx and surrounding tissues (STASKO N et al.); favorable effect on viral load by day 8 and on the median time until substained resolution of symptoms (-57 hours), compared to a sham device as placebo,

● Cysteamine HCl as a topical nasal treatment for prevention in exposed individuals, to mitigate existing infection and to limit the contagion in vulnerable populations (THOENE et al.)

● Covidgum (based on essential oils): reduction of viral load in exhailed air by at least 90 % (up to 99) % directly after chewing that gum; the reduction of the viral load persisted until 2 hours later. https://www.presseportal.de/pm/160209/5080056

● AOS2020, a novel sprayable acid-oxidizing solution containing pure and stable hypochlorous acid; in vitro> 99.8% virucidal efficacy in < 1 min against SARS-Cov-2; non-irritant for the mucosa in animal models (GIARRATANA et al.).

… and others (list not complete!).

It is also important to note that ethanol is known to be an effective virucidal agent, but it cannot be safely used in the nose (PELLETIER et al.). Moreover, an *in vitro* study showed that 21 % and 23 % ethanol is completely ineffective to reduce SARS-CoV-2 (STATKUTE et al.), indicating that the anti-SARS-CoV-2 effect of ethanol-containing Listerine products like Listerine Cool Mint and Listerine Advance depends on their additional ingredients. Interestingly, an in vitro assay on Vero cells found that ethanol at concentrations of 30 % was able to inactivate SARS-CoV-2 nearly completely (> 5.9 log10) within 30 s, whereas 20 % ethanol was only weakly effective (1.1 log10) (KRATZER et al.), suggesting a non-linear association with a threshold somewhere between 23 and 30 %.

IEBBA et al. proposed oral administration of special oral bacteria as local probiotics to counteract COVID-19 symptoms and cytokine storms. In their complex network analysis of oral microbiota and oral and serum cytokine profiles of hospitalized COVID-19 patients and healthy controls, they identified the following bacteria as beneficial and suited for probiotic administration: *Prevotella salivae; Streptococcus oralis; Rathia mucilaginosa; Gemella taiwanensis; Kallipyga gabonensis; Granulicatella elegans.*

Though they don't discuss that subject in their paper, one might hypothesize that reduction of pre-existing microbiota by oral antiseptics may improve the effect of subsequent administration of such beneficial probiotics since the antiseptic procedure may reduce the competition with the established microbiome, opening ecological niches.

In practice, there are, for example, recommendations for the use of pre-procedural antisepsis in dentistry (VERGANA-BUENAVENTURA and CASTRO-RUIZ), but these agents and their proposed concentrations are based so far preferentially on *in vitro* data with COVID-19 or experiences with other viruses.

CARROUEL et al. gave an excellent overview over the antiviral activity of reagents in mouth rinses against SARS-CoV-2, but also mostly based on experiences with other viruses, mechanistic aspects and *in vitro* data. They also analysed the WHO clinical trial register and found 17 trials about local procedures like mouthwash, gargle, nasal spray/irrigation; 9 of

them are about PCP-I, and only one each about chlorhexidine, hydrogen peroxide, CPC, Citrox, Cyclodextrin, essential oils, chlorine dioxide, nitric oxide. CEGOLON et al. focused exclusively on nasal antiseptics in their review.

STATHIS et al. presented another excellent review that included both mouthwash and nasal antiseptics. With regard to clinical trials about nasal administration, there were five trials about nasal spray or irrigation alone (without mouthwash/gargle), including PVP-I, saline, iota-carrageenan, NO-releasing solution = NORS, whereas 9 trials combine nasal rinse/spray with gargle. Unfortunately, most trials are small (range: 24 – 400 participants); the largest trial is about iota-carrageenan nasal spray four times a day in Argentinia (NCT04521322; see below, FIGUEROA et al.), followed by NORS or saline as nasal spray or nasal irrigation (NCT04442868; n = 300) from Canada and PVP-I as nasal spray and gargle (NCT04364802, n = 250) from US.

With respect to the well established literature about these subjects, it is *not* the intention of this review to collect laboratory data or recapitulate theoretical considerations why an agent is suggested to be promising for the purposes discussed here (for that purpose, see e.g. CARROUEL et al., CEGOLON e al., STATHIS et al.). **Thus, we will look only for results from clinical trials or, if available with regard to that subject, non-human primate models.**

There are already three ongoing systematic reviews in this field by the Cochrane Collaboration which focus on (i) administration of mouthwash and nasal sprays by Health Care Workers (HCWs) themselves (as a sort of PREP/PEP), (ii) their role in the protection of HCWs during aerosol-generating procedures, and (iii) administration to patients for the purpose to improve their outcomes and to reduce the infectivity with regard to the HCWs treating them.

Up to the review versions from September 16th, no results were found so far by the Cochrane authors. Several trials are ongoing, but the authors are concerned that they may not address sufficiently possible side effects e.g. with regard to smell or taste disturbances and possible unfavorable changes of the natural (protective) nasal and oropharyngeal microbiome.

COCHRANE REVIEWS:

BURTON MJ et al., **Antimicrobial mouthwashes (gargling) and nasal sprays administered to patients with suspected or confirmed COVID-19 infection to improve patient outcomes and to protect healthcare workers treating them.**

Cochrane Database Syst Rev 2020 Sep 16;9:CD013627.
doi: 10.1002/14651858.CD013627.pub2.

Version of Sept. 16th : 16 ongoing trials with nearly 1250 participants; among them: 14 RCTs

BURTON MJ et al., **Use of antimicrobial mouthwashes (gargling) and nasal sprays by healthcare workers to protect them when treating patients with suspected or confirmed COVID-19 infection.**

Cochrane Database Syst Rev. 2020 Sep 16;9:CD013626. doi: 10.1002/14651858.CD013626.pub2.PMID: 32936949 Review.

Version of Sept. 16th: 3 ongoing trials with nearly 700 participants; among them: 2 RCTs

BURTON MJ et al., **Antimicrobial mouthwashes (gargling) and nasal sprays to protect healthcare workers when undertaking aerosol-generating procedures (AGPs) on patients without suspected or confirmed COVID-19 infection.**

Cochrane Database Syst Rev. 2020 Sep 16;9:CD013628. doi: 10.1002/14651858.CD013628.pub2.

Version of Sept. 16th: 0 ongoing trials

In the absence of high-grade evidence from the Cochrane reviews, this review will also look for low-grade evidence which may be helpful in the meantime until high-grade evidence is available.

Methods

Pubmed will be searched continuously for [COVID and prophylaxis], [SARS-CoV and prophylaxis], [COVID and chemoprevention], [SARS-CoV and chemoprevention], Medxriv/Bioxriv will be searched among all new papers concerning COVID-19. Moreover, references from the selected papers or informations e.g. from press releases and internet sources will also be used if they are helpful to identify further studies.

The search will include both (i) papers about interventions in infected people and (ii) interventions in (presumably) uninfected people for the purpose of chemoprophylaxis (PREP and PEP). It will include interventions restricted to the nasal area/nasopharynx, restricted to the oropharyngeal area, or both (e.g. inhalation of decontaminating agents which are available for outpatients).

Inhalation of antiviral or other medications which are not generally available and easily accessible for outpatients or persons who are interested in chemoprophylaxis (e.g., NO inhalation or inhalation of NO producing formulations), or which are restricted to hospital settings (e.g. remdesivir inhalation) will not be subject of this review.

Note: this review will overlap a lot with the "early therapy paper", as far as local treatment options are concerned (**http://freepdfhosting.com/35f285c9f2.pdf).**

In contrast to usual practice, discussion of the results or methodological issues which apply to the underlying trial will already take place in the "result" section, and the "discussion" section will be restricted to items of more general relevance.

Note on VoCs (like B.1.1.7 and B.1.351)

In spite of their increased transmission dynamics, VoCs like B.1.1.7 and B.1.351 showed no increased environmental stability with regard to disinfectants, soap (surfactants), heat (56 degrees C) or surface stability (MEISTER et al. (2)). Exactly like in the case of the wildtype, 20 % (v/v) ethanol for 30 s was absolutely ineffective in the Vero cell assay, whereas 30 % ethanol (v/v) and higher concentrations were fully effective to reduce viral activity on Vero E6 cells below the limit of detection in this assay, independent of the virus strain.

The same applied to soap (30 s, 1 min, 5 min, 10 min) (VoCs may even be a little more sensitive to soap after 30 s, but this effect may also be only be by chance) and to heat (56 degrees), including the time-effect relationship of heat inactivation until nearly complete inactivation after 30 min, but little effect of 1 and 5 min.

These results rise the hope that the physicochemical properties of the VoCs don't differ from the original virus, and that methods that depend on antiseptic or disinfecting effects described herein are not influenced by the individual virus strain and VoCs. It is plausible that antiseptics and disinfectants act on the full virus and not particularly on the interaction between the Spike protein and its RBD on one side and the receptor (like ACE2) on the other side.

Therefore, the effect of pharmacological agents that act directly via inhibition or interaction with this binding process may be more sensitive to mutations of Spike/RBD than antiseptics or disinfectants with broad-spectrum virucidal activity.

ANDERSON ER et al. were the first who tested mouthwash (two formulations of 0.07 % CPC) not only against a wildtype strain (USA-WA1/2020), but also against three different VoCs (Alpha, Beta, Gamma) in the presence of human saliva and found that CPC was effective against all variants, reducing their activity below the limit of detection (>4 log10).

Results

PVP-iodine oral rinse (1 %)

In a small trial with only four patients, it was shown for the first time that PVP-iodine rinse actually reduces viral load in saliva (MARTINEZ LAMAS et al.). Among the four patients, two patients with low baseline viral load in saliva (100 - < 1000 copies/ml) didn't profit from PVP-I (except for a very small short-living decline around 2 hours later), whereas patients with high baseline viral load (> 10.000 and < 1.000.000 copies/ml) showed reductions between factor ~ 10 and > factor 100.000 after three hours.

The effect was not seen immediately after rinsing (5 minutes), but was evident after one hour and became stronger after two and three hours when the study was stopped (unfortunately). The time course of the RNA load curves from both patients with high baseline viral loads suggests that the effect of PVP-I lasts *much longer* than 3 hours, but the available data don't allow any estimation how long.

However, since these results are from saliva following PVP-I rinse, it is not clear whether the same would apply to PVP-I administration in the nasal tract? The time course of viral load following nasal PVP-I application (like nose drops, nasal spray or nasal douche) is still unknown. Moreover, there was no correlation between viral load in nasopharyngeal and saliva samples at baseline in this study.

One may wonder about the missing short-term effect of PVP-I (after 5 min). Maybe the RNA copies found at that point of time were from inactivated virus (there was no cell culture in this trial to assess the viability of virus from saliva). On the other hand, the long-term effect of PVP-I is surprising, since there is no depot effect of PVP-I expected in the mouth after rinsing (contrary to agents which may adhere very well to the mucosal surface like octenidin, chlorhexidine or carrageenan). However, since the results depend on only four patients, more data are needed until definite conclusions can be drawn.

One may hypothesize that viral RNA found 5 min after rinse was defective virus (RNA) as a consequence of PVP-I contact, but that there were still enough viral RNA fragments of a "quality" and size that they could be successfully replicated in PCR. PVP-I has an oxidizing effect. If this defective RNA is increasingly degraded in smaller pieces during the next hours, it may become increasingly difficult to replicate recognizable RNA from the residual fragments, and this may explain the gradual reduction of RNA copy number during the three hours time frame of that study.

In vitro experiments with a nasal spray based with 0.5% PVP-I (Nasidine) demonstrated that the formulation inactivated culturable virus in short timeframes (15 seconds to 15 minutes), but that it had no effect on PCR-detectable viral RNA *in vitro* (TUCKER et al.). TUCKER et al. conclude that PCR alone may not be adequate for viral quantification in studies with nasal application of PVP-I, and future studies should incorporate cell culture to assess viral viability. But it is also possible that such residual RNA may be cleared by other mechanisms *in vivo.* These observations may explain the clinical findings of MARTINEZ LAMAS et al. that RNA copy numbers were not yet reduced 5 minutes after the PVP-I procedure.

On the other hand, one may wonder why the defective virus is not replaced immediately by new virus from elsewhere, e.g. saliva. It was suggested that viral shedding in the salivary glands is an important source for COVID-19 content in saliva, and the high density of ACE2 receptors in salivary glands may support this view (DA SILVA PEDROSA M). Autopsy specimens eventually proved *in vivo* SARS-CoV-2 infection both in the salivary glands and oral mucosae (BYRD et al.). ABDULJABBAR et al. report that *"infection and inflammation of salivary glands are common among viral infections, particularly in the early stages, resulting in possible changes of salivary flow and content."*

The observed time course would still be plausible if PVP-I was supposed to have a depot effect, but this is probably not the case (which can also be watched by the quick decoloration of the mucosal surface and saliva after PVP-I administration even in the case of much higher PVP-I concentrations, as a proxy for its disappearance). So maybe viral shedding into the saliva in the salivary glands doesn't play such a dominant role as suggested. One may also ask how the virus can reach the salivary glands if not by blood in later and more disseminated stages of the disease.

In spite of all these uncertainties, the results from MARTINEZ LAMAS et al. are promising that PVP-I rinsing may have a longer lasting effect on viral load in saliva, at least 3 hours and maybe (much?) longer in people with baseline high viral load in saliva. Thus it is probable that PVP-I rinse reduces infectivity during exhalation, speeking or coughing.

Moreover, as noted by CHOUDHURY et al., PVP-I has not only a direct virucidal effect, but PVP-I of 0.5-10 % solution inactivates the ACE2 and CD147 receptors of host cells.

Povidone-iodine throat spray as PREP/PEP

An open-label parallel RCT among healthy male migrant workers (100 % men; mean age: 33 years; seronegative at baseline) quarantined in a large multi-storey dormitory in Singapore found a small, but significant protective effect of povidone-iodine (PVP-I) throat spray (3 times a day; 0.45 % Betadine; 270 microgram/day), administered for 42 days **(SEET et al.).** SARS-CoV-2 infection was confirmed by PCR (at any time) or antibody test on day 42.

Controls (n = 619) got 500 mg vitamin C per day (for 42 days) (PVP-I: n = 735). Confirmed SARS-CoV-2 by PCR or serology: 46.0 % (PVP) vs. 70.0 % (Vit. C). Relative risk ratio 0.66 (CI: 0.48 – 0.88), absolute risk reduction in case of the use of the PVP-I throat spray was 24 % (CI: 7 – 39 %).

Point estimates for adjusted ORs (depending on model, 6 different models were taken into account: between 0.36 and 0.40, some of them significant).

Symptomatic COVID-19: 5.7 % (PVP) vs. 10.3 % (Vit. C) (- 45 %). Symptomatic disease among those diagnosed with SARS-CoV-2: 12.4 % vs. 15.0 % (-17,3 %). No hospitalization, no death in any study arm (young age!). Since the swabs for PCR testing were taken from the nasopharynx, the results cannot be confounded by effects of the throat spray.

PVP-iodine (1 %) or CHX (0.2 %) gargle (effectiveness after 5 minutes)

While the study from MARTINEZ LAMAS et al. found no effect of PVP-I (1 %) oral rinse on viral load after 5 minutes in their small sample of 4 patients, ELZEIN et al. analysed Ct values 5 min after gargling with PVP-I 1 % (n = 25 patients) and CHX 0.2 % (n = 27) and found a significant increase of Ct values 5 min after gargling compared to the Ct values obtained before gargling. The mean difference of Ct values was +4.45 for PVP-I and +5.69 for CHX (5 min after gargle compared to before gargle), but the difference between PVP-I and CHX is not significant. There was no difference in the control group who gargled with distilled water (n = 9) before and after gargling.

This raises the question whether the missing effect of PVP-I 5 minutes after rinsing in MARTINEZ LAMAS et al. is only a statistical artefact due to their small sample size (n = 4 instead of n = 25 in ELZEIN et al.). In MARTINEZ LAMAS et al., Ct(N) changed by -0.38, -2.06, -0.34 and +1.59 (mean: -0.30) after 5 minutes. On the other hand, the procedures may have been different. While MARINEZ LAMAS et al. reported about “rinsing”, the patients from ELZEIN et al. had to gargle. Saliva specimens in ELZEIN et al. were generated from the throat

by coughing (“participants were asked to cough out saliva from the throat (2 ml))”, but this was the same procedure like in MARTINEZ LAMAS et al. who referred to the method in TO KK et al.: “all patients were asked to produce an early morning saliva sample from the posterior oropharynx (ie, coughed up by clearing the throat) before toothbrushing”. Thus there was no difference with regard to the collection of saliva samples. The only difference between both studies is “rinsing” in MARTINEZ LAMAS et al. compared to “gargling” in ELZEIN et al. though it is unclear whether “rinsing” also included some degree of gargling. Nevertheless, the sample size in ELZEIN et al. is much more robust and it is clear now that gargling with 1 % PVP-I or 0.2 % CHX reduces viral load (of live and/or dead virus) within 5 minutes by about +5 Ct.

On an individual base, 5 of the 25 patients in the PVP-I group (20 %) showed a decrease of the Ct value within 5 minutes after gargling (like 3 of 4 in MARTINEZ LAMAS et al.), but all 7 patients with an initial Ct value <= 27 showed an increase of the Ct value. In the CHX group, 3/27 (11 %) showed a decrease of the Ct value, and all 30 patients with an initial Ct auf <= 30 showed an increase.

However, a serious limitation of the ELZEIN study is that saliva was sampled only once after gargling, exactly after 5 minutes. There are no results about the long-term effects of gargling in that study. The 5 minutes interval was chosen because of the common procedures in dental offices; once patients had gargled, it may last about 5 min until dental treatment starts (e.g. because one has to wait due to local anesthesia). But both for longer lasting dental treatment and use of oral antiseptics in real life it would be important to know for how long Ct values are increased after gargling; when do they start to fall again, and when do they reach baseline values?

PVP-iodine (0.6 %) nasal and throat spray from a nose spray bottle for prophylaxis

See also AREFIN; not a study, but a personal report about an extremely exposed suregon and his also extremely exposed colleagues in a hospital in India of whom no one catched COVID-19 following routinely PVP-I prophylaxis several times a day from a simple nose spray bottle. A study of the group had found that PVP-I 0.6 % is more effective than 0.5 % or 0.4 % to achieve a negative PCR result 15 minutes later in COVID patients (AREFIN MK et al.).

In that study, AREFIN et al. compared nasal irrigation with 0.4, 0.5 and 0.6 % PVP-I and nasal spray with 0.5 % and 0.6 % PVP-I to distilled water in 189 COVID PCR+ patients. Endpoint was qualitative PCR (PCR+ or PCR-) 15 minutes after the procedure.

Negative PCR 15 min after nasal irrigation:

Controls (distilled water): 29.6 %;

PVP-I 0.4 %: 33.2 %; PVP-I 0.5 %: **92.6 %;** PVP-I 0.6 %: **85.2 %**

Negative PCR 15 min after nasal spray:

Controls: 7.4 %; PVP-I 0.5 %: 66.7 %; PVP-I 0.6 %: **81.5 %**

In summary, nasal irrigation was found to be a little more effective than nasal spray. However, for concentrations of 0.6 % nasal spray the difference is not large and not significant. For practical reasons (nasal spray is a much simpler procedure and can thus be repeated more often), 0.6 % nasal spray can be recommended, and that's what AREFIN and his colleagues decided to use for prophylaxis as described in the personal report of AREFIN in a separate paper (see AREFIN MK).

The only adverse event was nasal irritation in two patients (1 x 0.4 % nasal irrigation and 1 x 0.6 % nasal irrigation).

PVP-iodine (1.0 %) nasal spray and gargle for treatment of COVID-19

BLASI reported a single case of a 70-year old woman with COVID-19 with fever (38 C), intense headache; a profound asthenia confined her to bed. Subsequently, she suffered from muco-hematic nasal secretions and continuous non-productive cough, and her general condition worsened progressively. Treatment started with 1 % aqueous solution of PVP-I (inhalation through each nostril until the liquid is perceived in the throat; then gargling with the same solution für 60 s, twice a day). After 24 h, her fever started to decrease until body temperature normalized. After further 24 hours, all other systems disappeared except for the cough with progressively diminishing intensity. At day 3, she was fully recovered except for slight asthenia.

PVP-iodine (1 %) or Listerine gargle

In a registered trial from Malaysia (NCT04410159), gargling (for 30 seconds, 3 times a day) with 1 % PVP-I (Betadine, 10 ml/portion) resulted in viral clearance in 100 % of 5 COVID-19 stage 1 patients on day 6, compared to 80 % (4/5) with essential oils (Listerine "Original", 20 ml/portion), 20 % (1/5) with tap water (100 ml pro action) and 0 % in the controls (0/5) (MOHAMED et al.).

All of the patients were “early” patients, since stage 1 was defined as “an asymptomatic state start at the beginning of the first two days of infection”. Mild symptoms were already classified as stage 2 and those patients were not included. At the time of inclusion, all patients were asymptomatic and less than 5 days from diagnosis. In this study, 4 days after start of the intervention corresponded to 5-6 days after diagnosis.

Nasopharyngeal and oropharyngeal swabs were taken at day 4, 6 and 12 in the morning before the early morning gargle. In detail, among PVP-I users, all were negative at days 4, 6 and 12. Among essential oil users, one individual was still positive at day 4 and 6 and indeterminate at day 12. In the tap water group, three were positive or indeterminate at each of the days 4, 6 and 12. In the control group, 4, 5 and 4 were positive or indeterminate on days 4, 6 and 12. The difference between 1 % PVP-I group and control group was significant at all three time points ($p = 0.048$ or less). Ct-values from positive PCR were not documented (unfortunately).

The authors favor PVP-I and propose Listerine for those with contraindications against PVP-I. It is important to note that this study didn’t involve any method of nasal decontamination. It is therefore surprising to see such an effect simply as a consequence of gargling. However, there remain a lot of questions since nasopharyngeal and oropharyngeal swabs were taken, but the results were not shown separately for both. It is an interesting question whether (and how) oral/oropharyngeal gargle is able to promote viral clearance in the nasopharyngeal area? This study raises a lot of hopes, but much more data are necessary to understand what was really going on there.

With regard to Listerine, MEISTER et al. found in their *in vitro* assay that “Listerine Cool Mint” reduced SARS-CoV-2 until their limit of detection (about 3 log10) and competed with PVP-I and Dequonal that also reached the limit of detection, whereas CHX- and H_2O_2-containing mouthwash didn’t, and octenidine only in the formulation of Octenisept but not as Octenidol (MEISTER et al., STEINHAUER et al.). In an even more sensitive assay with a limit of detection after reduction of 5.5 – 6 log10, STATKUTE et al. found a reduction by 3 log10 for Listerine Cool Mint (what corresponds to the limit of detection in MEISTER et al. and is thus well in accordance with MEISTER et al.), whereas Listerine Advanced Gum Treatment was more successful and reduced SARS-CoV-2 below their own level of detection (> 5.5 log10). Listerine Advanced contains nearly as much ethanol as Listerine Cool Mint (23 % vs. 21 %), but Listerine Advanced contains 0.147 % ethyl lauroyl arginate HCl (LAE), a cationic surfactant. LAE offers an additional reduction of SARS-CoV-2 by 2 log10 compared to the essential oils (STATKUTE et al.). This may point to a general importance of surfactants, at least cationic surfactants, with regard to inactivation and local defense and against SARS-CoV-2.

Meanwhile “Listerine Cool Mint Mild” (without alcohol) was also found to be effective against SARS-CoV-2 *in vitro* (MEISTER et al., unpublished results, mentioned in KRAMER et al.).

PVP-iodine (1 %) oral rinse + gargle + nose drops + eye drops in outpatients with COVID-19

The most impressive results for local PVP-I administration were reported so far by CHOUDHURY et al., based on 606 patients. In the RCT with outpatients from Dhaka/Bangladesh, treated by telemedicine, 303 patients underwent mouthwash/gargle, nasal drops and eye drops with 1% PVP-I 4 hourly for 4 weeks (in combination with symptomatic home treatment as needed). The control group (303 patients) was advised to do the same, but with lukewarm water instead of PVP-I. PCR was done on day 3, 5 and 7 after randomization, and thyroid hormone levels (THS, T3, T4, FT4) were determined during the 4th week. 80 % of patients were males.

Patients were treated as outpatients because of hospital phobia. Thus there may have been a selection against severe symptomatic cases at randomization because hospitalization would have been unevitable for them. Thus the RCT is about patients who were still able to manage themselves at home at the time of randomization.

The procedure was instructed as follows:

“Care is taken to ensure the solution is distributed throughout the oral cavity for 30 seconds and then gently gargled or, held at the back of the throat for another 30 seconds before spitting out. Then 4-5 drops of 1% PVP-I is introduced to wash the nostrils by dropper and 2 drops in each eye. This application is done 4 hourly for 4 weeks.“ (4 times a day). (CHOUDHURY et al.)

Results PVP-I vs. lukewarm water:

- Hospitalization without oxygen support: 0.66 % vs. 4.62 %
- Hospitalization with oxygen support: 3.3 % vs. 20.79 %
- Death: 0.66 % vs. 5.61 %
- PCR + on day 3: 11.6 % vs. 96 %
- PCR + on day 5: 7.9 % vs. 88.4 %
- PCR + on day 7: 2.64 % vs. 70.3 %

No change in serum levels of THS, T3, T4 and FT4 were observed after 4 weeks.

The study demonstrates that local antiseptic treatment in early outpatients can be as effective, or even more effective, than systemic medications. Risk reductions for hospitalization, hospitalization with oxygen support and death were 84%, 84 % and 88 %.

Moreover, in a retrospective study with 1035 hospitalized patients from Pune/India (overall mortality: 7.73 %), use of PVP-I was the second most effective agent that was associated with survival, besides Vitamin D (survival > 94 %) (GHOOI et al.). However, there are no detailed data about the manner of PVP-I use and no further analyses (e.g. regressions) done.

Nasal saline irrigation with alkalinization (bicarbonate) or PVP-I starting immediately after diagnosis

BAXTER et al. report about a prospective cohort trial from Georgia/USA with patients aged at least 55 years who initiated nasal irrigation within 24 hours of a positive PCR test (patients with symptoms longer than 7 days before testing were excluded). Median 3.3 days of symptoms (IQR: 2-5) before enrollment.

Primary outcome was 28-day hospitalization for COVID-19. Patients were randomized either to 2.5 ml povidone-iodine or a half tea-spoon (betadine) or 0.5 teaspoon sodium bicarbonate, used with a pressure-based nasal irrigation system (either NAVAGE from Rhinosystems Inc. or Neilmed Sinus Rinse from Neilmed Inc). Patients should perform 2 nasal irrigations daily for 14 days (with another 14-day follow-up). The packages included saline pods/packets to prepare isotone solution with distilled water (the distilled was made available separately). In the study, all of the material was brought to the door of the patients at home)

The total content of the nasal rinse bottle was 240 ml. 2.5 ml of betadine (10 %) in 240 ml would thus correspond to only 0.1 % PVP-I concentration in the final solution.

37 patients were assigned to PVP-I, 42 to bicarbonate. There was no hospitalization in the PVP-I group and 1 in the bicarbonate group (altogether: 1/79 = 1.26 %). No death. Moreover, one patient in the bicarbonate group had a COVID-19 related ED visit but was not admitted.

During the time of the study, 19.33 % of all patients 50+ years had to be hospitalized according to the CDC. Taking this proportion as a control, the OR for hospitalization following the irrigation procedure was 0.054 (CI: 0.0074 – 0.38; p = 0.0036). Full diaries were only available from 62 patients; they reported about 1.79 irrigations on average by day.

Comparing PVP-I and bicarbonate, there were no statistical differences in symptoms and outcomes; however, symptom resolution in 14 days was more likely in the PVP-I group (77.8 % vs. 48.6 %, p = 0.0199).

The results are very impressive; however, it should be noted that this no RCT and there is also no real control group, matched by age and comorbidities, though the age limit for the CDC controls was 5 years lower in order to compensate some of these deficiencies. Testing occurred at a single location with a high proportion of minority and economically at-risk patients.

Originally, 158 matched controls (matched 1 : 2) were *"enrolled and identified respectively in Augusta, Georgia from September 23 to December 21, 2020 and followed 28 days. Due to contracting issues rendering control information unavailable, the COVID-19 Case Surveillance Public Use Data collected by the Centers for Disease Control was used as a control group for hospitalization outcomes."* (BAXTER et al.). Thus, originally the trial was planned as a RCT with 79 patients in the intervention group and 158 controls, but the concept of a direct control group could not be realized, and thus CDC data were taken as controls. This is of course a very serious limitation of that study. It would have been extremely interesting to know what happened to the 158 matched controls.

There are generally concerns that nasal irrigations in case of early COVID-19 infection may remove many important protective substances from the mucosal surface, e.g. interferons, defensins, lysozymes, antibodies (if already there), a problem that can be overcome by nasal spray though nasal spray may be less effective to contact all niches of the nasal tract. BAXTER et al.: *"The size variations in entire nasal cavity, rather than just anterior nares, supported the concept that full nasal cavity irrigation rather than just spray was worth testing."*

The BAXTER study now suggests that there is no need for such concerns; however, only a head-to-head comparison between pressured irrigation and nasal spray may answer this question, in a 7-arm RCT: PVP-I, bicarbonate and isotonic saline (as control) both as irrigation and as nasal spray, and a 7th arm without any of such intervention.

It is also surprising that PVP-I was so effective (and superior to bicarbonate with regard to symptom resolution) despite its very low concentration in the solution. According to LIANG

et al., the lower bound of virucidal effectiveness lies between 0.10 % and 0.17 %, and such low concentrations would demand longer contact times than can be achieved by irrigiation.

This is another study that supports the idea that early reduction of the viral load in the uppermost respiratory tract (e.g., by antiseptics) may result in favorable outcomes, similar like the concepts of LIANG et al. and MADAS et al.. BAXTER et al. write: *"Finally, the number of asymptomatic cases and the correlation of illness severity with viral load implied that even after PCR positivity, a window existed wherein lowering the viral load through irrigation could be clinically advantageous. The theory that pulmonary spread results from micro-aspiration of newly replicated viral particles is supported by the higher correlation between infection and obstructive sleep apnea than obesity, despite the increased ACE2 receptors in obese patients."*

PVP-I (1 %) mouthwash, gargle, nasal nebulization

GUENEZAN et al. reported about a small RCT from France with 24 early non-severe patients with a positive nasopharyngeal swab within the last 48 hours (n = 12 intervention group, n = 12 controls). *„Intervention consisted of 4 successive mouthwashes and gargles with 25 mL of 1% aqueous PI solution each (…), followed by one 2.5-mL nasal pulverization of the same solution into each nostril using an intranasal mucosal atomization device … followed by a massage of the nostril to help spread the ointment"* (control group: no intervention). The patients were trained during the first decolonisation session; then they practiced the procedure 4 times a day for 5 days.

Follow-up was done on day 1, 3, 5 and 7 and encompassed nasopharyngeal swabs (> 95 % taken by the same nurse) at least 3 hours after the last application of PVP-I. The swabs were used both for the quantification of viral RNA by RT-PCR (viral load) and also for assessment of viral titers by the dilution limit method on Vero cells. The latter allowed the quantification of live and potentially infectious virus.

There were no differences with regard to RNA copy numbers between the intervention group and the control group during the observation interval of 7 days; RNA copy number became lower from day to day in both groups without any trends in favor of the intervention or control group. (All patients had a good outcome and noone needed hospitalization). Unfortunately, despite randomization, the intervention group was on average 24 years

younger (33 vs. 57 years). Nevertheless, the PVP-I procedure seems to have no influence on the course of the RNA copy numbers over time.

With regard to viral titers in the Vero cell assay, 23 of the 24 patients were negative already by day 3. *However, „mean relative difference in viral titers between baseline and day 1 was 75% (95% CI, 43%-95%) in the intervention group and 32% (95% CI, 10%-65%) in the control group"* (GUENEZAN et al.).

The procedure, which was associated with quite high amounts of PVP-I in the nose, was associated with unpleasant nasal tingling, but everybody completed the study. TSH was found to be elevated above upper normal in 42 % of all patients from the intervention group after 5 days, but returned to baseline 7 – 12 days later, whereas no effect on T3 or T4 was observed.

The study is interesting because both RNA copy numbers (RT-PCR) and titers of viable virus were measured. This is an important study design because it allows to distinguish between viral RNA (that must not be infectious and may also be residual), and live, potentially infectious virus that may infect other persons, but what may also infect the lungs or reach other organs and contribute to the progression of the disease. The study was underpowered to detect significant results about the "live" viral titers; but it showed a trend that after 1 day after the start of the procedure, live virus in the nasopharynx was reduced stronger in the intervention group (by 75 % instead of 32 %), as theoretically expected. Unfortunately, there are no results from day 2. Of note, it was assured that swabs were taken not earlier than 3 hours after the last PVP-I procedure to avoid immediate influence on live virus by a very recent PVP-I exposure.

In contrast to this trend seen for live virus, the procedures 4 times a day had no effect on RNA copy numbers. This is not surprising because the infection of the epithelium was already established when the patients started with the PVP-I intervention. PVP-I cannot inhibit virus production inside the cells and the release of free viable virus from the cells. It can only start to act once viral particles are shedded. Like thymol, CPC or ethanol, PVP-I is assumed to destroy the viral envelope without degradation of viral RNA (MÜLLER LK et al.). Thus it is plausible that the PVP-I procedure cannot accelerate RNA clearance from the nasopharynx, but may, as indicated as a trend in that study, reduce the amount of live, infectious virus.

Of note, the null effect of the PVP-I procedure on viral RNA load was found in nasopharyngeal swabs taken at least 3 hours after the last administration of PVP-I. The situation may be different in the mouth, where a strong reduction of viral RNA was found 20 and (to a lesser extent) 60 minutes after PVP-I use (*see below*, YAJARAMAN et al.).

Hydrogen peroxide 1 % gargle

In full accordance with the disappointing *in vitro* results for H_2O_2-containing mouthwash (Cavex Oral Pre Rinse) in MEISTER et al. on Vero cells, GOTTSAUNER et al. showed *in vivo* that 1 % H_2O_2 (20 ml, gargling for 30 sec) is unable to reduce the RNA copy load in oropharyngeal specimens from COVID-infected patients (obtained by gargling with 0.9 % NaCl) 30 minutes later (PCR), compared to gargle specimens obtained directly before H_2O_2 administration.

However, it is unclear whether H_2O_2 had an influence on the survival of *viable* (infectious) virus, examined by virus culture on Vero cells (1/5 positive cultures from oropharyngeal specimens taken directly before H_2O_2 administration and 0/5 taken 30 minutes after H_2O_2 administration). There is a general methodological problem that RNA copy loads below 1000.000 RNA copies/ml (in PCR) in pharyngeal specimens hardly yield successful culture, and in the oropharyngeal specimens from this study, viral load was smaller except for a single case.

Hydrogen peroxide 3 % nasal wash, followed by hypertonic saline nasal wash

Whereas 1 % H_2O_2 seemed to have little effect on SARS-CoV-2 *in vitro* (Vero cells, see MEISTER et al.) and *in vivo* (GOTTSAUNER et al.), CAPETTI et al. reported about longer lasting effects of **3 % H_2O_2** in patients with long-lasting or reactivated nasopharyngeal PCR positivity (median time from exposure or symptom onset: 111 days). All patients were seropositive, though with comparatively low titers. The authors suggested 3 % H_2O_2 as “the most fit for mucosal cleaning”, and that it promotes destruction of RNA within 5 minutes through activation of free radicals (CAPETTI et al.).

At first, all seven PCR-positive patients cleaned both choanae with a micro-pump and hypertonic saline solution with pH 6 (Atomix; using the Atomix Wave kit). After that, they repeated the procedure with pure 3 % H_2O_2. Finally, they had to wash their mouth and gargle for 2 minutes with 3 % H_2O_2. During the following 14 days, they had to clean the nose with Atomix Wave (hypertonic solution). The H_2O_2 procedure was done only once at the beginning of the trial. Swabs were taken at 24, 48, 72 hours and, if still negative, at 7 and 14 days.

Though all 7 patients were PCR-positive before the first procedure, none of the patients was PCR-positive after 24 hours and 48 hours; one was weakly positive after 72 hours, four were

still negative after 72 hours but weakly positive at day 7, and two remained negative all the time and were still negative after 14 days.

Though the results are impressive, a lot of questions remain. Since all patients applied hypertonic saline solution daily (how often? once daily?) to the nose, it remains unclear whether the favorable effect can be attributed to one time H_2O_2 administration, daily Atomix administration, or both (once H_2O_2, then daily hypertonic solution). Second, the relevance of the results may be small in a population which is probably no longer infectious (they all had antibody titres); residual PCR positivity may be indicative of problems of the immune system to clear the virus completely and a sort of latency, but may be without any clinical relevance or need for containment.

The observation that the procedure caused only temporary interruption of viral shedding in 5/7 cases caused CAPETTI et al. to suggest that the virus may either continue to replicate in deeper mucosal strata, or in the bronchial epithelium (leading to reinfection of the upper respiratory tract by replicating virus from deeper areas of the respiratory tract). They propose to repeat H_2O_2 washing in a 14 day period (the epithelial turnover time) in order to investigate whether this is associated with more profound suppression of the virus. This may help to understand the source of the reappearance in nasopharyngeal swabs.

However, it would be very interesting to repeat the same procedure with COVID-infected patients during active infection in the early, infectious stage of the disease (first week after diagnosis). Moreover, it would make sense to establish a control group who only uses hypertonic saline solution without a single H_2O_2 procedure, and another group with neither H_2O_2 nor hypertonic solution. Maybe hypertonic saline solution alone is able to show the same favorable results?

However, if 3 % H_2O_2 procedure is obligatory to achieve the aim of long-lasting PCR negativity in nasopharyngeal swabs, one should consider its possible side effects on the nasal epithelium. The effect of H_2O_2 on the nasal epithelium hasn't been studied in detail so far, and the study of FELDMAN et al. gives cause for some concern. On the other hand, CARUSO et al. (2) showed that *"no damage was observed on oral mucous membranes or their microvilli after ongoing gargling treatment with H_2O_2 3%".* However, this is difficult to interprete since oral mucosa has no microvilli, thus it is unclear which mucosal location with microvilli had been examined (nasal microvilli?).

Beside of these open questions, CARUSO et al. (1,2) present a lot of mechanistic evidence why H_2O_2 administration is promising, and they proposed a starting dose for clinical trials which are urgently needed *"two puffs (about 0.28 ml) of 1.5% H_2O_2 nasal spray into each nostril two times daily combined with a mouth wash and gargling for 1 min with a 3% H_2O_2 solution two times daily."* (CARUSO et al. 1).

But there is a fundamental difference between the concepts of CAPETTI et al. and CARUSO et al.. Whereas CAPETTI et al. administrate 3 % H_2O_2 only once (and suggest that it may be repeated 14 days later in accordance with the renewal time of the nasal epithelium), CARUSO et al. propose two daily administrations, but only with half of that concentration (1.5 %). And whereas CAPETTI et al. didn't involve oral gargle except once directly after hydrogen peroxide nose wash, CARUSO et al. propose 3 % H_2O_2 for oral gargle (but only 1.5% for nasal administration) twice a day.

CARUSO et al. don't differentiate strictly between nasal wash and nasal spray; sometimes they write about "nasal wash" and then they eventually propose nasal spray. However, the difference between nasal wash and nasal spray may not be unimportant if one considers the possibility that nasal wash may wash out protective natural agents like immunoglobulines (IgA), defensins, interferon, lysozyme etc. from the mucosal membranes and may thus enhance susceptibility for infections for some time. WANG Z et al. demonstrated the important role of IgA in the local defense of the respiratory tract.

With regard to hypersaline solutions, it is unclear whether they can be really efficacious against SARS-CoV-2, because this virus is very salt-tolerant. Due to a strong negative electrostatic potential, SARS-CoV-2 RBD binds even stronger to ACE2 receptors at higher salt concentrations, and the SARS-CoV-2-RBD-ACE2-complex is stabilized independent of the salt concentration (PETER and SCHUG). Until there is clear clinical evidence for a real benefit of hypersaline solutions for nasal or pharyngeal spray or wash, one may suggest that it could be wise to prefer other methods of local antiseptic treatment.

However, STATHIS et al. pointed out that saline solutions and iota-carrageenan were so far the only antiseptics that showed efficacy against common coronavirus infections. They explain the efficacy of saline in the upper respiratory tract by increased availability of local chloride ions (from NaCl) that support the production of hypochlorous acid in epithelial cells. *In vitro,* the entry of chlorid ions into the cells and their conversion to hypochlorous acid (by peroxidase) was found to be necessary to achieve antiviral activity, pointing against a direct virucidal effect of saline solution (STATHIS et al.). If so, the high salt-tolerance of SARS-CoV-2 (which might point in question the usefulness of saline solutions) may be irrelevant in that context. Hypochlorous acid in the cells halts virus replication. "But once HOCl is used up, the virus will start to replicate again" (STATHIS et al.). Thus STATHIS et al. proposed to combine or alternate between antiseptic and saline nasal and oral rinses, because antiseptics and saline solutions depend on different mechanisms: NaCl suppresses viral replication (via HOCl) inside the cell, whereas antiseptics have a direct virucidal effect. They may work together – when used alternating – in the way that antiseptics inactivate free virus before its entry into the cells, or after its release from infected cells, whereas hypersaline solutions may suppress viral replication inside infected cells for some time.

Neutral electrolyzed water containing 0.0015% of reactive species of chlorine and oxygen (nasal and oral administration) for PREP (Esteriflu&Estericide)

GUTIERREZ-GARCIA et al. reported about an open-label RCT in Mexico City about nasopharyngeal and oropharyngeal rinses with "neutral electrolyzed water"(SES; pH: 6.5 – 7.5) in unvaccinated frontline health professionals without previous positive SARS-CoV-2-PCR in a general hospital (PREVECOVID-19).

170 volunteers were randomized (1 : 1). *"All members of the trial wore the adequate personal protection equipment at all times while performing their duties, as required by standard COVID-19 safety protocols."* The SES group performed three times a day oral and nasal rinses with SES for 4 weeks. All participants were monitored for COVID-19 symptoms and disease in a time-frame of 4 weeks. Persons with suggestive symptoms were immediately tested in the hospital's laboratory. All of the participants *"were previously trained to identify and report symptoms, as part of the intrinsic safety protocols of the hospital and the National Ministry of Health".* Mean age: 44 vs. 41 years (SES vs. controls), similar characteristics of professions (nurses vs. doctors) and sex; more comorbidities in the SES group (29.8 % vs. 16.5 %).

Study period: September – November 2020. Only the researchers who performed the statistical analysis were blinded.

Primary Endpoint: SARS-CoV-2 positive symptomatic cases (between the 14th day since their recruitment and the 28th day of follow up):

1 vs. 10 individuals, i.e. 1.2 % (SES) vs. 12.7 % (control) (p = 0.0039) (based on n = 163). Relative risk reduction: 90.6 %

Moreover, there were 6 symptomatic infections (6/85) in the control group, but only one in the SES group (1/85) within the 14 day interval after recruitment which are not included in the primary endpoint since the first two weeks were excluded before the final analysis (thus dropping the number from 170 to 163).

Supposing also a short-term effect of SES and including these individuals in the analysis would result in 2/85 vs. 16/85 symptomatic SARS-CoV-2 infections (2.4 % vs. 18.8 %) and a relative risk reduction of 87.5 % from the start of SES administration.

Because of the limited availability of PCR tests, testing was only performed in case of symptoms. Thus it remains unknown whether the SES procedure reduced the risk of asymptomatic infections too.

Procedures:

Three times a day:

Nasal cavity rinses with EsteriFlu: four vertical sprays in each nostril; it should be inhaled deeply at the time of each spray

Oral cavity rinses with ESTERICIDE Bucofaringeo: 10 ml as mouthwash and gargle during 60 sec, then spit out.

The three rinses were performed at the beginning of the day, at midday and at the end of the day, i.e. they covered the whole active time and not only the time window of the work shift as frontline HCWs.

Of note, the SES formulations from the RCT contained 0.0015% of reactive species of chlorine and oxygen. It was not simple ionized water.

No adverse effects were noted. The virucidal action of neutral SES on nonenveloped and enveloped viruses has been demonstrated *in vitro*; moreover, inhalation of nebulized SES by ambulatory COVID-19 patients (in combination with conventional therapy) was already found to reduce disease progression and improve the signs and symptoms after 24-72 h from first administration (References in GUTIERREZ-GARCIA et al.). *"Nasal goblet and ciliated cells are a likely initial invasion site and reservoir of SARS-CoV-2 virus since these cells have a high expression of ACE2 and TMRPSS2",* but *"it was also determined that salivary glands, epithelial cells of the tongue and fibroblasts of oral mucosa express ACE2, explaining that both the nasopharynx and oropharynx have the highest viral loads"* . *"It has been postulated that the REDOX potential of SES breaks chemical bonds and causes changes in surface proteins, destruction of the viral envelope, inactivation of viral enzymes, and destruction of viral nucleic acids",* in a similar way like hypochlorous acid, an active chlorine species present in the SES from that trial (GUTIERREZ-GARCIA et al.).

The same prophylactic effect might be achievable with other substances with oxidizing potential (e.g. povidone-iodine), but they may have an irritant effect on mucous membranes, what was not the case with SES. No one reported any adverse effects or discontinued the study (GUTIERREZ-GARCIA et al.).

A serious limitation of the study is the lack of placebo treatment in the control group. The authors explain this as follows: *"Nevertheless, it is important to mention that this possibility was evaluated and it was concluded that there was a high risk of spreading the disease by producing fomites with such rinses without an antiseptic effect (…). According to the World*

Health Organization, 'there is no evidence that regularly rinsing the nose with saline has protected people from infection with the new coronavirus'".

Any nasal and oropharyngeal cleaning may have some degree of a protective effect by simple reduction of the free viral load by mechanical cleaning (washing). Even if there is no true antiseptic effect, the early cleaning and reduction of the very initial viral load may reduce the latter below a threshold where the local immune system of the body (e.g. local interferons, defensins and others) may be able to clear the infection quickly on its own, so that the temporary contamination or short-term minimal infection remains undetected.

So we don't know from that study how much of the prophylactic efficacy of the SES is due to the very special formulations of Esteriflu/Estericide (that are different from "simple" ionized water because of a defined content of chlorine and oxygen), and how much of the prophylactic effect can be achieved by the same procedures with the same frequency e.g. with simple physiological saline solution, or "simple" ionized water without a defined content of chlorine and oxygen?

Nevertheless, the results are impressive even in the absence of placebo use in the control group. It seems to be much better than "no" nasal/oral/oropharyngeal procedures at all. In countries were Esteriflu/Estericide are not available, there may be similar formulations on the market for nasal spray and mouthwash/gargle, based on electrolyzed water, neutral pH, hypochlorite and hypochlorous acid. But these solutions are not fully identical to Esteriflu/Estericide. But if the antiviral effect of Esteriflu/Estericide is based on its oxidizing potential, similar formulations with reactive chlorine and oxygen species may work as well as Esteriflu/Estericide. They may then present a simpler and better tolerated (non-irritating) alternative to oxidizing nasal sprays and mouthwash/gargle based on povidone-iodine, that is contraindicated in people with certain comorbidities, may result in overdoses of iodine if used often and for a long time, and is much more difficult to handle (e.g. necessity to prepare fresh dilutions of the original formulation for administration as nasal spray or mouthwash in concentrations of 0.5 – 1.0 %). Without a direct comparative trial, one cannot speculate whether formulations like Esteriflu nasal spray and Estericide mouthwash/gargle are equally effective, even more effective, or less effective than the same procedures with 0.5-1.0 % PVP-iodine as nasal spray and mouthwash/gargle in the prevention or early treatment of COVID-19; however, in real life, they are simpler to use than PVP-iodine, including no risk of irreversible discoloration of clothes.

Chlorhexidine 0.12 % gargling

In a trial from Korea, viral load from saliva from two patients was measured at baseline and 1, 2 and 4 hours after gargling with 0.12 % CHX mouthwash (YOON et al.). The tests were done with each patient at day 3 and 6 of hospital stay, so that there are four series of measurements. At day 3, CHX gargle was successful in both patients after 1 and 2 hours (reducing viral load below the limit of detection). After 4 hours, ct values were still a little higher than at baseline, i.e. viral load was still a little lower.

At day 6, baseline viral loads were lower than at day 3 in one patient and higher in the other. After 1 hour, viral load was increased a little in one patient and decreased a little in the other patient. At 2 hours, viral load was decreased in both patients compared to baseline. After four hours, viral load was higher than baseline in one patient and went on to decrease in the other. Altogether, there were reductions of viral load compared to baseline in 3 of 4 cases after 1 hour, in 4 of 4 cases after 2 hours, and in 3 of 4 cases after 4 hours. But only 2/4 reductions after 1 and 2 hours were below the limits of detection.

Viral load was measured in saliva in this trial and thus it is unknown whether CHX gargle had any effect on viral load in oropharyngeal swabs.

However, *in vitro* studies about chlorhexidine showed only a very small effect (less than one order of magnitude virus reduction both with 0.1 % and 0.2 % CHX after 0.5, 1, 5 or 10 min, though increasing a little bit with increasing exposure time) (MEISTER et al., STEINHAUER et al., STATKUTE et al.). STATKUTE et al. found that Corsodyl (0.2 % CHX, 7 % ethanol) reduced SARS-CoV-2 only by factor 10 after 30 seconds of exposure – the worst results from all mouthwash products examined by their assay. ANDERSON et al. studied two CPC-containing formulations and 0.2 % CHX in the presence of human saliva for 30 seconds against SARS-CoV-2, including Alpha, Beta and Gamma, and found that CPC was more than 100 times more effective than 0.2 % CHX. This study is particularly important because it involved both critical variants and the presence of human saliva to simulate physiological conditions.

So there is reason to be reluctant about protective effects of chlorhexidine in spite of the promising results from YOON et al. Of course, a small effect can be expected even from the *in vitro* data.

Moreover, there are concerns about the effects of CHX on the physiological oral flora if used in higher concentrations, and its use as nasal spray is not well studied. It also cannot be used in the eye due to the risk of corneal damage (STATHIS et al).

Mouth rinse: 0.5 % PVP-I, 0.075 % CPC, 0.2 % CHX, sterile water as control

In a randomized controlled trial from Singapore, 16 patients with positive RNA results in saliva (3 ml) collected directly before mouth rinse, 4 participants performed mouth rinse with 0.5 % PVP-I (5 ml) (Betadine diluted with water), 6 with chlorhexidine (CHX) 0.2 % (15 ml), 4 with CPC (cetylpyridinium chloride; Colgate Plax; 20 ml) 0.075 %, and 2 with 15 ml sterile water (SENEVIRATNE et al.). Duration of mouth rinse: 30 sec.

3 ml of saliva were collected again 5 min, 3 h and 6 h after that mouth rinse and analyzed by PCR. Ct values were recorded and regarded as surrogate for viral load. Compared to mouth rinse with sterile water, significant increases of Ct value, analyzed by relative fold change analysis, were discovered 5 min and 6 h (but not 3 h) following CPC mouth rinse and 6 h following PVP-I mouth rinse.

At the first glance, this is the first trial which documents a favorable effect of CPC in the context of COVID-19, since *in vitro* data are missing so far for CPC, and the MEISTER study didn't include a CPC-containing formulation. In accordance with MARTINEZ LAMAS et al., this trial confirms a long lasting effect of PCP-I and extends its time frame now from 3 h to 6 h, well in accordance with expectations based on the time course in MARTINEZ LAMAS et al.. CHX 0.2% was found to be disappointing like in the *in vitro* study from MEISTER et al. and ANDERSON et al..

The first favorable results for CPC were not unexpected. HERRERA et al. had already proposed CPC for mouth rinse and gargle as an alternative to PVP-I, because it had been found to be effective in the prevention of other upper respiratory tract infections (usually caused by influenza, RSV, metapneumovirus, rhinovirus, adenovirus, human coronavirus) in a RCT with a CPC-based formulation for inhalation (HALO^{TM}; intraoral spray 3 x daily for 75 days) (MUKHERJEE et al.). CPC was found to be effective against MERS-CoV *in vitro*, and also against HCoV-229E (0.07 %; GREEN et al.), chosen as a surrogate for SARS-CoV-2, but there were so far no results with regard to "real" SARS-CoV-2. HALO oral antiseptic was suspended from the market in spring 2020 while it is tested against COVID-19 in order to avoid that people feel protected erroneously.

ANDERSON ER et al. confirmed the high virucidal acitivity (>4 log10) of two different formulations with 0.07 % CPC after 30 seconds, equal to 70 % alcohol. The results were confirmed separately for the variants Alpha (B.1.1.7), Beta (B.1.351) and Gamma (P.1). In that assay, a reduction of >4 log10 meant inactivation below the limit of detection. Most important, these results were obtained in the presence of human saliva. In contrast, 0.2 % CHX was more than 100 times less effective, with a reduction of less than 2log10.

Based on mechanistic considerations (destroying the virus capsid), quarternary ammonium compounds are promising candidates against COVID-19 (BAKER et al.). The envelope of SARS-CoV-1 and -2 has been described as "unusually stable" (SCHELLER et al.), so favorable experience with CPC with regard to other viruses may not automatically apply to COVID-19.

The SENEVIRATNE trial is now the first trial which suggests that CPC may be successful against COVID-19. On the other hand, the “unusual stability” of SARS-CoV may explain why CHX fails because of its direct effect on capsids and membranes which may depend on their lability or stability. However, in the YOON trial, CHX 0.21 % was quite successful for 1 – 2 hours.

Whereas these primary results are so far consistent with theoretical expectations or results from other trials, a more detailed look on the data raises some questions and shows a lot of limitations. First, in 19 from 36 COVID-19 positive patients, no positive PCR result was obtained from pre-rinse saliva; thus they were excluded from consideration within the trial. In those with positive salivary PCR pre-rinse, ct values ranged from 15.64 to 34.58 (mean: 27.73). The mean pre-rinse ct value in the four arms was very different and ranged from ~ 22.5 (PVP-I group) to ~ 32 (CPC group). This makes it hard to compare the four groups with one another. Even more surprising, in the control group (mouth rinse with 15 ml sterile water), ct values decreased steadily from ~ 27 pre-rinse to ~ 22 at 6 h after rinse, indicating a rise of viral load. It remains unexplained why rinsing with sterile water can rise the viral load during the next six hours compared to pre-rinse viral load. If there was a reduction of viral load 5 min after rinse (compared to pre-rinse) and then again a rise of viral load, one could explain this effect by mechanical removal of viral particles as a simple consequence of rinsing, but this was not the case here. Lowest viral load was found *before* rinse. The significant differences in the relative fold change analysis for PCP-I at 6 h and CPC at 5 min and 6 h were found in comparison to the sterile water control group. Without the unexplained rise of viral load in the control group, the differences would probably not have become significant. With only two patients, the control group was extremely small and the rise of viral load may simply be by chance.

In contrast to the graphs in papers from other trials (e.g. MARTINEZ LAMAS et al.), figure 1 in SENEVIRATNE et al. doesn’t show the time course of ct values for individual patients. This makes it even more difficult to interprete the results. If one compares the time course of ct values within the trial arms, instead of comparing the fold change with the control group, the conclusions are quite different. Then PVP-I was most successful after 3 h, since the lowest ct value of the sample increased from ~ 16 to ~ 19, whereas after 6 h, the distribution of the 4 ct values, and the lowest ct value, were similar to those pre-rinse. This is in contrast with the comparison with the control group based on fold changes where PVP-I was found to be most successful only after 6 h.

In the CHX group, there were only minor changes, and the lowest ct values were lower than pre-rise after 5 minutes and 6 hours. The results are well in accordance with the suggestion that CHX is ineffective with regard to COVID-19.

In the CPC group, the changes were also small and the best results were obtained 5 min post-rinse. The lowest ct values were obtained 3 and 6 h post-rinse, even lower than pre-rinse.

Except for the steady, though statistically insignificant reduction of ct values in the control group (indicating a rise of viral load following sterile water rinse), all changes after 5 min, 3 h and 6 h post-rinse, compared to pre-rinse and to one another within the same study arm, show no clear trend and show a distribution with can be best explained by chance (see fig. 1 in SENEVIRATNE et al.). Judging the time course of each arm for itself (instead of comparing fold change of the trial arms to fold change of the control group), the mouth rinse procedure seems to be ineffective at each time point and for each agent. The significant results were only obtained because viral load rose in the two patients of the control group for unexplained reasons. To understand what was going on in that trial and why the mouth rinse procedure was seemingly ineffective, one would have to see the individual curves of ct values within the time frame of that trial. Thus the most important informations are lacking in the documentation of the trial results.

The authors report that the participants had to rinse the mouth. Nothing is said about gargling. Maybe the procedure was really ineffective because the mouth wash didn't reach the oropharynx as much as one would expect if the participants are asked to gargle? Even gargling is far suboptimal to reach the oropharynx, but mouth wash without gargling is definitely inferior to gargling. If so, then the most important result of this study would be that mouth rinse alone is ineffective and that one needs at least to gargle (and/or spray); moreover, in this case the study would not allow any conclusions with regard to the agents which were used in that trial, including CPC.

In vitro data with CPC and SARS-CoV-2 (instead of other coronaviruses) are promising. STATKUTE et al. found maximal effectivity against COVID-19 (reduction below the level of detection, i.e. > 5 log10, for two or their three CPC-containing mouthwash solutions: Dentyl Dual Action (0.05 – 0.10 % v/v CPC; isopropyl myrisate; *Mentha arvensis* extract) and Dentyl Fresh Protect (0.05 – 0.10 % v/v CPC; xylitol). However, SCD Max (0.07-0.10 % v/v CPC; 0.05 % sodium citric acid; sodium monofluorophosphate) was less efficacious and reduced SARS-CoV-2 only by 3 log10, but this is still comparable to the "top products" in the MEISTER study which used a less sensitive assay with a limit of detection in the range between 1.4 and 3 log10, thus SCD Max still competes with the "top products" from MEISTER et al. Nevertheless, STATKUTE et al. conclude that the different results for different CPC-containing products (with similar CPC concentrations) suggest "that the exact formulation is important; thus, individual mouthwash formulations should be empirically tested for antiviral activity, rather than basing decisions on the 'major' antimicrobial component".

In their in vitro assay on Vero cells, MUNOZ-BASAGOITI et al. found reductions of 3 log10 after 2 min (1 : 1 ratio with virus-containing suspension) for Perio Aid Intensive Care (0.05 % CPC, 0.12 % CHX) and Vitis CPC Protec (0.07 % CPC). CPC blocked viral entry and inhibited the fusion of target cells. MUNOZ-BASAGOITI et al. proposed also the use of CPC nasal sprays *"to fully achieve the prophylactic potential of this approach."* They mentioned that the

antibacterial activity of CPC in saliva lasts for 3 – 5 hours *in vivo*, but it is not known whether this applies to SARS-CoV-2 too.

This seems to be an important limitation for the practical application of study results from mouthwash formulations if the exact formulation from the study is unavailable (or only available with difficulties, e.g. internet business instead of local shops) and one has to be unsure whether similar formulations are as effective as the tested formulation. However, the differences between the three CPC-containing products would have probably been undetected in an assay as used by MEISTER et al. Taken all evidence from in vitro data together, CPC-containing products can be regarded now as at least as effective as the "top products" from MEISTER et al. and STEINHAUER et al. (PVP-I, Listerine Cool Mint, Dequanol, Octenisept), and some CPC-containing formulations and Listerine Advance may even be superior.

Mouth rinse + gargle: 2 % hydrogen peroxide + 0.133 % chlorhexidine (therapeutic)

MUKHTAR et al. reported about an unblinded RCT from Qatar involving oral rinsing and gargling three times daily with a mixture of 2 % hydrogen peroxide and 0.133 % CHX in hospitalized patients, admitted due to COVID complications or comorbidities in the context of a COVID-19 infection (intervention group: n = 47; control group: n = 46). Treatment duration: 2 weeks. If indicated, the patients got antivirals, antibiotics, steroids in addition to hydroxychloroquine and convalescent plasma transfusion. Mean age was 49 years, Start of treatment: mean 5.5 years since onset of symptoms (range: 1 -14 days).

Mouth rinse and gargle: at least 30 seconds, using 15 ml of a mixture of 10 ml of 0.2% CHX (oral rinse) plus 5 ml of 6% hydrogen peroxide, making a final concentration of 2 % hydrogen peroxide and 0,133 % CHX.

The intervention was based on the hypothesis of a "dysbiosis model" elicited by SARS-CoV-2 with some interaction between the virus and the local microbiota. The authors suggest a " tit-for-tat interaction with the microbiota, especially species with the potential to benefit from dysbiosis, as each side can manipulate the other, either directly or through inducing the host's immune response." (MUKHTAR et al.).

MUKHTAR et al. suppose that oral inhalation is a larger risk than nasal breathing; "given the lack of natural filtering capacity in the former and the protection provided by the high levels of nitrous oxide produced, which proved to inhibit viral replication". They assume that "an

existing dysbiosis can facilitate contracting the viral, leading to developing the infection and then the disease's progression to yield worse outcomes" and thus tried to target the oral microbiota by the use of mouthwash.

Mouthwash resulted in better improvement of symptoms after 2 days of treatment, higher rate of PCR negativity by day 5 of treatment in combined naso-/oropharyngeal swabs and less intubation/mortality (all $p < 0.05$), and insignificant trends with regard to shorter hospital stay (8.11 vs. 9.43 days), less progression of oxygen requirements and clinical categories.

There was no linear relationship with the duration of use and the observed improvement suggested better potential in an earlier stage of the disease. However, it was only a adjuvant to common treatment protocols for the hospitalized patients.

In detail, severity scores and oxygen demands on admission were a little (but not much) higher in the intervention group compared to the controls, and steroids were more often used in the intervention group, HCQ and convalescent plasma a little less, but the differences in the treatment regimens weren't large.

3 patients of the control group, but no patient from the experimental group progressed to intubation. Two patients in the control group (both among the 3 intubated) and no patient from the experimental group died. During 60 days follow-up, there was an additional death due to COVID-19 in the control group (day 54), rising the deaths until day 60 to 3 : 0.

At day 5 after start of mouthwash, 13.3 % vs. 0 % of naso-/oropharyngeal swabs were PCR-negativ (intervention vs. control group). At day 15, these rates were 34.9 v. 20.5 %. At day 5, the differences between both groups (based on CT value categorization) became marginally significant ($p = 0.047$).

The authors suggest that mouthwash/gargle influenced and reset complex interactions between the virus and local microbiota instead of only a simple direct virucidal activity. It is well established that microbial dysbiosis in the female genital tract enhances the risk for viral infections (e.g., HIV, HPV, HSV) and also for bacterial STI infections, and the persistence and progression of viral infections like HPV, and that the production of hydrogen peroxide by genital bacteria plays an important role in the antiviral and antibacterial defense and maintenance of microbial health and stability. A similar concept is suggested now by MUKHTAR et al. for oral/oropharyngeal COVID-19, whereas they regard the nasal mucosa as less relevant.

While this concept can be regarded at the moment only as a hypothesis, it may offer explanations for observations from other studies that are difficult to understand, e.g. the suppression of COVID-19 RNA in oral samples for several hours following a virucidal intervention (like PVP-I) without a depot effect (by mucosal adhesion) which is suggested to have only a short-time effect on the viral load until new viral particles derived from higher or lower airways pass the oropharynx or oral cavity and settle down there. Moreover, if one

considers a more indirect effect of the mouthwash on SARS-CoV-2, mediated by oral/oropharyngeal microbiota, it doesn't matter that CHX was found to have only a small virucidal effect on SARS-CoV-2 *in vitro.*

Reduction of SARS-CoV-2 in oral mouth secretions and respiratory droplets (PVP-Iodine, hydrogen peroxide, CHX) (avoidance of infectiousness)

JAYARAMAN et al. evaluated the effect of mouthwashes on SARS-CoV-2 burden in "whole mouth fluid" (WMF) and respiratory droplets (RD) of hospitalized COVID-19 patients before and after use of PVP-Iodine, hydrogen peroxide (HP) and chlorhexidine (CHX) mouthwash. They used both quantitative RT-PCR and (in some cases) Rapid antigen test (RAT).

The following situations were studied (early morning, unstimulated):

20 and 60 min after 1% PVP-I

20 and 60 min after 1.5 % hydrogen peroxide (RAT besides RT-PCR)

90 and 180 min after 1.5 % hydrogen peroxide

90 and 180 min after 0.2 % CHX

Respiratory droplets showed higher reductions of viral burden than WMF samples (92 % vs. 50 %; p = 0.008).

Statistically significant reductions of viral burden were found 20 min and 60 min after PVP-I mouthwash, 20 minutes (but not 60, 90 and 180 min) after hydrogen peroxide mouthwash and 90 (but not 180) minutes after CHX. There were reductions of > 1log10 (factor 10) at 20, 60 and 90 min after PVP-iodine, hydrogen peroxide and CHX.

Moreover, it was found in that study that Rapid Antigen Tests (RAT) are more appropriate than PCR to evaluate the efficacy of mouthwashes. RT-PCR will detect both live and dead virus, whereas RAT is better suited to detect the infectious state. This limitation of the RT-PCR may explain, for example, the conundrum from the MARTINEZ LAMAS trial that very recent administration of PVP-I was unable to reduce viral load (measured by PCR) 5 minutes after the antiseptic intervention, contrary to the theoretical assumption that efficacy of such a strong antiseptic should be maximal at that early point of time. *"The unbiased detection of*

viral RNA by RT-PCR irrespective of active viral infection can be problematic" (JAYARAMAN et al.).

Unfortunately, antigen tests were only performed on a subset of six patients (all from the hydrogen peroxide group) in the JAYARAMAN study. Since hydrogen peroxide has to be regarded as suboptimal due to the results from other trials (*in vitro* trials and *in vivo* in GOTTSAUNER et al.), it would have been interesting to compare RAT and quantitative RT-PCR in patients who used PVP-I.

Whereas only 13/36 patients were PCR-positive at baseline, all 6 patients who were tested with RAT were positive at baseline. Three became RAT-negative after hydrogen peroxide treatment (PCR: 2 x decrease by 1.1 log10; 1 x slight increase by +0.5 log10); three stayed RAT-positive (PCR: 3 x increase by 1.2 – 2.4 log10). However, all three negative RATs were found after 20 min and all three positive RATs were found after 90 min, thus the result may simply correlate with a time effect that HP is able to reduce live virus within 20 min, but not within 90 min, below the limits of detection by that test.

With regard to qRT-PCR and WMF, both PVP-I and HP reduced viral burden by >1 log10 and <2log10 after 20 and 60 min, and HP and CHX after 90 min, but not after 180 min (-0.93 log10 for HP and -0.43 log10 for CHX).

Using qRT-PCR, the effects were stronger on viral load in respiratory droplets: essentially 100 % reduction in 100 % of participants for PVP-I and HP after 20 min, well in accordance with the RAT results mentioned above.

Summarizing all specimens from the same time point, independent of the sort of mouthwash, reduction of viral load (by qRT-PCR) in WMF was constant at about 1.5 log10 during the first 90 min (20, 60, 90 min) and then decreased to 0.68log10 at 180 min (preferentially by the drop of efficacy of CHX).

With regard to respiratory droplets, there was a steady decrease from 2.93log10 (100 % efficacy compared to baseline) after 20 min, 2.02 log10 after 60 min, 1.53 log10 after 90 min, but interestingly 1.81 log10 after 180 min (thanks to hydrogen peroxide). This drop of efficacy seems to be reproduced by the RAT results: all (n = 3) negative at 20 min, but already positive (n = 3) at 60 min.

CHX performed badly both in WMF and RD after 180 min (0.43 and 0.59 log 10 reduction). However, the study was not powered to compare directly the efficacy of the three different mouthwash formulations with one another.

Despite a lot of limitations, the study showed for the first time that mouthwash is able to abolish viral load in respiratory droplets c*ompletely* after 20 min (at least in case of PVP-I and HP; CHX: untested) and *to a high extent (but not completely)* also after 60 min (PVP-I and HP;

CHX: untested); after 90 and 180 min, the effect is still strong in HP, but not in CHX (not tested for PVP-I). Comparing and correlating RAT and qRT-PCR results, the data allow the conclusion that both PVP-I and HP are “fully sterilizing” on respiratory droplets at 20 min, but no longer at 60 min.

Most important, these favorable results were obtained by simple mouthwash; “patients were instructed to swish 15ml of the mouthwash for 30 seconds with cheeks pouched“; there was no gargling (B. GITA, pers. comm.).

Perioplus+ mouthwash (cyclodextin 0.1 %; citrox 0.01 %)

In a RCT with 176 adult PCR-positive participants, either asymptomatic or with mild symptoms and not more than 8 days from symptom onset at the time of inclusion, mouthwash with bioflavonoid-based Perioplus + (β-cyclodextrin 0.1% and citrox 0.01%; 30 ml per mouthwash) for 1 min had a significant beneficial effect on salivary viral load 4 hours after the initial dose, by reducing viral load by 71 % (CARROUEL et al. 2).

The second dose 4 or 5 hours later maintained the lower level in the verum group and the difference compared to placebo. However, three daily rinses had a beneficial effect on the salivary viral load 7 days after the initial intake preferentially in adults with high salivary viral loads at baseline, while the trial provided unclear evidence for the general population independent of the initial viral load.

In summary, Perioplus+ provided only modest benefit compared with placebo in reducing viral load in saliva, preferentially in patients with high salivary viral load.

The study offers a RCT-based proof of principle that mouthwash can actually reduce salivary viral load not only for a short time (as may be necessary for e.g. a dental treatment session) but also for hours, and, if repeated 3 times a day, also long-term in persons with initially high viral loads; however, the effect of Perioplus+ on the salivary viral load is only modest (less than 1 log10, i.e. less than 90 %), and it remains unknown whether such a modest reduction of viral load may be of any clinical significance on symptoms, outcome or infectivity.

It would have been interesting to compare Perioplus+ to other mouthwash types within the same RCT study design. Nevertheless, compared to “stronger” mouthwash, Perioplus+ has the advantage that its ingredients are not supposed to disturb the physiological microbiome of the oral system.

Acetic acid inhalation (therapeutic)

PIANTA et al. reported about their small controlled trial of acetic acid inhalation in mild COVID outpatients at home. 35 ml of vinegar (with a content of 6 % acetic acid) was given into 500 ml of boiling water and inhaled for 10 minutes twice a day, either with a simple apparatus for inhalation or with head and nose above a saucepan and some cover over the head (in that case, one needs protection for the eyes). The authors calculated a concentration of 0.34 % acetic acid in this situation.

There were two groups; all patients got hydroxychloroquine. The control group, who didn't inhale acetic acid, got lopinavir/ritonavir beside of HCQ. Thus this trial compared acetic acid inhalation to lopinavir/ritonavir in patients who got HCQ as basic treatment. Again, this was only a very small trial. There were 14 patients in the acetic acid group and 15 patients in the lopinavir/ritonavir group. No side effects of acetic acid inhalation were reported.

	Acetic acid N = 14	Lopinavir/Ritonavir N = 15
hospitalization	0	1
improvement of symptoms after 15 days	100 %	92,9 %
PCR-negative at day 15	80 %	53,8 %*
total number of symptoms (cumulative for all patients)	47	50
symptoms still present on day 15	17 %	38 %
dto., but calculated without symptoms associated with smell and taste**	12,5 %	34,1 %
		*of 13 patients

**which may take some time to recover because they are neurological symptoms

The number of participants was too small for statistical testing; however, taking all results together, there is an obvious trend that acetic acid inhalations are superior to lopinavir/ritonavir – an antiviral that is well known for its unpleasant side effects (mostly gastrointestinal) in the case of short term treatment; in larger clinical trials with lopinavir/ritonavir, there are usually some patients who stopped taking it because of these side effects. And in the WHO Solidarity trial, it was found to be absolutely ineffective in hospitalized patients with regard to important outcomes (like mortality) (WHO Solidarity Trial Consortium). However, uselessness in hospitalized (and thus more progressed) patients doesn't mean evitably that it is equally ineffective if started early in outpatients.

Nasal irrigation with hypertonic solution (with or without surfactant)

KIMURA et al. reported about interim results from a RCT evaluating nasal irrigations in mild or moderate outpatients, starting shortly after diagnosis (median symptomatic days before diagnosis: 2 – 2.5 days). There were three groups of patients: 17 controls (no intervention = NI), 14 patients performed twice daily irrigation with 250 ml hypertonic saline solution* (salt concentration not given) (= HTS), and 14 patients performed the same with hypertonic saline solution with 1 % surfactant** (= HTSS). Participants also performed scheduled nasal (mid-turbinate) swabs (results not available to far) and recorded daily temperatures and symptom scores over 21 days. All three groups were similar in structure based on statistical calculations, but there was a trend to older age (~ + 6 years) and more comorbidities in the NI group.

*240ml of distilled water with 2 packets of NeilMed brand buffered salt (NeilMed Pharmaceuticals; Santa Rosa, CA).

**½ teaspoon (2.5 ml) surfactant (Johnson's Baby Shampoo; Johnson & Johnson Inc.; New Brunswick, NJ)

Significant differences in median days to symptom resolution were found for nasal congestion (NI: 14 days, HTS: 5 days, HTSS 7 days; p = 0.04) and headache (12 vs. 3 vs. 5 days, p = 0.02) and (statistically insignificant) trends with regard to cough (14 vs. 7 vs. 6 days) and quicker symptom improvement "compared to yesterday" (14 vs. 7 vs. 7 days).

There are no reports about hospitalizations or progression to severe/critical disease, so obviously this didn't happen in either group. However, altogether 9 patients (3 in each group) are lost to follow up. Nothing is said about their fate. This is a serious limitation as long as it is not clear whether they were lost as a consequence of a bad outcome.

There were no improvements in the irrigation group compared to the control group with regard to "altered smell/taste", "think clearly", "sleep well", "breath easily", "walk/climb stairs", "accomplish daily activities", "work inside home", "muscle/joint pain"; and with regard to "fatigue" only in the HTS group, but not in the HTSS group.

Thus it looks that only symptoms of the upper respiratory tract (like nasal congestion, cough) and its close proximity (headache) seem to improve following nasal irrigation. As a neurological symptom, a quick relief from smell/taste alterations cannot be expected. More general or more distant symptoms don't seem to be influenced by the procedure.

Moreover, there is no evidence that the addition of the surfactant is more effective than hypertonic saline solution alone. For 7 items, median time until symptom resolution was the same in HTS and HTSS, for 4 items it was shorter in HTS compared to HTSS by 2 – 7 days, and for 3 items it was longer in HTS compared to HTSS by 1.5 – 4 days.

The means of the median days until symptom resolution for 13 symptoms (except "compared to yesterday") was 10.1 days in the control group, 8.5 days in the HTS group and 9.0 days in the HTSS group. Thus the overall difference isn't large and it becomes evident that the quicker improvement is confined to local symptoms. Excluding nasal congestion, headache and cough from the analysis, the means of the median days until symptom resolution for 10 symptoms are 9.0, 9.6 and 9.9 days. Thus it is evident that the procedure has no effect on general symptoms of the disease. Excluding smell/taste as another local symptom (though its quick resolution cannot be expected due to its neurogenic origin), the association stays the same (9.0, 9.3 and 9.7 days).

Whereas the highest individual symptom score in the control group was 6.0 in one participant by day 7 (and quickly improved), there was one participant in each intervention group with higher symptom scores (between 7 and 7.5), and the high scores lasted longer than in the individual from the control group.

Though the results of the intervention group were statistically significant with respect to nasal congestion and headache, the overall results are not so impressive. Maybe one cannot expect more than local symptom improvements from such a procedure; however, at least in theory, as mentioned above, it was suggested that local antiviral (antiseptic) effects in the upper respiratory tract may reduce overall viral load and its distribution to the lungs and other parts of the body, thus reducing general symptoms and accelerating their resolution. But this didn't happen in this study.

However, this is only an interim analysis and one has to wait until the full results, including viral loads, are available. Until more data are available, one may consider the possibility that

the procedure accelerates relief of local symptoms without altering the course of the disease and its prognosis, though the latter would be most important. General symptoms don't seem to be affected by the procedure, including symptoms which are associated with lung disease ("breath easily").

As discussed elsewhere in this paper, there are concerns that nasal irrigation may be unfavorable because of the removal of protective natural agents like immunoglobulins, interferons, lysozymes, defensins from the surface of the mucosa. To find out whether this is true, one should repeat the same study with the same agents, but administrating them as a nasal spray in an additional study arm, with nasal irrigation as comparator. It would be interesting to see whether this makes a difference.

KIMURA et al. point to caution with regard to nasal irrigation for patients who cannot isolate themselves well: irrigation can potentially disperse viral particles or contaminate surfaces in the vincinity, and virus can remain viable on plastic or metal surfaces for days. They enrolled only patients into their study who could self-isolate and perform irrigation in a separate bathroom.

A detailed description of the saline water procedure (nasal irrigation and gargle) and its theoretical background and experiences in other respiratory diseases were given by PANTA et al. (and also SINGH et al.). However, PANTA et al. gave no concrete recommendations about the salt concentrations except for quoting a range from 1.5 to 3.0 %, and they present no clinical study results in the context of COVID-19. They consider the possibility that saline solution may be superior to PVP-iodine because the latter *"may injure pharyngeal mucosa due to its cytotoxic effects, altering microbial flora dynamics thereby, enabling the settling, entry, and invasion of bacterial pathogens and viruses."* However, they don't consider that the cytotoxic effect of PVP-I is concentration-dependent, and, as mentioned elsewhere is this paper, PVP-I should not exceed 1.25 %. As TSUDA et al. showed in a RCT, topical application of 10 % PVP-I in the oral cavity did not disrupt the balance of the oral microbiota.

With regard to COVID-19, it was found meanwhile that 1.5 % NaCl inhibited SARS-CoV-2 *in vitro* by 100 % in Vero cells, not by destroying the virus itself, but by rapid depolarization of the cell membrane. Since the membrane potential is critical for viral entry, hypertonic saline impairs one or several steps of the viral intracellular cycle. The effect is dose-dependent; 1.2 % NaCl inhibited SARS-CoV-2 by 90 %, so 1.5 % have to be regarded as the minimal dose for complete inhibition (MACHADO R et al.). The authors recommend inhalation of hypertonic saline solutions of 1.5 % NaCl or greater for treatment of COVID-19.

Xlear nasal spray (nasal spray with xylitol + grapefruit seed extract)

GO et al. presented a case series of three symptomatic COVID-19 patients with mild to moderate risk for severe disease/bad outcomes because of underlying comorbidities (ages: 16, 38 and 60 years) who were treated with Xlear nasal spray as an adjuvant to their ongoing treatment for seven days (twice per nostril four times a day every six hours for seven days). They showed rapid clinical improvement and a shorter time until negativization of repeated intranasal swab tests (all negative at day 7 instead of an average of 14 days as supposed as common by the authors), without any safety issues.

However, with only three patients (only one of them in a higher risk category) and in the absence of controls, it is impossible to decide whether the same favorable course would also have occurred in the absence of the Xlear intervention. Except for the quick negativization of PCR tests, there was no immediate cure of symptoms after initiation of Xlear. Most apparent was a comparatively quick improvement of ageusia and anosmia that usually takes a longer time. *In vitro* experiments demonstrated meanwhile a high virucidal activity of Xlear nasal spray against SARS-CoV-2, and the active component seems to be the grapefruit seed extract. Xlear achieved complete inhibition of viral infectivity *in vitro* in the concentration of 90, 80 and 60 %, and still a reduction of 2.17 log10 viral titer in the 20 % condition (FERRER et al.).

Taffix nose spray (Nasus Pharma, Israel) (for prophylaxis)

At the end of November 2021, the double-blind placebo-controlled RCT NP-003 was termed prematurely because of superiority of the tested Taffix nose spray compared to placebo.

Taffix is a powder nasal spray with HPMC, citric acid, sodium citrate, benzalkonium chloride and menthol. It was developed against various viral infections of the upper respiratory tract. The powder from the spray (hypromellose) forms a thin acidic gel layer above the nasal mucosa within 50 seconds after administration that persists for 5 hours and blocks viruses from infecting nasal cells (MANN BJ et al.).

The study is based on 521 unvaccinated and healthy partcipants (18 – 65 years) with negative SARS-CoV-2 serology at inclusion (n = 260 Taffix, n = 257 placebo) from Bulgaria. Following randomization, the participants were examined twice weekly for the occurence of cold or infection of the upper respiratory tract. In case of symptoms suggestive of COVID-19, a PCR test was performed. At the end of the study period, a second antibody test was performed (if positive, a PCR test was also performed).

Results:

Symptoms of infection of any upper respiratory tract: 38 (Taffix) vs. 67 (placebo) (p = 0.002) (14.6 % vs. 26.1 %)

COVID-19 infection: 7.3 % (Taffix) vs. 11.3 % (placebo) (risk reduction: 35 %). The difference is only a trend since it missed significance (p = 0.079)

Source (results so far unpublished in a scientific paper; 17.12.2021):

https://www.finanzen.net/nachricht/aktien/nasus-pharma-gibt-klinische-daten-bekannt-die-die-wirksamkeit-von-taffix-einem-intranasalen-antiviralen-schutz-gegen-erkaeltungen-und-infektionen-der-oberen-atemwege-belegen-10779927

The modest results from that RCT contrasts to the more favorable results from a mass-gathering of the ultra-orthodox community in Bney Brak city that topped Israel's COVID-19 infection rate and mortality (SHMUEL et al.). In the prospective users survey, 243 members of that community that participated in two days prayers (supposed to become a "superspread event") were followed for 14 days. 83 of the 243 participants used Taffix throughout holiday's prayers and the following two weeks (ITT); 81 used it regularly (PP); two used it rarely if at all. 160 participants did not use Taffix.

COVID-19 infection within/after 14 days:

0/81 from the PP, 2/83 (2.4 %) from the ITT and 16/160 (10 %) from non-users. OR for Taffix users: 0.22, risk reduction 78 % (CI: 1- 95 %). No side were effects reported.

In vitro, Taffix reduced viral RNA from three VoCs (Alpha, Beta, and Delta) by 99.9 % in a VeroE6 cell assay (MANDELBOIM et al.). This impressive *in vitro* result then translated to only a ~ 35 % risk reduction of symptomatic, PCR-confirmed COVID-19 in the NP-003 RCT.

Thus it would be very interesting to compare Taffix to a carrageenan-based nasal spray instead of placebo in a RCT setting of people with high risk to acquire COVID-19.

Iota-Carrageenan nasal spray (for prophylaxis)

Carrageenan forms a protective gel-like layer on top of the mucosal lining and inactivates most of the viral particles which settle down on the mucosal surface, but without damaging

the normal physiological microbiota there (since carrageenan is no antiseptic/ decontaminant), but providing a sort of physical barrier against viral entry into the cells. Carrageenan is a sulphated polysaccharide which cannot penetrate mucosal membranes (HUI KK). Its efficacy against SARS-CoV-2 was demonstrated *in vitro* (MOROKUTTI-KURZ et al., VEGA et al., JANG et al.; for these references and a more detailed discussion about carrageenan see the "early therapy paper").

VARESE et al. studied the antiviral activity of iota-carrageenan (in 0.9 % NaCl) against SARS-CoV-2 on Calu-3 cells (that are very similar to human respiratory epithelial cells and thus provide a much more adequate assay compared to Vero cells). Whereas 0.06 microgram/ml was inefficient, 0.6 microgram/ml was associated with a reduction of SARS-CoV-2 replication by a little more than one order of magnitude, 6 microgram/ml with a reduction between 2 and 3 orders of magnitude, and both 60 mg/ml and 600 mg/ml with at least 4 orders magnitude.

Iota-Carrageenan (I-C) nasal spray was studied for prophylaxis in a placebo-controlled double-blind RCT from Argentinia (FIGUEROA et al., NCT04521322, CARR-COV-02). The trial was performed in late summer 2020 before the start of vaccinations and before the occurrence of VoCs in Argentinia.

The spray contained 1.7 promille I-C (in 0.9 % NaCl) (the product is available on the market in Argentinia). Participants were hospital personnel (~ 49 % physicians) dedicated to care of COVID-19 patients (working in a "COVID hot zone"). I-C sprays was administered four times a day (1 puff for each nostril) over a period of 21 days. Primary endpoint was clinical COVID-19, confirmed by PCR.

The RCT encompassed 394 participants with similar baseline characteristics between I-C and placebo group. Placebo was nasal spray 0.9 % NaCl. Mean age of participants: 38.5 years.

12 of the 394 participants developed symptomatic, PCR-confirmed COVID-19, 2/196 vs. 10/198 (1.0 vs. 5.0 %). Incidence of PCR-confirmed COVID-19 was 1.0 % vs. 5.0 % (OR 0.19; CI: 0.05 – 0.77; p = 0.03).

40 participants underwent a PCR test because of symptoms that were compatible with COVID-19. 31 tests were negative (7.6 % of all participants in the I-C group and 8.6 % of the placebo group).

Business day losses were lower in the I-C group (0.5 % vs. 2.0 %, p < 0.0001, censored at day 21). No hospitalization. There were no differences in side effects like headache or rhinorrhea or suspension because of intolerance between the I-C and the placebo group.

In a sensitivity analysis, individuals who presented symptoms < 7 days after randomization (i.e. who may have been infected before the first carrageenan administration) were excluded. In that calculation, risk reduction was 95 % (CI: 6.0 – 99.7 %, p = 0.04; OR 0.05; CI:

0.003 – 0.9, p = 0.04). This may be explained because the first case in the I-C group developed symptoms 2 days after randomization, the other one 4 days after randomization, what makes it highly probable that at least one individual and maybe also the second individual catched the infection prior to randomization.

However, there are some limitations of that study. Asymptomatic participants were not PCR tested; thus this study doesn't allow conclusions about prevention of asymptomatic infections. Antibody testing was not performed. Only one PCR test was performed between 48 and 72 hours after symptom onset. Altogether, 8.6 % vs. 13.6 % had symptoms that might be associated with COVID-19, but only 1.0 % vs. 5.0 % had PCR-confirmed COVID-19. In summary, there was a reduction of symptomatic disease by 37 %, and this consists of 12 % reduction of PCR-negative symptomatic disease and 80 % (or even 95%) reduction of PCR-positive disease.

In 2014, KOENIGHOFER et al. demonstrated in two randomized double blind placebo controlled trials that iota-carrageenan nasal spray had significant effects in acute common cold. It shortened the duration of the disease, the number of relapses and accelerated virus clearance. 46 % of the patients in that study suffered from human rhinovirus, 25 % from human coronavirus, and 14 % from influenza A virus. Most important, the effects of iota carrageenan were much more pronounced in coronavirus infections than in other infections.

Finally, it was already shown that iota and kappa carrageenan in saline irrigation solutions are safe and non toxic and have no detrimental effects on epithelial barrier structure and ciliary beat frequency. Moreover, kappa carrageenan increased the transepithelial electrical resistance and suppressed IL-6 secretion (RAMEZANPOUR et al.). There are already nasal sprays available with both iota and kappa carrageenan – a combination which seems to make sense.

What about VOCs, FRÖBA et al. demonstrated *„by using a SARS-CoV-2 spike pseudotyped lentivirus particles (SSPL) system and patient-isolated SARS-CoV-2 VOCs to infect transgenic A549ACE2/TMPRSS2 and Calu-3 human lung cells“* that *„Iota-carrageenan exhibits antiviral activity with comparable IC_{50} values against the SARS-CoV-2 Wuhan type and the VOCs“* Alpha, Beta, Gamma and Delta; thus it *„might be effective for prophylaxis and treatment of SARS-CoV-2 infections independent of the present and potentially future variants.“*

Inosine-glutamyl-cysteinyl-glycine disodium solution (Molixan) inhalation

A controlled trial (registered: ISRCTN34160010) from a Russian hospital showed preventive effectiveness of inhalation of Molixan solution (inosine-glutathione; for parenteral use for the treatment of viral hepatitis) mixed with 4 % potassium chloride solution in HCWs, four times a day for five minutes, every 4 hours, for 14 days (DUBINA et al.).

1.0 ml inosine-glutathione solution (produced for parenteral use) and 0.25 ml potassium chloride solution were mixed before each inhalation to yield a solution with a content of 21.3 mg/ml glutathione, 8.7 mg/ml inosine in 107 mM potassium solution, administered as aerosol by a personal handheld nebulizer (Nebzmart, MicroBase Technology, Taiwan).

99 HCWs who were highly exposed to COVID-patients performed this procedure for 14 days, whereas a control group of 268 similarly exposed HCWs from the same hospital did not. The participants were selected randomly. Mean age was 27 years; 69 % female, 51 % nurses. All participants and controls were PCR- and sero-negative at baseline.

During the study period, 2/99 (2 %) HCWs of the inhalation group and 24/268 (9 %) from the control group were found to have been infected either by PCR or IgG/IgM testing ($p = 0.02$). Hazard ratio 0.23. Among the two positive cases in the inhalation group, one was detected as positive on day 6 of the intervention and the other 6 days after the intervention was stopped (it was confined to a time frame of 14 days).

10.5 % of HCWs were already SARS-CoV-2-positive when the study started; they were not included in the study.

No serious side effects were reported. It is suggested that inosine inhalation has antiviral effects through the incorporation of inosine into the double-stranded viral RNA and through potentiation of immune system sensing (DUBINA et al.). The authors suggest that this procedure may be also very effective for treatment.

Though the procedure is time-consuming (20 minutes per day + time for preparation of the final solution for nebulization) and thus not easy to replicate, it is a proof of principle for the effectiveness of nebulization procedures in PREP (or PEP). A serious limitation of that study is that the mean age of the participants was quite young (27 years) and it would be interesting to see whether the procedure is also effective in elder persons and when administered over a longer period of time.

Interferon alpha 2 b inhalation

In contrast to the favorable results of IFN beta inhalation of SNG 001 (SYNAIRGEN, so far unpublished), **IFN alpha 2 b inhalation** treatment starting after admission to hospital in a Chinese study was less impressive (HAO et al.). The inhalations shorted shedding time of SARS-CoV-2 significantly (10 vs. 13 days, p = 0.014), but this association became insignificant after propensity-score matching (12 vs. 15 days, p = 0.206). Among patients who did not use glucocorticoids, virus shedding time was 13 vs. 12 days (IFN vs. control); in mild cases, virus shedding was 9 vs. 12 days (p = 0.089).

The outcomes showed a trend to be more favorable both in the unmatched and matched IFN groups compared to the controls, but the control groups had a higher portion of critical patients on admission. Even after propensity-score matching (n = 32 IFN and n = 32 control), the percentage of critical patients was 12.5 % in the IFN group, but 28.1 % in the control group. The percentage of mild patients was similar (56.3 vs. 53.1 %). Thus lower rates of ARDS (43.8 % vs. 59.4 %, p = 0.211), mechanical ventilation (25.0 % vs. 37,5 %, p = 0.281), ECMO (12.5 % vs. 18.8 %, p = 0.491), ICU admission (21.9 % vs. 50 %, p = 0.019) and higher discharged rates (78.1 % vs. 62.5 %, p = 0.171) and lower hospital time of discharged patients (16 vs. 21 days; p = 0.084) in the IFN group (after matching) may be in part due to confounding by a smaller portion of critical patients on admission among those who got IFN. However, the difference between ICU admission rate between both groups is so large what this cannot be explained solely by the portion of critical patients, indicating a true preventive effect of IFN inhalation for this outcome. Altogether, the results for IFN alpha 2b inhalation are rather disappointing. But there may be trends for a small advantage, and the study was probably underpowered to find out whether these small effects are significant.

Inhalative interferon beta (SNG001) was found to be highly effective to prevent progression to bad outcomes like ventilation or death (OR 0.21), and was able to shorten the time of hospitalization and recovery in a placebo-controlled trial. However, the data are from press releases from the producer (SYNAIRGEN), and the results from the study haven't been published so far. The interim results reported of 101 patients (outpatients and hospitalized patients) who used nebulized SNG001 or placebo once daily for 14 days. OR for death or ventilation was 0.21 (CI: 0.04 – 0.97). Hazard ratio for full recovery, defined as unlimited ability for activities, was 2.19 (CI: 1.03 – 4.69) under IFN inhalation. There were 6% deaths in the placebo group and no deaths in the SNG001 group. According to SYNAIRGEN, SNG001 was effective independent of the duration of the disease until the inhalations were started (https://www.aerzteblatt.de/nachrichten/114853/COVID-19-Inhalatives-Interferon-beta-erzielt-in-erster-Studie-gute-Wirkung).

TaibUVID inhalation/nebulization

Besides oral intake of TaibUVID (a combination of *Nigella sativa* powder, chamomile powder and lots of natural honey), EL SAYED et al. reported about favorable effects of TaibUVID inhalation and described in detail the preparation of the inhalation (10-15 g *Nigella sativa* seeds, 2-4 g *Anthemis hyalina*, 2-5 g costus, 500 ml water, 1 ml clove oil). The inhalation therapy *"alleviated respiratory manifestations e.g. cough and respiratory difficulty and was life-saving in some cases."* (EL SAYED et al.). However, since only 13 patients used the inhalation therapy (all of them in combination with oral TaibUVID), it is impossible to evaluate the effects of the inhalation therapy on its own.

Discussion

In summary, there are so far favorable results for (i) PVP-I oral rinse and gargle (both 1 % PVP-I), (ii) "original" Listerine gargle, (iii) 3 % H_2O_2 administration into the nose (once), followed by daily nasal wash with hypertonic saline solution, (iv) chlorhexidine 0.12 % after and 2 hours, but only with regard to saliva samples, (v) diluted acetic acid inhalation, (vi) inosine-glutathione aerosol inhalation and (vii) interferon beta (SNG 001) inhalation.

These activities resulted in (i) a few (1-2) or several (> 3) hours of strong reduction of RNA copy number in saliva in persons with initially high salivary viral (RNA copy) load (PVP-I); (ii) acceleration of viral clearance in oropharyngeal/nasopharyngeal swabs in recently diagnosed asymptomatic infected persons (PVP-I, Listerine); (iii) long-time suppression (several days) of PCR positivity in nasopharyngeal swabs in people with long-lasting or reactivating PCR-positivity, and possible in definite viral clearance in some of them (3 % hydrogen peroxide and hypertonic saline nasal wash); (iv) a quicker amelioration of symptoms and PCR results in infected persons with mild disease (acetic acid inhalation), (v) better outcomes with regard to death, ventilation, recovery, hospital discharge in ill patients (IFN beta SNG001 inhalation), and (vi) preexposure prophylaxis with a hazard ratio of 0.23 in highly exposed HCWs (inosine-glutathione inhalation, but with the need for a time-consuming procedure).

However, these are all very small trials and punctual observations in special situations, and they don't enable any generalization or far-reaching conclusions. Much more of these "small points" on an empty map have to be collected until a more complete picture may arise what can be achieved (or *not* achieved) by local antiseptic/decontaminating methods, and what are the best methods, and *when*, *where (nasal vs. oral)*, *how (drops, spray, gargle, irrigation, inhalation/nebulization), how often* and *using what concentration* should they be applied, both with regard to treatment, avoidance of progression, viral clearance and reduction of infectivity on one side and chemoprophylaxis in exposed people on the other hand. There are too many variables and aspects which will make it very difficult to draw final conclusions as long as the multiplicity of variables is not addressed in large and multi-armed trials.

It is surprising, somehow unexpected and promising that simple decontamination methods may accelerate viral clearance so effectively (like in MOHAMED et al.) in freshly diagnosed patients, and that they also offer a chance for viral clearance in some patients with chronic or reactivated PCR positivity (CAPETTI et al.), though it is not clear in the latter trial whether the same could have happened spontaneously too. In contrast to these doubts with regard to the CAPETTI trial, the effect of PVP-I gargle on viral clearance in MOHAMED et al. was impressive and statistically significant and hard to explain by chance if one considers both “no gargle” and “tap water gargle” as controls.

However, it is not absolutely unplausibel that local decontamination methods may eradicate the virus more effectively than systemically (orally) administered antiviral agents as long as the infection is confined to the nasal/naso-/oropharyngeal area. If the infection has already disseminated into the lungs or other organs, local decontamination will come too late. It may then still contribute to the local eradication at the site where decontamination took place, but local PCR negativity won’t help a lot when infection persists elsewhere. It is already well known that specimens from the lower respiratory tract (e.g. BAL) are PCR-positive for a longer time than those from the upper respiratory tract, and the same seems to apply for gastrointestinal specimens (e.g. stool). In the case of a more generalized infection, local decontamination in the nose and pharynx area may still make sense in order to reduce infectivity and possibly local symptoms, as demonstrated by KIMURA et al., but it seems unlikely that it will still have a fundamental effect on the further course of the disease which is now dominated by viral infections of lungs or other organs, and possibly already signs of hyperinflammation, cytokine storms and coagulation disorders.

That said, the results from MOHAMED et al. raise the hope that local decontamination may play an important role in the early stage of the disease, and that it may be strong enough to eradicate the infection in some or many cases, if practised early enough. Much more and larger trials are urgently needed, but since PVP-I gargle (1 %) is cheap and easily available and well tolerated for people without contraindications, it should be recommended in the meantime to everybody with suspected or confirmed COVID-19 infection. Listerine Cool Mint may be an alternative for those who don’t like PVP-iodine or who have contraindications. In general, it is not recommended to use alcohol-containing mouthwash more than once a day in the long run (especially in smokers). However, in the case of suspected or confirmed COVID infection, exposure to Listerine 3 times a day would not be a chronic condition but restricted to at most 2 weeks, thus it seems feasible. Fortunately, the alcohol-free version of Listerine, Listerine Cool Mint Mild, was also found to be effective (KRAMER et al.).

Whereas little attention had been directed towards oral gargle in clinical trials, data for nasal decontamination are even more sparse. A detailed systematic review of the pre-COVID evidence for nasal irrigation with regard to upper respiratory tract infections is given by SINGH et al.; however, since COVID-19 has a very special pathogenesis and immunopathogenesis, there is a need to be careful with regard to results which were

obtained in the context of other URT infections. Thus the trials which are reported by SINGH et al. were not included in this living review which is limited to trials with COVID-19, but the SINGH review is very valuable because it summarizes all pre-COVID evidence in a systematic manner.

But as the primary port of entry in most cases, nasal decontamination is suggested to be of prior significance for chemoprophylaxis in COVID-19, and any form of local chemoprophylaxis should involve both the nasal tract and oropharyngeal gargle/spray, or inhalation/nebulization as a single procedure (e.g., MADY et al.). So one may really wonder how MOHAMED et al. achieved their good results in early asymptomatic COVID patients solely by oropharyngeal decontamination, without any procedure for the nose. May oropharyngeal gargle impede the expansion of the nasal/nasopharyngeal infection downwards in the airways? This sounds very optimistic and probably too simple, but the results from MOHAMED et al. are statistically significant and the study had a control group, thus the evidence from MOHAMED et al. is *comparatively* better than evidence from many other trials mentioned above.

The dominant problem which impairs the interpretation of the trial results is the difference between PCR positivity and the presence of viable, infectious virus. It is accepted meanwhile that the detection of viral RNA by PCR must not necessarily mean that this RNA is from a viable and replication-competent virus. For example, it is generally communicated that one was unable so far to culture SARS-CoV-2 from PCR-positive specimens more than 10 days after diagnosis; maybe the virus is captured by antibodies at that time and unable to replicate. (However, in a study from Sweden, virus was cultured from 3 patients 11 days after the onset of symptoms and from one immunocompromised patient 16 days after symptom onset, though this must not necessarily contradict what is said about infectivity if one takes the time of diagnosis as starting point, except for immunocompromised patients. Moreover, culture included sputum specimen besides nasopharyngeal specimens) (GLANS et al.).

Thus PCR positivity is no prove for infectivity especially in the case of high Ct values, and PCR positivity directly after administration of a strong antiseptic like PVP-I may not necessarily prove the presence of viable virus independent of Ct value.

Detection of viable, infectious virus is methodically demanding and expensive due to the need for special laboratory security levels (level 3 or more). Moreover, as GOTTSAUNER et al. pointed out (based on data from WÖLFEL et al., ref. 36 in GOTTSAUNER), it is difficult to culture virus from specimens with a viral load below 1.000.000 RNA copies/ml (“hardly yielding successful culture”). This is a serious methodical limitation, since the inability to culture virus from oral/pharyngeal samples (e.g. saliva or swabs) doesn’t mean that viable virus is really absent. The problem to distinguish between defective virus and infectious virus

in the aftermath of a decontamination procedure is a main obstacle for the interpretation of trial results.

As a consequence, one has to take RNA copy load or PCR Ct number as proxies for viral load. After decontaminating procedures which are supposed to inactivate the virus, it is then a pure matter of safety to assume that it represents (or may represent) viable and infectious virus, though this may be too pessimistic and not true, since RNA copies may stem from virus that is defective and inactivated. This problem will persist until new methods will be able to detect viable, infectious virus also in specimens with low viral RNA copy load.

The urgent need to distinguish between defective virus/RNA and viable/infectious virus is demonstrated very well in the study from MARTINEZ LAMAS et al., where one may wonder why 5 minutes after PVP-I administration, RNA copy load is as high as before PVP-I gargle, whereas it was reduced during the next 1-3 hours and beyond. Since the virucidal effect of PVP-I even in very low concentrations is established very well *in vitro* (much better than for any other agent discussed here), and PVP-I inactivates SARS-CoV-2 within seconds (for details and references, see the “early therapy paper” mentioned above), it is not plausible that all viral particles can survive the PVP-I gargling procedure for five minutes and more and become eliminated only slowly during the next hours. Like hydrogen peroxide, PVP-I also has an oxidizing effect. So one may ask whether the RNA detected 5 minutes after PVP-I gargle was RNA from defective virus, but RNA was still intact enough to be replicated in the PCR procedure, whereas the reduction in the RNA counts during the next 1-3 hours may correspond to an increasing degree of full degradation of that damaged RNA; the longer the time since the PVP-I intervention, the more of the original RNA may be degraded so much that it cannot be recognized as COVID-19-derived RNA any more during PCR.

Accessibility of different areas of the upper respiratory tract by antiseptics

In the case of local antisepsis to kill the virus, reduce viral load, reduce infectivity and suppress the expansion of the infection beyond the area of its entry, it is important to cover all possibly infected or virus-carrying/-shedding mucosal areas of the upper respiratory tract by the disinfectant/antiseptic.

As far as the epithelium of the nasal cavity and nasopharynx is concerned, nose drops, nasal spray and nasal irrigation (nasal lavage, nasal douche) are simple methods. Because of expression of high levels of ACE2 and TMPRSS2 proteins in this area, decontamination of the nasal area seems to be most important, both for early treatment and also in the case of prophylaxis/PEP.

The role of the oropharynx as the primary area of infection and port of entry seems to be less clear; however, one should not forget about mouth breathing.

In an inoculation experiment with Syrian hamsters, oral inoculation was established successfully, but the animals developed only subclinical infection with mild pneumonia and without weight loss, in contrast to hamsters which were inoculated by the same viral dose intranasally. Lung histopathology score and viral load in the lungs were lower, whereas virus shedding in oral swabs and faeces was similar to hamster that were infected intranasally (LEE et al.). The results suggest the importance of both infection routes (nasally and orally), though the nasal route seems to be more critical with regard to severe pneumonia and pulmonary viral load (at least in the animal model), whereas there seem to be no differences in oral viral load which may be more relevant with regard to infectivity to contacts e.g. by speaking or coughing. If may be a sort of oversimplification, but one may hypothesize that antiseptic treatment of the nasal area is more in favor of someone's own protection, whereas oral antisepsis (oral spray, gargle) is more in favor for the protection of others, maybe with regard to speaking, coughing or preprocedural antisepsis in dentistry.

Anyway, the oropharynx has to be passed when virus particles or mucosal infection expands downwards from the nasal area/nasopharynx in the direction of the lower airways. Thus, beside the nose, the oropharynx is another area which is accessible to local interventions. It can be reached by gargling or throat spray.

Antiseptic treatment of the oropharynx may be especially important to reduce infectivity for some time, especially with regard to speaking/coughing (infectivity by the droplet route), whereas exhalation over the nose may play a dominant role for infectivity by the aerosol route. Whereas infection as a result of speaking, singing, crying, coughing, sneezing, intimate kissing, i.e. the *droplet-dominated route*, may be acquired in a short time contact with an infectious person, possibly in a short moment, the *aerosol-dominated route* (preferentially via nose, secondarily via small droplets which become smaller and more aerosol-like when they dehydrate as long as they are still in the air) may need some time until aerosol (and thus viral) concentration accumulated beyond an infectious threshold in a closed and badly ventilated room.

The regions of the respiratory tract below the oropharynx cannot be reached *directly* by home-based methods. Lozenges (and swallowing saliva with the contents of lozenges) may still reach a small area below the anatomical borders which can be reached by direct contact during gargling (see LIMB et at). However, PVP-I cannot be recommended to be swallowed (whereas carrageenan could be). Therefore, at the moment, the only possibility to reach deeper parts of the airways (below the oropharynx) are deep inhalations of acetic acid as described by PIANTA et al., but there are some other promising candidates for nebulization/inhalation procedures for which so far no clinical study results are available, but they are subject to ongoing clinical tests (e.g., iota-carrageenan inhalation, interferon inhalation SNG001).

Thus it is an important question which methods for the application of antiseptics/ disinfectants are most suited in order to reach as much mucosal area of the oropharynx and nasal tract as possible.

These questions are reviewed in detail in a German paper (accessible: **http://freepdfhosting.com/1c7f0ba1e1.pdf).**

To shorten this here, only the final results are reported:

Oropharynx

In order to reach the posterior parts of the oropharynx well, throat spray is more effective than gargling in most people. However, due to differences in the individual capabilities to gargle deeply, and also for anatomical differences (e.g. size and position of the tongue, individual differences in the shape of the pharyngeal area), there are some people for whom gargling is superior to spraying. As a consequence, though throat spray seems to be superior for most cases, the combination of gargling, followed by spray (to create a small „depot" of the agent which is not spit out directly) seems to be the most effective method (included in the review were: PATEL et al., LIN et al., LIMB et al.). Moreover, since ACE2 expression at comparatively high levels was also found in the oral mucosa (SRINIVASAN et al.), gargling/mouthwash combined with spray might be most effective to reach the oral and oropharyngeal area. As shown in the table below, based on the results of LIMB et al., gargling was found to be the least effective method in all situations, both for oral cavity and oropharynx:

Effectiveness in the oral cavity during the first 10 minutes after application:

Lozenges, chewable tablets >>> spray >>> gargle

Effectiveness in the oral cavity, cumulated over 120 minutes:

lozenges >> chewable tablets >> spray >>> gargle

Effectiveness in the oropharynx during the first 10 minutes:

spray >> lozenges, chewable tablets >>> gargle

Effectiveness in the oropharynx, cumulated over 120 minutes:

spray >> chewable tablets >>> gargle, lozenges

(according to LIMB et al., based on scintigraphic measurements, cumulated over time)

MOOSAVI et al. mention at least three different pathways how COVID-19 may be present in saliva:

● COVID-19 enters the oral cavity together with liquid droplets that are frequently exchanged between the lower and upper respiratory tract

● if COVID-19 is present in blood, it can access the mouth via gingival crevicular fluid

● by viral shedding from the salivary glands following the infection of these glands (via ACE 2)

Moreover, it is noteworthy that ACE2 receptors are abundant on the tongue.

Though it is not the intention of this paper (that is intended to focus on *in vivo* evidence) to review the wealth of *in vitro* data on mouthwash, a brief overview over available *in vitro* data based on MEISTER et al., STEINHAUER et al., STATKUTE et al., DAVIES et al., MUNOZ-BASAGOITI et al., ANDERSON ER et al. suggests the following ranking

First rank (very high effectiveness, > 5 log 10)

Listerine Advanced Gum Treatment, some CPC-containing formulations (Dentyl Dual Action, Dentyl Fresh Protection); OraWize+ Aqualution Systems (0.01-0.02% stabilised hypochlorous acid);

Possibly also first rank (unsure): Dequonal and Octenisept** (not tested in the extremely sensitive assay of STATKUTE et al.); 0.07 % CPC (> 4log10 below the limit of detection in ANDERSON et al.).

Second rank (high effectiveness, > 2.5 log10)

Other CPC-containing formulations (like SCD Max, Perio Aid Intensive Care*, Vitis CPC Protec); PVP-Iodine (0.5 %), Povident (0.58 % PVP-I; close to the border of the first rank); Listerine Cool Mint and Listerine Cool Mint Mild (without alcohol); Listerine Total Care (close to the border to the first rank), Dequonal and Octenisept** (if not rank 1), Listerine Advanced Defense Sensitive (alcohol-free, 1.4 % dipotassium oxalate)

Low effectiveness (about ~ 1 log10 or less):

Octenidol, chlorhexidine 0.12 or 0.2 % (see also ANDERSON et al.), formulation with 1 % H_2O_2 or 1.5 % H_2O_2 (like Peroxyl Colgate)

No effectiveness at all:

21 % and 23 % ethanol (even 70 % ethanol needs 30 seconds for complete inactivation of SARS-COV-2; see BIDRA et al.); possibly also Peroxyl Colgate

* 0.5 % CPC + 0.12 % CHX

** not very recommendable because of bitter taste for many hours and high potential of mucosal irritation; not suited for long-term use; should not be aspirated into the lungs

However, XU et al. demonstrated in their own *in vitro* experiments that supposed antiviral effects of mouthwash in the experiments mentioned above may be in part due to cytotoxic effects of the mouthwash formulations themselves; thus the cytotoxic effects of mouth rinses should be considered when assessing their antiviral activities *in vitro:* “Published studies on reduction of SARS-CoV-2-induced cytotoxic effects by antiseptics do not exclude antiseptic-associated cytotoxicity” (XU et al).

Since diluted Listerine Original and diluted CHX 0.12 % showed no cytotoxic effects, but inhibited SARS-CoV-2 following 50/50 dilution, XU et al. regarded both mouth rinses as "suited candidates to reduce viral spread". Colgate Peroxyl (1.5 % hydrogen peroxide in the original solution) and 10 % PVP-I were able to inactivate SARS-CoV-2 completely in much higher dilutions (5 % vs. 50 %) than Listerine and CHX, but were found to be cytotoxic. On the other hand XU et al. pointed out that "despite our finding that commercially available mouthwashes had some degree of cytotoxicity, these formulations are well tolerated in clinical use."

With regard to local antiseptics e.g. as mouthwash, there is also a need to distinguish between rare/one-time use (e.g. as pre-procedural mouthwash and gargle in dentistry) or short-term use several times a day for a few days for postexposure/ring prophylaxis or in case of a suspected or proven COVID infection, in contrast to long-term use several times a day for the purpose of long-term PREP e.g. in HCWs or teachers. Some agents like ethanol and hydrogen peroxide may induce mucosal inflammation if used several times a day over 2-3 months (O-DONNELL et al.), and *" it will be important to ascertain whether a repeated daily rinse with mouthwash would have any detrimental impact on the stromal tissue lining"* (O'DONNELL et al.).

Nasal cavity, nasopharynx

More studies are published concerning the accessibility of various parts of the nasal tract. However, most of them focus on the paranasal sinuses for therapeutic purpose in the context of chronic sinusitis. Taking all available evidence together, nasal irrigation seems to be superior to nasal spray in order to better reach the posterior parts of the nasal tract, though even nasal spray will reach the nasopharynx. Nasal drops seem to be inferior to both nasal spray and nasal irrigation (BLEIER et al., DJUPESLAND et al., JIRAMONGKOLCHAI et al., LARN et al., PYNNONEN et al., SMITH and RUDMIK, VAN DEN BERG et al., WORMALD et al.).

Whereas nasal irrigation may reach more parts of the nasal tract better than nasal spray, the solution is quickly removed from the body, flowing out through the other nostril. Nasal spray will offer a sort of short-time depot for the agent inside the solution. This suggests that a combination of irrigation and spray may be optimal. However, contrary to the usual procedure in nasal disorders (at first, nasal spray for reduction of the swelling of the nasal mucosa followed by irrigation a while later), for the antiseptic procedure, irrigation should

be followed (at least a few minutes later or at a different point of time at all) by nasal spray to generate a small depot of the antiseptic agent.

Though irrigation seems to be superior to reach all parts of the nasopharyngeal tract, there is one problem with regard to COVID-19. Irrigation may weaken the local immune defense by removing immunoglobulins (IgA, IgG), lysozyme, interferons, defensins and other relevant molecules from the surface of the nasal or nasopharyngeal mucosa. Both local IgA and local interferons play an important role in the local fight against the virus (for IgA, see WANG Z et al.).

For this reason, it seems to be reasonable to prefer nasal spray instead of nasal irrigation as long as there is no clear evidence for superiority of nasal irrigation in the context of COVID-19. The situation is special since local immunity is so important to prevent or dampen infection with this virus. But it is generally not recommended to perform nasal irrigations in the long run (statement of German pulmonologists: https://www.lungenaerzte-im-netz.de/news-archiv/meldung/article/nasenspuelung-nicht-dauerhaft-sondern-nur-bei-akuten-infekten-anwenden).

Moreover, nebulisation was shown to be more effective than spray or irrigation for far-reaching and more complete distribution of agents within the nasal tract (LOU H et al., MOFFA et al.), especially with regard to the nasopharynx, but also all („hidden“) areas of the nasal vestibulum (MOFFA et al.).

As far as nasal drops/spray/irrigation are concerned which one has to be prepare individually (like in the case of PVP-I dilution), isotonic saline solution (0.9 % NaCl) should be preferred.

The mode of administration of nasal spray may also be important. It is commonly recommended in the user instructions of nasal spray bottles to lean the head forward a little (about 22°) and hold the spray bottle upright in the hand, inserting it 5 mm into the nostril. However, BASU et al. showed *in silico* and in replica-based simulations, that a more oblique direction along the “line of sight” (the view onto the ostiomeatal complex, acting as the mucociliary drainage pathway for the sinuses) is more effective. On the other hand, this study focussed on the treatment of sinonasal diseases and it remains unclear whether this applies also for antiseptic nasal spray which should be able to reach as many areas of the nasal and nasopharyngeal mucosa as possible. This study was not about COVID-19 prevention and treatment, though the authors discuss their results in the light of a possible nasal vaccination strategy.

Another critical aspect of nasal decontamination is the choice of the correct dosage, especially with regard to the ciliary epithelium of the nasal tract which is very sensitive e.g. with regard to the ciliary beat frequency. Any damage of the epithelium or reduction of ciliary beat frequency must be avoided. Damage may increase susceptibility for the virus. In uninfected people, it may increase the risk of primary infection in case of contamination, and in already infected people, it may ease the spread of the virus and the increase of viral load.

SARS-CoV-2 itself damages motile cilia both *in vitro* and *in vivo* (Syrian hamster model), and cilia loss may play a role in COVID-19 pathogenesis, because it impairs the clearance at the site of viral replication what could facilitate viral spread within the airways. For example, decreased cilia movements in the trachea are suggested to slow transport of released virions towards the pharynx and instead facilitate viral access to deeper regions of the bronchial tree. If this process self-perpetuates, the virus will eventually reach the alveoli and trigger pneumocyte damage (ROBINOT et al.). Thus any damage of cilia as a consequence of antiseptic procedures has to be strictly avoided.

As far as the nose is concerned, only methods and concentrations should be used of which one is sure that they have no deleterious effect. For example, in the case of PVP-I, the concentration should not exceed 1.25 %, since this is the highest concentration for which no reduction of the ciliary beat frequency was found (PELLETIER et al.). The next step, 2.5 %, is already critical in this respect. According to PELLETIER et al., *"Povidone iodine concentrations of 2.5% and above are toxic to nasal mucosa, upper airway respiratory cells, and ciliated epithelia."*

Thus it seems to be save to create a concentration between 0.5 % to 1.0 %. Within that range, the exact concentration doesn't seem to matter, so one doesn't have to work extremely exactly (for details and references, see the "early therapy paper" mentioned above). In this respect, it is surprising that the SHIELD Study (NCT04478019) uses 10 % PVP-I for nasal administration by swab sticks (combined with 0.12 % chlorhexidine gargle).

However, there remain open questions with regard to PVP-I which can only be answered by clinical trials. YAN and BLEIER caution that the enormous amount of mucus secretion of the nasal mucosa will dilute a PVP-solution within 5 minutes by more than a half. This problem cannot be overcome by a higher PVP-I concentration because of its ciliotoxicity.

The oropharynx is less sensitive in this respect; for biological reasons, it must be much more robust against environmental influence (like nutrition, drinks or so), and there is no respiratory (ciliary) epithelium in the oropharynx and hypopharynx. On the way downward, respiratory epithelium restarts upon the vocal fold in the larynx.

In their WHO trial register analysis mentioned above, CARROUEL et al. discovered 11 trials which include mouthwash and/or gargle:

Oral:

1 x mouthrinse with Citrox + Beta-Cyclodextrin (NCT04352959) (see belos)

1 x rinse and gargle: distilled water (control) or hydrogen peroxide or essential oils (Listerine Zero alcohol) or CPC or chlorine dioxide (NCT04409873)

1 x gargle with essential oils (Listerine) or PVP-I or tap water (as control) (finished, published, see MOHAMED et al.) (NCT04410159)

Oral + nasal:

1 x mouthrinse (0.12 % CHX) + nasal swab sticks with 10 % PVP-I (NCT04478019)

1 x oral + nasal rinse with 0.5 % PVP-I or 0.12 % CHX (NCT04344236)

1 x gargle + sinus rinse with sinus rinse bottle with PVP-I 0.23 %; or 0.6 % PVP-I gel-forming nasal spray (without gargle) (NCT04449965)

1 x mouthwash + sinus rinse (= nasal rinse) with 0.23 % PVP-I (NCT04393792)

1 x gargle + nasal lavage: PVP-I (0.2 %) or hydrogen peroxide (1.0 %) or Neem or 2 % hypertonic saline or distilled water (as control) (NCT04341688)

2 x gargle + nasal spray: (i) 1 x nitric oxid (+ nasal irrigation) (NCT04337918); (ii) 1 x PVP-I 0.33 % (NCT04478019)

1 x mouthwash, gargle and nasal spray; 1 % PVP-I (NCT04371965)

Only nasal:

1 Nasal spray (with nose spray bottle): 0.5 % PVP-I or 2 % PVP-I (NCT04347954) (no mouthwash/gargle)

Interestingly, no trial uses throat spray, though the combination of spray and gargle/mouthwash has to be regarded as superior compared to mouthwash or gargle alone because gargling doesn't reach the regions behind the anterior palatal arch.

Of note, *in vitro* data for the nasal spray formulation "Nasodine" indicate that this special formulation is more effective than 0.5% PVP-I alone (prepared in saline) at both the clinically relevant 15 second and 5 minute time-points (TUCKER et al.).

Prophylactic procedures

There are so far no results from PREP/PEP trials which involve nasal or oropharyngeal decontamination procedures, but a few trials with PVP-I decontamination in (e.g. in hospital staff) are ongoing. The DUBINA trial with inosine-glutathione inhalation is not about decontamination, but it presents so far the best evidence for successful PREP by local measures.

The MENG trial from China combined interferon nasal drops (3 times a day) in all 2944 clinical staff members with thymosin injections in those who were exposed most, and there was no single infection during the maximum of the local epidemic in the trial population. Since thymosin was found to be ineffective in prophylaxis in another trial (LIU X et al.), it is suggestive that the beneficial effect of the prophylactic regimen can be attributed to interferon nasal drops alone.

But in the absence of a control group it remains doubtful whether the MENG trial demonstrated a prophylactic effect of the regimen at all. There are no clues how many infections would have been expected without the prophylactic intervention. In the absence of PCR testing and retrospective antibody testing, too many questions remain unanswered and it is impossible to draw any valid conclusions from that very early trial. Nevertheless, interferon beta 1a inhalation (in the formulation of SNG001 from Synairgen) was highly effective in treatment: In a placebo-controlled phase II trial with hospitalized patients, inhalation of nebulized SNG001 reduced the risk of ventilation or death with an Odds Ratio of 0.21 (CI: 0.04 – 0.97), and recovery was more quickly (HR 2.19; CI: 1.04 – 4.69), especially with regard to the recovery from shortness of breath. Among 101 patients altogether, there were 3 deaths (6 %) in the placebo group, but no death in the verum group.

(https://www.aerzteblatt.de/nachrichten/114853/COVID-19-Inhalatives-Interferon-beta-erzielt-in-erster-Studie-gute-Wirkung).

So far unavailable and without approval in the EU, interferon drops / spray / inhalation was not included in the “results” section of this paper. Moreover, it is not an antiseptic, disinfectant or another method of direct decontamination, but it helps the body to fight against the virus also in a local, mucosal membrane related manner. In some countries outside the EU, like Russia, interferon nose drops or spray or formulations which are suited to prepare inhalation/nebulization are accessibly for everyone (OTC) in pharmacies, e.g. as medications against influenza or influenza-like infections.

The concept of local interferon administration may be interesting not as a substitute, but as an adjunct to local decontamination. If a local COVID-19 infection cannot be controlled, if it

persists or expands, the local interferon response may be too weak to fight against the infection successfully. In these cases, the balance between the virus and the local interferon response seems to be moved in favor of the virus. There were also reports from animal experiments with local interferon response in the respiratory tract following infection with COVID-19 is comparatively weak and late (compared to other URT infections), and this may contribute to the fact that COVID-19 is a comparatively dangerous infection.

The failure of early hydroxychloroquine treatment, also in the case of PEP, may be the consequence of the inhibiting effects of hydroxychloroquine on interferon-related genes. Though there is no doubt that hydroxychloroquine has inhibitory activity against COVID-19 *in vitro*, this favorable effect seems to be counteracted *in vivo* by the suppression of interferon-related genes. The "anti-interferon" effect of hydroxychloroquine may explain very well the time course of HCQ administration in the PEP trials from BOULWARE and MITJA et al. (for details and references, see the "chemoprophylaxis paper" **http://freepdfhosting.com/863ed84c7f.pdf).** In fact, in contrast to HCQ, umifenovir (Arbidol) induces interferon production (FAN et al.).

Though both hydroxychloroquine and Arbidol show antiviral effects against SARS-CoV-2 *in vitro,* their difference with respect to their effectiveness in PEP may be explained by their contrasting effects on local interferon production: ↑ in case of Arbidol, ↓ in case of hydroxychloroquine. Arbidol showed to be highly effective in PEP in a dose-dependent manner (YANG C et al., ZHANG et al.), with about 95 % protection if therapeutic doses (200 mg TID) are given and moderate protection (OR 0.214) in the case of prophylactic doses (200 mg daily).

If the failure of HCQ in spite of its undoubted inhibitory activity against SARS-CoV-2 stresses the important role of local interferon production on the course of the early infection, an optimal concept against the local infection of the upper respiratory tract should involve both decontamination procedures (to weaken the "viral" side of the fight between virus and interferon), and administration of local interferon by nose spray or (probably better) inhalation/nebulization (in order to strengthen the "interferon/local immune response" side). Such a concept is probably highly effective for PEP and also for early treatment as long as the infection hasn't disseminated beyond the upper respiratory tract.

Besides of interferon, lactoferrin is another natural agent with functions in the local immune response, and contrary to interferon and inosine-glutathione for inhalation, it is already accessible.

SERRANO et al. reported about a complex treatment of symptomatic COVID-19 outpatients with liposomal lactoferrin. All 75 infected patients got "Lactoferryn TM Forte drinkable" (Sesderma laboratories) and a zink-based formulation orally. Those with nasal congestion,

dry cough and headache also took lactoferrin mouth spray and nose drops, and those with breathing difficulties got Lactoferryn aerosol using the Nanomist Nebulizer SES.

After 5 days of treatment, except for some patients with smell or taste reduction and very few patients with tiredness, all symptoms were either lost completely or reduced to “mild”. No progression or hospitalization was reported. Similar to interferon, lactoferrin is neither an antiseptic nor another sort of decontaminant, but has antiviral properties.

Though the results look extremely promising for a population of outpatients with moderate symptoms, there are serious limitations. Treatment started after COVID-19 had been confirmed by a positive IgM/IgG test. The patients were reported to be heavily symptomatic (but not as severely that they had to be hospitalized yet), but with a positive IgM/IgG result, the patients may already have been in the phase of their recovery.

Moreover, infected people with bad prognosis and progression would have already been hospitalized at the seropositive stage of the disease. Positive IgM/IgG test in combination with non-hospitalization may suggest a selection bias in favor of patients *without* potential for serious progression and critical prognosis, even in the absence of lactoferrin- and zinc-based interventions. Thus, without a control group, it is impossible to decide how much of the improvement is due to the lactoferrin/zinc therapy and how much improvement would have occurred spontaneously. And since all of the patients got systemic (oral) liposomal lactoferrin and zinc, it is unclear how far local treatment (mouth spray, nose drops and inhalation) contributed to the favorable results. Though the results are highly promising, the only consequences one can draw from that trial are to repeat it (i) in an early patient setting based on PCR positivity, and (ii) to establish a control group.

Conclusions

In summary, local decontamination procedures of the nasal and oropharyngeal area but also local interferon administration may play an important role in chemoprophylaxis and early treatment of COVID-19 disease, and the strong focus of the research on hospitalized, later stage patients seems to have overlooked so far the enormous potential of early application of these methods at a time when the infection is still localized in the nasal/nasopharyngeal and/or oropharyngeal tract. However, there are so far no results from ongoing trials with the prophylactic use of these methods (the DUBINA PREP trial is not about decontamination), and very limited data for the use in infected people.

Since it is difficult to distinguish between defective and infectious virus in specimens taken in the aftermath of such procedures, final decisions on the effectiveness of such methods can only be based on clinical parameters like outcomes or improvement of symptoms in controlled trials as far as infected patients are concerned, or infections, symptomatic disease, outcomes of disease or seropositivity as far as prophylactical trials are concerned.

With regard to ongoing prophylactical trials (which usually involve health care workers [HCWs]), there is a need to be reluctant even if they prove to be successful. It is well known meanwhile that the infection dose is of high importance with regard to successful (or failed) infection, symptomatic disease, number and severity of symptoms, and general severity score and outcome of the disease. In prophylactical trials with HCWs, it is highly probable that HCWs wore at least some sort of protection (e.g. surgical masks), and in the case of successful infection and positive PCR or (later) seropositivity, they were probably exposed only to a small amount of virus (even in a high exposure event), since most of the virus was kept back by PPE. In the presence of PPE, a high exposure (risk) setting may turn to a low infection dose setting (for the role of masks and infection dose, see CHENG et al.). So any favorable results for chemoprophylactic methods in trials with HCWs (independent whether they are local or systemic methods) may not apply to situations when individuals are exposed directly and without PPE/masks to high viral loads and infection doses because of close or possibly intimate contact to highly infectious people or even super-spreaders.

The higher virucidal effectiveness of surfactant-containing Listerine Advanced compared to Listerine Cool Mint (with essential oils) by about 2 log10 offers a rationale for the use of surfactant-containing nasal sprays, mouthwash/gargle and throat spray. However, more data are needed and it might be critical to use the “right” surfactants that don’t have the potential to irritate the mucosal lining.

In the more distant future, inhalation of monoclonal antibodies may offer a very effective treatment for early COVID-19 disease. In a hamster model, inhalation even of low doses of such antibodies resulted in a reduction of viral burden in the respiratory tract below the detection limit, and mitigated lung pathology (PIEPENBRINK et al.). Most important, local delivery of antibodies to the respiratory tract is dose-sparing and thus cheaper compared to the conventional parental route. Moreover, antibody inhalation may work both in prevention and treatment. However, this method is mentioned only briefly here because it is not available yet, though there is an urgent need for improved methods of local treatment, and such progressive methods should experience the same accelerations in translational and clinical research and approval like vaccinations. Moreover, antibody therapies are very sensitive to escape mutations of SARS-CoV-2.

This limitation doesn’t seem to apply to the concept of DE VRIES et al.. They developed a lipopeptide **[SARSHRC-PEG4]2-chol** for nasal administration. It prevented SARS-CoV-2 transmission in a relevant animal model (ferrets) during a 24-hour period of intense direct contact.

This is the first successful prophylaxis of SARS-CoV-2 transmission in an animal model and provided complete protection. The lipopeptide fusion inhibitor blocks membrane fusion as the first critical step of infection.

The lipopeptide was administered once daily. 100 % of the control ferrets became infected. It was also found that the lipopeptide is equally active against CoVs like B.1.1.7 and B.1.351. It has a long shelf life and does not require refrigeration. The authors propose to advance the lipopeptide fusion inhibitor to human use by translating into a safe and effective nasal spray or inhalation for SARS-CoV-2 prophylaxis. Unfortunately, this will still take a lot of time until approval.

Provisional recommendations

The *in vivo* trial results so far available alone are too sparse to generate recommendations solely upon them. The best evidence so far has been provided by the RCT from CHOUDHURY et al., and the use of 1 % PVP-I.

Combining the clinical trial data from this living review with *in vitro* data discussed in the "early therapy paper", the following *provisional* recommendations can be given:

People with suspected or confirmed COVID-19 infection and their contacts should gargle thrice a day with ~ 1 % PVP-iodine or Listerine Cool Mint (the latter especially for those for whom PVP-I is contraindicated or unwanted). Based only on *in vitro* results (and thus outside the scope of this review), Dequonal, Listerine Advanced; Octenisept or CPC-containing mouthwash may be another alternative.

Based on theoretical assumptions (and thus also outside the scope of this review), it would be probably advantageous to combine this procedure with nasal spray of 0.5 – 1.0 % PVP-I diluted in isotonic saline solution, or iota-carrageenan-based nasal spray, in both the infected or suspected index patient and his contacts (e.g., family members).

The concept with the best evidence so far is based on 1 % PVP-I and includes mouthwash (30 sec), gargle as deep as possible (30 sec; alternative: throat spray), nose drops and eye drops four times a day, started as early as possible.

For people with long-lasting or reactivated naso-/oropharyngeal PCR positivity, the CAPETTI procedure may be recommended: once 3 % hydrogen peroxide as nasal spray or irrigation, combined with gargle with the same solution, followed by hypertonic saline nasal irrigation once a day for 14 days. 3 % H_2O_2 procedure may be repeated after 14 days, the turnover interval of the nasal epithelium. It is very plausible, but not verified in a clinical trial, that the PVP-I procedure described above may also be effective in this situation.

To avoid infectiousness, mouthwash (1.0 % PVP-iodine or 1.5 % hydrogen peroxide) – even in the absence of gargling - has a sterilizing effect on respiratory droplets for at least 20 minutes, but the fully sterilizing effect lasts less than 60 minutes (when Rapid Antigen Tests turned positive again, whereas viral load was still reduced a lot – but not completely – according to qRT-PCR).

If a potential exposure is expected, or to reduce possible residual risks in spite of use of PPE, or if a risky contact in the absence of the possibility to wear adequate PPE is forseeable, but again based only on theoretical assumptions (and thus outside the scope of this review), it would be probably advantageous to administrate a carrageenan-based nasal spray ***before*** the possible exposure, and PVP-I-based nasal spray, throat spray and gargle directly ***after*** exposure ("peri-exposure prophylaxis"). For gargle/throat spray, based on *in vitro* evidence, also Dequonal, Listerine Cool Mint, Listerine Advanced, Octenisept or CPC-containing mouthwash/spray can be used instead of PVP-I.

As already mentioned above, Carrageenan would form a protective gel-like layer on top of the mucosal lining and inactivate most of the viral particles which settle down on the mucosal surface (without damaging the normal physiological microbiota there since carrageenan is no antiseptic/decontaminant), but providing a sort of physical barrier against viral entry into the cells, whereas later, after the potential exposure, the PVP-I procedure would inactivate all the remaining viral particles which survived carrageenan exposure somehow (for more details about carrageenan and *in vitro* evidence, see the "early therapy paper"). Carrageenan is a sulphated polysaccharide which cannot penetrate mucosal membranes (HUI KK). PVP-I has a direct virucidal effect, but it also inactivates ACE2 and CD147 receptors of host cells.

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„Early therapy paper“: **http://freepdfhosting.com/35f285c9f2.pdf**

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