Early results of chemoprophylaxis trials against COVID-19

URL for download: http://freepdfhosting.com/863ed84c7f.pdf

R. Steinmeyer (2020 – August 2021)

WARNING:

None of the studies mentioned here so far is specifically about Delta, Gamma or Lambda, or from a population / setting / time interval where / when one of these VoCs was prevalent or dominant.

Because of different biological behavior (e.g., earlier and quicker rise and higher viral load in Delta) it may be the case that different agents that were found to be successful for prophylaxis in the past, don't work any more at all, or less efficiently, in the context of the new VoCs.

This means that even prophylactic agents that seemed to be quite or very effective so far, may fail now. Until evidence specifically for Delta, Gamma or Lambda becomes available, all prophylactics mentioned here have to be regarded as experimental in the context of these variants. Effectively, one would have to start research (and trials) on COVID-19 PREP and PEP right from the beginning again.

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.

Introduction

Whereas first results about the efficacy of the COVID vaccines, particularly the mRNA-based vaccines, from late 2020 gave some reason for hopes that the SARS-CoV-2 pandemic can be overcome by these vaccinations, it became evident until the middle of 2021 that the vaccines of the first generation, including the mRNA vaccines, are definitely unable to do so.

There are several reasons for these disappointments. All vaccines of the first generation are based on the Wuhan sequence of SARS-CoV-2, and meanwhile there are several variants of concern that partially escape from the immunity generated by these vaccines. Very critical variants with regard to immune escape are (as of July 2020) Beta (see MADHI et al. for Vaxzevria) and Lambda. But also Delta causes a lot of problems and breakthrough infections, combined with its high infectiousness and high viral load in the upper respiratory tract of infected people, whether vaccinated or not. Moreover, during the first days of the infection, the nasopharyngeal (NP) viral load of the Delta variant is exactly the same in fully vaccinated and unvaccinated people, proving that there is no sterilizing effect at all. It is only since about day 5-6 that the NP viral load declines more quickly in vaccinated infected people compared to unvaccinated, but at that point of time, most of the infected people will already be isolated. This points to the high infectiousness of the Delta variant even in vaccinated people once they got infected.

Whereas the vaccines still offer a relatively good (but already reduced) protection against very serious outcomes like deaths, there are now many breakthrough infections even in fully vaccinated people with the Delta variant, even in the case of mRNA vaccines, and the incidence data from Israel clearly demonstrate that even high vaccination rates exclusively with mRNA vaccines are unable to prevent a new large and quickly rising wave of infections.

Based on experiments with Rhesus macaques, it was clear from the beginning that Vaxzevria won't be able to generate sterilizing immunity in the upper respiratory tract (VAN DOREMALEN et al.), whereas in similar experiments with the mRNA vaccines from Moderna and Biontech/Pfizer, the sterilizing effect of two doses was not perfect, but close to perfect (VOGEL AB et al., CORBETT et al.). So the hope was that at least a full vaccination (2x) with mRNA vaccines will offer nearly-sterilizing immunity, at least with virus loads so low that one would no longer be infectious in case of a breakthrough infection.

But the experiences with early breakthrough infections with Alpha und Beta destroyed the hopes of a nearly sterilizing immunity, and meanwhile, Delta made it very evident that all of the vaccines, not only the vector-based ones, lack sterilizing immunity. As a consequence, someone with a complete vaccination series may get infected (though the risk is lower than for an unvaccinated person), and he may also be infectious to others, particularly in the case of Delta with its high viral loads also in vaccinated people.

Sterilizing vaccines aren't expected on the market in the near future. It is assumed that vaccines have to be administered intranasally in order to generate a sterilizing effect in the uppermost respiratory tract. Such vaccines are under development, but until early August 2021, only 9 of 110 vaccines in clinical phases involve intranasal administration: 2 of them were in clinical phase 2, 1 in phase 1 /2, the remainder all in phase 1. The two vaccines from phase 2 are from China and Iran and thus improbable for quick approval and access in Europe even if they finally succeed. As a consequence, it will take a long time until intranasal vaccinations will become available. And it is also not guaranteed that they will really offer fully sterilizing immunity; they only may have the potential to do so.

Such vaccines of the second generation would need much more financial and governmental support to accelerate their development. 2020 has shown what is possible if there is enough political power and financial support, and that it is possible to generate a new vaccine within one year until its delivery to the first people. Now the pressure is gone (exactly spoken: *felt to be gone*), politicians and stakeholders feel good amidst the AstraZeneca, Moderna and Biontech/Pfizer vaccines and hope (at best) for their updates e.g. based on the Beta variant (the vaccine generation 1.2), but new vaccines (generation 2) will get less support and will need much longer time until approval, production and delivery to people. But life and society won't be able return to full normality until fully sterilizing vaccines are available. That's why the pressure is still there (not much less than in 2020), but it is harder to understand and feel now.

The aim of the currently available vaccines is no longer *to prevent infection (with a relative high grade of confidence),* and it is no longer *to prevent the spread of the infection (once acquired),* but the aim of the vaccines is now primarily *to prevent severe cases of COVID-19,* ICU, intubation, death. But even the latter is now less sure than it was before Delta, and the number of severe cases, hospitalizations and deaths of fully vaccinated people is rising. Of course, the risk of very severe disease or death in fully vaccinated people is dominated by very old or immunocompromised people, but not exclusively limited to them. This is not unsurprising since MUELLER L et al. showed that about one third of inhabitants of a care facility for old people, most of them > 80 years, were unable to develop neutralizing antibodies following 2 doses of Biontech/Pfizer. Moreover, it is clear now that people with certain immunosuppressive or immunomodulating therapies fail to develop antibodies at all, or only very low titers.

Another important aspect that is not limited to very old or immunocompromised people is waning immunity. Starting a few weeks after the second dose, the strength of the protection wanes from month to month, and this process is accelerated in the elderly (> 60 years) and was demonstrated very well in studies from Israel. That's why Israel decided to give everybody from 60 years on a third dose – just 7-8 months after vaccinations had started.

In summary, (partial) escape variants, the lack of sterilizing immunity (particularly with regard to Delta), and quickly waning protection in the elderly (and originally low or missing protection in some special subgroups like very old or immunocompromised people) limit the efficacy of the vaccines, and the situation increasingly worsens with the rise of the Delta variant and the elapsed time since the last vaccination dose.

So no one can be sure to be really protected by the vaccine, even if fully vaccinated. Whereas healthy younger people may accept the risk of a breakthrough infection, because

they know that they won't get severe disease (but maybe Long Covid?), older people or those with relevant comorbidities must take a lot of care to avoid breakthrough infections, because they can be dangerous and life-threatening for them. There is no return to "normal life" for them. Of course, the risk is still (much) lower as if they were unvaccinated, but the risks in case of breakthrough infections in older people are substantial: In Germany, RKI reported in July 2021 that 26 – 27 % of all breakthrough infections in people of 60years + had to be hospitalized – compared to about 2 % of the younger breakthrough patients (RKI, weekly reports from Thursday).

Since non-pharmacological protections are lifted and fully vaccinated people get their full freedom back, the situation becomes now risky particularly for older (fully) vaccinated people and – of course – everybody who is unvaccinated or only partially vaccinated.

With the lifting of many restrictions, whether for all people or only for the vaccinated and cured people, the governments withdraw from the protection of the people, including the vulnerable people (and people who are vulnerable despite full vaccination), and it is now the responsibility of each person on its own, how many risks he/she will accept, and to care for own protection. Moreover, it will be much more difficult to avoid certain contacts, events and meetings in the future than it was during "protected" lockdown-like times.

In this situation, chemoprophylaxis becomes an important second pillar for protection – besides vaccination. In some way, it must become a substitute for the loss of protection by legal restrictions from the government. Vaccinated people will now attend mass gatherings with many other vaccinated people (without any testing before) – but in the absence of sterilizing immunity, this is just a gradual difference to mass gatherings between unvaccinated people (it may be statistically *substantial,* but in principle, it is only *gradual*). The difference is, that the risk of infection is gradually smaller, and, once infected, the risk of severe disease is substantially smaller – but it still exists and it cannot be neglected, particularly for the elderly.

This new situation makes chemoprophylaxis now much more important than it was before. As already mentioned, prophylaxis must reduce and close the gap that is generated by the lifting of the legal restrictions and lockdown measures. Everybody is now responsible for himself. Even if fully vaccinated with the "top" vaccines (or "cross vaccination"), the risk of COVID-19 is real, and the risk of severe disease despite full vaccination increases with age, comorbidities and elapsed time since the last dose of the vaccine.

Chemoprophylaxis may have many faces. It may be permanent / long-time (e.g. for professional reasons in highly exposed professions, interrupted only by holidays) or short-time and situation-dependent for protection in the context of a special risky event that may last only a few minutes or a few days. It may be preexposure prophylaxis and peri-exposure prophylaxis (PREP + PEP for a few days) if the risky situation is foreseeable, or post-exposure prophylaxis, if it was not foreseeable, e.g. if someone is suddenly informed to be a contact person of an index case, or in case of being a household contact of an index case, or in case of a spontaneous decision to join a mass event.

Chemoprophylaxis may be systemic (like tablets of even non-COVID heterologous vaccinations), local (nasal/oropharyngeal), or a combination of both.

If there is effective chemoprophylaxis, this will give some degree of freedom back to both unvaccinated and vaccinated people. For **unvaccinated people** who have the chance to get vaccinated, vaccination should always have priority compared to chemoprophylaxis. Nobody should forego vaccination because he feels protected well enough by chemoprophylaxis. Vaccination (with the current "top" vaccines) should always have priority, and situation-dependent chemoprophylaxis would then be an adjunct in the sense of a double protection strategy particularly for those who need this second protection (like elderly).

However, there are people with **contraindications against COVID vaccines.** Beside the need to adhere to non-pharmacological restrictions, now self-generated and no longer installed by the government, chemoprophylaxis is their only chance if they cannot avoid contacts. This is now (in Delta times) even more important because it is evident now that even vaccinated people can be infectious (with high viral load and high infectious dose). For example, one can no longer feel absolutely "safe" in a hospital (as a patient) because all of the staff is vaccinated.

Moreover, **convalescent people** may get reinfected by new variants, particularly when immunity from the first infection wanes. DHILLON et al. performed a systematic review and meta-analysis about 577 cases of reinfections published until March 16, 2021, including 81 studies (72 of them with good quality) with 577 cases from 22 countries and mean age of 46.2 years (males: 45.8 %; 31.0 % comorbidities). Average duration between first infection and reinfection was 63.6 days. Most worrying, there were 10 cases of ICU admission because of reinfection instead of 3 in case of first infection; 9 vs. 2 of 577 needed mechanical ventilation following reinfection. There were ten death among the 577 reinfections; respiratory failure was the most common reason for death (7/10). It must be noted that these are "historical" data from the time before the arrival of the Delta variant. It is probable that the situation worsened since then.

As already pointed out, even **fully vaccinated people** are still endangered not only by simple infection (asymptomatic or more often symptomatic), but also severe disease particularly if they are older or suffer from relevant comorbidities. To this group, the vaccinations don't give so much freedom back as originally expected in late 2020 and very early 2021. The latter group has still to think about every step they take: can I accept the (residual) risk?

If this (residual) risk can be substantially diminished by situation-dependent chemoprophylaxis, this would offer a lot of freedom to these groups of vaccines. Protected by short-time prophylaxis, even older people with residual risks for severe disease despite full vaccination may be free to attain celebrations, mass gatherings, events; they may resume travelling and meeting their old contacts.

In summary, since it is clear now that the once-celebrated vaccines are suboptimal, at least for the elderly and particularly in the context of Delta, there is a need for "double protection", at least for some groups of people, not necessarily for the whole society.

Besides individual protection both for unvaccinated people and vaccinated people with residual risks (e.g. because of age), chemoprophylaxis may have an effect on the whole society and the course of the epidemic within a country:

For example, when the Delta wave started to rise in Germany in July and early August 2021, the R value, as calculated by the RKI in its daily reports, oscillated around 1.10. This was of course enough to start exponential growth and the fourth wave.

If chemoprophylaxis (e.g. used by contacts of index cases) could reduce the number of new cases only by about a little more than 10 %, this would have been enough to keep the R value in such a situation below 1.0 and to avoid an exponential growth and a new wave. This may no longer work with high R values like 1.5 or 2.0, but with R values just a little above 1.0, the avoidance of some cases by chemoprophylactic procedures may be sufficient to bring the R value down to less than 1.0, particularly if chemoprophylaxis is practiced in the context of outbreaks or in contact settings (as a sort of PEP), or in association with risky mass gatherings.

As will be mentioned below (see Table 3 in BEN-ZUK et al.), it is a pity that Europe, and particularly the European Union with its EMA, ignores the field of chemoprophylaxis nearly completely. EU and EMA rely completely on vaccines. **What do they have to offer to their inhabitants if there is a fully vaccine-resistant variant of COVID-19? How do they want to avoid the overload of the health system and triage in such a situation?**

Everybody knows that new variants come so quickly and generate new waves so fast that it is impossible to adapt the vaccines to that variant, to approve these vaccines by the EMA, and to deliver them so quickly to the whole population, before this escape variant hits the population.

So in case of a vaccine-resistant variant, the EU and EMA have nothing to protect the population, except for the reintroduction of the non-pharmacological restrictions with all of their limitations, particularly in the context of highly infectious variants that can be catched by simply “passing by”.

Many scientists meanwhile assume that vaccine-resistant variants *will arise;* this is no longer regarded as a panic scenario or worst case scenario, but it is accepted meanwhile that it is well plausible that something like that will happen earlier or later. High vaccine hesitancy in countries where enough vaccines for everybody are available, and lack or paucity of vaccines in poor countries (with their low vaccination rates) both offer an ideal ecosystem for the evolution of fully resistant variants.

It is a scandal and irresponsible that EU and EMA don’t prepare for such a situation.

What about chemoprophylaxis in a situation with vaccine-escape variants, one must be aware that it may happen that a few agents of chemoprophylaxis may work not or less effective against such a new variant (see the WARNING on page 1). It depends on the exact mechanism how a prophylactic agent acts against the virus. If the vaccine escape variant can also escape from that special mechanism, then that special chemoprophylactic agent won’t work in the context of that variant. Thus it is important to have several methods of chemoprophylaxis available which act differently on the virus, its entry or its replication. Nevertheless, local antiseptic measures (like povidone-iodine or CPC) or local measures based on physical protection (barriers) like iota-carrageenan or clay-based methods like Bentrio® will be effective in any case; they don’t depend on the sequence of the virus. Thus it is too simplistic to pretend that chemoprophylaxis may also fail in the case of a vaccine-

escape variant. If one has a portfolio of chemoprophylactic agents, the majority of them will still be effective then, and one has only to take care to avoid single chemoprophylactic agents that can be circumvented by the variant virus.

Vaccination and PREP are not competing with one another; they have to complement one another. It is time that politicians and those who decide about strategies and financial funds for research do recognize this reality, and that the field of chemoprophylaxis research is supported and acknowledged to the same extent as the field of vaccination research.

In this situation, it is hard to understand that impressive results from chemoprophylactic trials like – for example – Sepsivac (see JAISWAL et al.) or Umifenovir (ZHANG et al.) are ignored. For example, the impressive results for Umifenovir PEP were already published on February 26th in 2020. Of course there is an obvious need to replicate them in an animal model, maybe mouse, hamster, ferret or macaque, because animal models allow a more detailed analysis of the underlying mechanisms and how it works *in vivo*, and whether there is a rationale to explain the high protective effectiveness found in the study. Nothing of that happened within more than a full year. No one is interested in a cheap and well tolerated agent (old enough to be no longer protected by patent laws) that has the potential to prevent symptomatic disease by 90 % and more in a PEP situation.

The same applies to Ambroxol that was never tested in an animal model for COVID prophylaxis or a clinical trial of PREP/PEP, though the *in vitro* background for its effectiveness in prophylaxis is excellent and much better than for Bromhexine, though the latter already proved to be successful in PREP (MIKHAYLOV et al.). Moreover, ambroxol is very safe (safer than bromhexine) and better suited for long-term intake (PREP) and also available for inhalation. Ambroxol has the potential of a top agent for prophylaxis (at least as an interim until new COVID-specific prophylactics are developed by the industry), but was ignored so far.

In an own analysis of the WHO registered trial database (ICTRP) until May 13th 2020, **88 prophylactic trials** (PREP or PEP), including unspecific vaccinations (like BCG or measles), but not COVID vaccines, were found, with about 200.000 participants altogether (German paper, available: **http://freepdfhosting.com/bedd8b1c79.pdf ;** shortened english version: **http://freepdfhosting.com/9686575098.pdf).**

86.4 % of the 88 trials were randomized, 56.8 % restricted to health care workers (HCWs) and another 19.3 % included HCWs and other risk groups (e.g., household contacts). 34 trials were planned to be finished until end of October 2020.

Most worrying, 65.9 % of all trials were about CQ/HCQ, followed by BCG vaccination (6.8 %). The strong focus on CQ/HCQ poses a high risk if CQ/HCQ fails in prophylaxis, because alternatives are subject only of 1 – 3 trials each, many of them small or of low quality (e.g., not RCTs), and many other agents which are suggested to be potential candidates for PREP/PEP are not investigated in clinical trials at all.

MANOHARAN et al. analysed registered chemoprophylaxis trials in a similar time interval, including about two additional weeks (until May 26, 2020). They found 76 chemoprophylatic

study registrations that planned to enroll altogether 206,367 people with a median size of 490. 82.9 % of the trials were randomized (altogether 197,010 patients with a median size of 600 for the RCTs). 97 % of trials were underpowered to detect a 30 % effect size at the 80 % level. Only one study had an adaptive design. Outcomes were tested in 46 % of the trials by PCR, in 6.6 % by serological testing and in 14.5 % by both methods. 65.8 % of the trials were dedicated to HCWs (n = 52; 49 x PREP, 3 x PEP), 20.3 % to PEP in close contacts. Older adults were subject only of 3 (3.8 %) of the studies (long-term care facilities), while only 2 (2.5 %) of the studies in the general population included older adults. 59 of the prophylactic trials study HCQ or CQ (77.6 %). This proportion is higher than in the own analysis (65.9 %) because the MANOHARAN study didn't include vaccinations like BCG. Lopinavir/ritonavir was the second most frequently studied agent in their analysis.

MANOHARAN et al. criticized underpowering of many studies and their inability to detect clinically meaningful protection, making many trials of marginal importance. They see a need for international coordination mechanisms and collaboration and the use of adaptive platform trials *"that will allow structured entry and exit of candidate agents and rapid stand-up of trial infrastructure."*

SALLARD et al. chose a very similar approach for their review about clinical trials for prophylaxis (PREP or PEP), but they also included EudraCT repository, the anticovid platform and the covid-nma platform in their search. Until July 5th, **112 prophylactic trials** were registered according to the review by SALLARD et al., but the authors also included trials with convalescent plasma or monoclonal antibodies (contrary to the own analysis from May 13th).

88 % of the 112 trials were randomized. Again, it was found that 62 % were still about (hydroxy)chloroquine, followed by BCG (11 %) (non-specific vaccines altogether: 13 %). The proportion of trials with CQ/HCQ decreased only slightly from 68 % before May 2020 to 52 % for trials registered in May or June, in spite of many doubts with regard to the effectiveness and risks of CQ/HCQ. SALLARD et al. suppose that many of these "late" trials were designed before evidence and opinions with regard to CQ/HCQ became much more critical and cautious.

A more recent overview about ongoing and registered prophylactic trials with HCQ is given by MONTI et al., also including details about dosing regimes. Until October 15th, there were 77 registered trials about HCQ prophylaxis, 92 % of them randomized and 71 % recruiting health care workers. 58.5 % of the trials plan to use a loading dose.

A systematic review by SMIT et al. (2) analysed clinical trials of PREP or PEP from two clinical trial registries (ICMJE, ICTRP) up to December 13th 2020, but restricted their search to RCTs. 117 RCTs met their inclusion criteria, 85 on PREP, 29 on PEP and 3 on both PREP and PEP. 72 trials targeted HCWs alone, 15 RCTs targeted close contacts of index cases alone.

Only 7 of the trials were completed so far, 57 either recruiting or ongoing, 38 not yet recruiting and 5 suspended or prematurely ended. The low number of completed trials is

disappointing, since the own analysis from May 13th found that 34/88 prophylactic trials (RCTs and non-RCTs) were planned to be completed until the end of October 2020.

Similar to former analyses of trial registries (see above), HCQ or CQ was still dominant (n = 69 = 59 %: n = 63 about HCQ/CQ alone = 53.8 %, n = 6 in combination with antivirals, antibiotics, antiseptics or anthelmintic drugs).

18 trials (15.4 %) study non-COVID-vaccines, among them 12 about BCG. 10 RCTs study antivirals/antiretrovirals, 7 study vitamin D or supplements like lactoferrin, probiotics, quercetin, and 7 study anthelmintic or antiprotozoal drugs.

From 7 completed RCTs, 5 already reported results until December 13th, and all of them focused on HCQ (ABELLA, BARNABAS, BOULWARE, MITJA, RAJASINGHAM). All of these trials will be discussed in the HCQ section of this paper. SMIT et al. conclude that none of the 5 studies established a prophylactic effect of HCQ against COVID-19. As will be discussed below in the HCQ section, there may be special subgroups who may profit from HCQ prophylaxis, but these are not the people who need chemoprophylaxis at most (=the elderly).

The study from SMIT et al. (2) offers an opportunity to compare their results with the own analysis from May 13th, looking for the progress with regard to "new" prophylactic agents introduced into "new" prophylactic trials within the 7 months between May 13th (own analysis) and December 13th (SMIT et al.).

Besides HCQ/CQ, BCG (including VPM1002), nitazoxanide, ivermectin and antibodies, the following drugs, vaccines or supplements were subjects of RCTs (excluding suspended RCTs) in the analysis from SMIT et al. (2) (REC = recruiting; NYR = not yet recruiting):

● Azithromycin + HCQ (PREP, Jordan, 200 HCW, NYR // PEP, USA, 5000 contacts; NYR)

● BACMUNE (MV130) (PREP, Mexico, 3321 HCW, NYR) *[BACMUNE (MV130) is a bacterial preparation that contains a mixture of Gram + and Gram - inactivated bacteria]*

● Bromhexine + HCQ (PREP, Mexico, 140 HCW, enrolling)

● Bromhexine alone (PREP, Russia, 50 HCW, completed) **(see below: MIKHAYLOV et al.)**

● Darunavir/cobistat (PEP, Spain, PEP CoV-2 Study, 3040 contacts; ongoing)

● Emtricitabine/tenofovir (CoViPrep, PREP, Argentinia, 1378 HCW, NYR // PREP, Columbia, 950 HCW, NYR // PREP, Spain, 4000 HCW including HCQ arm and combined arms; REC)

● Favipiravir (PEP, Canada, outbreaks in long-term care, n = 760, REC)

● GLS-1200 nasal spray (PREP, USA, 225 HCW, REC) [quinine topical nasal spray; G protein-coupled receptor agonists]

● Icosapent ethyl (PREP, Montevideo, 1500 HCW, NYR) [a special omega 3 fatty acid]

● Inosine-glutamyl-cysteinyl-glycine disodium inhalation (PREP, Russia, 100 HCW, completed, *results see below)*

● Iota-Carrageenan nasal spray (PEP, Argentinia, 400 HCW, REC // "PREVICHARM" PREP/PEP, Spain, nursing homes: staff and residents; n = 1930; NYR)

● Lactobacillus coryniformis K8 (Probiotic) (PREP, Spain, 314 HCW, REC)

● Lactoferrin (PREP, Egypt, 200 HCW, NYR // PREP, Peru, 336 HCW, NYR)

● Levamisole, Isoprinosine or both (PREP, Egypt, 100 HCW, NYR)

● Lopinavir/ritonavir (PEP, Canada, 1220 HCW/contacts, REC // PREP, France, 1200 HCW incl. HCQ arms; active trial // PEP, Switzerland, 300 contacts/HCW, REC)

● Mefloquine (PEP, Spain, 200 contacts, ongoing)

● MMR vaccine (Crown Coronation and a small trial with 200 HCW in Egypt; Crown Coronation: PREP, USA and international; 30000 HCW; REC)

● Nitric oxide releasing solution (PREP, Canada, 200 HCW/contacts, REC)

● NO (nitric oxide) inhalation (PREP, USA, 470 HCW, REC)

● Oral Polio vaccine or NA-831 or combination (PREP, USA, general population, enrolling) [NA-831 is a small neuroprotective molecule]

● Peginterferon lambda alpha-1a s.c. (PEP, USA, 164 contacts, REC)

● PUL-042 Inhalation (PEP, USA, general population; 200; REC)

● PVP-Iodine nasal decolonization (Swab) + 1.2 % CHX gluconate oral rinse (PREP, USA, 84 HCW, REC)

● PVP-iodine nasal spray and gargle (PREP, USA, 250 HCW/patients, REC)

● Quercetin (PEP, Turkey, 50 contacts; REC)

● RUTI vaccine (PREP, Spain, 315 HCW, NYR) *[made of detoxified, fragmented Mycobacterium tuberculosis cells, delivered in liposomes]*

● Sepsivac (Mycobacterium w) (PREP, India, 4000 HCW/contacts, NYR)

● Sofosbuvir/Daclatasvir (PREP, South Africa, 1950 HCW, NYR)

● Tranexamid acid (antifibrinolytic) (PEP, USA, 100 contacts, NYR)

● Vitamin D (PREP, Canada, 2414 HCW, NYR // PREP, Iran, 1500 HCW/contacts, 1500, REC // PREP, UK, 4400 young adults, NYR)

● Zinc + HCQ (PREP, Tunisia; 660 HCW, NYR)

Only the agents that are marked in yellow were newly introduced into prophylactic trials within the seven months between May 13th and December 13th (this doesn't exclude that they were already subject of therapeutic trials in May, e.g. Favipiravir). For 6 of them, recruitment hasn't started until December 13th.

A more recent paper (BEN-ZUK et al.), submitted in May 2021, adds a trial with **doxycycline + zinc** (100 mg/day doxycycline + 15 mg/day zinc) from Tunisia to the list (NCT04584567) and mentions a few more trials with interferons (n = 5), nitazoxanide (n = 5) and NO (n = 5).

Including completed trials and a trial in combination with iota-carrageenan, there are now 10 prophylactic trials about ivermectin. Since HCQ (alone) was not subject of this paper, ivermectin took the top position of prophylactic trials (n = 10), followed by nitazoxanide, interferons, NO (each n = 5) (immunizations like BCG or MMR were not part of their statistics). But as already mentioned above, HCQ is a combination partner with bromhexine in a prophylactic trial from Mexico.

Interestingly, from the 36 identified prophylactic studies in Table 3 of BEN-ZUK et al. (some including both prophylaxis and early treatment), only 2 are from EU countries (LPV/R from France, Emtricitabine/Tenofovir from Spain) and 4 are from non-EU-countries (the completed and published Bromhexine trial from Russia [MIKHAYLOV et al.], 2 x NO from UK, 1 x LPV/R from Switzerland).

This exemplifies what EU and EMA think about COVID prophylaxis and what may happen when a vaccine-resistant strain arrives and once again – like in 2020 – one has nothing but non-pharmacological methods of which it is wellknown that they are not sufficient in many settings (household settings, work places etc.) and may fail in case of the new highly infectious variants.

The first version of the **Living Guideline to Prevent COVID-19 from the WHO** was published on March 2nd, 2021. It discusses only (!) HCQ for prophylaxis and mentions no other agent in a prophylactic context. It concludes with a strong recommendation not to administer HCQ prophylaxis, based on 3 RCTs about PREP and 3 RCTs about PEP. Based on these trials, WHO calculated 1 fewer death (2 instead of 3) per 1000 who take HCQ PREP/PEP and 1 fewer hospitalization (4 instead of 5) per 1000, but no fewer cases of lab-confirmed COVID-19 and 34 instead of 15 /1000 cases of discontinuation because of adverse effects (but see correction by SCHILLING et al., making this difference insignificant).

These recommendations and results are not surprising and are in general accordance with the "HCQ chapter" below, except that there are some hints that very young HCW populations (like those in India) may profit a little more from HCQ PREP with regard to the outcome "lab-confirmed infection". However, due to the extreme low COVID mortality and hospitalization rate in these young HCW populations (in many studies with mean ages of less than 30 years), this must not put in question the conclusions of the WHO with regard to mortality and hospitalization.

Nevertheless, there is also serious criticism against this guideline (see SCHILLING et al.), but this is not based primarily on the efficacy results for HCQ as such (SCHILLLING et al.: *"It is reasonable to conclude already that hydroxychloroquine does not provide high prevention or early treatment efficacies. Vaccines are rightly the priority")* but the insufficient evidence on which the recommendations were based (6 very heterogenous RCTs with different outcomes), and the very high grading of certainty for this recommendation (that is based on so weak evidence), and because of the recommendation to stop all already *ongoing* trials of HCQ prophylaxis immediately.

Nevertheless, the WHO guideline is mentioned here primarily not because of HCQ (that will be discussed in detail below), **but to demonstrate that the WHO was unable at that point of time to recommend any (!) prophylactic agent.** The guideline from March 2nd was still valid on August 16th, 2021. According to WHO, there ***is*** no (evidence-based) prophylaxis (sensu PREP or PREP) available so far.

This paper will summarise already available evidence from trials for chemoprophylaxis in the sense of preexposure prophylaxis (PREP), postexposure prophylaxis (PEP) or both ("peri-exposure prophylaxis"). It will be restricted to finished and published trials or similar informations with the aim to analyze the effects of a given agent on chemoprophylaxis. It is not primarily about candidates for chemoprophylaxis because of indirect evidence, e.g., retrospective analysis of COVID prevalence or severity in people who took some prescribed agents for other reasons in large health system databases. Such data may provide very precious hints on candidates for chemoprophylaxis; however, they are no trials on chemoprophylaxis, and the underlying disease (because of that the agent was prescribed) may have influenced the outcome (like COVID incidence or severity) as a confounder. This doesn't exclude the possibility that some results from such studies are mentioned here, but it is not the intention to collect data from such studies systematically. Some aspects and candidates are also discussed elsewhere in a separate paper:

Chemoprophylaxis against COVID-19 is needed more urgently than ever before

available from: http://freepdfhosting.com/9686575098.pdf

(**no longer updated since August 2020) (!)**

and some of them also in:

Early unspecific systemic and local therapeutic options in COVID-19 disease

available from: http://freepdfhosting.com/35f285c9f2.pdf

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.

available from: http://freepdfhosting.com/66b45bc8c1.pdf

A potential strategy to overcome COVID-19: combination of COVID vaccines with type-1-biased immunomodulation, e.g. by inactivated mycobacteria – a strategy of "double protection"

available from: http://freepdfhosting.com/437d9e1634.pdf

There is an important multinational ongoing project with more than 40 highly specialized authors that will continuously monitor the evidence for prophylactic drugs in the form of a living systematic review and network meta-analysis:

BARTOSZKO JJ et al., Prophylaxis for covid-19: living systematic review and network meta-analysis.

The first version was published on February 26th as a preprint. Updates will be published from time to time and there is also a website:

https://www.covid19lnma.com/

That project is a very high-qualitative approach to the subject of chemoprophylaxis with very strict inclusion criteria: any included trial MUST be a RCT; it must randomize at least 100 persons or have at least 20 events of the pre-defined outcomes (laboratory-confirmed infection; composite endpoint of suspected, probable or laboratory-confirmed infection; hospitalization, death).

Until January 19th 2021, only 9 RCTs met the inclusion criteria; 6 about HCQ and 3 about ivermectin (IVM) alone or in combination with local carrageenan administration.

For HCQ, no important preventive effect was found, but it probably increases adverse effects.

For IVM (with and without carrageenan), favorable effects were reported from each of the three RCTs, but the authors "*are very uncertain if Ivermectin with or without iota-*

carrageenan reduces the risk of SARS-CoV-2 infection and mortality because of serious risk of bias, very serious imprecision and the effect estimates are likely to change substantially with additional evidence from ongoing trials". (BARTOSZKO et al. preprint).

In the final publication (in BJM) BARTOSZKO wrote about IVM*: *"Because of serious risk of bias and very serious imprecision, it is highly uncertain whether ivermectin combined with iota-carrageenan and ivermectin alone reduce the risk of SARS-CoV-2 infection".

Moreover, "*no other drug has been studied in large enough trials to make any inferences regarding effects of prophylaxis for covid-19." (BARTOSZKO et al.)*

Content of this paper

(List of potential chemoprophylactic agents in the same order as they are mentioned here):

(RCTs are mentioned if available)

Mycobacterium w (Mycobacterium Indicus pranii) injection

Prolectin-M (food supplement, galectin antagonist)

Neem capsules (Azadirachta indica) (RCT: NESARI et al.)

Withania somnifera (Ashwagandha) (RCT: CHOPRA et al.)

Cannabidiol

***Supplemental:* No protective effect of HIV PREP?**

***Supplemental:* various common nutritional supplements** (RCT: SEET et al.)

***Supplemental:* Influenza or MMR vaccination?**

***Supplemental:* Bamlanivimab** (RCT: Blaze II)

***Informational:* intravenous ozonized saline therapy**

***Informational:* Ramipril (RAAS inhibitor) (no effect)**

***Informational:* preexisting aspirin prescription**

***Informational:* Vitamin D prophylaxis?**

Discussion

Subjective ranking

Important note on new COVID-19 variants (VoCs)

The studies about prophylaxis and/or (early) COVID treatment that were published so far preferently refer to COVID-19 disease and infection associated with the virus variants that were circulating in 2020 and persisted to do so in some regions of the world in early 2021.

While studies from China may be dominated by patient populations infected by the original Wuhan virus and its sequence, most of all studies from the world are expected to refer to populations and cohorts infected by the virus variants that became dominant worldwide in 2020 (with the D614G mutation).

However, there are concerns now that some drugs may be less efficient (or even inefficient) in people infected with VoCs. This applies particularly to two different groups of drugs:

● Entry inhibitors that may – in the worst of all cases – lose their efficacy because of changes (e.g., conformational changes) of the Spike, particularly the RBD of the Spike

● immunomodulators that enhance the early innate immune response to viral infections, particularly the early interferon response in the respiratory tract (important for the early control of the local infection, to reduce and prevent replication and thus expansion to the lungs and dissemination into the body).

GUO K. et al. observed that emerging SARS-CoV-2 variants evolved to resist the antiviral IFN-I and IFN-III response and confirmed the evasion of innate immunity for B.1, B.1.1.7 and B.1.351 isolates. This weakens or eliminates the interferon pathway, i.e. the early interferon reponse, of the innate immune response.

As a consequence, drugs (or vaccines) that strengthen the early innate immune reponse or stimulate early interferon production may become inefficient with regard to the prevention (prophylaxis) or early treatment of infections with VoCs like those examined by GUO K et al..

There are already first hints that MMR vaccination in young children had a small to moderate effect to protect these children from COVID-19 in 2020, but that this protective effect was completely lost after the rise and dominance of B.1.1.7 in Germany (see **Supplement** in: **http://freepdfhosting.com/437d9e1634.pdf**).

Heterologous vaccinations, particularly with live or live-attenuated vaccines (like BCG, MMR or oral polio), are expected to train the innaty immunity and therefore to stimulate the very early local interferon response in the respiratory tract immediately after viral infection. But beside vaccinations, there are also drugs that stimulate interferon production in the respiratory tract (e.g. umifenovir), and this may contribute to their prophylactic and/or early therapeutic efficacy besides of a direct antiviral effect.

It is questionable now whether these drugs or heterologous vaccinations retain their prophylactic or early therapeutic effectiveness in the presence of the new VoCs. For example, umifenovir could be affected both because of its function as an entry inhibitor and because of its effect on early interferon release, if VOCs resist to the latter.

As a consequence, all drugs that act either as an entry inhibitor or on the early innate immune response/early interferon response in the respiratory tract, should be re-examined in the context with the VoCs. Until then, it is doubtful whether they act still as well as some studies mentioned here in this paper showed in the past.

It is not necessary and would be too time-consuming to replicate the clinical studies. As far as their function as entry inhibitors is concerned*, in vitro* studies with cell cultures, particularly human epithelial cells, should be performed - both with the VoCs and the conventional virus variants. The direct comparison between the effect of the drug on VOCs vs. conventional variants may allow conclusions whether there is need for concern about its clinical efficacy in the context of VoCs, or not.

With regard to drugs that influence the early interferon response or act as immunomodulators on the early immune response following infection, animal models

should be used (e.g. hamsters, ferrets) to examine whether these drugs act differently in animals infected by VoCs compared to conventional variants.

Only after studies of that kind are published one will be able to understand what drugs and methods for prophylaxis or early treatment can still to be used in an epidemic context that is dominated by VoCs (or, as far as individual treatment is concerned, in cases when it is proven or probable that the patient is infected by a VoC), or whether these drugs and methods have to be discarded now in the context of the VoCs, even if they were shown to be successful or very successful in the past.

LEE J et al. studied the effects of viral entry/TMPRSS2 inhibitors and viral RNA-dependent RNA polymerase inhibitors (RdRp inhibitors) on B.1.1.7 and B.1.351 in direct comparison to early SARS-CoV-2, both on Vero E6 cells (missing TMPRSS2 expression) and Calu-3 cells (highly expressing TMPRSS2).

They studied four different TMPRSS2 inhibitors (camostat, nafamostat, aprotinin, bromhexine), two RdRp inhibitors (remdesivir, EIDD-2801 = molnupiravir) and EIDD-1931 (an active form of EIDD-2801), niclosamide and ciclesonide.

In summary, this "*in vitro analysis of viral replication showed that the drugs targeting TMPRSS2 and RdRp are equally effective against the two variants of concern."*

As expected, TMPRSS2 inhibitors showed no antiviral effects in the Vero cell assay. No substantial changes in the antiviral effectiveness on Calu-3 cells were found. This is explained because TMPRSS2 cleaves the Spike protein at the S2' cleavage site, and B.1.1.7 and B.1.351 have no sequence changes at this site or close to it, i.e. the original sequence of this region is conserved in both VoCs from that study.

Moreover, the efficacy of the two representative RdRp inhibitors (remdesivir and molnupiravir) was also not affected by the VoCs. The same applied to niclosamide and ciclesonide, suggesting *"that the potential targets of these drugs lie outside of the substituted amino acids in the two variants."* (LEE J et al.).

However, looking at the results of the Calu-3 cell assay in detail (Fig. 3 in Lee et al.), some differences can be noted, but all of them were too small to reach the level of significance:

Camostat and EIDD-2801 were a little less effective against B.1.351 at higher concentrations, nafamostat and aprotinin at lower concentrations, ciclesonide at middle concentrations. Bromhexine was generally less effective than all of the other agents with regard to inhibition of infection (with 50 – 60 % inhibition at the highest tested concentration compared to 80 – 100 % for all other agents at the highest concentration), and the efficacy of bromhexine against B.1.351 was a little lower across the whole spectrum of tested concentrations (e.g., 50 vs. 60 % at the highest concentration) compared to the wildtype and to B.1.1.7, whereas no difference was found between wildtype and B.1.1.7. Nevertheless, the difference with regard to B.1.351 was insignificant.

Interestingly, remdesivir was more effective against B.1.1.7 and B.1.351 in low concentrations compared to the wild type, whereas no difference at high concentrations is visible. The same seems to apply to niclosamide in the middle of the spectrum of tested concentrations.

Taken together, there are subtle differences with regard to the variants and it remains unclear whether they are by chance or whether they are of some smaller relevance, but missed significance just because of statistical power. Most importantly, there is no general trend that VoCs are less sensitive to the wide spectrum of drugs from that study.

Umifenovir (Arbidol)

Apart from COVID vaccines themselves, non-COVID vaccines like Mycobacterium w *(Sepsivac,* see below) and some evidence about Ivermectin, especially if administered locally (oral mucosa) in combination with carrageenan nose spray, the most impressive results about chemoprophylaxis have been presented so far by ZHANG et al., using umifenovir in therapeutic doses for PEP in exposed HCWs and household contacts of infected people.

The paper from **ZHANG J et al.** was already published on February 26th 2020 on the ChinaXiv Server. Though it was the most successful study for a long time, and the first proof of concept that chemoprophylaxis may actually work, it got no recognition and reception in the western world (e.g., no citation in other early papers about that subject). It was eventually published online on May 30th in “Current Medical Science”.

The ZHANG preprint from ChinaXiv is a historical paper, because it was the first paper about successful chemoprophylaxis of COVID-19 – posted on ChinaXiv about two weeks before WHO accounced the COVID-10 outbreak a pandemic.

In their retrospective, non-randomized trial, ZHANG et al. compared the incidence of new symptomatic COVID-19 infections among exposed HCWs and household contacts of infected people who took either Arbidol or oseltamivir for prophylaxis in a PEP setting. Compared to taking oseltamivir or nothing, HCWs who took umifenovir reduced their risk of COVID-19 infection by 95 % (point estimate; OR: 0.049; CI: 0.003-0.727; p = 0.0276) and household contacts of infected people by 99 % (point estimate; OR: 0.011; KI: 0.001 – 0.125; p = 0.0003). Compared to umifenovir, intake of oseltamivir was associated with an OR of 20.446 (CI: 1.407 – 297.143; p = 0.0271) (data from the ChinaXiv Paper).

Though the trial involved only 124 HCWs and 66 members from 27 families, the results became significant (HCWs) or even highly significant (household contacts). The usual dose for Arbidol was 200 mg TID, and household contacts took it for 4 – 14 days (mean: 7.1 days).

In their final publication in Current Medical Science, ZHANG et al. calculated Hazard Ratios instead of Odds Ratios. The HR for household contacts was 0.025 (CI 0.003-0.209; p = 0.0006), offering 97.5 % protection, and for HCWs the HR was 0.056 (CI: 0.005-0.662, p = 0.0221), offering 94.4 % protection.

Meanwhile, oseltamivir was found to be ineffective against COVID-19 (TAN and JIN). Thus retrospectively, one can argue that oseltamivir was a sort of placebo. Then this trial was "pseudo"-placebo controlled. Moreover, during the early phase of the epidemic in China, people didn't know what helps better against COVID-19. Both oseltamivir and umifenovir were expected to have some preventive effect against the influenza-like disease, based on past experiences with influenza. Therefore it is improbable that there was a systematic bias between those who chose umifenovir and those who chose oseltamivir. They possibly decided to take what they had already available at home, or they bought it according to their personal preferences. This mimics a sort of randomization. In summary, with the knowledge we have meanwhile about umifenovir and oseltamivir, one may call this trial retrospective, "pseudo"-placebo-controlled, "pseudo"-randomized.

In another retrospective trial, even low doses of Arbidol (200 mg per day) and less consistent use (6.7 days on average during the last two weeks before COVID onset) proved to be very successful ($p < 0.001$) for prophylaxis in HCWs **(YANG C et al.),** but the effect was smaller than in the study of ZHANG et al. where most participants took 600 mg per day (200 mg TID):

Among the 82 infected HCWs from the YANG study, 23.2 % had taken any Arbidol within the last two weeks before disease onset, whereas among 82 uninfected HCWs, this quote was 56.5 % (OR = 0.214, KI: 0.109 – 0.420, $p < 0.001$).

Bearing in mind that the thresholds for hospitalization were extremely low in China, 36.8 % of the infected 19 HCWs who had taken Arbidol prophylactically (and then therapeutically, with higher dose) were hospitalized, compared to 65.1 % (41/63) infected who had not taken Arbidol (OR = 0.313, sign.). After age-matching, this difference lost significance, but became a strong trend ($p = 0.091$), probably as a consequence of underpowering. Four of the 63 infected HCWs without Arbidol and none of the 19 infected HCWs with Arbidol prophylaxis developed severe pneumonia. Arbidol didn't delay viral clearance after age-matching (duration of positive throat swab: $r = - 0.240$; $p = 0.056$).

Whereas oseltamivir is recognized meanwhile to be ineffective with any regard to COVID-19, the effectiveness of Arbidol in the treatment of manifest infections is still open to debate. Among six early trials, one small trial showed no effect (LI Y et al.), one trial showed favorable effects only in patients with non-severe disease (XU K et al.), and four trials showed favorable effects (ZHU Z et al., DENG L et al., CHEN W et al., LIU Q et al.). LIU Q et al. found a reduction in mortality of 81 % following adjustment. Taken together, these data are far from any "breakthrough" which may be interesting for the media, but the results are more favorable than what was heard about HCQ or Lopinavir/ritonavir during the last months, and even Remdesivir is regarded now as uneffective or only a little effective, at least for patients that progressed so far that they needed hospitalization.

However, in a systematic review and meta-analysis, HUANG et al. found no advantages of umifenovir except that it's safe and offered a higher viral clearance rate at day 14. In particular, there was no advantage with regard to the combined endpoint [death or ICU transfer] (RR 1.20; CI: 0.61 – 2.37). However, for unknown reasons, the large retrospective trial from LIU Q et al. was not included. Since it is also not mentioned in the discussion or reference section, it was probably overlooked. LIU Q et al. don't report about ICU transfers, but about death. Combining (ICU + death) cases from six studies from the meta-analysis

from HUANG et al. (n = 283 Arbidol, n = 301 controls) with death cases from LIU Q et al., the combined endpoint from these seven studies is evidently in favor of Arbidol (bad outcome: 33/540 = 6.1 % in the Arbidol group vs. 75/548 = 13.7 % in the control group).

JOMAH et al. analysed the results of 8 therapeutic trials with Arbidol and found that 5 of the 8 trials showed favorable results, including reduced mortality and earlier viral clearance.

WANG Z et al. reported lower mortality (0/36 instead of 5/31 = 16.1 %) in hospitalized patients who got 400 mg Arbidol TID for a median of 9 days. Moreover, their discharge rate from hospital at the time of the study was higher. NOJOMI et al. compared Arbidol monotherapy (200 mg TID) with Lopinavir/Ritonavir monotherapy (but both groups got 400 mg HCQ once at day 1) in a RCT with hospitalized patients in Iran, and Arbidol was found to be superior with regard to clinical, laboratory, virological and radiological outcomes; however, the study was underpowered to examine mortality (1/50 death in the Arbidol group, 2/50 in the L/R group).

FANG et al. compared “Lianhuaqingwen without Arbidol” with “Lianhuaqingwen + Arbidol” in hospitalized patients from Wuhan with moderate and severe disease. Whereas there were no significant differences for outcomes like PCR conversion, CT improvement and hospital stay in severe patients between both groups, the differences became significant ($p < 0.01$) for all three outcomes in moderate patients in favor of the combination.

With regard to death, no significant differences were found, maybe because of the small number of deaths. However, there was a trend in favor of the combined therapy. There were 3/49 deaths in the LQ group and 3/113 deaths in the combined group (moderate + severe patients combined). With regard to severe patients only, there were 3/18 (16.7 %) deaths in the LQ group and 2/45 (4.4 %) in the combined group.

With regard to recurrence (re-positivity), a study with 23 re-positive patients from China found that treatment of the primary COVID-19 infection with Arbidol was associated with a significantly lower likelihood of testing re-positive (adjusted HR: 0.178; 95% CI: 0.045-0.709; $p = 0.0144$) (ZHOU et al.). In a meta-analysis of two other studies with altogether 86 cases of recurrence and 426 cases without recurrence, HOANG found a significantly reduced risk of recurrence in patients who had gotten arbidol (OR 0.48; CI: 0.25 – 0.92). The same applied to steroid (OR 0.48, sign.), but not to lopinavir/ritonavir (OR 1.17, n.s.) and chloroquine/HCQ (OR 1.24, n.s.).

Surprisingly, in a retrospective study with hospitalized patients from China, early umifenovir – in contrast to early hydroxychloroquine – was not associated with prevention of aggravation and shortening of improvement time (SU et al.). However, that study focused on HCQ and the authors didn’t even discuss the seemingly unfavorable results for umifenivor. As shown in their Fig. 1, umifenovir was preferentially given to severe or critical patients; (about 64 % of all patients who got early umifenovir were in a severe/critical stage, in contrast to about 8-9 % who got early HCQ).

Finally, AMANI et al. performed a systematic review and meta-analysis about Arbidol in patients with COVID-19 until May 2021. They included 16 studies (14 from China, 2 from Iran; only 5 are RCTs) and found no significant benefit of Arbidol compared to other antiviral treatments with regard to PCR negativity or secondary outcomes like CT improvement,

cough alleviation, hospital stay. Serious outcomes like ICU, intubation or mortality were not subject of their analysis, but it was mentioned that Arbidol is associated with lower mortality compared to oseltamivir. Though not analyzed in detail, the combination of Arbidol and TCM, particularly Lianhuaquinwen and Shufeng Jiedu capsules seems promising. Interestingly and in contrast to their disappointing results about therapy, AMANI et al. also mention that *"recent findings from two studies have suggested its efficacy and safety for prophylaxis".*

But one has to respect that most of these studies were about early experiences with Arbidol with the "early" SARS-CoV-2 at the beginning of the pandemic in China when it was dominated by the Wuhan strain. It is completely unknown how Arbidol would act now at the times of more aggressive variants. With regard to these limitations, the data on Arbidol have to be regarded as "historical". There are so far no *in vitro* data about the antiviral activity of Arbidol against VoCs.

WANG X et al., based on their own *in vitro* results, concluded that umifenovir must be very effective against (early, wild-type) SARS-CoV-2; however, the doses which were given in most trials (200 mg TID) might have been too small (they recommend at least 800 mg per day) and this may explain why some clinical results were not as favorable as expected from laboratory data.

In 2020, there wre three registered trials about Arbidol prophylaxis, one large, but only observational trial with 1000 participants (ChiCTR20000295920; PEP; high-risk contacts and HCWs), one small randomized trial which compares HCQ and Arbidol (CHICTR2000029803; PEP, 320 participants, close contacts) and a non-randomized trial with 500 HCWs with Arbidol in combination with Jinyebaidu granules (CHICTR2000029728). Thus all three trials have serious limitations (two are not randomized and the randomized one is rather small), and since all three trials are from China, it is doubtful whether they can be completed successfully.

In summary, whereas the role of Arbidol/Umifenovir in therapy of manifest COVID-19 or hospitalized patients is still unclear and evidence is limited, two retrospective trials found umifenovir as highly effective in post- or periexposure prophylaxis in HCWs (two trials) and household contacts (one trial). Since both trials reached results of high statistical significance, there cannot be any doubt any more that umifenovir is effective in chemoprophylaxis. Of course, the first retrospective cohort study (ZHANG et al.) with a hazard ratio of 0.056 (CI: 0.005-0.662, p = 0.0221) and OR of 0.049 (CI: 0.003-0.727; p = 0.0276) showed better results for HCWs than the second one (YANG C et al.) with its OR of 0.214, KI: 0.109 – 0.420, p < 0.001). However, this difference is plausibel with regard of the dosage. Whereas most participants in ZHANG et al. took 600 mg per day (200 mg TID), the prophylactic dose in YANG C et al. was only 200 mg per day, and only infected (symptomatic) people took the therapeutic dose of 600 mg. Thus the difference between the more favorable results of ZHANG et al. compared to YANG et al. seems to be due to a simple dose-effect relationship, and the therapeutic dose of 200 mg TID seems to be more effective than 200 mg once a day, though also the latter showed a highly significant protective effect, but inferior to 200 mg TID. Thus, in spite of these differences, the results of ZHANG et al. and YANG et al. are very well compatible with one another, and one should be satisfied that even low dose umifenovir shows significant prophylactic effectiveness. Moreover, the results from

ZHANG et al. are highly transparent because they present individual data (including dose and duration of Arbidol intake and family situation) for each participant, as far as the household contacts are concerned.

The molecular mechanisms of the anti-COVID activity of umifenovir (as an inhibitor of viral entry) were analysed by PADHI et al.. Unfortunately, there are no reports about umifenovir in animal models as far as COVID-19 is concerned (until October 4th, 2020). It was found in a ferret model that Arbidol down-regulates proinflammatory cytokines induced by influenza (IL-10, TNF-alpha, IL-8, IL-6) and alleviates influenza-induced lung lesions (WANG Y et al.).

DADRAS et al. describe how Umifenovir can be synthesized cheaply in large amounts.

Interferon alpha nose drops and thymosin alpha 1 s.c.

2944 HCWs from a hospital in Hubei province applied interferon α Type 1b nose drops four times a day (2-3 drops/nostril) during the peak of the local COVID epidemic. Among them, 529 HCWs were exposed to high COVID risks (isolation wards, fever clinics), and they got weekly injections (s.c.) of 1.6 mg thymosin-α1 alongside of interferon nose drops. The other HCWs were of low risk of exposure to COVID-19. At the end of the trial (28 days), none of them had acquired confirmed or presumed COVID infection or any other respiratory infection **(MENG et al.).** However, there was no control group. Furthermore, the authors didn't estimate or model how many infections would have to be expected without that intervention, based on experiences from other hospitals in this epidemic region und during that time frame. This makes it very difficult to evaluate the effect of this intervention.

In a clinical trial with hospitalized patients, thymosin alpha1 was found to be very effective to increase the number of CD8+ and CD4+ T cells in older patients with COVID-19, and people with lower levels of CD8+ and CD4+ profited most from thymosin (LIU Y et al.). This effect is already well known from the administration of thymosin alpha 1 in other severe infections, e.g. ARDS because of CMV in renal transplant patients (JI et al. 2007).

In the clinical trial mentioned above, thymosin alpha1 (10 mg/day for at least 8 days) reduced mortality in severe COVID-19 patients from 30 % in the control group to 11.1 % in the intervention group, and the need for mechanical ventilation from 22.5 % (9/40) to 0 % (0/36) and for non-mechanical ventilation from 27.5 % (11/40) to 5.56 % (2/36) (LIU Y et al.).

Taken together, 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. However, if there is a real protective effect, it is more probable that it is a consequence of interferon administration in all participants than of thymosin injection in the high risk participants. On the other hand, since this study didn't rely on COVID PCR but on symptomatic disease, it is still possible that thymosin inhibited the development of symptomatic disease in participants who got infected, so that their infection remained undetected. 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 early trial.

The rationale for nasopharyngeal administration of type-1-interferon for prophylaxis and early treatment is decribed in detail by LEE AC et al.

What about **interferon spray**, there is already some good experience with interferon alpha 2b from an old RCT where participants who used interferon alpha 2b nasal spray and a control group without interferon were inoculated with common human coronavirus (a human challenge trial) (TURNER et al. 1986). There were less symptomatic infections in the interferon group (41 vs. 73 %), and, more importantly, the mean total symptom score was much lower (9.2 vs. 23.4) ($p = 0.003$). And interferon beta (SNG001) inhalation was proved to have a strong beneficial effect in the treatment of COVID-19 patients at various stages of the disease (MONK et al.).

In vitro, interferon beta and interferon lambda pretreatment decreased SARS-CoV-2 replication in a reconstructed bronchial epithelium model. A too weak natural early interferon response following SARS-CoV-2 infection of ciliary epithelia seems to play a central role in the pathogenesis of COVID-19 (ROBINOT et al.) and may be a rationale for local interferon administration for chemoprophylaxis and early treatment.

However, it was suggested that interferon increases ACE2 expression. This would make a possible role in prophylaxis and early treatment very doubtful. But ONABAJO et al. found that interferon induces a novel, transcriptionally independent truncated isoform of ACE2 (called deltaACE2), which is unable to bind the SARS-CoV-2 spike protein. In contrast to deltaACE2, ACE2 is not an interferon-stimulated gene.

Impaired type I IFN production or signaling is associated with severe COVID-19, offering a rationale for the treatment with recombinant IFNs. In a Syrian hamster model, intranasal IFN-α administration was only effective if administered very early, i.e. one day pre-infection or one day post-infection (BESSIERE et al.). In that case, weight loss (as a proxy for disease severity in hamsters) and viral lung titers were decreased, compared to hamsters without IFN administration. In hamsters, symptoms appear three days after inoculation. IFN-α administration starting at the onset of symptoms (three days) had no impact on the clinical course of the infection; however, there were no signs of enhanced disease with late IFN. Since the clinical course of COVID-19 progresses much more quickly in hamsters than in humans (viral replication, lung pathology progress; peak of virus replication in the lungs of hamsters on day 2 or 3), IFN treatment at day 3 in hamsters correlates with „late“ treatment that was associated with upregulation of IL-6, CCL2 and TNF alpha, whereas such upregulations were not observed in the „early“ treatment group that simulated PREP or PEP settings. The hamster study supports the concept that intranasal IFN administration is effective in PREP or PEP.

With regard to **thymosin**, a retrospective study with 435 hospital staff in China (January 25th – March 25th,2020) found that use of thymosin (different products and different dosages) as preexposure or postexposure prophylaxis was not significantly associated with reduced COVID risks (LIU X et al.). There was 1 confirmed infection in 57 staff members without thymosin prophylaxis (1.8 %), 2 infections in 101 persons in the PREP group (2.0 %) and 3

infections in 277 persons in the PEP group (1.1 %). Some participants had side effects including fever for 1 – 2 days, and side effects were more common in persons who also used interferon for prophylaxis.

According to *in vitro* studies, it cannot be expected that thymosin has significant effects on normal peripheral blood lymph cells (e.g., the number of T cells) in healthy people. As long as the healthy person's own immunity levels maintain a dynamic balance and T cells play normal cellular immune functions, thymosin seems to be without effect in agreement with laboratory data (LIU X et al.). This may explain why thymosin seems to be quite successful in therapy (see above), but seems to fail in prophylaxis.

However, the thymosin prophylaxis study from LIU X et al. has a lot of limitations. Nearly all of the hospital staff was young (18 – 40 years, only few participants > 40 years) and dominated by young nurses. Moreover, between 66 and 81 % took Jinyebaidu Granules, between 60 and 69 % took Abidor (Umifenovir), between 12 and 25 % took Lianhuaqingwen Capsules, between 8 and 10 % alpha interferon, and between 7 and 10 % took Oseltamivir, and more rarely some other drugs which were also supposed to be of some prophylactic effectiveness. Thus this study doesn't allow conclusions about a possible role of thymosin for chemoprophylaxis in older people when the immune system is no longer as well balanced as in people under the age of 40 who dominated the LIU X trial.

The PROTHYMOS RCT will study the role of thymosin alpha 1 prophylaxis of severe COVID-19 in cancer patients undergoing active cancer treatment (Eudract 2020-006020-13) (BERSANELLI et al.). The rationale behind the use of thymosin in that context is described in detail in that paper. The study will examine the incidence of serious COVID-19 within 8 weeks from randomization.

In the era of vaccinations, thymosin may also increase efficacy of vaccines, particularly in frail individuals. It is already known from RCTs that thymosin α1 enhances the immunogenicity of influenza vaccines in immunocompromised patients.

Lactoferrin

Lactoferrins disrupt the primary attachment of coronaviruses, mediated by heparan sulfate interactions, and may have the potential for a pan-coronavirus inhibitor (LEBLANC and COLPITTS).

SERRANO et al. reported about a sort of ring prophylaxis in 256 contacts (family members) of 75 infected people with moderate or severe disease. Contacts got liposomal lactoferrin (Lactoferryn TM Forte drinkable, Sesderma laboratories), 64 mg 2 – 3 times a day (128 – 192 mg/d). This is half of the dose that was given to infected patients who were treated by lactoferryn- and zinc-based agents as outpatients (including lactoferrin mouthspray and nose drops for patients with nasal congestion, dry cough and headache and lactoferrin aerosol using the Nanomist Nebulizer SES for those with breathing difficulties).

None of the 256 contacts was reported to be infected; however, there are no informations whether any examinations (PCR, antibody testing) were performed. Follow up was 30 days, so the results probably relied on the absence of symptoms until then. More details were not given with regard to the contacts.

Whereas the results are impressive for a PEP setting, even if they document only the absence of symptomatic disease and not necessarily absence of infection, one has to be careful since treatment of the symptomatic infected outpatients (as index patients) and their family members started *after* the index patients had a positive IgM/IgG test. The index patients were heavily symptomatic (but not as severely that they had to be hospitalized), but with a positive IgM/IgG result, the index patients were probably already beyond the phase of high infectiousness when their own treatment (and the prophylactic treatment of their family contacts) began. Maybe they were already progressed beyond the stage of infectiousness when LF therapy and prophylaxis started. However, some contacts may already have been infected at the time of start of LF prophylaxis, and within their incubation period, and LF may have prevented progress to symptomatic disease?

In a study with 36 patients from Sweden, virus culture both from nasopharyngeal and sputum samples was unsuccessful in all patients with SARS-CoV-2 specific IgG-titers above 1 : 40, neutralizing titers above 1 : 10, or negative PCR (GLANS et al.). No antibody titres were mentioned in the SERRANO trial, and treatment didn't start directly following positive IgG/IgM-diagnosis but in a time frame within the next two days (*"Day 0: treatment had not yet begun").* It is therefore probable that most or all index patients were no longer infectious at the time when lactoferrin treatment was started. This is a serious limitation of that study with regard to PREP, but it may still indicate effectiveness of the regimen in PEP.

Without a control group, it is impossible to estimate how many (symptomatic) infections among family members would have occurred without the lactoferrin intervention. It is urgently necessary to repeat a lactoferrin trial in a prophylaxis setting at a time when the infectiousness of index persons is still higher. Also lactoferrin treatment of the patients themselves might have reduced their infectiousness.

Moreover, liposomal lactoferrin was applied in the trial. This is difficult to access in other countries, because it is not yet available on the local markets. But it is unknown whether normal lactoferrin without liposomalization will be helpful too and to a similar extent? It was found that liposomalization enhanced the anti-inflammatory effects of lactoferrin (ISHIKADO A et al.).

Thus there is a need for more trials with liposomal lactoferrin and also with non-liposomal lactoferrin (which is much more easy to access in an acute situation). The SERRANO et al. trial was performed by SESDERMA, the producer of the liposomal lactoferrin products. However, all 75 moderate or severe patients were treated successfully, their symptoms improved quickly, there are no hints that anyone had to be brought to hospital, and all were alive 30 days after treatment start. This is an excellent starting point for further (independent) (!) research.

As WANG Y et al. pointed out in their detailed review about different mechanisms how lactoferrin may act against COVID-19, the assumed effects of LF *"on SARS-CoV-2 are based on the effects of LF on other viruses, and there is currently a lack of direct research on the effects of LF on SARS-CoV-2."* They also hint to problems in applying LF in the clinical setting:

"For example, it remains unknown which state of LF is more effective in treating SARS-CoV-2, namely unsaturated vs. saturated, human-derived vs. bovine-derived, whereas the combined metal, specific dosage and route of administration have yet to be clearly determined …"

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 ($p = 0.02$). Among the two positive cases in the inhalation group, one was detected as positive on day 6 of the intervention and the other one 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 assume 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.

Iota-Carrageenan nasal spray

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 already shown *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 %, I-C vs. placebo). 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 protective effects of iota carrageenan were much more pronounced against coronavirus infections compared to other infections. This may offer a possible explanation why I-C spray reduced SARS-CoV-2-PCR negative symptomatic disease only a little (12 %), compared to PCR+ symptomatic disease.

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.

Povidone-iodine throat spray

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 possible effects of the throat spray on PCR performance.

See also **AREFIN;** not a study, but a personal report about an extremely exposed doctor 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.

Hydroxychloroquine

The first report about potential chemoprophylactic effectiveness came from South Korea and was originally posted on April 11th on Medxriv. **LEE et al.** reported from a long-term care facility from South Korea where two employees were found to be infected: a social worker who worked some time in spite of her symptoms before COVID diagnosis, and a caregiver who possibly got her infection on a different pathway since she had no close or relevant contact to the infected social worker. 189 inhabitants of the long-term care facility (mean age: 80 years) and 22 staff members took 400 mg HCQ a day (without a loading dose because there were many small people there, many of them about 40 kg). Within the next two weeks, there were no new infections among the 211 people who got HCQ. Again, there was no control group, and both infected women worked usually with face masks, but it is not sure whether they wore them all of the time at work. So maybe the inhabitants and the other staff were protected solely because of the face masks, therefore it is hard to guess whether (and how many) infections would have occurred in the absence of HCQ PEP. The authors point to these limitations and that's why they regard their paper only as a sort of communication and not as a trial.

And the seemingly favorable results could not be replicated during a serious outbreak of COVID-19 in a home care facility in Northern Italy in spring 2020. 42 PCR-negative inhabitants got HCQ prophylaxis as PEP **(AGOSTINIS et al.),** but 15 of them became PCR positive and 5 died. In contrast, none of 15 patients who got amiodarone prophylaxis became PCR +. During April, HCQ was used as prophylaxis (200 mg 2 times a day for 5 days); but from May 6th, prophylaxis was done with amiodarone (200 mg twice a day for 10 days).

However, there is no control group for inhabitants with the same risk of infection without prophylaxis. And whereas the results seem to suggest a favorable effect of amiodarone

compared to HCQ, it is important to note that amiodarone started to be administered as prophylaxis at a time when the outbreak was already under control, thus it is impossible to base any conclusions about amiodarone prophylaxis upon these data.

(Amiodarone was chosen as a substitute after the dispense of HCQ because it is “a cathionic drug that accumulates in the lumen of organelles with an acidic interior and increases lumenal pH similarly to hydroxychloroquine, a property that explains its interference with the processing of Ebola virus spike protein”. It also “inhibited SARS-CoV-1 infection acting after the delivery of the viral genome into the cytoplasm of the target cell, a property not known for hydroxychloroquine”, it inhibits “the expression of tissue factor by endothelial cells and has displayed antithrombotic activity in an animal model”) (AGOSTINIS et al.).

Moreover, many papers were published where COVID prevalence or severity were reported from patients who took HCQ for autoimmune or rheumatic disease in the COVID era. Most of the studies point against a protective effect of HCQ from acquiring symptomatic COVID disease or hospitalization.

For example, **MACIAS et al.** reported about the 7-week incidence of COVID-19 during the peak of the first wave of COVID epidemic in Spain in 722 patients with autoimmune/rheumatic disease. 290 of them got HCQ as regular treatment for their underlying disease, mimicking a chemoprophylaxis setting. 1.7 % of patients who took HCQ and 1.2 % of those who didn’t take HCQ were infected during these 7 weeks, and 1 of the 290 HCQ patients and 2 of the 432 non-HCQ patients were transferred to hospital (none of them needed ICU). However, there were no serological tests and not all of the presumed COVID 19 cases could be confirmed by PCR testing because of lack of material.

An update for the same study population of 722 patients after 17 instead of 7 weeks of observation **(MACIAS et al. (2))** found an incidence of 3.4 % of clinically diagnosed COVID-19 in the HCQ group and 3.0 % in the group without HCQ. PCR-confirmed COVID-19: 1.4 % vs. 1.4 %. Hospitalization: 1.0 % (HCQ) vs. 0.9 %. No ICU, no death. Median age was 56 vs. 58 years.

In a retrospective cohort study from Spain with 919 individuals with autoimmune disease with HCQ treatment and 1361 controls without HCQ, there was no difference with regard to confirmed COVID-19 (1.7 % vs. 1.9 % in the control group) and hospitalization (0.4 vs. 0.3 %). Suspected COVID-19 was more common in the HCQ-group (6.1 vs. 4.3 %) **(LOPEZ DE LA IGLESIA et al.).** This study has the same limitations like that of MACIAS et al.

In another study from Spain, encompassing 319 patients with autoimmune disorders regularly taking chloroquine or HCQ and matched control patients without CQ/HCQ, COVID-19 prevalence was even higher in the CQ/HCQ group (5.3 % vs. 3.4 %), indicating a lack of protection of regular administration of CQ/HCQ **(LAPLANA M et al.).**

In a retrospective population-based cohort study from South Korea, attack rates of COVID-19 in patients with RA or SLE were compared between those who underwent HCQ therapy within 14 days before a COVID test and non-users **(JUNG SY et al.).** Among 2066 patients with RA or SLE, 31.4 % were treated with HCQ, most of them got 200-400 mg/day as recommended for the treatment of their underlying rheumatic disease. COVID 19 attack rate was 2.3 % (15/649) in HCQ users and 2.2 % in non-users (31/1417). Interestingly, there was an insignificant trend for a lower attack rate (compared to non-users) in patients < 60 years

(uOR 0.66; CI: 0.26-1.69; adjusted OR: 0.69; 0.25-1.92) and for a higher attack rate (compared to non-users) in patients 60 years or older (uOR 1.61; 0.69 – 3.75; aOR: 1.37; 0.54 – 3.47), an observation that was recapitulated in HCQ PREP trials *(see below).*

In another study from South Korea, nationwide health-insurance data were used to correlate results from COVID testing from 20 January 2020 to 15 May 2020 with pretreatment with HCQ for at least 30 days until the date of SARS-CoV-2 testing (total of 216,686 adult individuals who had been tested; 743 were pretreated with HCQ; among them: 695 >= 3 months, 611 >= 6 months) **(BAE et al.).** Median daily dose of HCQ was 200 mg, range 100 – 800 mg.

Prevalence of positive tests was 2.2 % in HCQ users and 2.7 % in non-users (OR 0.79; CI: 0.48 – 1.20). Following propensity score matching: 2.2 % vs. 3.1 % (p = 0.18; aOR 0.69; CI: 0.40 – 1.19; but after adjusting for region, aOR was 0.80 (0.42 – 1.52)). Mortality was 0/16 among HCQ users (0 deaths, 16 infections) compared to 140/5865 infected non-users (2.4 %), but after propensity-score matching, mortality was 0 % vs. 0 %.

Long-term intake of HCQ was associated with an insignificant trend for a small protective effect (>= 3 months: aOR 0.69; CI: 0.39 – 1.22; p = 0.20; >= 6 months: aOR 0.59; CI: 0.32 1.07; p = 0.08).

Interestingly, the patients who took HCQ for rheumatic disease did worse compared to the total group who took HCQ: OR 0.93, aOR 0.85; aOR adjusted for region 1.07; aOR >= 3 months: 0.85; aOR >= 6 months 0.88; all ORs n.s.). This may suggest that patients who took HCQ for other reasons than rheumatic disease may profit from HCQ, but this is not discussed in BAE et al. Moreover, it is not given how many of the 743 patients who took HCQ did that because of rheumatic disease; this makes it impossible to calculate the effect for those who took HCQ for other reasons than rheumatic disease.

In summary, this study shows that HCQ doesn't reduce the risk of a positive SARS-CoV-2 test in people who take HCQ for rheumatic disease; however, there may be a signal that there may be a prophylactic effect in those who take HCQ for other reasons, but this was not further examined in that study. One may argue that the kind of indication for HCQ may be independent of its prophylactic effect on SARS-COV-2 testing. However, different indications may result in different dosing regimens, and, as will be shown below, there are hints that dosing has some influence on its prophylactic effect, but not in a simple way of "the more the better".

In contrast to the disappointing results for CQ/HCQ (like for example in the study from BAE et al.), rheumatic patients who received biologicals had a reduced risk of severe disease in a large study from Spain (0.48 % severe disease instead of 2.75 % in the general population; hospitalization: 0.48 % vs. 3.7 %), and IL-6-inhibitors were especially effective to reduce the risk of COVID-19 infection (OR 0.10; p = 0.05) whereas some other agents increased the risk (Rituximab beyond the limits of significance; IL-12/23 inhibitors) **(SANTOS et al.).** This study shows that some medications for people with rheumatic disease may actually decrease the risk of symptomatic COVID-19 infection or severe disease, but, based on other studies from Spain, this doesn't apply to CQ/HCQ.

Based on data from a health administration database from Catalonia between January 1st and April 30th 2020, no effect of chronically taking CQ/HCQ on COVID risk was found (based on 6746 patients with active prescriptions for CQ/HCQ and 13492 controls). The COVID incidence was 1.4 % in the exposed cohort and the same in the control cohort. Incidence rates: 12.05 vs. 11.35 cases/100,000 person days; HR of infection: 1.08; CI: 0.83 – 1.44). The risk of hospitalization showed a trend to be higher in the exposed cohort (0.6 % vs. 0.4 %; HR 1.46; CI: 0.91 – 2.34; p = 0.10) **(VIVANCO-HIDALGO et al.).**

Results from an Israeli healthcare database confirmed a null effect of *continuous* hydroxychloroquine or colchicine treatment with regard to the results of COVID PCR testing: among 14.520 people tested, 13.203 were negative and 1317 were positive. 0.25 % of all test participants took HCQ (0.23 % positive, 0.25 % negative) und 0.49 % took colchicine (0.53 % positive, 0.48 % negative) **(GENDELMAN et al.).**

In a study from South Korea with 219961 subjects tested for COVID-19 **(HUH et al.)** (7341 COVID-19 positive, matched with 36705 controls; 878 patients with severe COVID-19, matched with 1927 mild-to-moderate patients), pre-diagnostic use of HCQ (for any reason) was not associated with risk for COVID-19 (aOR 0.94; CI: 0.53-1.66), but with an insignificant trend for more severe disease (aOR 3.51: CI: 0.76 – 16.22), but the latter may be confounded by underlying diseases. The same applied to Azithromycin (aOR for test positivity: 0.58 [CI: 0.30-1.12], but aOR for severe disease: 2.03 [0.39 – 10.60]).

Another study from Spain compared the probability of hospital admission because of COVID 19 in 3951 patients with inflammatory rheumatic diseases; 16.8 % (666) of them got CQ or HCQ. Their risk of hospitalization because of COVID-19 was not smaller than the risk for patients without CQ/HCQ (HR for CQ/HCQ: 0.95; CI: 0.5 – 2.1). CQ/HCQ doesn't seem to reduce the risk of infection or the severity of the disease in people with inflammatory rheumatic disease, at least with regard to the endpoint "hospital admission" **(FERNANDEZ-GUTIERREZ et al.).**

KONIG et al. reported about COVID-19 infected patients with SLE from the Global Rheumatology Alliance Registry. Until April 17th, 80 patients with SLE were reported to be infected with COVID-19, 51 of them used HCQ/CQ. There was no difference in the proportion of hospitalization between users (57 % = 29/51) and non-users (55 %; 16/29). 33 % of the SLE patients on CQ/HCQ and 45 % of those without CQ/HCQ needed any form of oxygen support.

Based on a large health care data set, 0.29 % of 26.815 SARS-CoV-2-positive people in Portugal were found to be chronically treated with HCQ (at least 2 g per month on average), compared to 0.36 % of 333.489 negative persons (p = 0.04). After adjustment for age, sex, chronic corticosteroids/ immunosuppressants, the aOR for SARS-CoV-2 infection in people with chronic HCQ treatment was 0.51 (0.37 – 0.70) **(FERREIRA et al.).** But the authors didn't examine dose-effect relationships which would be interesting in a study which found such a protective effect. In China, a retrospective study of 27 patients with autoimmune rheumatic disease in families where COVID-19 was diagnosed found a strong protective effect for patients who took HCQ (OR 0.09; p = 0.044; CI: 0.01 – 0.94, p = 0.044) instead of other anti-rheumatic medications. However, this analysis is based on very small numbers of patients taking HCQ, explaining the extremely large CI **(ZHONG et al.).** Mean age of the rheumatic patients who took HCQ was 49 years.

A retrospective cohort study with 32.109 rheumatic patients (US Veterans Health Administration) found only small effects of chronic HCQ use (COVID-19 incidence: 0.3 % in users vs. 0.4 in non-users; OR 0.79; CI: 0.52 – 1.20; p = 0.27), whereas overall mortality (COVID and NON-COVID) was decreased significantly (OR 0.7; p = 0.0031) **(GENTRY et al.).** There were 31 active COVID infections in rheumatic patients taking HCQ (what corresponds to the portion of 0.3 %) and 78 in patients not taking HCQ. Rates of hospitalization (29.0 % vs. 24.4 %) and ICU care (22.4 vs. 21.1 %) were similar; but there were no deaths in the HCQ group compared to 9 % (7/78) deaths in the non-HCQ group, but this difference is not significant (p = 0.19).

In a study with 159 patients with COVID-19 from U.S. (22 % SLE, 80.5% rheumatoid arthritis, 1.5 % both), there was no reduced risk among HCQ users compared to other immunosuppressants **(SINGER et al.).** And data from the NHS of the UK showed that people with SLE/rheumatoid arthritis/psoriasis have a slightly increased risk of death from COVID-19 (HR = 1.19; CI: 1.11 – 1.27) compared with people without one of these diseases. **UGARTE-GIL et al.** conclude: *"antimalarials neither prevent severe acute respiratory syndrome coronavirus 2 infection nor reduce its severity".* The data from the NHS are independent from the mode of treatment; however, since many of these people take HCQ, a protective effect of HCQ would not be in accordance with a significant Hazard Ratio of 1.19.

Finally, a large study examined the influence of HCQ on COVID mortality in people with rheumatic arthritis or Lupus in England **(RENTSCH et al.),** based on a large dataset representing 40 % of the general population in England. Among 194.637 patients with RA or SLE, 15.7 % received at least 2 prescriptions of HCQ in the six months before March 1st, 2020. There were 547 COVID deaths in that group, 70 among HCQ users. Cumulative mortality was 0.23 % (HCQ users) and 0.22 (non-users), and adjustments didn't change the result (HR for death: 1.03; CI: 0.80 – 1.33). A case report from Turkey showed that even young adults under HCQ treatment because of rheumatic arthritis may develop severe COVID-19 and need mechanical ventilation quite early (day 6 after start of fever and muscle pain) **(GÜRSOY et al**.). In a small study from New York with patients with rheumatic disease who were hospitalized because of COVID-19, chronic HCQ use (because of the underlying disease) was *not* associated with less severe presentation and better outcomes (aOR for mechanical ventilation: 1.5; CI: 0.34 – 6.38; aOR for in-hospital mortality: 0.77; CI: 0.13 – 4.56) **(PHAM et al.).**

Taking into account the large sample size of the RENTSCH trial, there is now overwhelming evidence that CQ/HCQ treatment for autoimmune diseases has no protective effect with regard to COVID-19 infection or COVID-19 outcomes/mortality in people with that underlying disease. This warns that the chances of a successful role of HCQ in COVID PREP are probably small; however, the studies with RA or SLE patients didn't analyse different dosages, and there may be still a small chance that dose or age may matter (for age, see the age signal in JUNG SY et al. mentioned above).

These results were also corroborated by a large study from Denmark that encomprised all persons (!) in Denmark who got HCQ prescriptions in 2020 and 2019, matched by age and sex with controls, based on complete health data from the Danish national health registries (KAMSTRUP et al.). Databases of that size, collecting all health informations from a patient, are rare worldworde. Most countries would be unable to perform such a study. Study period was February 27th until November 27th, 2020.

Altogether, there were 5488 HCQ users who were matched with 54486 non-users as controls. 3.43 % of the HCQ group and 3.72 % of the control group had a positive test result. 82.11 % of the HCQ group and 78.74 % ($p < 0.1$) had at least one SARS-CoV-2 test during the study interval.

After adjustments, HCQ use (for non-COVID-19 indication) was not associated with confirmed SARS-CoV-2 (HR 0.90; CI: 0.76 – 1.07).and the result remained robust in propensity-score matched sensitivity analysis. uHR was 0.92 (CI: 0.79 – 1.07). Sensitivity analysis: HR 1.01 (CI: 0.92 – 1.31).

There were 175 hospital admissions within 14 days of a positive test among the ~ 60000 participants. OR was 1.44 (0.78 – 2.65) for HCQ users.

This well-designed study shows clearly that HCQ prescriptions have a null effect on the risk of SARS-CoV-2 positivity and hospitalization. It is noteworthy that the mean age of the study participants was 57.4 years. Thus this study doesn't exclude the possibility that HCQ may have some prophylactic effect in very young populations.

There are many ongoing prospective trials about HCQ/CQ in PREP or PEP for HCWs, and some of them are very large. If there are impressive and significant interim results that clearly show a high protective effect (not necessarily 100 %), one would expect that such results would have already been announced and celebrated by conventional and social media as "big breakthrough". Beside of high media coverage, it would also be necessary for ethical reasons to communicate such results to the public, provided that they are actually statistically robust, in order to give other exposed HCWs the chance to protect themselves by such methods, given that millions of HCWs are under high risk worldwide. Moreover, if the success is definitely evident, it could be possible to unblind the trials and allow participants in the placebo arm to switch to the verum.

Nothing of that happened so far; instead, as will be described below, one large trial was prematurely stopped (BOULWARE et al.). However, according to an online questionnaire to asymptomatic physicians, Indian physicians preferred the recommended (ICMR) HCQ regimen; it appears to be safe and associated with a high level of adherence. Adverse effects were similar in those who took the ICMR regimen (5.9 %) compared to those without prophylaxis (6.5 %) (BAVDEKAR et al.).

The first hints for favorable effects of HCQ prophylaxis came from an undated paper from the Indian Ministry for Health and Welfare. HCWs in three hospitals in New Delhi who cared for COVID patients experienced fewer infections with SARS-CoV-2 themselves if they took HCQ for prophylaxis. The protective effect is reported to be smaller in HCWs who cared for the general population. Moreover, another observational study mentioned in that paper found that, among 334 HCWs altogether, those 248 who took HCQ for prophylaxis showed lower incidence of SARS-CoV-2 infection after median 6 weeks of follow-up than those who didn't take HCQ.

However, no precise results were given there or published in a scientific paper. It is not possible to estimate the quantity of protection and the statistical significance of the results from this paper from the Ministry (for critics, see also: BMJ India correspondent, and TANDON et al. with regard to HCQ use for prophylaxis in the general population).

In the first half of June 2020, there was a first published report about PREP in HCWs in an Indian hospital **(BHATTACHARYA R et al.).** Following an outbreak among HCWs in that hospital (altogether 28 infections among HCWs), quarantine and COVID testing, there was a chance to compare PCR positivity rates between 54 HCWs who had opted voluntarily for HCQ PREP according to the recommendations of the Indian Ministry, and 52 HCWs who didn't take HCQ during the critical time interval. 7.5 % of the HCWs who took HCQ were found to be infected, compared to 38.5 % who didn't take HCQ ($p < 0.001$). Among the 55 HCWs who had contact with symptomatic infected people (staff), these quotes are 9.38 % vs. 54.55 %, and among 92 HCWs with face-to-face contacts, they were 7.84 % vs. 39.02 %.

In spite of the impressive and highly significant results, one has to be careful because this was not a randomized trial and one cannot exclude selection and recall bias; for example, one cannot exclude the possibility that HCWs who opted for HCQ were more fearful and thus more careful in their behavior or use of protection. Moreover, both cohorts were very young (mean age: 26.5 years in die HCQ group, 27.7 years in the control group) and comorbidities were rare. The authors themselves warn that they cannot prove a causal relationship between HCQ PREP and COVID incidence in their cohort.

In a second case-control study among Indian HCWs, **CHATTERJEE et al.** found an adjusted OR of 0.44 (CI: 0.22 – 0.88) in HCWs who took at least 4 maintenance doses of HCQ (following loading dose). In India, a loading dose of 400 mg BID and then 400 mg weekly were recommended to HCWs. The trend between the number of maintenance doses and risk reduction of COVID-19 was highly significant ($p < 0.001$). Six or more maintenance doses were associated with a risk reduction of more than 80 %.

Adjusted ORs: only loading dose „and irregular recall of maintenance": aOR 1.87 (CI: 0.82 – 4.24); 2 – 3 maintenance doses (MDs): aOR 2.34 (CI: 1.23 – 4.83), 4 – 5 MDs: aOR 0.44 (sign.) and more than 5 MDs: aOR 0.04 (0.01 – 0.16).

Taken together, 45.5 % of 378 COVID cases among HCWs and 51.75 % of 373 controls had taken any HCQ (OR 1.28, n.s., $p = 0.087$). Unadjusted ORs: loading dose „and irregular recall of maintenance": OR 1.27 (n.s.); 2 – 3 MDs: OR 1.65 (n.s.), 4 – 5 MDs: OR 0.55 (sign.), more than 5 MDs: OR 0.19 (sign.).

Among the combinations, HCQ + vitamins was most successful (OR 0.21; CI: 0.08 – 0.52), whereas there was a trend that HCQ + azithromycin + vitamins is unfavorable (OR 1.36; CI: 0.71 – 2.64) (for comparison: HCQ alone: OR 0.85; CI: 0.62 – 1.17).

The participants of that study were quite young (mean age of cases and controls: 34.7 vs. 33.5 years). Compared to HCWs > 50 years (Ref., OR = 1.00), the protective effect was more pronounced in the youngest group (18 – 25 years; OR 0.62; n.s.) and the following age group (26 – 33 years; OR 0.81; n.s.).

KHURANA et al. reported about a COVID-19 outbreak among HCWs in a tertiary hospital in Delhi. 94 HCWs were infected (mean age: 36 years), 87 were not infected (mean age: 34.3 years). Only 52.1 % of infected HCWs had any symptoms. The authors compared the 22 HCWs who had taken a full course of prophylactic HCQ to the 159 who had taken either an incomplete course or no HCQ at all and found a significant risk reduction for those who took the full course ($p = 0.012$).

However, there was no plausible dose-effect relationship: the proportion of infections was 27.3 % among the 22 HCWs who took the full course, 70.6 % among 68 HCWs who took an incomplete course of HCQ, but only 44 % among the 91 HCWs who hadn't taken any HCQ at all. The risk of exposure to infected patients was similar for infected and uninfected HCWs, but infected HCWs more often preferred surgical face masks instead of N95 respirators. With this confounder and without multiple logistic regressions, the results from KHURANA et al. are difficult to interprete. There are no informations whether HCQ impacted the severity of the disease; however, there were only three hospitalizations (one ICU, no death) in the quite young cohorts at all. It remains unclear whether HCQ may have contributed to that favorable outcome.

The first randomized placebo-controlled PREP trial with HCQ was disappointing (**COVID-PREP** study) **(RAJASINGHAM et al.).** It dealt with PREP in HCWs with ongoing exposure in US and Canada who were randomized to 400 mg HCQ once or twice weekly for 12 weeks (n = 494 and 495; placebo: n = 494). Placebo was folic acid.

Primary endpoint was confirmed or probable COVID-19. Compliance was controlled by HCQ whole blood concentrations. The trial included 1483 HCWs (79 % reported aerosol-generating procedures). Median age 41 years, follow up: 311 years; 97 persons developed confirmed or suspected COVID-19.

Incidence rates for either laboratory-confirmed or symptomatic compatible illness were 0.27 events per person-year (HCQ once weekly) or 0.28 events (HCQ twice weekly) compared to 0.38 events in the placebo group (Hazard Ratios: 0.72; CI: 0.44 – 1.16 for once weekly and 0.74, CI: 0.46 – 1.19 for twice weekly; % of participants with confirmed or suspected COVID-19: 5.9 %, 5.9 % and 7.9 %).

Median blood HCQ concentration was 98 ng/ml in the „once-weekly group" and 200 ng/ml in the „twice-weekly group", and HCQ concentrations did not differ significantly between those who developed COVID-19 (154 ng/ml) and those who did not (133 ng/ml, p = 0.08).

Because of its strict methodology, the randomized, double-blind and placebo-controlled trial design, the high number of highly exposed participants and laboratory control of compliance, this single trial outcompetes the combined evidence from all Indian PREP reports taken together.

A limitation of that study is that among the 97 „cases", only 18 had a positive PCR test, 38 a negative PCR test (but most of them tested before occurence of symptoms) and 42 no PCR test at all.

The point estimates of the hazard ratios were only a little lower in those who reported full adherence at 80 % or more of the surveys (once weekly: 0.66, twice weekly: 0.68; both without significance; 5.7 % vs. 5.7 % vs. 8.5 %). Side effects were more frequent in the HCQ arms and dose-dependent.

There were nine hospitalizations in the placebo arm, three in the low-dose arm and eight in the high-dose arm. No ICU, no death.

The authors calculated that plasma concentrations of HCQ were too low, and they calculate that even daily dosing might be not enough, but suggested that daily dosing may still be an option that may be worth trying.

One important limitation of that study was that enrollment was stopped early after the interest of participation in the trial declined following negative reports about HCQ, resulting in inadequate power of the trial. The risk reduction of 0.11 per person-year means that nine highly exposed HCWs would have to take HCQ prophylaxis for one full year to avoid one case of COVID-19.

There were no subanalyses for different age-groups. Such sub-analyses would be important with regard to the age-dependent trends seen in CHATTERJEE et al. (PREP) and BOULWARE et al./WISEMAN et al. (PEP, see below).

Though the results from the COVID PREP trial (= RAJASINGHAM et al.) are disappointing, they are still better than those from PEP trials of similar quality (placebo-controlled RCTs) like BOULWARE et al. and MITJA et al. (see below) as long as one looks at the point estimates. Both the COVID PREP trial and the BOULWARE PEP trial were stopped prematurely and didn't include as many participants as originally planned and calculated as necessary for statistical robustness. Thus, statistical insignificance of the trends found in COVID PREP and BOULWARE et al. doesn't mean that they represent a *true* null effect; instead, the insignificance of the trends may simply be a result of underpowering because of the early stop of recruitment.

Taking this into account and based on the point estimates, the risk reduction of 28 % in the once-week group in the RAJASINGHAM trial (which rises to 34 % in participants with very good adherence) is still superior to the relative risk reduction of 16.8 % in the BOULWARE PEP trial, and 11 % risk reduction in the MITJA PEP trial. Though all results are very disappointing, PREP seems to work still better than PEP (in accordance with theoretical assumptions). This difference seems to be even stronger if one considers that the HCQ doses were much higher in the PEP trials compared to COVID PREP, including a high loading dose and daily intake. Taking the different doses into account, it becomes even more evident that PREP works better than PEP, and this is well in accordance with HIV PREP vs. HIV PEP and seems to be a general phenomenon which applies to chemoprophylaxis of viral infections. If so, this may have consequences for other methods (agents) of COVID chemoprophylaxis which were shown to work in PEP (like umifenovir/Arbidol) and for which it can be assumed now that it is probable that they may work even better in a PREP setting.

Finally, a possible limitation of the RAJASINGHAM study has to been considered. Folic acid was given as a placebo. However, there are hints that folic acid supplementation (like in pregnant women) has a protective effect on its own (see ACOSTA-ELIAS and ESPINOSA-TANGUMA), and folic acid is already subject of a prophylactic trial (PACTR202005599385499). If folic acid has really a prophylactic effect, even if it is small, the effect of HCQ prophylaxis would be stronger than suggested by the COVID PREP trial. Folic acid binds to furin-protease and the spike:ACE2 interface of SARS-CoV-2, and its level was lowest among severe patients compared to mild or moderate patients (KAUR et al.). However, SKIPPER and BOULWARE discuss the use of folic acid as a placebo in a separate paper and see no problem, e.g. because of its low dose in placebo tablets.

A second placebo-controlled PREP RCT was reported from Mexiko **(ROJAS-SERRANO et al.).** In contrast to RAJASINGHAM et al., participants took 200 mg HCQ daily for 60 days (or placebo). In spite of ist favorable results, the trial was terminated early because of the lack of new participants after the reputation of HCQ was damaged in July 2020. Participants were highly exposed HCWs who cared for severe COVID patients. Only 127 participants (PCR-negative at baseline) could be included (62 HCQ, 65 placebo) (originally, 400 participants were planned to be randomized). Median age was only 31.5 years (31.0 years in the HCQ group).

1.6 % from the HCQ group and 9.2 % from the placebo group developed symptomatic, PCR-proven COVID-19 (1 : 6), but this difference missed significance (p = 0.09; aHR 0.18; n.s.). There was no case of severe disease and no hospitalization in that study. As will be discussed later, the favorable results from that study may have been associated with the young age of the participants. In the HCQ group, one individual initially sero-negative became sero-positive during the study period in the absence of a positive PCR test, suggesting a case of asymptomatic infection.

A third placebo-controlled PREP RCT was reported from Pakistan (CHEER trial, **SYED et al.).** In that trial with exposed HCWs, HCQ prophylaxis showed no favorable effect at all. In two dosing regimens, there was even a trend for higher risks. About 200 HCWs (exposed to COVID patients to a similar, but not identical extent) were randomized to three dosing regimens (group 1: 400 mg twice a day at day 1 as loading dose, then 400 mg weekly; group 2: 400 mg once every 3 weeks without a loading doese; group 3: 200 mg once every 3 weeks without a loading dose; group 4: placebo; n = 48; 51; 55; 46). The medication or placebo was given for 12 weeks. As common in that region of the world, the mean age of the participants was quite young (range: 28.2 to 32.0 years in the four groups).

	Group 1 / group 2 / group 3 / **controls**			
PCR + during the study time	31.3	37.3	14.5	**15.2 %**
PCR + at the end of week 12	6.3	5.9	1.8	**6.5 %**
Symptoms compatible with COVID	33.3	54.9	23.6	**30.4 %**
Illness with outpatient observation	20.8	27.5	10.9	**13.0 %**
Positive serology at the end of 12 weeks (IgM+ or IgG+ or both)	29.2	41.2	16.4	**23.9 %**

In direct comparions, the lowest dose regimen (200 mg/3 weeks) fared a little better than the controls, but both 400 mg regimens fared worse, without a plausible dose-effect relationship. No participant developed severe or critical disease or needed hospitalization (however, this is not surprising because of the young age). Enrolment began on May 1st, 2020, thus the study was performed before the arrival of VoCs.

The 200 mg regimen was tolerated as well as placebo, whereas side-effects were more prevalent in the 400 mg regimens. One cannot exclude the possibility that the 200 mg regimen has a small protective effect, but the study was underpowered to show this with certainty.

REVOLLO et al. reported about HCQ PREP in a hospital from Badalona/Spain; frontline HCWs were invited to participate in PREP (day 1: 400 mg BID; day 2-5: 200 mg BID, thereafter maintenance dosing of 200 mg weekly). HCWs were classified as high-, moderate- and low-risk occupational exposure according to their contacts to COVID-19 patients. PCR was performed in case of suspicious symptoms; all hospital HCWs were screened by SARS-CoV-2 serology at the end of the local epidemic.

69 HCWs received HCQ PREP, 418 did not (all worked in the same hospital at the same time). Altogether, 16.6 % of the 487 HCWs had positive nasopharyngeal PCR during the study period and 17.9 % had IgG antibodies after the epidemic. No one had received antiviral or immunomodulatory treatment. HCWs with HCQ PREP had higher crude rates for positive PCR (23.2 % vs. 15.6 %) and positive serology (28.3 % vs. 15.4 %). Median time from PREP initiation to PCR-based diagnosis was 14 days (IQR: 7 – 23 days).

After risk stratification (COVID-19 cases had an average higher exposure than controls), the rates of PCR positivity were 22.9 %, 22.5 % and 15.3 % in the high, moderate and low risk PREP group (no PREP: 15.6 %). The rates of seropositivity were 23.8, 15.8 and 16.4 % in the three PREP groups (no PREP: 15.4 %).

A propensity-score analysis with 1:1 matching allowed complete adjustment and resulted in an aOR (PREP vs. non-PREP) of 0.77 (CI: 0.35 – 1.68) for positive PCR and 1.43 (CI: 0.62 – 3.38) for positive serology. Covariate imbalance did not remain after matching (REVOLLO et al.).

Since there are suggestions that HCQ inhibits trained immunity and the expression of IFN-stimulated genes, REVOLLO et al. point out that their *„results are very robust in the identification of an absence of PrEP efficacy of hydroxychloroquine",* but that "*the possibility of increasing the risk of infection is not concordant and the interpretation must be very cautious."*

Unfortunately, the authors didn't report whether there was any association between HCQ PREP and the severity of symptoms or severity of the disease. Though insignificant, the lower aOR (0.77) for PCR positivity (PCR tests were performed only in case of suspicious symptoms) compared to the aOR (1.43) for seropositivity (IgG test was performed for all hospital staff at the end of the epidemic) might hint to the possibility of a lower risk of *symptomatic* COVID-19 in PREP users in case of infection (as demonstrated by IgG some time later), thus it would have been interesting to look closer on symptoms and severity grades of the disease in that study. Interestingly, the MITJA PEP trial (see below) found also that people on HCQ had an increased risk of seropositivity following adjustment. In MITJA et al., the phenomenon nearly reached significance (aRR 1.6; CI: 0.96 – 1.69).

Combining the MITJA and REVOLLO trial, the effect would probably become significant, and then raise the question why people on HCQ PREP/PEP have a higher risk of seropositivity, but not of symptomatic infection/PCR positivity? Is this a result of the immunomodulatory effect of HCQ, maybe the downregulation of IFN-stimulated genes or the inhibition of

trained immunity? If trained immunity (which creates no antibodies) is inhibited to some extent, the antibody response may be stronger, or the possibility may rise that antibodies will be produced even in the case of a mild, asymptomatic or subclinical infection? HCQ seems to influence the balance between trained immunity and the induction of IgG response (in favor of the latter). Maybe the reduced response of trained innate immunity and the reduced expression of IFN-stimulated genes decrease the chance that the infection is stopped before the induction of IgG production, i.e. more cases of early infection progress until a stage when IgG production is induced?

It is also suggested that the high prevalence of IgG in the HCQ group is the consequence of early activation of adaptive immune response (YANG A et al. 2

Beside these hypotheses, considering both the REVOLLO and the MITJA results, the increased risk of seropositivity doesn't seem to be a chance finding.

In a placebo-controlled RCT of HCQ PREP from three hospitals in Barcelona with 269 HCWs (142 HCQ group, 127 control group), there was 1 confirmed COVID infection after one month in each group **(GRAU-PUJOL et al.).** There were 3 suspected cases during that time interval in each group. The study was underpowered because it was performed at a time when incidence decreased. HCQ was given 400 mg daily during the first four days, then 400 mg once weekly. Though insignificant because of the very small number of cases, this trial was unable to demonstrate any protection by HCQ. It is mentioned here only for the purpose of completeness, but not really helpful.

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 HCQ (400 mg at day 1, then 200 mg daily for altogether 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) (ICQ: n = 432). Confirmed SARS-CoV-2 by PCR or serology: 49.1 % (HCQ) vs. 70.0 % (Vit. C). Relative risk ratio 0.70 (CI: 0.44 – 0.97), absolute risk reduction in case of the use of HCQ was 21 % (CI: 2 – 42 %).

Point estimates for adjusted ORs (depending on model, 6 different models were taken into account: between 0.34 and 0.39, some of them significant).

Symptomatic COVID-19: 6.7 % (HCQ) vs. 10.3 % (Vit. C) (- 35 %). Symptomatic disease among those diagnosed with SARS-CoV-2: 13.7 % vs. 15.0 % (- 8.7 %). No hospitalization, no death in any study arm (young age!). Whereas the mean age in the whole study was 33 years, it was only 30.6 years in the HCQ arm because of the exclusion of participants who were assumed to have contraindications against HCQ (e.g., because of ECG results).

In a small retrospective study of HCWs in a gastroenterology department from a tertiary-care hospital in India (based on PCR and IgG), 6 of 117 participants had taken HCQ in adequate doses (according to the Indian recommendations); none of these 6 HCWs tested positive for COVID-19. Among the 111 participants who didn't take any HCQ prophylaxis, or in inadequate doses, COVID positivity by PCR and/or IgG was 34.2 % (no p value calculated) **(KUMAR GOENKA et al.).** No median or mean age is given, but 78.6 % of the 117 HCWs were

<= 40 years old and 48.7 % were <= 30 years, thus the HCW population was quite young, as common in India. Because of the low number who took HCQ according to the Indian recommendations, this study has little impact on the issue of HCQ PREP.

In a retrospective case-control study among altogether 3100 HCWs at a tertiary care centre in India, 506 tested positive for SARS-CoV-2 (16.3 %; 45 % of them asymptomatic), and 253 who tested negative were matched as controls **(DEV et al.).** Among other factors of increased or reduced risk, HCQ intake was associated with lower risk of COVID infection. Fewer cases took HCQ prophylaxis than controls (31 % vs. 42 %; RR: 0.74; CI: 0.61 – 0.90, p = 0.003, NNT = 9). Moreover, the difference in the number of HCQ doses between both groups was significant (p = 0.0009). The adjusted OR according to the number of doses of HCQ was 0.92 (0.86 – 0.99), though it is not clear how this was calculated.

But it is important to note that the HCW population was quite young (cases: mean 32 years, controls: 30 years). There was no subgroup analysis for the efficacy of HCQ PREP in different age groups. The small, but significant preventive effect of HCQ in this study is in accordance with some other studies that found modest preventive effects in HCW populations dominated by very young HCWs.

In an observational study from India, HCQ as PREP in HCWs at high-risk of exposure was studied between June and October 2020. Dosing: loading dose 400 mg twice a day, followed by 400 mg once weekly up to 16 weeks **(BHATT et al.).** There were 927 full-time hospital-based HCWs, of whom 731 initially started HCQ; 196 did not. Mean age: 27.5 years (range: 20 – 52 years).

22.8 % of the HCQ group and 15.3 % of the non-HCQ group tested positive (PCR +) (p = 0.220).

All COVID positive HCWs irrespective of use of HCQ were either asymptomatic or had mild disease and fully recovered, but the study population was quite young.

Though there were no grade 3 or 4 adverse effects, many participants discontinued taking their weekly HCQ. At week 16, there were only 5 participants in the HCQ group. Many infections in the HCQ group (n = 32) occurred in week 1 (n = 731 participants on HCQ) and week 2 (18 infections, 565 HCW on HCQ); the incidence was low in weeks 4 – 6 (4 cases/week, when 470 – 432 HCWs were on HCQ) and rose then up to 64 cases in one week after many HCWs had given up HCQ.

Even if one takes the bad adherence into account, HCQ doesn't seem to have any preventive effect in that study then otherwise the SARS-CoV-2 rate must have been smaller in the HCQ group (who took HCQ at least part of the 16 weeks) than in the control group wjo never took HCQ. But the serious lack of adherence didn't allow to calculate exact RRs or HRs.

PEP:

Whereas all Indian „results", the US/Canadian COVID-PREP trial (RAJASINGHAM et al.) and the REVOLLO and GRAU-PUJOL trials from Spain mentioned above are about PREP,

BOULWARE et al. reported on June 3rd the first ever results of a randomized, placebo-controlled trial with HCQ for PEP. High-dose HCQ (800 mg loading dose, 600 mg 6 – 8 hours later, then 600 mg/day for the next four days) was started within 4 days following exposure as PEP for highly or moderately exposed HCWs (exposed to infected patients or infected staff without adequate PPE) or household contacts or partners of infected people. The index persons had to be proven COVID-19-positive by PCR. COVID-19 incidence of the participants was based on reported symptoms within 14 days; PCR testing was performed only in a few of them, so the calculations are based mainly on symptoms (and thus cannot exclude the possibility of asymptomatic infections). Placebo was folic acid too.

The trial was stopped following the third interim analysis on May 6th, when 11.8 % of participants in the HCQ group and 14.3 % in the placebo group were assumed (by the criteria mentioned above) of being infected. The risk difference of – 2.4 % (i.e. 16.8 % relative risk reduction) was insignificant and not in an acceptable relation to the side effects (40.1 % vs. 16.8 %), so the trial was stopped. Infected people in the HCQ group didn't profit from milder disease; instead, they had on average more symptoms, but this may be due to the adverse effects of HCQ. The risk of hospitalization was the same for both groups (each: 1 person). The median symptom-severity score (on a scale from 0 to 10; higher scores indicating greater severity) was 2.8 in the HCQ group and 2.7 in the placebo group.

However, there was an association between risk reduction and the time between exposure and first intake of HCQ. Day 1: 6.5 % vs. 12.7 % (HCQ vs. placebo), relative risk: - 49 %; day 2: 12.0 % vs. 17.0 %, relativ risk: - 29 %; day 3: 12.2 % vs. 14.5 %; relative risk: - 16 %: day 4: no risk reduction at all. None of these differences reached statistical significance, but the tendency is striking. Thinking backwards in time, these results still offer the chance that an earlier start of HCQ (like in the case of PREP) may possibly offer better results. Thus the possibility of better results with regard to PREP (compared to PEP) would not be absolutely incompatible with the results of BOULWARE et al., though experiences with HCQ in people with rheumatic or autoimmune diseases point to the opposite (see above). But it has to be noted again that none of the associations mentioned above reached significance because the trial was underpowered to analyse the associations between COVID incidence and the latency time from exposure to first HCQ intake.

The combination of HCQ with zinc or vitamin C intake showed no better outcome than zinc or vitamin C alone. Increased risks for this combination (HCQ + zinc: RR 1.23, n.s.; HCQ + vitamin C: RR 1.60, CI: 1.12 – 2.28) may be confounded if participants with the highest risk of infection had a higher probability to take zinc or vitamin C.

Moreover, there is a striking age gradient: young participants (18 – 35 years) profited more from HCQ (11.9 % vs. 18.6 % placebo) than middle-aged (36 – 50 years: 11.9 vs. 15.2 %), and older participants (> 50 years) had an increased risk of COVID-19 in the HCQ group (11.5 % vs. 5.5 %). Though none of these differences was significant and the trial was underpowered to resolve this question, the trend is very obvious.

Interestingly, household contacts did profit much more (14.4 % vs. 20.8 %) than HCWs (11.3 vs. 12.2 %), though the overall risk for household contacts was higher. Again, there was no significance.

Unfortunately, in spite of these striking (though insignificant) differences between subgroups, no multivariate analysis was performed. Maybe there are subgroups who may profit a lot from HCQ PEP and others who get harmed by it (aside of harms from adverse effects)? Or the striking trends are simply the result of confounding by uneven distribution of the other variables and would get attenuated by multivariate statistics? Of special interest is the question of an increased risk for people above 50 years.

Future studies and papers about HCQ in PREP and PEP should perform similar sub-analyses, but combine them with multivariate calculations to resolve these urgent questions which suggest that HCQ PEP may be (very?) helpful to some people and (very?) harmful to others. If only young people profit from HCQ, this wouldn't help a lot because older people need PEP much more because of their higher risk of severe or critical disease in the case of infection.

The „age effect" is especially striking because it was found in PREP (CHATTERJEE et al.) and in PEP (BOULWARE et al.). In both trials, the „age effect" didn't reach statistical significance. However, since the trends in both trials point to the same direction, this has to be seen as a warning that those who need PREP/PEP at most (the elderly), will profit less (or not at all) from HCQ chemoprophylaxis, and the profit – risk ratio of HCQ chemoprophylaxis may be age-dependent at the expense of those with the highest need of protection. There may be also some associations between the effect of HCQ and the ageing of the mitochondria; mitochondria of young people are more adaptable and resilient (SHEAFF RJ).

In a prospective open label (not-randomized) control trial from India, HCQ PEP showed moderate effectiveness **(DHIBAR et al.).** The trial participants were "asymptomatic non-HCW individuals who had direct contact with laboratory confirmed COVID-19 cases (family members, friends, colleagues, relatives; *contacts without personal protective precautions*) or who had undertaken international travel in last 2 weeks". They were given the option for taking HCQ prophylaxis (800 mg on day 1, divided into 2 doses of 400 mg 12 hours apart, followed by 400 mg once weekly for 3 weeks). Total cumulative dosis was 2000 mg. PEP: n = 132, controls: n = 185. Home quarantine (2 weeks), social distancing and personal hygiene were identical in both groups. Follow-up was 4 weeks (by telephone or physically if required). 7 patients were not fully compliant to take all HCQ tablets. But they were included in the analysis for the HCQ group. However, from originally 325 patients, 8 were lost from follow-up. They were not included in the analyses.

Altogether, there were 50/317 (15.8 %) cases of new onset COVID 19 (including „probable COVID-19" without a positive COVID-19 test): 10.6 % in the PEP group and 19.4 % in the control group (p = 0.033; RR 0.59; CI: 0.33 – 1.05); number needed to treat to prevent one case was 12. There were no serious adverse reactions in the HCQ group.

Definitive COVID (i.e. PCR-positive, with or without symptoms) were 7.6 % vs. 15.1 % (p = 0.041) of participants; NNT = 14. Probable COVID (symptoms suggestive of COVID-19 but negative PCR or no PCR performed) were 3.0 % vs. 4.3 % (p = 0.552).

In 17 of 317 participants, no PCR could be performed. If they are excluded, the incidence of definitive COVID-19 was 7.7 % vs. 16.5 % (p = 0.023).

42 % of all 50 new onset COVID cases (including probable cases) were symptomatic. Incidence of new onset symptoms: 4.5 % (HCQ) vs. 8.1 % in the control group (p = 0.209). No one needed oxygen or life support. Symptoms were not significantly different between HCQ and control group.

This was not a placebo-controlled study but dependent on the voluntary consent of people who met the criteria of the study as mentioned above either to take HCQ or to take not; people with contraindications for HCQ were directly assigned to the control group. All asymptomatic patients who fulfilled the criteria and were in contact to the medical institute participated in the study. There was no self-selection about study participation, only about HCQ intake for those who had no contraindications. PCR was performed in all symptomatic participants, but also in asymptomatic participants after 5 – 14 days. COVID-19 cases were defined as PCR-positive, whether symptomatic or not. Only 3.1 % of participants had a history of international travel. Mean age was 37.2 years, only 8.7 % had relevant comorbidities. The authors point to important differences between HCWs and non-HCWs (all participants in their study were non-HCWs), because HCWs usually wear some sort of PPE when they are in contact with patients, whereas private contacts don't. Thus they see a need to distinguish between HCWs and non-HCWs in PEP studies. Whereas the study design of BOULWARE et al. excluded the possibility to detect asymptomatic COVID infections (PCR only in symptomatic cases), the DHIBAR trial allowed to do so. This is an important difference, because 58 % of all PCR-proven infections in DHIBAR et al. were asymptomatic.

However, in contrast to these comparatively favorable results of DHIBAR et al., **MITJA et al**. showed in their large controlled trial of PEP and preemptive therapy as "ring prophylaxis", that neither PEP nor preemptive therapy are successful. With its trial design and large number or participants, this trial is even more important than the trial of BOULWARE et al..

MITJA's trial encompassed 2314 participants, among them 2000 PCR-negative when the trial started (PEP participants) und 314 with positive PCR, but without significant symptoms (preemptive therapy). The verum participants got HCQ (800 mg at day 1, 400 mg the following six days); for the control group, there was no placebo (in contrast to BOULWARE et al. who used folic acid as placebo). Participants were exposed health care workers or household contacts of infected people (in the sense of ring prophylaxis), and workers and residents of nursing homes.

Altogether (n = 2314), 6.2 % of participants in the control arm and 5.7 % of participants in the HCQ arm developed PCR-confirmed symptomatic COVID-19 (adjusted RR: 0.89; CI: 0.54 – 1.46).

If one restricts analysis to the 2000 participants who were PCR-negative in the beginning (1042 in the control arm and 958 in the HCQ arm), the rates for PCR-confirmed symptomatic COVID-19 were 4.3 % (control) and 3.0 % (HCQ). Whereas these data look like a little success, the adjusted risk ratio shows the opposite (aRR 1.45; CI: 0.73 – 2.88). And if one considers a more inclusive outcome (either symptoms compatible with COVID 19 or PCR positivity), the risk was a little higher in the HCQ compared to the control arm (18.7 % vs. 17.8 %; aRR 1.04; 0.77 – 1.81). Finally, at day 14, 14.3 % of the participants in the HCQ group, but only 8.7 % of the control group were seropositive (IgM and/or IgG), and this difference became nearly

significant (aRR 1.6; CI: 0.96 – 1.69) *(the same effect was observed in the REVOLLO PREP trial; see above).*

If one looks at the 313 persons who were PCR-positive at the beginning (about half of them got HCQ as preemptive therapy), 22.2 % of the HCQ group and 18.6 % of the control group got PCR-confirmed symptomatic COVID-19 (aRR 0.96; CI: 0.58 – 1.58).

Altogether, 12 persons from the control arm and 11 from the HCQ arm were taken to hospital. 8 vs. 5 died. However, there are no informations how many of them were from the PEP vs. therapy arm.

Among PEP participants, the median time lag between exposure and assignment to HCQ or standard care was 4 days. The RR was 0.89 if HCQ started until day 3, 0.93 if started on day 4-6 and 4.09 if started at day 7 or later. All these RRs are insignificant. Unfortunately, the time interval up to 3 days is not divided into shorter intervals; this would have been helpful to compare with the results of BOULWARE et al./WISEMAN et al. which suggested that very early PEP on day 1 or 2 may have some protective effect, and the results of BOULWARE et al./WISEMAN et al. showed a time effect that was reminiscent of HIV PEP.

Except for the open question of the effect of very early PEP (especially on day 1), the results from MITJA et al. destroy all hopes about HCQ PEP in a real word setting (a possible favorable effect on day 1 would not meet the requirements of a real word PEP setting, but would be of academic interest).

Whereas the results from BOULWARE et al. and MITJA et al. are regarded as disappointing, it is understood meanwhile that these are valuable data which may still offer the chance to identify subgroups or situations where HCQ PEP may be effective, and that there is a need for a more detailed analysis of the datasets. Thus, a re-analysis of the datasets from BOULWARE was planned in a separate study (see WISEMAN et al. (2)), and as soon as the datasets from MITJA et al. are made available, the MITJA data will also be re-analysed by the same methods (WISEMAN et al.). Maybe some aggregation of both datasets, as far as possible, may help to understand more about HCQ PEP or the principles of COVID-19 PEP in general. The new study will address all the critical aspects and open questions mentioned above and will allow much deeper understanding about the chances and limits of HCQ PEP.

A re-analysis of the supplementary data from BOULWARE et al. by YANG et al.(2,3), based on Cochran-Armitage analysis of trend, found a significant protection of HCQ against symptomatic COVID-19 in a time-dependent manner ($p = 0.0496$), taking into account the time lag for the delivery of the drug of about 2 days because of mailing.

Another re-analysis of the original BOULWARE dataset was reported by WISEMAN et al. (2). After requesting additional data, WISEMAN et al. found that 52 % of participants received the HCQ medication 1 - 2 days after intended overnight delivery, and 19 % of all participants received it outside the four-day window calculated from exposure (i.e. they are outside the original inclusion criteria for the study). Taking that into account, many participants started taking HCQ later than originally calculated by BOULWARE et al. If there is a time-dependent effect, this difference could have attenuated the calculated preventive effect in the original dataset from BOULWARE et al. significantly.

For participants who started HCQ *really* within 1 – 3 (elapsed time) days after exposure, COVID incidence was 9.6 % vs. 16.5 % (placebo), RR 0.58 (0.35-0.97), p = 0.044, NNT 14.5. If HCQ started > 3 days, there was no risk reduction (RR 1.22; CI: 0.72 – 2.04). A separate analysis with HCQ given within 2 (elapsed time) days of exposure found an ever stronger effect (RR 0.35, 95%CI: 0.13 – 0.93; p=0.0438).

With regard to an age-dependent effect, early start of HCQ was preventive in younger people (18 – 45 years; RR 0.53; CI: 0.29-0.97; p = 0.0448, NNT 11.5), but late start (>3 days) was not (RR 1.02; CI: 0.55 – 1.89). In older adults (> 45 years), the effect of early HCQ was small and insignificant (RR 0.75; CI: 0.27 – 2.05) (early cohort).

The RRs in the early cohort were 0.53 (18 – 35 years), 0.52 (36 – 50 years) and 2.8 (> 50 years), but all insignificant. The authors discerned a boundary between 42 and 48 years and found the significant result (RR 0.53, as shown above) for the younger adults (18 – 45 years).

There was significant reduction with early prophylaxis (days 1-3) in household contacts (RR 0.35; CI: 0.13-0.89, p = 0.025, NNT 5.7), but insignificant in HCWs (RR 0.74; CI: 0.4-1.38).

A higher protective effect in household contacts may be associated with the lack of advanced PPE, hygiene training and multiple high-risk exposures in household contacts.

Gender, folate, zinc or vitamin C intake had no effect on the results. While folate as a placebo seemed to be “neutral” and thus suited as a placebo, the use of zinc and vitamin C was balanced between verum and placebo group so even if their effect is not neutral, it would not confound the study results. WISEMAN et al. note, *“At earlier stages, any effect associated with HCQ appears independent of zinc, evidenced by the lack of synergy we observed between HCQ and zinc.”*

The effect of HCQ was more pronounced in people who had no comorbidities. Among the comorbidities, asthma attenuated the preventive effect of HCQ at most. There were no differences in the severity of symptoms in infected participants between early and late prophylaxis cohorts.

RRs in subgroup analyses from WISEMAN et al.:

Early HCQ*, 18 – 45 years: RR 0.54 (0.29 – 0.97), p = 0.0448

Early HCQ, 46 – 90 years: RR 0.75 (0.27 – 2.05), n.s.

Late HCQ, 18 – 45 years: RR 1.02 (0.55 – 1.89), n.s.

Late HCQ, 46 – 90 years: RR 1.87 (0.68 – 5.13), n.s.

Early HCQ, household contacts: RR 0.35 (0.13 – 0.89), p = 0.025

Early HCQ, HCWs: RR 0.74 (0.40 – 1.38)

Late HCQ, household contacts: RR 1.17 (0.55 – 2.49)

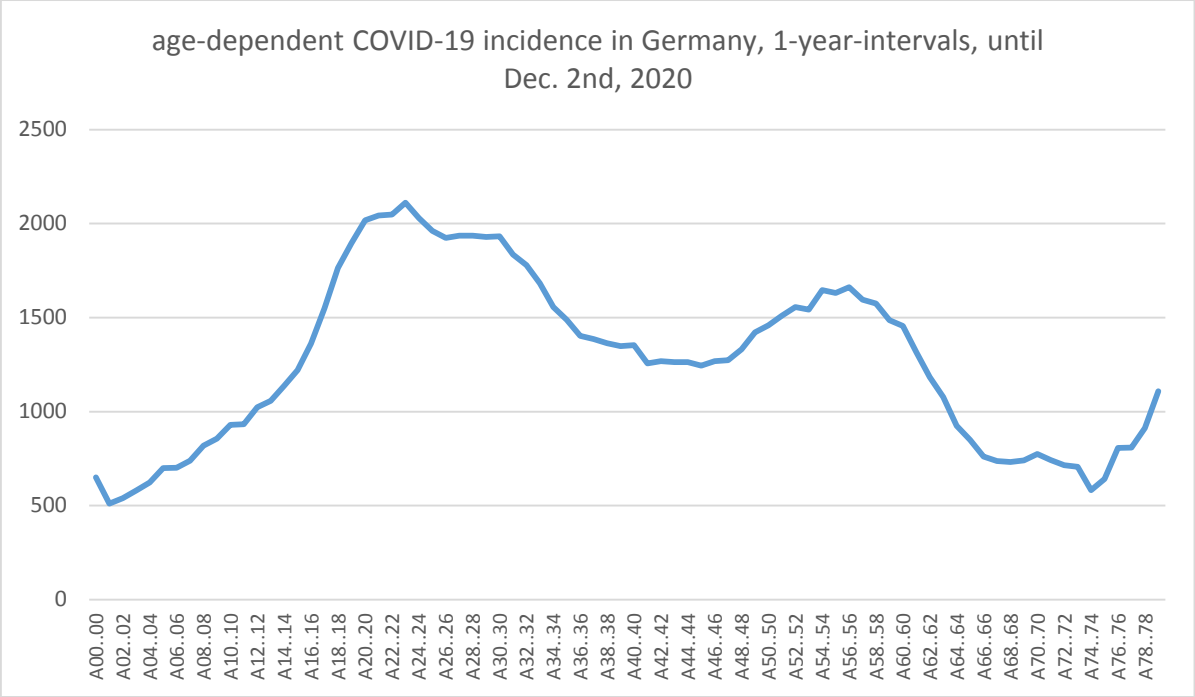
Late HCQ, HCWs: RR 1.23 (0.61 – 2.49)

*Early HCQ: day 1-3 (elapsed time), late: day 4-6; all results from the ITT population

In summary, young household contacts (up to 45 years) profited most from HCQ prophylaxis if started within 3 days after exposure. HCQ starting > 3 days was useless and possibly risk-enhancing. However, there was no significant preventive effect even in the case of early start for those who need prophylaxis at most: the elderly and those with comorbidities. Most worrying, though insignificant, there was an increased risk (RR 2.8) for participants > 50 years, even in case of early start of HCQ.

Though insignificant, this has to be communicated as a warning not to use HCQ for prophylaxis in the middle-aged and elderly (50 years and beyond), and this is in accordance with other trials as already mentioned above. The reason for that difference is not clear and speculative. It is improbable that it may be related to the antiviral effect of HCQ, because the target of the antiviral activity is the virus itself or its life cycle. However, as already mentioned, HCQ impacts the innate immunity, may cause lymphopenia (KELLENI) and reduces the expression of interferon-stimulated genes. It inhibits trained immunity at the functional and epigenetic level (ROTHER A et al.)

Maybe these immunosuppressive effects are more pronounced in elderly, increasing the risk of getting infected after exposure. Both “trained” innate immunity and interferon I response act as very early defense against viral infections of the respiratory tract. If they are suppressed by HCQ, the risk of an active infection (that is diagnosed by PCR and may provoke symptoms) may rise. This may be the mechanism how HCQ enhances COVID-19 risks, even if given early. However, the balance between a favorable antiviral effect and an unwanted immunosuppressive effect seems to be age-dependent, and starting somewhere a little below 50 years (maybe between 42 and 48 years as suggested by WISEMAN et al.), the immunosuppressive effects start to dominate. From that age on, HCQ prophylaxis must be avoided. It is tempting to speculate that age-dependent epigenetic changes either in the “trained” innate immune system pathway or the interaction between HCQ and interferon-stimulated genes may be the reason. Interestingly, the “age window” shortly before 50 years or between 42 and 48 years (when HCQ prophylaxis turns from being preventive to increasing risks) is exactly the age when age-dependent COVID incidence rates start to increase again (after a first maximum in young adults). The same mechanisms that make people more susceptible to COVID-19 starting at the end of the 40’s may be responsible for the effect why early HCQ PEP, though preventive in younger adults, may increase the risk of COVID-19 infection in older adults.



Age-dependent COVID-19 incidence in Germany; range: 0 – 79 years (80 years and more not shown); source: RKI, survstat@rki 2.0 (analysis from Dec. 3rd, 2020).

The graph shows two maxima; whereas the first maximum may be related to lifestyle and behavioral aspects in young adults, it is unplausible to explain the rise of the incidence in the second half of the 40's and early 50's by lifestyle aspects. A similar bimodal curve is found for other viral infections with complex interactions with immunity, e.g. oral HPV 16/18 in the US (like NHANES study). Thus it is improbable that it is only by chance that early HCQ prophylaxis turns from favorable to unfavorable (risk-enhancing) at exactly the same age when COVID incidence starts to increase again in the general (i.e. not „HCQ-protected") population.

Moreover, the difference between the (comparatively) more favorable effect of HCQ PEP in the BOULWARE trial (especially after critical re-analysis by WISEMAN et al.) compared to MITJA et al. (where the overall preventive effect of HCQ was found to be smaller with a RR of 0.89) may be associated with older mean age in the MITJA trial (48 years vs. 42 years in BOULWARE). Eventually, the null-effect (or even unfavorable effect) of late start of HCQ PEP (RR > 1.0 in all subgroups of WISEMAN et al. for day 4 – 6; RR 4.09 in MITJA et al. for > 6 days, though insignificant) is well in accordance with the disappointing effects of HCQ in the treatment of COVID-19, at least if given alone.

Meta-analyses about prophylactic/early HCQ

GARCIA-ALBENIZ et al. combined the results from the three prospective RCTs in a meta-analysis: the PREP trial from RAJASINGHAM et al. and the PEP trials from BOULWARE et al.

and MITJA et al.. They found a significant protection with a pooled risk ratio estimate of 0.78 (95% CI: 0.61-0.99).

With regard to MITJA et al., they took the unadjusted rates of 4.3 % (control arm) and 3.0% (HCQ arm) from table 2 in MITJA et al. (resulting in a RR of 0.69 for the HCQ arm compared to the control arm in MITJA et al.), instead of the adjusted RR of 1.45 from the last row in table 2 of the MITJA paper. GARCIA-ALBENIZ et al. conclude: *"The available evidence indicates that HCQ reduces the risk of COVID-19 by about 20%. Yet the findings from the randomized trials were widely interpreted as evidence of lack of effectiveness of HCQ, simply because they were not statistically significant when taking them individually."*

LADAPO et al. included in their meta-analysis RCTs concerning PREP, PEP and early treatment of outpatients. Besides of the three prophylaxis trials mentioned above and already meta-analysed by GARCIA-ALBENIZ et al., they also included a second MITJA trial (early treatment) and the SKIPPER trial (early treatment too; references see in LAPADO et al.). Endpoints were different in these five studies, what makes it doubtful whether it makes sense to combine them in a meta-analysis (endpoints: new COVID-19 infection in BOULWARE et al. and RAJASINGHAM et al., hospitalization in the MITJA early treatment trial, death in the MITJA prophylaxis trial, hospitalization or death in the SKIPPER early treatment trial). Median or mean ages ranged from 40-42 years except for the MITJA prophylaxis trial (49 years). Altogether, 5577 patients were included, and BOULWARE et al. and RAJASINGHAM et al. contributed most of all participants. The risk reduction associated with HCQ use was 24 % (RR 0.76; CI: 0.59 – 0.97) with regard to COVID-19 infection, hospitalization or death, similar to the results from GARCIA-ALBENIZ et al.

Though not a RCT, the aOR for PCR positivity (as a proxy for symptomatic disease according to the study design) of 0.77, though insignificant, in the retrospective control study of HCQ PREP from REVOLLO et al., following propensity score matching without evidence for residual confounding, is in very good agreement with the results (RR 0.78 and RR 0.76) from the two meta-analyses from GARCIA-ALBENIZ et al. and LAPADO et al., and strengthen their results by adding 487 more participants.

The meta-analysis of **KASHOUR Z et al.,** limited to prophylactic RCTs (PREP and PEP) until October 6th, found only an insignificant effect of HCQ on the risk of COVID-19 infection (pooled RR: 0.85; 0.69 – 10.4), based on 5 RCTs (ABELLA, BOULWARE, MITJA, GRAU-PUJOL, RAJASINGHAM) and 2725 participants who took HCQ and 2287 controls. In contrast to other meta-analyses, KASHOUR et al. looked also for the risk of hospitalization in the prophylactic trials. The pooled RR was 0.81 (CI: 0.45 – 1.44) based on 2806 patients on HCQ and 2389 controls in the same five RCTs. Moreover, in three RCTs with COVID-19 outpatients, HCQ was associated with a similar risk reduction for hospitalization (pooled RR 0.80; CI: 0.46 – 1.39). Combining prophylactic trials with outpatients trials (n = 8) didn't change the overall result (pooled RR 0.80; CI: 0.54 – 1.20).

KUMAR J et al. based their meta-analysis on only 3 prophylactic studies (ABELLA et al., BOULWARE et al. and RAJASINGHAM et al.) and found a RR of 1.04 (CI: 0.58 - 1.88) for the risk of infection.

HERNANDEZ et al. performed a systematic review and meta-analysis until December 8th, 2020 and included 5 RCTs (n = 5579; 4 of them placebo-controlled) and one cohort study (n = 106).

PCR-posivitity: RR 1.01 (CI: 0.88-1.16)
COVID-19 infection: RR 0.98 (CI: 0.78-1.22)
all-cause mortality: RR 0.73 (0.27-1.99)

No different effects were found if distinguished between HCQ as PEP or PREP. However, quality of evidence was judged as low for all outcomes. The meta-analysis included the following RCTs: RAJASINGHAM and ABELLA for PREP, MITJA, BOULWARE, BARNABAS for PEP and BHATTACHARYA as cohort study.

The qualitatively very high-grade network meta-analysis from a large study group of more than 40 specialists **(BARTOSZKO et al.)** found no "important" effect of HCQ prophylaxis. They included 6 RCTs (ABELLA, BARNABAS, BOULWARE, GRAU-PUJOL, MITJA, RAJASINGHAM) (until January 19th 2021, published February 26th 2021):

● HCQ had no effect on laboratory-confirmed infection (OR 1.03; CI: 0.71 – 1.47); risk difference per 1000: + 2 (CI: -18 - + 20); moderate certainty

● HCQ had no significant effect on suspected, probable or laboratory-confirmed infection (OR 0.90; CI: 0.58 – 1.31); risk difference per 1000: -15 (CI: -64 - + 41); low certainty

● HCQ had no significant effect on hospitalization (OR 0.87; CI: 0.42 – 1.77; risk difference per 1000: - 2 (CI: -3 - + 4); high certainty

● HCQ had no important effect on mortality (OR 0.70; CI: 0.24 – 1.99; risk difference per 1000: - 1 (CI: -2 - + 3); high certainty

● HCQ was associated with increased adverse effects (OR 2.34; CI: 0.93 – 6.08; risk difference per 1000: +19 (CI: -2 - + 70); moderate certainty.

It is noteworthy that this network meta-analysis differed from all meta-analyses mentioned above by an aggregated control group (standard care or placebo), aggregated from all included 9 RCTs (including the ivermectin RCTs). This may explain the differences between the ORs from other meta-analyses that included nearly the same studies. A sort of "universal control group" from all 9 included studies (HCQ + IVM) was created that is characterized by the following probability of events:

● laboratory-confirmed infection: 65 / 1000

● suspected, probable or laboratory-confirmed infection: 167 / 1000

● hospitalization: 5 /1000

● death: 3 / 1000

● adverse effects: 15 / 1000

However, one may ask whether such an aggregation of control groups from individual studies to an universal control group is actually a progress compared to conventional meta-analyses? In the case of COVID-19, this results in mixing of controls from very different epidemiological backgrounds (e.g. risk factors, PREP or PEP, HCWs of different extents of exposure, community contacts, family members; background incidence etc.).

According to the conventional meta-analyses (excluding BARTOSZKO et al. with their different methodology), there may be a true significant or borderline significant effect for prophylactic or early HCQ. However, it is very small and probably too small to be balanced with side effects and risks. Nevertheless, after statistical significance was shown in the meta-analyses, it is a proof of principle that chemoprophylaxis does actually work (at least to some extent) even under the strict conditions of a RCT. Moreover, the protective effect would have been larger if the WISEMAN analysis of the BOULWARE trial had been taken into account, which was not possible since the re-analysis was published later.

This knowledge allows two consequences:

● this may be a starting point for combination prophylaxis: what combination partner may improve the outcome of HCQ: zinc? low dose doxycyclin (25 mg/day)? or both? interferon inhalation (to compensate for the anti-interferon effect of HCQ)? Quercetin (or ECGC, green tea polyphenols) and zinc? antiparasitic agents?

● since we now have a proof of principle that chemoprophylaxis actually works under RCT conditions (though weakly, but in principle it works), the results of the retrospective non-RCT PEP trials with umifenovir (Arbidol) (ZHANG et al., YANG et al.) should be reconsidered more seriously. They seem to be discarded because of their retrospective and non-randomized design. However, if one considers that HCQ inhibits the early natural interferon response following viral infection, whereas umifenovir promotes interferon (FAN et al.), it is now absolutely plausible to assume that umifenovir can outcompete HCQ in chemoprophylaxis. Compared to retrospective HCQ trials with a similar design like ZHANG's Arbidol trial, i.e. the HCQ trials of BHATTACHARYA et al., CHATTERJEE et al. and KHURANA et al., the ZHANG trial was the most effective. If umifenovir outcompetes HCQ in retrospective non-RCTs, why shouldn't it outcompete HCQ in a RCT? Based on general experiences with comparions between retrospective control studies vs. RCTs, it is well probable that the protective effect of umifenovir in a RCT would be lower than expected from ZHANG et al., but it is likely that the results will be better than those from the HCQ trials.

At the same time of these meta-analyses, which showed **a small, but significant effect** of HCQ in prophylaxis and early treatment, another placebo-controlled RCT of HCQ PREP was published, confined to HCWs at risk, which showed a **null effect of HCQ PREP** in spite of the high dose regimen of 600 mg daily for eight weeks (ABELLA et al.). Because of futility following an interim analysis, the RCT was discontinued, so that only 64 participants in the HCQ group and 61 participants in the placebo group could be evaluated.

Compared to BOULWARE et al., the method of ABELLA et al. was more rigorous because it included nasopharyngeal PCR testing at baseline, at week 4 and 8 and at any time in case of relevant symptoms. Altogether, there were 4 cases of PCR-proven infection in the HCQ group (6.3 %) and also 4 cases in the placebo group (6.6 %). 6 of the 8 cases were symptomatic (2 placebo cases, 4 HCQ cases), no one needed hospitalization. However, the participants were quite young (mean age: 33 years), so the benign outcome is not unexpected. No further cases of infections during the study period were found by antibody testing. Interestingly, infections in the HCQ group were detected on average earlier (weeks 1, 4, 5, 6) than in the placebo group (weeks 1, 8, 8, 8). This may be a chance finding, or may point to some effects of accumulating doses of HCQ over time because of its long half-life. On the other hand, there is a fundamental difference to the PREP trial of RAJASINGHAM et al., which showed a moderate success: in RAJASINGHAM et al., the doses were 400 mg or 800 mg weekly, and 800 mg/week was not superior to 400 mg. In ABELLA et al., the weekly dose was 4200 mg, more than tenfold compared to the most successful dose regimen in RAJASINGHAM et al.

With regard to a mere antiviral, one might suggest that higher doses must be able to increase efficacy. However, since HCQ is not a mere antiviral but also an immunomodulating agent which suppresses some pathways of the immune system, including the interferon I response which is so important in the earlier phases of the disease (e.g., HADJADJ et al.), there is no automatism that higher doses might be more effective. Depending on a possibly dose-dependent balance between wanted antiviral effects and unwanted immunosuppressive effects (unwanted in a prophylactic setting! – this is very different from treatment of advanced disease when immunosuppression may become important), it is not implausible that low dose HCQ may be more efficient in prophylaxis than high-dose HCQ.

Thus the RAJASINGHAM trial and the ABELLO trial are so extremely different from one another that it is not justified to combine them in a meta-analytic manner. Because of their rigorous methodology, ABELLO et al. show that a daily dose of 600 mg cannot be recommended for prophylaxis. However, this doesn't mean that much lower dose regimens like that of RAJASINGHAM et al. must be completely ineffective too.

For comparison:

RCTs:

RAJASINGHAM	PREP (HCWs)	400 mg/week	moderate effect
		800 mg/week	moderate effect, not better than 400 mg/week (minimally worse)
ROJAS-SERRANO	PREP (HCWs)	200mg/day (60 days)	strong effect (1.6 vs. 9.2 % COVID-19, but underpowered and Insignificant). Median age only 31.0 years in HCQ arm.
ABELLO	PREP (HCWs)	4200 mg/week	null effect

SYED (CHEER)	PREP (HCWs)	400 mg weekly or 400 mg every 3 weeks or 200 mg every 3 wks.	null/bad effect (despite young age: 30.6 years); trend for **more infections** in the 400 mg regimes; possibly small protective effect of the 200 mg regimen (unsure, underpowered)
MITJA	PEP	800 mg day 1, 400 mg day2-5	moderate effect (PCR-confirmed) null effect for "PCR-confirmed or symptoms compatible with with COVID-19" more cases of seropositivity in HCQ group
BOULWARE	PEP	1400 mg day 1, 600 mg day2-5	small effect (based on symptoms, regular PCR tests only in some cases) moderate effect if started on days 1 or 2 (day 1 > day 2) moderate effect in subgroups (young participants, household contacts) negligible effect in HCWs, no protection in older participants WISEMAN et al. re-analysis: Moderate – strong effect if starting within three (better: two) days of exposure (up to 65 % protection), particularly in younger adults (up to 45 yrs), household contacts and people without comorbidities; no protective effect > 3 days **warning:** probably increased risk of infection in older adults, even if started early (age limit seemingly somewhere between 45 and 50 years)
BARNABAS	PEP	400 mg day 1-3 200 mg next 11 days	slightly increased risk of infection (aHR ~ 1.2). Very rigorous study design with daily swabs
SEET	PEP	400 mg day 1	slightly, but significantly reduced risk

		200 mg days 2-42	(relative risk reduction: 30 %). Young men, mean age 33 years in the whole study, 30.6 years in the HCQ arm. Relative risk reduction of symptomatic disease: 35 %.

Prospective non-randomized open label trials

DHIBAR	PEP (non-HCWs)	800 mg day 1 400 mg per week (week 1-3) total 2000 mg	41 % risk reduction of probable or confirmed COVID-19 53 % risk reduction of PCR positivity 44 % risk reduction of new onset of symptoms

Retrospective observational PREP studies for HCWs from India (high risk of bias)
(recommended dose regimen: 400 mg BID loading dose, then 400 mg/week):

CHATTERJEE	highly effective in case of optimal compliance (> 80 % risk reduction). Predominantly young participants. Age-dependent effect (the younger, the more effective). But: paradoxical effect in case of irregular intake (increased risk), no plausible dose-effect relationship (making also the favorable results mentioned above more questionable)
KHURANA	moderate effect (HR ~ 0.62) in case of optimal compliance, but unplausible dose-effect relationship in participants with less compliance (higher risk in participants who didn't take the full course of HCQ compared to control group)
BHATTACHARYA	highly effective (HR ~ 0.20), but very young HCW population (mean age < 28 years)
KUMAR GOENKA	(highly?) effective; 0 % vs. 34.2 % infections in HCWs; however, only 6 HCWs in the HCQ group (thus not significant); comparatively young HCW population
DEV	small protective effect (RR 0.74; CI: 0.61 – 0.90) in a young population of HCWs (mean age: 30 – 32 years); but significant dose-effect relationship
BEHERA	retrospective case-control study; young HCWs (mean: 29 years). uOR 0.58, aOR 0.56 (but not significant); endpoint: PCR positivity
BHATT	prospective observational study; young HCWs (mean: 27.5 years);

OR/HR could not be calculated because of bad adherence during the study time, but more PCR-confirmed infections in the HCQ group, thus RR/OR/HR must be > 1.0

Retrospective observational PREP study for HCWs from Spain
(recommended dose regimen: 400 mg BID loading dose, then 200 mg BID for day 2-5, then 200 mg once weekly as maintenance dose)

REVOLLO higher crude rates of PCR positivity and seropositivity in the PREP group due to differences in the risk of exposure; after robust propensity-score matching:
aOR for PCR positivity: 0.77 (CI: 0.35 – 1.68) (PREP vs. no PREP)
aOR for IgG positivity: 1.43 (CI: 0.62 – 3.38)

Retrospective observational PEP study from Bulgaria
(HCQ 200 mg qd **+ Zinc** up to 50 mg qd for 14 days)

SIMOVA symptomatic disease: 0/156 in HQ+Zinc group vs. 3/48 in those who refused to take the proposed prophylaxis (all: HCWs with close contact to COVID-19 patients)

Interestingly, there seems to be no or even a negative dose-effect relationship. In ABELLO et al., the high dose (4200 mg/week) was associated with a null effect, whereas in RAJASINGHAM et al., 800 mg/week were not superior (and even a little bit less effective) than 400 mg/week. Similar effects were observed for the *treatment* of COVID-19: In a meta-analysis of 26 studies, DI CASTELNUOVO et al. found a small, but significant reduction of mortality in HCQ-treated patients (pooled risk ratio: 0.79; CI: 0.67 – 0.93) which vanished when daily dose was > 400 mg/day (pooled risk ratio: 1.10) or total dose was > 4400 mg (pooled risk ratio: 1.10).

At the end of September 2020, **LEWIS et al.** performed a meta-analysis about the 4 prophylactic RCTs (!) that were available at that point of time **(ABELLA et al., BOULWARE et al., MITJA et al., RAJASINGHAM et al.)** and found

● a relative risk (RR) of developing COVID-19 of 0.82 (CI: 0.65 – 1.04; moderate certainty) (if only those patients with proven PCR+ were analyzed, the RR for infection was 0.97, CI: 0.64 1.47),

● a RR of 0.72 (0.34 – 1.50; moderate certainty) for hospitalization (the favorable trend is mostly based on RAJASINGHAM et al. with their RR of 0.61, whereas the RR for hospitalization in BOULWARE and MITJA are close to 1.0)

● and a RR of 3.26 (CI: 0.13 – 79.84) for mortality (low certainty; only based on MITJA et al. with one death among 322 persons in the HCQ group and no death among 350 persons in the control group),

● whereas the risk of adverse effects was increased for those who took HCQ (RR 2.76; CI: 1.38 – 5.55, moderate certainty), though there was no evidence for increased risk of arrhythmia (RR 0.71; CI: 0.29 – 1.73).

These results are based on a total of 4921 participants, an average age of 40.7 years, 61.2 % women, 32.4 % had chronic comorbidities; 81.7 % were HCWs, 14.2 % household contacts. Three studies were about PEP, one about PREP. 3 studies were placebo-controlled.

In subgroup analyses, weekly dosing was associated with less adverse effects than daily dosing, and there were slight insignificant trends that weekly dosing may be a little more effective than daily dosing, and PREP a little more effective than PEP. No subgroup analyses for different age groups were presented.

Most important, the trial with the most rigorous design, a double-blind household-randomized multicenter RCT of HCQ PEP from US (NCT04328961), found no protective effect at all **(BARNABAS et al.).** Participants were close contacts who were recently (< 96 hours) exposed to persons with diagnosed SARS-CoV-2 infection. Unfortunately, the BARNABAS trial was published too late so that it could not be respected in the LEWIS meta-analysis. Otherwise, the small insignificant protective effects from that meta-analysis would have been attenuated further.

"Close contacts were defined as either household contacts (residing in the same residence or prolonged exposure in a confined space) of an index person diagnosed within the past 14 days or health care workers who cared for an index case without appropriate personal protective equipment". (BARNABAS et al.); study visits via telehealth.

Median age: 39 years (IQR: 27-51), 60 % females, 82 % household contacts, 18 % HCWs.

Dose: HCQ 400 mg/d (2 x 200 mg) for 3 days, followed by 200 mg/d for 11 days; placebo: 500 mg/day vitamin C for 3 days, followed by 250 mg/day.

Primary outcome was PCR-confirmed SARS-CoV-2 by self-collected mid-turbinate swabs daily (day 1-14) for PCR testing. (Participants were SARS-CoV-2 PCR negative at enrollment).

Among 689 participants who were available for analysis, there was no significant difference in SARS-CoV-2 acquisition by day 14 (53 vs. 45 events in the control group; aHR 1.10; CI: 0.73-1.66, p > 0.20). Overall cumulative incidence was 14.3 %. Based on a sensitivity analysis with a CT cutoff of 38, aHR was 1.26 (CI: 0.81 – 1.95).

There were more adverse effects in the HCQ group (16.2 vs. 10.9 %, p = 0.026). The median delay between exposure and first dose of HCQ or vitamin C was 2 days (IQR: 1-3 days).

Thanks to daily self-collection of swabs, this was the most rigorous HCQ PEP trial reported so far, and outcompetes other trials about the same subject. *"In preplanned analyses, aHRs were not significant within subgroup for type of contact, time between most recent contact and first dose of study medication, duration of contact, number of contacts enrolled within the household, quarantine status, index symptoms, and number of adults or children in the household*" (BARNABAS et al.).

In a secondary analysis including infections up to day 28, aHR was 1.16 (CI: 0.77 – 1.73; 58 vs. 48 infections).

With regard to *symptomatic* infection (cumulative incidence at day 14: 11.1 % instead of 14.3 % for positive PCR), aHR was 1.27 (CI: 0.79 – 2.03).

SARS-CoV-2 infection by day 14: aHR 1.10 (CI: 0.73 – 1.66)

dto., Ct not higher than 38: aHR 1.26 (CI: 0.81 – 1.95)

SARS-CoV-2 infection by day 28: aHR 1.16 (CI: 0.77 – 1.73)

Symptomatic disease: aHR 1.27 (CI: 0.79 – 2.03)

(all p > 0.20; HCQ vs. controls).

The BARNABAS trial avoided serious limitations which applied to the BOULWARE and MITJA trial. BOULWARE et al. didn't assess SARS-CoV-2 status at baseline and relied primarily on self-reported flulike symptoms as primary endpoint (no objective virological endpoint), whereas MITJA et al. had an unblinded, uncontrolled design with infrequent PCR testing for incident infections until day 14 and was underpowered to assess incident infection (BARNABAS et al.). Frequent testing is needed to detect transient infections (BARNABAS et al.).

With regard to the important question of the time lag between exposure and first intake of HCQ, BARNABAS et al. point out: *"Limitations of the study include the average of a 2-day window between most recent exposure and initiation of study medication owing to remote recruitment and shipping times; PEP should be given as soon as possible after exposure to prevent infection. Delays in index testing and receipt of results meant that some index cases may have had infection for several days before the enrollment of their close contacts into this trial, during which time transmission could occur."*

Though the BARBABAS results are extremely disappointing, they still leave open the possibility of a protective effect of HCQ PEP it started very early.

In summer 2021, **STRICKER and FESLER** published a meta-analysis that was restricted to **PREP in HCWs from India.** Based on 11 studies (most of them already mentioned above), they found

● 10 % absolute risk reduction (25 % instead of 35%; relative risk reduction: 28.6 %) for any weekly intake of HCQ, based on 3489 HCWs who took HCQ and 4127 who did'nt

● if one confines the analysis to those who took at least six weekly doses (5 studies: n = 1273 HCWs while the number of controls is the same), the absolute risk reduction is 14 % (21 instead of 35 %) and the relative risk reduction in 40 %.

STRICKER and FESLER calculated an adjusted risk ratio of 0.56 (CI 0.37 – 0.83, p = 0.0040) for the former group (any HCQ intake) and an aRR of 0.25 (0.13 – 0.50, p < 0.0001) for those who took at least 6 weekly dosis, but is in unclear how these adjustments were performed that they yielded so favorable results, whereas unadjusted relative risk reductions were only 28.6 % and 40 %.

All studies were retrospective cohort studies (no RCTs), and HCQ had to be taken weekly according to the Indian ICMR protocol (altogether 7616 participants or conrols). The ICMR protocol introduces some unifomity in the intake of HCQ and makes the results better comparable. However, compliance is always a critical matter In HCQ PREP studies, and STRICKER and FESLER themselves point to limitations due to retrospective design and subject homogeneity.

Whereas these results look surprisingly good at the first glance, the relative risk reduction for the total group (< 30 %) is well in accordance with some earlier meta-analyses mentioned above like LAPADO et al. and GARCIA-ALBENIZ et al. Moreover, this meta-analysis was restricted to PREP, and HCWs in India are comparatively young. Many single studies about HCQ PREP in HCWs from India had mean ages around or below 30 years. So the results are well in accordance with the hypothesis that HCQ may actually have some preventive effect in young adults. Nevertheless, only RCTs can offer definitive results, and this meta-analysis should be only understood as a signal In favor of a moderate effect of HCQ PREP in HCW populations that are dominated by young adults.

Some additional aspects about HCQ:

As far as HCQ is concerned, the situation became more delicate meanwhile also for some other reasons: LI G et al. discovered *in vitro* large differences in the antiviral activity against SARS-CoV-2 between both stereo-isomers of CQ and HCQ. S-HCQ was more effective by 60 % compared to R-HCQ (in CQ, the difference was also found, but smaller). The available CQ/HCQ drugs in therapy and trials are racemous (50/50). So from now, any trial with HCQ would only make sense if S-HCQ is applied, but at first, this would have to be made available on the market in large amounts. Moreover, whereas HCQ showed *in vitro* antiviral activity in simple assays with Vero cells (that don't express TMPRSS2), it failed to do so with human respiratory epithelia (KORMAN).

Finally, ROSENKE et al. tried HCQ for prophylaxis and treatment (starting 12 hours after inoculation) in animal models (Syrian hamsters, rhesus macaques), including very high dosage in the Syrian hamster model. Except for some small, but significant advantages in the total symptom score for the macaques, there was no other benefit from HCQ, e.g. with regard to viral load or lung damage. In summary, HCQ failed in animal models of both chemoprophylaxis and treatment.

Moreover, in an animal experiment (ferrets) with early therapy (starting 1 day after virus inoculation), HCQ was also very disappointing: Tenofovir/Emtricitabine was evidently superior to lopinavir/ritonavir (L/R), which was only a little superior to HCQ, and both L/R and HCQ were of comparatively small effect compared to placebo, whereas

Tenofovir/Emtricitabine was most successful (PARK SJ et al.), as expected from *in vitro* results (JOCKUSCH et al.).

These results are very well in line with clinical data: whereas lopinavir/ritonavir was found to have little effect on COVID-19 so far (at least alone and not as part of complex combination therapies), and was already abandoned in the WHO SOLIDARITY trial, tenofovir as disoproxil fumarate (but not as tenofovir alafenamide) in combination with emtricitabine was found to have some preventive effects in a large retrospective study which encompassed about 75 % of all HIV infected people in Spain who receive ART (nearly halving the risk of symptomatic COVID-19 disease compared to the general population or to HIV-infected individuals under other ART regimes), and there was no ICU case or death among the 20 COVID cases among 12.395 HIV-infected people in the Madrid area who took TDF/Emtricitabine as part of their ART regime (DEL AMO et al.).

All of these results are well in accordance with the ferret data which suggested that tenofovir is evidently superior to lopinavir/ritonavir and HCQ.

Eventually, HCQ was tested for PREP in macaques and was not able to confer protection against acquisition of infection (MAISONESSE et al.). However, macaques represent only a model for mild and early disease, not for severe or critical disease, because SARS-CoV-2 infection doesn't result in cytokine storms and serious hyperinflammation in macaques. But in a therapeutic situation, neither HCQ alone nor HCQ + azithromycin were able to show significant effects on viral loads in infected macaques, independent of whether administration started after or before viral peak (the latter may simulate a PEP setting).

Taken together, HCQ showed no or only little positive effects in hamsters, ferrets and macaques, and it also failed as PREP or early therapy/PEP in macaques.

RAKEDZON et al. reviewed the disastrous history of HCQ research in the context of COVID-19 and called it a scientific failure. However, they discussed "prophylaxis" only in the context of the BOULWARE trial; the open question of PREP was not subject of their review.

The discrepancy between promising *in vitro* results of antiviral activity on one side (as far as simple assays like Vero cells are used; not in human respiratory epithelial cells; *see below*) and failure in PEP or early treatment on the other (as demonstrated by MITJA et al. and BARNABAS et al. as far as PEP is concerned) may be explained by the anti-interferon properties of HCQ/CQ (GIES et al., KASHOUR and TLEYHEJ). The local interferon type I response is an important protection in the early phase of the viral infection and contributes to the local defense against the virus (this was also the rationale for the use of interferon nose drops as chemoprophylaxis in the MENG trial mentioned below).

The interferon response is the most important mechanism to combat replication of RNA viruses. The recognition of double-stranded RNA results in the secretion of type-I interferons. These interferons then stimulate the expression of the ISGs, inluding multiple antiviral proteins. SARS-CoV-2 differs in this respect from SARS-CoV; it is more sensitive to IFN-alpha and IFN-beta treatment; it triggers ISG expression much stronger; in infected human lung tissue, SARS-CoV-2, but not SARS-CoV, induces type I, II and III interferons. The reason for this difference is probably an enhanced expression of viral M protein in SARS-CoV compared to SARS-CoV-2, since the M protein is a known inhbitor of type 1 interferon expression (GROSSEGESSE et al.).

The inhibition of interferon production by HCQ/CQ may be detrimental and may also explain the time trend demonstrated by BOULWARE et al./WISEMAN et al.: If started shortly after exposure, the antiviral activity of HCQ may be sufficient at least in some of the cases to interrupt the expansion of the viral infection in a very early stage, so that no interferon is needed to combat against the virus at all. If HCQ is started a little later, viral load (that doubles about every 6 hours) may already be too high to be controlled or reduced by HCQ *alone*, and the suppression of the local interferon response may impair the local immunological reaction and control of the viral infection. Though statistically not significant in BOULWARE et al./WISEMAN et al. and MITJA et al., this hypothesis can even explain why HCQ PEP may increase the risk of symptomatic COVID infection if started several days after exposure (BOULWARE et al.: day 4: 14.5 % in HCQ group vs. 12.4 % in placebo group; MITJA et al.: RR 4.09 if started > 6 days after exposure). Beside of its anti-interferon effects, it was also reported that HCQ causes lymphopenia (KELLENI).

Moreover, this hypothesis is in accordance with results from therapeutic trials with interferon which showed that early administration of interferon reduces bad outcomes (like mortality) a lot, whereas late administration may even increase mortality (DAVOUDI-MONFARED et al., WANG N et al.). Morever, it was found that Arbidol induces interferon production (FAN et al.). Though both hydroxychloroquine and Arbidol show antiviral effects against SARS-CoV-2 *in vitro,* the difference with respect to their effectiveness in PEP may be explained by their different effects on local interferon production: ↑ in case of Arbidol, ↓ in case of hydroxychloroquine.

In vitro studies that compared pre- and post-infection treatment with CQ or HCQ showed less antiviral activity in the post-infection treatment experiments (KASHOUR and TLEYHEJ), another argument that may explain the time-dependent results in BOULWARE et al./WISEMAN et al., MITJA et al.. Moreover, this is in accordance with suggestions that HCQ may be more effective in PREP than in PEP.

Beside inhibition of interferon-stimulated genes, also TMPRSS2 may play an unfavorable role with regard to the antiviral properties of HCQ. AGARWAL et al. gave a pharmacokinetic explanation for the discrepancy between favorable *in vitro* studies on Vero cells and the failure to replicate these results *in vivo* in animal models and most clinical studies: a *" failure to achieve adequate drug concentrations at the target site and attenuation of its inhibitory effect due to the presence of TMPRSS2 in airway epithelial cells."* In fact, the *in vitro* results of HCQ on Vero cells could not be replicated in reconstituted human airway epithelial cells; in that case, HCQ was unable to affect apical viral titers and to protect epithelial integrity. TMPRSS2 attenuates the inhibition of SARS-CoV-2 by HCQ; but TMPRSS2 is not expressed in Vero cells, thus explaining the favorable results in the Vero cell assay. From a pharmacological point of view, addition of a TMPRSS2 inhibitor (like Camostat, but also bromhexine or ambroxol) may overcome this limitation of HCQ (AGARWAL et al.).

HCQ only blocks endosomal entry of the virus into the host cell, but not TMPRSS2 mediated membrane fusion. The failure to inhibit membrane fusion limits the effectiveness of HCQ on blocking SARS-CoV-2 entry into the host cell (YANG A et al.(3)).

Finally, especially with regard to prophylactic use, it is important to note that chloroquine is considered as mutagenic, genotoxic and in some situations also co-carcinogenic. It is not

clear whether this applies to HCQ too, because these questions haven't been studied sufficiently for HCQ. But the strong similarities between CQ and HCQ and their metabolism make it improbable that HCQ is completely safe in this respect (see GIRI et al.). Moreover, there are psychiatric side effects which may become relevant especially in long-term prophylactic use, but they are reversible following withdrawal (TALARICO et al.).

One may also wonder why none of the larger prophylactic trials mentioned above combined HCQ with zinc supplemention, an obvious combination with regard to the ability of HCQ to act as an ionophore. There is an ongoing trial with HCWs from the Tunisian Military (NCT04377646) that analyzes this combination vs. HCQ alone. This trial has the potential to investigate whether zinc has an additional effect compared to HCQ prophylaxis without zinc. However, there are no results available so far. But there is a report from a Bulgarian cardiological hospital system where 204 HCWs with close contacts to COVID-19 patients were offered HCQ prophylaxis (200 mg qd for 14 days in combination with zinc up to 50 mg qd). 156 of the 204 HCWs took the prophylaxis, 48 refused to do so. There were no symptomatic infections in the PREP group, whereas 3/48 (6.3 %) who didn't take prophylaxis became symptomatic and tested positive for COVID-19 (SIMOVA et al.). Moreover, among 38 COVID-19 positive HCWs (half of them symptomatic), 33 took 500 mg Azithromycin (qd), 200 mg HCQ (tid) and up to 50 mg Zinc (qd) as outpatients. Symptoms abolished between day 2 and 4, no one needed hospitalization, and all were PCR negative at day 14. Among five HCWs who used other regimens without HCQ, two needed hospitalization and three tested still positive at day 14 (SIMOVA et al.). So one may ask whether the combination of HCQ and zinc may be responsible for these favorable results. If so, this would be in contrast to hospitalized patients, for whom a study from Egypt showed that the addition of zinc to HCQ had no effect (neither favorable nor unfavorable) compared to HCQ alone (ABD-ELSALAM et al.).

Unfortunately, this SIMOVA study was only presented in a short letter to the editor, and there are no demographic data about study participants and controls. It would have been important to know about the age structure of participants and non-participants.

Final conclusions (December 9th): It is still possible that **HCQ PREP** may reduce COVID risks according to some studies (e.g. RAJASINGHAM as a RCT and some retrospective control studies with high risk of bias). Dose and age may play a role, and high doses don't seem to be helpful (see ABELLO et al.). Moreover, HCQ prophylaxis seems to be harmful in older individuals, beyond an age limit around 45 years. Addition of Zinc may enhance the preventive effect of HCQ PREP (SIMOVA et al.).

In contrast, after the trial from BARNABAS et al. it became more evident than before that **HCQ PEP** doesn't work. Because of daily PCR testing, the BARNABAS study was much more rigorous than any other PREP or PEP study reported before. HCQ PEP may even increase the risk of infection or symptomatic disease, especially in older people. However, the results (including WISEMAN et al.) may still offer the option that very early start of HCQ (e.g. at the day of exposure or next day) may have some protective effect, and even BARNABAS et al. point to this possibility as quoted above.

It is also unknown so far whether the combination with zinc may have a synergistic favorable effect in the case of PEP.

● any HCQ prophylaxis must be avoided in people > ~ 45 years (probably increased risk of infection)

● HCQ PEP is ineffective in most constellations; but it might have a moderate preventive effect if started very early after exposure (at best, the day of exposure or the day after exposure) in young adults < ~ 45 years. Household contacts seem to profit more than HCWs.

● HCQ PREP may have a small to moderate preventive effect in younger populations, but only in low – moderate doses and not in high doses

● It is still possible that combination of HCQ + Zinc may be more effective, but more data are needed and even in that case, there is a need to look at the possibility of different effects in different age groups. Since zinc supplementation increased the risk of COVID infection in older men in a study based on the UK Biobank (LOUCA et al.), risk-enhancing effects of HCQ and zinc might be synergistic. Age- and sex-dependent analyses of study data are mandatory.

● HCQ chemoprophylaxis (PREP/PEP) is not suited, and probably dangerous (risk-enhancing), for those who need chemoprophylaxis at most: the elderly. (It is unclear, but well possible, that this applies to HCQ+zinc too).

Thus it depends on the individual perspective whether HCQ chemoprophylaxis may be regarded as helpful or dangerous. An employer in the health care system e.g. in India may indeed profit a little bit if he recommends HCQ PREP to his young HCW staff because this may reduce COVID-associated absentism to a small or moderate extent. For him, the balance may be favorable.

However, the most important perspective about chemoprophylaxis is the perspective of people of increased risk of severe COVID, hospitalization, ICU, intubation, death, i.e. the perspective of older people or those with comorbidities. From the perspective of those people who need chemoprophylaxis at most, it is evident now that chemoprophylaxis with HCQ, even if started early, is of null effect and probably dangerous, increasing the risk of infection or symptomatic disease beside its risk of adverse effects.

HCQ for COVID 19 PREP in pregnancy ?

In a paper from 2021, FESSLER and STRICKER recommended HCQ PREP in pregnancy, despite limited evidence. However, since most pregnant women are quite young, there is a reasonable chance that HCQ may actually reduce the risk of COVID-19 despite its disappointing results for prophylaxis in older people.

A big problem for COVID prophylaxis in pregnancy is that other alternatives that may be more effective (e.g. ivermectin) are strictly contraindicated in pregnancy. FESSLER and STRICKER gave an overview about potential alternatives (atovaquone, ivermectin,

nitazoxanide, tafenoquine, mefloquine), but except for nitazoxanide, all other agents are contraindicated or critical during pregnany. For nitazoxanide, proper dosing for the purpose of PREP is unclear. And macrolides may be associated with an increased risk of birth defects. Based on FDA approved medications, there seems to be no alternative to HCQ for COVID PREP during pregnancy, because HCQ is regarded as safe in that situation.

Special note for HCQ PREP/PEP studies that will be published in the future:

Because of the age signal mentioned above, any study about PEP or PREP (but also treatment) should perform sub-analyses for different age groups and analyse the efficacy of HCQ separately for each age group. Adjustments or Cox regressions that include also age are not enough; one really needs to see the HR, RR or OR for each age group separately.

In most studies, this is probably not shown because the studies were underpowered to find significant results for each (age) subgroup. The size of a study is usually calculated that one may find a significant result for the total intervention group (if the intervention works), compared to placebo or no intervention, but not for subgroups. Journals and peer reviewers don't like to see insignificant trends. I assume that this is the reason why only few studies about prophylaxis or treatment present subgroup analyses for different age groups.

However, the question whether HCQ prophylaxis is only favorable for young adults and possibly/probably harmful to older adults is so important that even results without statistical significance should be reported. Though insignificant on their own, they are then available for meta-analyses or systematic reviews that may be able to resolve the question whether there is such an age gradient or not. The age gradient is not implausible; it may be explained by the ageing of mitochondria or the waning innate immunity with increasing age. Since HCQ dampens innate immunity, older people with (naturally) reduced innate immunity might be more prone to the immunosuppressive effects of HCQ (e.g., weakening of the interferon response, lymphopenia).

The age gradient might be based on the same or similar mechanisms like the "time gradient" (exposure - first dose) mentioned above: age- or time-dependent changes of the balance between antiviral activity on one side and immunosuppressive effects on the other side may explain both effects.

Chloroquine nasal drops: THAKAR et al. reported about a small study with chloroquine nasal drops from India. They seem to be effective for prevention if administered before the infection is established, while "no significant differences in clinical and virological outcome" were found in patients with mild but established COVID-19. Moreover, nasal chloroquine was associated with local irritation in 7/30 patients.

Ivermectin

PREP:

In a hospital-based matched case-control study about HCWs in an Indian hospital in September and October 2020, ivermectin provided some protective efficacy **(BEHERA et al.).** Cases and controls were PCR-positive and PCR-negative HCWs, and 186 case-control pairs were matched by profession, gender, age and date of diagnosis. Exposures were classified as intake of Ivermectin and/or HCQ and/or vitamin C and/or other prophylaxis. There were 904 tested staff members; 234 tested positive and 670 negative. There were 186 COVID cases for whom matched controls were available. Mean age was 29 years (60.75 % < 30 years). 9.7 % of cases were admitted to hospital.

Ivermectin intake was recorded for 77 controls and 38 cases; those who took two doses of Ivermectin (0.3 mg/kg with a gap of 72 hours) had a 73 % reduction of COVID risk (uOR 0.27; CI: 0.15-0.51) for the following month. A single dose of Ivermectin was not associated with significant risk reduction.

Among 372 participants, 102 controls and 67 cases had taken any form of prophylaxis (54.8 vs. 36.2 %).

Ivermectin: 77 (41.4 %) of the controls and 38 (20.4%) of the cases (p < 0.001) *(final publication:* 76 : 41)
Single dose: unadjusted OR 1.23 (0.43 – 3.50; p = 0.70); aOR: 1.30 (0.44 – 1.385, p = 0.63)*
Two doses: uOR 0.27 (0.14 – 0.47, p = 0.00); aOR: 0.27 (0.15 – 0.51, p = 0.00)*
Vitamin C: 38 (20.4 %) of the controls and 29 (15.6 %) of the cases (p = 0.22)
uOR 0.72 (0.42 – 1.27; p = 0.23); aOR 0.82 (0.45 – 1.57; p = 0.58)*
(500 mg once daily: n = 54; twice daily: n = 13)
HCQ: 12 (6.5 %) of the controls and 7 (3.8 %) of the cases (p = 0.25)
uOR 0.58 (0.23 – 1.48; p = 0.26); aOR 0.56 (0.19 – 1.63; p = 0.29)* (400 mg once a week)

*adjusted for COVID duties, household type, physical activity as a proxy for social contacts (e.g. sharing of gymnasium equipment), vitamin C prophylaxis, HCQ prophylaxis.

AIIMS concensus statement about IVM prophylaxis in HCWs (not in pregnant women; need for contraception):
- Body weight: 40 – 60 kg: 15 mg ivermectin; 60 – 80 kg: 18 mg Ivermectin; > 80 kg: 24 mg Ivermectin;
- second dose (as above) at day 4 (72 hours apart);
- subsequent dose: once a month dose (as above) on every 30th day after the last dose
- taken on an empty stomach with water (at least 2 hours before the next meal)

(BEHERA et al.)

This study was followed by a report from a prospective study from the same group **(BEHERA et al. (2))** that is based now on a larger number of HCWs. There were 1147 HCWs without IVM prophylaxis, 186 HCWs with a single dose of IVM and 2199 who took two doses.

Again, a single dose was not associated with protection (RR 1.07; adjusted RR 1.04), though no trend for an increased risk is recognizable in that larger data set; the two dose regimen was associated with an unadjusted RR of 0.18 (0.13 – 0.2; $p < 0.001$) and an aRR of 0.17 (0.12 – 0.23; $p < 0.001$).

Incidence of COVID infection (PCR-confirmed) during the full month of follow-up was 2 % vs. 11.7 % (IVM vs. controls), whereas symptoms suggestive of COVID-19 (with or without PCR confirmation) occurred in 6 vs. 15 % of participants.

The background of that prospective study was the increasing number of SARS-CoV-2 in HCWs in a large hospital in India in early September 2020, which negatively impacted the health care service. According to the available literature at that point of time, IVM prophylaxis was offered in that hospital (prospective cohort study). Outcome was assessed exactly one month after the first dose. Mean age in that study was 30.6 years (53.4 % < 30 years). 72.2 % of participants were involved in direct management of COVID patients.

No subgroup analysis was performed in order to find out whether age influences the prophylactic efficacy of IVM. However, the IVM group was biased towards more older participants (> 40 years and > 50 years) compared to the control group.

BEHERA et al. (2) point out that they were unable to randomize their prospective study for ethical reasons. Unfortunately, this will have the consequence that this large and impressive study has to be completely ignored by the BARTOSZKO group, and, as a consequence, by the WHO, since the results from the BARTOSZKO group will inform the WHO and will be the basis for decision-making of the WHO.

In a prospective controlled trial (source: **NCT04425850)** from Argentinia with 229 HCWs (131 treatment, 98 control), the following combined prophylaxis regimen was studied:
„1 drop IVM buccal drops (6mg/ml) + 6 sprays iota-carrageenan nasal spray (0.17mg/spray) (1 x each nostril + 4 oral cavity) both repeated 5 times per day + PPE“ vs. „PPE only“ in the control group.

There were 0 vs. 11.2 % PCR-positive COVID-19 infections within 28 days ($p < 0.0001$). No adverse effects (serious + non-serious) in the intervention group.

MORGENSTERN et al. studied the effect of IVM prophylaxis for HCWs in an observational and retrospective cohort study (no RCT!) in two medical centers from the Dominican Republic. IVM was given at a weekly oral dose of 0.2 mg/kg. The study was performed in summer 2020. 713 HCWs were included in the analysis, of which 326 had adhered to the prophylaxis program. The other 387 HCWs were assigned to the control group. After PPS matching to a 1 : 1 ratio in order to homogeneously match the risks (including exposure levels, gender, role), 271 participants of each group were evaluated. Participants were assigned to the IVM group if they had taken at least 2 doses (not more than 14 days apart) during the 28 day study interval. 51.5 % took 4 doses, 40.4 % 3 doses, 7.2 % 2 two doses; 1.4 % managed to take a single dose and then reported a COVID infection. Mean age was 35.9 years, 79 % females.

SARS-CoV-2 (positive PCR) was observed in 1.8 % of the IVM group compared to 6.2 % of control participants (5 vs. 18; risk reduction 71 %); the HR for a positive PCR test was 0.26 (CI: 0.10 – 0.71; Cox regression analysis). Clinical progression that needed hospitalization was observed in no participant from the IVM group, but 2 individuals (0.7 %) from the control group (as soon as SARS-CoV-2 was detected, outpatient treatment started with 0.4 mg/kg IVM once and azithromycin 500 mg every 24 hours for 5 days; after hospitalization, IVM therapy was intensified and azithromycin was given for 7 days). No death.

In the unmatched cohort (326 IVM : 387 controls), SARS-CoV-2 PCR + was found in 1.8 % vs. 5.7 %, clinical deterioration in 0 vs. 0.8 % (0 vs. 3 individuals).

In the matched cohort, there were 5 infections in the IVM group and 18 in the control group:

Week 1: 7 : 3
Week 2: 6 : 1
Week 3: 4 : 1
Week 4: 1 : 0

There was no infection between days 16 and 28 in the IVM group. Kaplan-Meier analysis showed that IVM prevention started to become significant after the second dose at day 8. **The authors assume that a second dose may be needed for a substantial prophylactic effect, well in accordance with the BEHERA study mentioned above.**

In fact, 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 no protective effect of a single dose of oral IVM (12 mg once) for the study interval of 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) (IVM: n = 617). Confirmed SARS-CoV-2 by PCR or serology: 64.5 % (IVM) vs. 70.0 % (Vit. C). Relative risk ratio 0.93 (CI: 0.71 – 1.18), absolute risk reduction in case of the use of IVM was 5 % (CI: - 10 to + 22 %).

Point estimates for adjusted ORs (depending on model, 6 different models were taken into account: between 0.73 and 0.89, all n.s.).

Symptomatic COVID-19: 5.2 % (IVM) vs. 10.3 % (Vit. C) (- 49.5 %). Symptomatic disease among those diagnosed with SARS-CoV-2: 8.0 % vs. 15.0 % (- 46.7 %). Interestingly, the results suggest that one dose of IVM is unable to reduce the risk of SARS-CoV-2 acquisation, but may reduce the risk of symptomatic disease. With regard to the short half-life of IVM on one side and the observation interval of 42 days (after the single IVM dose) on the other side, this is another argument that IVM's effect in COVID-19 it not based on direct antiviral activity (in accordance with laboratory data that suggest that much higher doses/concentrations of IVM would be needed for a direct antiviral effect), but instead immunomodulatory. In contrast to a direct antiviral effect, an immunomodulatory effect may last much longer than the presence of relevant concentrations of the drug in the body.

PEP:

In a randomized prospective study from Egypt **(NCT04422561; SHOUMAN W)** in a PEP setting ("contacts") *("asymptomatic family close contact of confirmed COVID -19 patient will receive prophylactic ivermectin and will be followed up for 14 days for any symptoms & diagnosis of COVID -19") …*

… 7.4 % from 203 persons of the Ivermectin arm compared to 58.5 % from 101 controls developed symptoms compatible with COVID-19 within 14 days after enrollment (HR 0.13). All cause mortality was 0 % in both groups. No PCR results were reported.

Doses were given according to body weight as mentioned above (twice, 72 hours apart, day 1 and day 3; 40-60 kg: 15 mg; 60-80 kg: 18 mg; >80 kg: 24 mg).

https://clinicaltrials.gov/ct2/show/results/NCT04422561

In detail, the trial included 340 contacts of 83 confirmed cases; 228 in the IVM arm, 112 in the control arm; lost to follow-up: 36 cases; analysed: 203 from the IVM arm and 101 from the control arm. Mean age was 40 vs. 38 years (IVM vs. control), median 38 vs. 35 years.

In the ivermectin arm, there were 13 cases (6.4 %): 8 mild, 4 moderate, 1 severe (but it is said in the text that there were 15 cases with symptoms, i.e. 7.4 %) (unexplained discrepancy of the data).

In the control arm, there were 59 cases (58.4 %): 31 mild, 21 moderate, 7 severe.

Risk of a contact to become a moderate or severe case: 2.5 % vs. 27.7 %

Risk of a contact to become a severe case: 0.5 % vs. 6.9 %

Days until symptoms appeared: 3.0 days vs. 4.3 days (mean) (median: 2 vs. 4 days); none in the IVM group developed symptoms > 6 days

Chest CT suspect: 7 vs. 28 patients (3.4 % vs. 27.7 %) (CT was performed only in those who developed symptoms)

Complete blood count suspect: 12 vs. 53 patients (5.9 % vs. 52.5 %) (performed only in those who developed symptoms)

Side effects of IVM were reported in 11 (5.4%) of contacts taking IVM: diarrhea (1.5%), nausea (1%), fatigue (1%), sleepiness (0.5%), abdominal pain (0.5%), heart burn (0.5%), tingling and numbness (0.5%) and lastly burning sensation (0.5%). No serious side effects.

The "protection rate", i.e. the probability *not* to develop symptomatic COVID-19 in the contacts, was 92.4 % overall, but differed between subgroups (<= 60 years: 93.8 %; > 60 years: 84.0 %; males: 94.3 %, females: 90.7 %; comorbidities no: 95.5 %; yes: 83 %).

In general, the risk of symptomatic disease was higher for contacts when the index case was severe; this applied to both groups: 14.8 % (IVM) vs. 71.1 % (controls). The protection was

stronger in contacts less than 60 years old (6.2% infected compared to 58.7%; RR 0.106) than > 60 years (16% infected compared to 55.6%; RR 0.29).

Though all subgroups profited from IVM prophylaxis, younger (<= 60 y) profited more than older contacts, males more than females, and people without comorbidities more than people with comorbidities, and all these associations were highly significant. Based on these calculations, older women with comorbidities would profit least of all and younger men (< 60 years) without comorbidities most of all. However, IVM was highly advantageous in any subgroup despite these minor (though significant) differences and this is in strong contrast to HCQ which seems to have a null effect or (more probably) a deleterious effect on the infection risk in people > ~ 50 years.

In a study from Peru, Ivermectin (0.2 mg/kg body weight) was given on day 1 to persons who qualified as contacts and who were PCR-negative **(AGUIRRE-CHANG et al.).** Participants who presented symptoms within the first 3 days of taking Ivermectin were excluded because it was assumed that these people must have contracted COVID-19 prior to initiation of this study. Daily follow-up was 21 days from taking Ivermectin. However, among 33 participants, no one had to be excluded because of symptoms during the first three days. No participant exhibited symptoms, there was no case of COVID-19 disease. However, there was no control group. 7 of 40 potential participants had been excluded in the beginning because of a positive PCR (leaving 33 persons as study participants). It remains unclear how high the risk of COVID symptoms would have been for the 33 participants in the absence of Ivermectin prophylaxis. Of note, 19 of 33 participants were > 65 years.

In a Syrian hamster model, Ivermectin treatment had no effect on viral load after intranasal inoculation of SARS-CoV-2, but was able to prevent deterioration of clinical pathology greatly (DE MELO et al.). In that trial, IVM was administered once (s.c.) at the time of intranasal inoculation with SARS-CoV-2, thus this animal trial reproduced a prophylaxis setting rather than a treatment setting.

In an **ecological study, HELLWIG and MAIA** proposed that countries with routine mass drug administration of prophylactic chemotherapy including Ivermectin (IVM) against parasitic infections (most common in Africa) might experience lower incidences of COVID-19. IVM is widely used prophylactically in mass drug administration campaigns against filiariasis and chocerciasis.

The correlation between Ivermectin use and COVID incidence was found to be highly significant both for Africa and in a worldwide context. HELLWIG and MAIA suggest that Ivermectin inhibits SARS-CoV-2 replication, resulting in lower infection rates, but other unknown inhibitory effects have to be considered after serum levels of IVM declined. The association between low COVID-19 incidence and Ivermectin prophylaxis programs (on country level) became stronger (statistically more robust) as the pandemic progressed (from $p < 0.01$ on April 15th to $p < 0.001$ on June 5th), and remained at that level until the end of the study (October 20th).

Comparing only African countries (Ivermectin prophylaxis, n = 22; prophylaxis without use of Ivermectin n =3; no prophylaxis n = 28, including North Africa), the lower incidence in IVM-

using countries was also significant (p = 0.017). HELLWIG and MAIA reported that in the countries that use IVM prophylaxis, the portion of the population who participates in prophylaxis ranges from 30 to 90 %. However, they didn't find an association between COVID incidence and the participation rate in IVM prophylaxis, nor between different prophylactic IVM dosing regimes. They conclude: *"This becomes less surprising once we consider Ivermectin's relatively short half life, meaning that the added effect of any higher dose would not be prolonged. Instead, we hypothesize that there is an as of yet unknown pathway that can be triggered with lower, proven safe doses."* (Plasma half-life of IVM in humans after oral administration: ~ 18 hours).

However, a missing relationship with both the coverage rate of IVM prophylaxis and the IVM dose, both unexplained, raises questions. HELLWIG and MAIA speculate that there might be an unknown mechanism that may inhibit SARS-CoV-2 replication after serum levels of Ivermectin decline (maybe an immunomodulatory effect as already proposed above).

Moreover, all countries with IVM prophylaxis belong to equatorial Africa or neighbouring regions, with Mali, Niger, Chad, Sudan as the northernmost countries with IVM prophylaxis, and Mozambique as the southernmost. If one excludes northern countries (Morocco, Libya, Tunisia, Egypt, Algeria) and countries from the south of Africa from the data of their Fig. 3, it becomes evident that the association would be attenuated. Logically, IVM prophylaxis is restricted to countries more central (closer to the equator) in Africa, and thus one should prefer to compare IVM prophylaxis vs. "no prophylaxis" only in countries of the same geographical latitude. In equatorial and para-equatorial Africa, the high risk and load of infections of any kind, not only filiariasis and onchocerciasis, may have some impact on the immune system that may train the innate immune response, perhaps in a similar way like BCG vaccines or mycobacterial strains *(see below)*, thus differences in the trained innate immunity may also offer a hypothesis to explain a lower risk of diagnosed (i.e. symptomatic) COVID infections or death in the more tropical (equatorial) parts of Africa.

To decide between the "IVM hypothesis" and the "trained immunity hypothesis", individual-based data would be needed, e.g. comparing COVID incidence or history of COVID-related symptoms and seroprevalence in those who took IVM for prophylaxis compared to those who didn't. But such studies are not available so far.

Because of these doubts, a re-analysis of the data from Africa was done based on Worldometer incidence data as reported by HELLWIG and MAIA (date of the own analysis: December 7th). For IVM prophylaxis, the same countries were included like in Fig. 3 in HELLWIG and MAIA. The 3 countries with other prophylaxis regimens than IVM were ignored since the other prophylactic agents could possibly have an own effect against COVID-19, as already discussed by HELLWIG and MAIA.

From the countries without a prophylaxis program against parasitic infections (blue color in Fig. 3), only those countries were included that are (i) continental and (ii) within the limits of the geographical (latitudinal) range of the countries with IVM program. Islands were excluded since they may have a different COVID epidemiology due to better possibilities of isolation, as demonstrated very well by New Zewland or Taiwan. Moreover, there were no islands in the IVM group. Including islands into the analysis may thus introduce an unnecessary bias.

The following “no prophylaxis” countries were excluded because they are outside the latitudinal belt that includes countries that have IVM prophylaxis programs: West Sahara, Morocco, Algeria, Tunisia, Libya and Egypt in the north and Lesotho and Swaziland in the south (South Africa was not included in HELLWIG and MAIA).

The mean incidence (cases/1 Mio.) was 513.2 in the 22 countries with IVM prophylaxis programs and 2145.5 (relative risk: 4.18) in 16 countries without prophylaxis programs within the “IVM prophylaxis belt”. Median was between 332 and 440 in the IVM group and between 1542 and 1628 in der “no prophylaxis group”.

In a stricter analysis, a few more countries were excluded because they are situated only marginally (to a small part) inside the “IVM prophylaxis belt”: Mauritania, Namibia and Botswana. Mean incidence in the “no prophylaxis group” of the remaining 13 countries was 1640.9 (relative risk 3.2), median 806. Thus the difference (RR) becomes smaller if one restricts the analysis to countries that are situated more precisely within the “IVM prophylaxis belt”.

However, since COVID incidence is strongly dependent on the availability of testing and the regional testing strategies, mortality may be an indicator that is less prone to testing bias. The same analysis was repeated with mortality data from WORLDOMETER (Deaths/1 M Pop). Mortality/1 Mill. Pop. was 10.15 (mean) in 22 countries with IVM prophylaxis and 25.26 in 16 countries from the “IVM belt” without any prophylaxis program (relativ risk 2.49). Median was between 6 and 8 in the “IVM countries” and between 15 and 19 in the 16 “no prophylaxis” countries. Excluding the borderline countries Mauritania, Namibia and Botswana, mean became 22.3 (relativ risk 2.2) and median 10 for the “no prophylaxis countries”.

Thus, both (i) stricter geographical limitation to the tropical and subtropical belt where IVM prophylaxis is used by many countries, and (ii) use of death rates instead of incidence rates attenuated the original findings from HELLWIG and MAIA, but the signal detected by HELLWIG and MAIA could still be found in all analyses. Though effects of better trained innate immunity due to the high exposure to various infectious agents may still play a role and may explain why the associations become less strong if one confines the analyses to the tropical/near-tropical belt, the differences in COVID incidence and mortality between countries with and without IVM prophylaxis remain to be striking; as a consequence, the critical re-analysis of the African data supports and strengthens the original hypothesis of HELLWIG and MAIA.

Eventually, TANIOKA et al. found that COVID mortality was lower in African countries where onchocerciasis is endemic and IVM may be used for prophylaxis, based on data until January 15th, 2021. They compared 31 onchocerciasis-endemic countries with 22 non-endemic countries and also took differences in life expectancy between the countries into account. Both morbitity and mortality were statistically significantly lesser in the onchocerciasis endemic countries. Recovery and fatality rates were not statistically significantly different, but this may due to difference in COVID testing and quality of the health system. As TANIOKA et al. pointed out, *“in areas where ivermectin is distributed to and used by the entire population, it leads to a significant reduction in mortality.”* Moreover, Ivermectin was allowed for controlled use in South Africa on January 27th, 2021 and may have contributed to

the ongoing decrease of COVID-19 incidence and mortality since then despite the delay of the start of vaccinations (TANIOKA et al.).

KORY et al. reported about "natural experiments" with IVM administration in South America (Peru, Brazil, Paraguay). In regions with IVM programs, a sudden and persistent drop of death cases was observed (see fig. 4 – 7 in KORY et al. for examples from Peru and Paraguay). Because IVM programs were established only on a regional level, the development of the incidence or mortality can be compared to neighbouring or structurally similar areas or towns without IVM prophylaxis. In areas with IVM prophylaxis in Paraguay, the IVM campaign was officially termed "deworming program" in order to avoid conflict with the country-wide recommendations of the government *not* to use IVM. For the Para region in Brazil, where early ambulatory treatment (including IVM) was recommended, deaths rates (per million inhabitants) were much lower during the second wave (EMMERICH).

Metaanalysis of Ivermectin prophylaxis from IVMMETA, https://ivmmeta.com/

Until January 12th, the IVMMETA website summarized 10 prophylactic trials with IVM (among them 3 RCTs). Single doses ranged from 2 to 28 mg (in the RCTs: 12, 18 and 28 mg), but no association between the size of the single dose(s) and the relative risk reduction of COVID-19 is detectable.

Altogether, IVM prophylaxis reduced the risk of COVID-19 by **90 % (RR 0.10, CI: 0.04 – 0.23),** based on 3663 participants from the 9 of the 10 trials (one trial was an ecological/ epidemiological study: HELLWIG and MAIA).

Update February 24th: the inclusion of the prospective study from BEHERA et al. (2) rises the number of participants to 7011 and gives a RR of 0.11, CI: 0.05 – 0.23).

The results from each of the 11 trials were significant. The point estimate of the RR ranged from 0.00, 0.01, 0.04, 0.05 and 0.09 (2x) to 0.17, 0.20, 0.21, 0.22 (ecol. study) and 0.50.

Update April 23rd: 3 further studies were added, giving a total of 14 studies:

● the ecological study from TANIOKA et al. (outcome death; RR 0.12),

● the RCT from SEET et al. (outcome severe disease: RR 0.50; outcome infection: RR 0.94) – in that study, only a single dose of 12 mg was given for prophylaxis

● the retrospective propensity matched study from MORGENSTERN et al.; outcome infection: RR 0.26; hospitalization: RR 0.20 (0 % vs. 0.7 %, but "continuity correction due to zero event (with reciprocal of the contrasting arm)"

In their meta-analysis, IVMMETA combined the "cases" outcomes of the 10 former studies (+ the "death" outcome in BERNIGAUD et al.) with the "death" outcome from TANIOKA, "severe" outcome from SEET and "hospitalization" outcome from MORGENSTERN and found a RR of 0.15 (CI: 0.09-0.25), based on 5059 participants on IVM prophylaxis and 6723

controls. The RR would have been higher if they had taken the “case” outcome from SEET et al.

One of the two “worst” trial in this series (RR 0.50) was the BEHERA PREP trial, a matched case-control study described in detail above, which showed a significant protective effect only after a second dose (uOR 0.27 and aOR 0.27), whereas there was no protection after a single dose in that trial, resulting in a RR of 0.50 for the total IVM group.

The other “bad” result is from the SEET trial that showed only a 6 % reduction of the infection risk (and 50 % for hospitalization). However, a single dose of 12 mg was given in the SEET trial, while the observation time was 42 days. Thus the results are very well in accordance with the BEHERA study that a single dose of IVM is not sufficient for prophylaxis.

As the IVMMETA website states (accessed April 23rd), based on these 14 studies, the probability that IVM is ineffective in prophylaxis is now about 1 : 16000. Combined with 38 studies about early or late treatment of COVID-19 with IVM (in most studies, in addition to other agents), the probability that IVM is ineffective in COVID-19 is now 1 : 85 trillion.

If one restricts the analysis to the three early RCTs (738 participants), but excluding the SEET RCT that seems to be characterized by underdosing, the point estimate of the RR is 0.107 (risk reduction 89 %), thus there is no striking difference in the effect size of the RR between RCTs and non-RCTs – an amazing observation since it is a common phenomenon that effect sizes are attenuated if analyses are restricted to RCTs, compared to Non-RCTs. Interestingly, this doesn’t seem apply to IVM prophylaxis – an indicator for the robustness of the trial results. RCTs were associated with point estimates for RR of 0.05, 0.09 and 0.20, well in the range of Non-RCTs.

The 3 RTCs are:

SHOUMAN (Egypt), see above, RR 0.09

ELGAZZAR et al. (Egypt), RR 0.20 (see below)

NCT04701710 (Argentinia), RR 0.05 (calculated for moderate/severe disease) (see below) (= CHALA et al. in Table 1 of BARTOSZKO et al)

These were also the three RCTs that were included in the network meta-analysis of BARTOSZKO et al. *mentioned above* (from the version from February 26th 2021).

Overview over all 10 trials mentioned on the IVMMETA website until February 24th:

1. RCTs

1.1 PEP: SHOUMAN (Egypt), 203 IVM/101 controls; contacts;

2 doses (15 – 24 mg depending on weigth days 1 + 3); outcome: symptomatic disease compatible with COVID-19 (no PCR):

7.4 % (IVM) vs. 58.5 % (controls); HR 0.13. Adjusted OR 0.087 ($p < 0.001$). **Adjusted risk reduction for symptomatic disease: 91 %**

1.2 PREP+PEP: ELGAZZAR et al. (Eygpt), multicenter double-blind RCT, 100/100 persons; HCWs (PREP) + household contacts (PEP); (personal protective measures in both groups). Comparatively old population (mean ~ 57 years mean in both groups; 75 % vs. 72 % males).

2 doses (0.4 mg/kg days 0 and 7).

PCR-confirmed infection rates: 2 vs. 10 %; **risk reduction: 80 % for PCR-confirmed infection.**

1.3 PREP: NCT04701710 (Argentinia; IVERCAR-Tuc). 117/117 HCWs; mean age: 39.6 vs. 38.4 years. All groups received "standard biosecurity care".

IVM oral drops: 2 drops of 6 mg = 12 mg every 7 days + iota-carrageenan: 6 nasal sprays per day; treatment regimen for 4 weeks.

PCR-confirmed symptomatic disease: 4 vs. 25, 3.4 % vs. 21.4 % ($p = 0.0001$) (OR 0.13; CI: 0.03-0.40), **risk reduction: 84 % (for PCR-confirmed symptomatic disease)**

Mild symptoms: 4 vs. 16
Moderate symptoms: 0 vs. 6
Severe symptoms: 0 vs. 3

Adjusted risk reduction for moderate/severe disease was calculated as 94.7 % ("adjusted for zero (with reciprocal of the contrasting arm))"

Added after February 24th

1.4 PREP: SEET et al., Singapore, healthy migrant workers quarantined in a large multi-storey dormitory; 617 vs. 619 controls (controls got 500 mg vitamin C per day). Mean age 33 years, 100 % men.

IVM dose: once 12 mg. Observation time: 42 days

New infection diagnosed by PCR or serology: 84.5 % vs. 70.0 %, relative risk ratio: 0.93; **risk reduction for infection (asymptomatic or symptomatic): 7 %**

Point estimates for adjusted ORs (depending on model, 6 different models were taken into account: between 0.73 and 0.89, all n.s.).

Symptomatic COVID 19: 5.2 % vs. 10.3 %; **risk reduction for symptomatic disease: 49.5 %**

2. Non-RCTs

2.1 PREP: NCT04425850/CARVALLO et al. (Argentinia, IVERCAR pilot study); observational; 131/98 HCWs;

„1 drop IVM buccal drops (6mg/ml)* + 6 sprays iota-carrageenan nasal spray (0.17mg/spray) (1 each nasal + 4 x oral cavity buccal) repeated 5 times per day + PPE“ (for 14 days) vs. „PPE only“ in the control group. IVM drop was applied 5 min after carrageenan to the tongue. Foods and liquids had to be avoided one hour before and one hour later (CARVALLO et al.)

*There were 30 drops in 1 ml, 1 ml equals 6 mg. 5 drops per day = 35 drops per week = 7.5 mg/week.

0 vs. 11.2 % PCR-positive infections within 28 days. **Risk reduction was calculated to 96.3 %** ($p < 0.0001$) “adjusted for zero (with reciprocal of the contrasting arm)” (IVMMETA).

“The effect is likely to be primarily due to ivermectin - the author has later reported that carrageenan is not necessary”. (IVMMETA).

2.2 PREP: CARVALLO et al. (Argentinia. IVERCAR: further study following the pilot trial); multicenter observational trial; 788/407 HCWs (separate study from NCT04425850, based on the success of the former study):

“A modification of the initial protocol was performed for ease of medication delivery. Carrageenan application was reduced to 4 x a day at the same total dose, and Ivermectin was administered as once per week dose of 12mg.” (CARVALLO et al.). PPE in both groups. (Increased weekly IVM dose compared to the pilot trial)

0 vs. 58.2 % PCR-positive infection within 3 months (i.e. may include asymptomatic infections).

Risk reduction for PCR-positive infection was calculated to 99.9 % ($p < 0.0001$) “adjusted for zero (with reciprocal of the contrasting arm)” (IVMMETA).

“The effect is likely to be primarily due to ivermectin - the author has later reported that carrageenan is not necessary”. (IVMMETA).

2.3 PREP: BEHERA et al. (India); HCWs, 186 pairs COVID+ cases/COVID - controls (retrospective matched case-control-study). Mean age was 29 years (60.75 % < 30 years). 9.7 % of cases were admitted to hospital.

Outcome: PCR +. **Dosing: 0.3 mg/kg.**

2 doses (72 hours apart): uOR and aOR 0.27 (sign.);
1 dose: no effect (uOR 1.23, aOR 1.30, n.s.).

No reports about effect of IVM on the risk of symptomatic disease, severity, hospitalization. May include asymptomatic infections.

The IVM 2 dose regimen was more effective than HCQ prophylaxis (uOR 0.58, aOR 0.56, n.s.) in the same trial.

Added after January 12th

2.3a PREP: BEHERA et al. (2) (India); HCWs; 1147 controls; 186 with 1 dose IVM; 2199 with 2 doses. Prospective cohort study.

Outcome: PCR +. **Dosing: 0.3 mg/kg.** Follow-up: the following month

2 doses (72 hours apart): uRR 0.18; aRR 0.17 (sign.) ($p < 0.001$). HR 0.15 (CI: 0.11 – 0.21).
1 dose: no effect (uRR 1.07, aRR 1.04, n.s.).

Symptoms *suggestive* of SARS-CoV-2 infection: 6 % vs. 15 % (IVM vs. controls)

Adverse effects: only 1.8 % of the participants (mild and self-limiting)

2.4 PREP: BERNIGAUD et al., care home residents (median age 90), 69/3062 controls (median age of controls: 86.2 years). 69 residents in a single care home were treated for scabies outbreak + 52 staff members.

2 Doses (0.2 mg/kg on day 0 and 7) for scabies prophylaxis.

10.1 % of the 69 residents had probable or certain COVID-19 and 7.7 % of 52 staff members; no serious cases, no deaths. 10 of 11 cases were minimal (no hospitalization, no oxygen support). Residents in comparable care homes: 22.6 % COVID-19 and 4.9 % deaths.

Calculated risk reduction: 55.1 % (p =0.01) for COVID case and 99.4 % (p = 0.08) for death; "adjusted for zero (with reciprocal of the contrasting arm)" (IVMMETA). However, there are discrepancies in data presentation *"Parmi eux, 22,6 % [95 %IC 16,3-28,9] ont eu la COVID-19 vs. 1.4 % EHPAD-A"* (EHPAD-A is the code for the care home with scabies), giving a crude risk reduction of 94 % for COVID-19, well in concordance with the risk reduction of mortality (4.9 vs. 0 %). Maybe there were herd effects in EHPAD-A that those residents not on IVM

profited also from less circulating infections, and the 1.4 % quote refers to all EHPAD-A inhabitants including those without IVM?

2.5 PREP: ALAM et al., observational control study; 58/60 HCWs. Mean age ~37 years in both groups.

Dose: 12 mg once per months for 4 months. Outcome: PCR- or CT-confirmed symptomatic COVID-19 (no routine testing of asymptomatic participants).

6.9 % vs. 73.7 % COVID-19 (4 vs. 44 cases); **risk reduction 90.6 %** ($p < 0.0001$).

The 4 infected participants from the IVM group "had mild symptoms with low grade fever, dry cough and weakness." No reports about symptoms/severity in the control group. Older participants (> 40 years) profited from IVM as much as younger participants (COVID+: 6.8 % vs. 7.1 % for > 40 years).

With regard to their monthly dosing regimen, ALAM et al. point out that, whereas plasma half life is only 16-18 hours, there is "wide tissue distribution, time length ranging from 4 days up to 12 days due to its high lipid solubility". Moreover, IVM has also immunomodulatory effects (that may explain effectiveness beyond the time of direct antiviral action).

2.6 PREP: VALLEJOS (Argentinia), press report, IVERCAR prophylaxis (IVM + carrageenan, see above), HCWs 371/502, no details on dose regimen given. But 241 took IVM + Carrageenan, 130 only IVM.

Cases: 6 vs. 38, 1.6 % (IVM) vs. 7.6 % in controls. No hospitalization in the IVM group; unknown for control group. **Risk reduction: 79 %.**

Added after February 24th

2.7 PREP: MORGENSTERN et al., retrospective cohort study from the Dominican Republic. 271 HCWs on IVM were homogeneously matched to 271 HCWs without IVM prophylaxis.

IVM: weekly oral dose of 0.2 mg/kg. Observation time: 28 days.

Participants were assigned to the IVM group if they had taken at least 2 doses (not more than 14 days apart) during the 28 day study interval. 51.5 % took 4 doses, 40.4 % 3 doses, 7.2 % 2 two doses; 1.4 % managed to take a single dose and then reported a COVID infection.

Positive PCR: 1.8 % vs. 6.2 %; Hazard ratio for a positive test: 0.26 (CI: 0.10 – 0.71). **Risk reduction: 74 %.**

Hospitalization: 0 vs. 0.7 %

In the unmatched cohort (326 IVM : 387 controls), SARS-CoV-2 PCR + was found in 1.8 % vs. 5.7 %, clinical deterioration in 0 vs. 0.8 % (0 vs. 3 individuals).

3. Ecological/epidemiological studies

3.1 PREP: HELLWIG and MAIA; ecological study (see above). Effect of IVM prophylaxis for parasitic infections on registered COVID infections on country level. Outcome: registered COVID-cases on Worldometer.

Risk reduction for countries with IVM prophylaxis compared to those without was 78 % within Africa ($p < 0.02$) and 80 % ($p < 0.001$) worldwide.

Own analyses with more stringent criteria for the selection of African countries and more recent data confirmed the results for registered infections and extended the analysis to registered COVID deaths, an outcome that may be more robust and less sensitive to testing strategies in African countries.

Not amenable to meta-analyses because the study is not based on individual patient data. And if IVM prophylaxis is implemented in a country, this doesn't mean that all people participate (range: 30 – 80 %). If anti-parasitic IVM prophylaxis is effective against COVID-19, any participation less than 100 % results in underestimation of the protective effect of IVM on COVID-19.

Added after February 24th

3.2 TANIOKA et al., ecology study (see above). COVID mortality in African countries; retrospectice study of 31 onchocerciasis-endemic countries (that may use community-directed treatment with ICM) compared to 22 non-endemic countries. COVID mortality was calculated to be 88.2 % lower in the endemic countries (RR for death: 0.12)

Discussion of the IVM study results (14 studies until April 23rd)

Excluding the ecological/epidemiological studies from HELLWIG and MAIA and TANIOKA et al., there are 12 studies that allow to calculate risk reductions based on individual participants and controls. Four studies were RCTs. Three non-RCTs were about IVM buccal drops ("on the tongue") in combination with carrageenan nose (+ oral) spray. In two of the four trials with IVM drops, the effective risk reduction for that regimen was 100 %, whereas it was only 79 % in the unpublished trial where 130 of 371 participants took only IVM and 241 IVM + carrageenan. Without publication and more data, the possible role of the addition of carrageenan remains unclear. In the only RCT about the combined regimen (NCT04701710), the risk reduction was 84 % with regard to symptomatic disease, but

effectively 100 % with regard to moderate/severe disease (though statistically calculated to 94.7 % after adjustment).

The authors of the CARVALLO trials stated later that they assume that the protective effect seems to be largely due to IVM (cited in: IVMMETA website). However, if one calculates the combined RR from the four trials (based on IVMMETA), weighting each study according to the number of participants, then the combined RR for the combined regimen is 0.08 (n = 2531) compared to RR 0.23 (n = 1132) for the 5 studies with IVM tablets without carrageenan (based on the studies that were listed in IVMMETA until the end of January, and the RR would be still higher if one would add the SEET and MORGENSTERN studies). If one exchanges the endpoint of the IVERCAR RCT from “moderate/severe disease” (RR 0.05) to “any symptomatic PCR-confirmed disease” (RR 0.16), the combined RR for the four studies about IVM + carrageenan rises only from 0.08 to 0.09. Thus there is still evidence that IVM drops + carrageenan nose spray + carrageenan throat spray is more effective than IVM tablets without the carrageenan procedure.

Meanwhile, FIGUEROA et al. showed in a placebo-controlled RCT from Argentinia, that iota-carrageenan nasal spray (4 times a day) reduces the risk of COVID infection in exposed HCWs by 80 % (details see above). Thus it is very questionable whether the high efficacy of a combined prophylaxis regimen of IVM and carrageenan nasal drops is largely due to the effect of IVM. Theoretically, also the opposite may be true now. In reality, both IVM and carrageenan nasal spray seem to contribute both to the favorable results of the combined regimen. That said, the effect of IVM alone seems to be smaller as suggested by the studies with the combined regimen, or meta-analysis that include both the combined regimen, IVM-only studies and studies where IVM was added to another systemic medication.

An important observation from studies is that IVM prophylaxis not only reduces the risk of COVID PCR positivity or symptomatic disease, but also the risk of severe disease/hospitalization in those people who became infected or symptomatic despite IVM prophylaxis (MORGENSTERN et al. SEET et al., IVERCAR-TUC trial, BERNIGAUD et al.). As far as results are reported stratified by severity, there was no severe case, hospitalization or death in any of the IVM arms, in contrast to control arms in the 9 non-ecological trials summarized on IVMMETA until end of January 2021. The added studies until end of April 2021 don’t change this conclusion. However, only few studies reported about such outcomes, and it is impossible to meta-analyse them. Moreover, many of these studies were performed in young populations with low risk of serious outcomes, at least at the times before the VOCs arrived.

In contrast to HCQ, there seems to be no strong age effect. One study showed favorable results for IVM (80 % risk reduction) though the mean age was about 57 years (ELGAZZAR et al.). Moreover, IVM worked very well in a care facility with a median age of 90 years (BERNIGAUD et al.). Whereas the risk reduction for disease was only 55.1 % in that setting, all cases were reported to be mild and/or without hospitalization and oxygen support, and there was no death among the 7 very old patients who got COVID-19 despite IVM prophylaxis (for scabies).

IVM is generally well tolerated, without severe side effects and much less concerns than HCQ.

Most trials were about PREP in HCWs; one trial was about PEP, another combined PREP and PEP. With an adjusted risk reduction of 91 % for symptomatic disease in the PEP trial

(SHOUMAN), and an overall risk reduction of 90 % based on 3663 patients from 9 studies (PREP + PEP combined), IVM in PEP doesn't seem to be inferior to IVM in PREP. Unfortunately, ELGAZZAR et al. didn't perform subgroup analyses about PREP (HCWs) and PEP (household contacts).

But there are still uncertainties about the dose. In countries where IVM drops are available, the IVERCAR regimen (the simplified version from the larger study from CARVALLO et al. with 12 mg once weekly) should be preferred because the data are impressive and the study cohorts were large. And the FIGUEROA RCT confirmed a favorable role of carrageenan nasal spray.

With regard to IVM tablets, all dose regimens in the trials were successful with two exemptions: in the PREP trial of BEHERA et al., a single dose of 0.3 mg/kg was not effective at all, whereas the repetition of that dose 3 days later yielded a preventive effect with an aOR and uOR of 0.27. In the SEET PREP RCT, a single dose of 12 mg IVM was ineffective to reduce the risk of PCR- or serology-confirmed infection, but it halved the risk of symptomatic COVID-19. On the other hand, a monthly dose of 12 mg (once monthly!) was highly successful with a risk reduction of 90.6 % (ALAM et al.). Taking this into account, one might propose 0.3 mg/kg at day 0 and 3 and then 0.3 mg/kg once every month, until more evidence is available. The initial two doses of that regimen are identical to the very successful PEP regimen of SHOUMAN (91 % risk reduction based on symptomatic disease).

ELGAZZAR et al. used higher doses (+ 33 %), 0.4 mg/day also twice (days 0 and 7), without better results (80 % risk reduction) compared to SHOUMAN, but this was based on PCR+ and the population was quite old (mean 57 years), thus the results cannot be directly compared to SHOUMAN. Since IVM not only reduces incidence of PCR+, but also severity of the disease, PCR+ may occur more often than symptomatic disease, i.e. risk reduction for PCR+ may be lower than risk reduction for symptomatic disease (e.g., SEET et al.). Both studies (SHOUMAN and ELGAZZAR et al.) are disproportionately important because of their RCT design. Nevertheless, though the prophylactic efficacy of IVM is well established now, more trials are needed to understand better about the most optimal dosing regimens, especially with regard to long-term PREP. For PEP, two doses (day 1 + 3) seem to be enough.

For PREP, the question is whether 0.3 mg/kg at day 1 and 3 and then 0.3 mg/kg once every month, or whether 0.2 mg/kg (or more according to body weight) once a week is better? As shown by BEHERA et al. and SEET et al., a single dose of IVM is not enough.

In summary, a high prophylactic effect of IVM of about 85 % risk reduction is now established based on the IVMMETA metaanalysis, based on 14 studies (12 non-ecological) with 5059 participants in the IVM group and 6723 controls, among them 4 RCTs. However, outcomes were different in the studies, and a stronger homogenization of the outcomes (by taking the "infections" from SEET et al. and MORGENSTERN et al. instead of severe disease/hospitalization) would have worsened the RR for "infections/cases"). Moreover, the combined use with local administration of carrageenan (like the IVERCAR regimen) improves the results (RR for the combined regimen 0.08 – 0.09). Taking that into account, the effect of IVM prophylaxis alone must be weaker than the RR 0.15 that was calculated on the IVMMETA site.

If one excludes the single-dose arms (BEHERA et al., SEET et al.) that showed no effectiveness against infection/positive test, results from RCTs don't differ from non-RCTs. Each of these studies showed significant results. The results from these studies are

corroborated by an ecological studies (HELLWIG and MAIA) and its extension to mortality and a more stringent design for the selection of countries (presented here), but also by the ecological study from TANIOKA et al. and the favorable results for IVM in early treatment settings. From a biological and virological point of view, there is some continuity along the line PREP / Peri-EP / PEP / early treatment. Agents that work in early treatment may also work in prophylaxis, and *vice versa.* In IVMMETA, early treatment with IVM was associated with an "improvement" (varying endpoints between different studies) of 81 % (RR 0.19; 0.09 – 0.38), based on 18 studies (11 RCTs) with 1008 patients who got IVM and 934 controls presented on IVMMETA up to April 23rd.

However, in the therapeutic setting, IVM is often combined with one or several other agents, so these results cannot be compared directly to its administration as a single agent for prophylaxis. But as shown above, even for the purpose of prophylaxis a combined regimen like IVERCAR seems to be more successful.

Most important, IVM prophylaxis works also in older and very old age groups – in contrast to HCQ that seems to increase the risk of infection in older people. Moreover, if infected despite IVM prophylaxis, the disease is milder or more often asymptomatic, even in very old people as shown by BERNIGAUD et al.. There were no reports about severe cases, hospitalizations or deaths among them who took IVM prophylaxis in those studies that reported about such outcomes. However, there are still uncertainties about the minimal dose that is necessary to elicit the full prophylactic effect of IVM. Based on its half-life and virucidal effect, the long-term effect of IVM prophylaxis (as shown e.g. by ALAM et al. with its administration once a month) is surprising and far beyond theoretical considerations; maybe it can be attributed to its immunomodulatory effect that may exceed its mere antiviral effect. The same applies to the successful two-dose regimen at day 1 and 3, when the next dose is given after a full month. This regimen is also not in accordance with the half-life of IVM.

One may hypothesize that this is mediated by effects of Ivermectin on innate immune responses, since IVM is also effective in the treatment of diseases like rosacea that are characterized by dysregulation of innate immune responses, thus IVM seems to help to restore a dysregulated innate immune response (see also: http://freepdfhosting.com/437d9e1634.pdf).

Besides the TMPRSS2 pathway that is not addressed by HCQ (what remained undetected in experiments with Vero cells), innate immunity might be the second cause for the **HCQ-IVM-conundrum.** This conundrum describes that, at first, HCQ was found very effective *in vitro* against SARS-CoV-2 (Vero cells), and gave reason for high expectations, but later, it failed or yielded disappointing or weak results in prophylaxis and clinical trials for treatment. In contrast, there was not much hope with regard to IVM because *in vitro* data suggested that one would need much higher concentrations of IVM for antiviral activity against SARS-CoV-2 than can be achieved *in vivo,* and, in contrast to HCQ, half-life is quite short. IVM still looked interesting, but only as a partner in a multidrug regimen.

Now we see that HCQ is worse than originally expected based on early *in vitro* and pharmacokinetic data, whereas IVM seems to be more effective than originally expected (also based on *in vitro* and pharmacokinetic data). Besides their antiviral activity, both agents are immunomodulators. However, whereas HCQ weakens the innate immune response

(inhibits the stimulation of interferon-stimulated genes and may also cause lymphopenia), IVM seems to stabilize the innate immune response and correct for dysregulations – otherwise it wouldn't be effective against rosacea. Thus the HCQ-IVM-conundrum may be based, in part, on opposite effects of HCQ and IVM on the innate immune system.

Of note, IVM was able to inhibit SARS-CoV-2 in Vero cells, but **not** in human-derived airway epithelial cells (DINESH KUMAR et al). Thus any prophylactic or therapeutic effect that was seen in studies with IVM cannot be attributed to a direct antiviral activity, supporting the view that its effect in SARS-CoV-2/COVID-19 is immunomodulatory, not antiviral.

In their meta-analysis for Ivermectin prophylaxis on the outcome **"symptomatic COVID-19",** KORY et al. found an OR of 0.073 (CI: 0.044 – 0.123) based on 4 observational studies (BEHERA, CARVALLO-1, CARVALLO-2, ALAM) and an OR of 0.079 (CI: 0.047 – 0.125) for 3 RCTs (ELGAZZAR, SHOUMAN; CHALA).

Recommendations for Ivermectin-based PREP/PEP from the FLCCC Alliance:

FLCCC Alliance = Front Line COVID-19 Critical Care Alliance

https://covid19criticalcare.com/

I-MASK+ Protocol of the FLCCC Alliance (accessed April 25th, 2021) for prophylaxis:

PROPHYLAXIS PROTOCOL

Ivermectin as prophylaxis for high risk individuals (PREP): 0.2 mg/kg* per dose — one dose today, 2nd dose in 48 hours, then one dose every 2 weeks

Ivermectin as PEP: 0.2 mg/kg per dose— one dose today, 2nd dose in 48 hours

Vitamin D3 1,000–3,000 IU/day

Vitamin C 1,000mg twice a day

Quercetin 250mg/day

Zinc 30 – 40 mg/day

Melatonin 6mg before bedtime

(may become updated)

(KORY P et al.)

Highly recommended for further reading about IVM:

KORY P et al., Review of the Emerging Evidence Demonstrating the Efficacy of Ivermectin in the Prophylaxis and Treatment of COVID-19. Doi: 10.31219/osf.io/wx3zn

https://covid19criticalcare.com/

EMA advised against the use of ICM for the prevention or treatment of COVID-19 outside randomised clinical trials (March 2021).

But in the EU, Ivermectin was approved for prophylaxis and treatment of COVID-19 on a country level in **Czechia** and also in **Slovakia** on January 26th, 2021 (first for six months):

https://ockbgkekw6vfmhahzvs46a7ewy-adwhj77lcyoafdy-www-health-gov-sk.translate.goog/Clanok?covid-19-27-01-2021-ivermectin

EMA statement:

EMA advises against use of ivermectin for the prevention or treatment of COVID-19 outside randomised clinical trials

22/03/2021

EMA has reviewed the latest evidence on the use of ivermectin for the prevention and treatment of COVID-19 and concluded that the available data do not support its use for COVID-19 outside well-designed clinical trials.

In the EU, ivermectin tablets are approved for treating some parasitic worm infestations while ivermectin skin preparations are approved for treating skin conditions such as rosacea. Ivermectin is also authorised for veterinary use for a wide range of animal species for internal and external parasites.

Ivermectin medicines are not authorised for use in COVID-19 in the EU, and EMA has not received any application for such use.

Following recent media reports and publications on the use of ivermectin, EMA reviewed the latest published evidence from laboratory studies, observational studies, clinical trials and meta-analyses. Laboratory studies found that ivermectin could block replication of SARS-CoV-2 (the virus that causes

COVID-19), but at much higher ivermectin concentrations than those achieved with the currently authorised doses. Results from clinical studies were varied, with some studies showing no benefit and others reporting a potential benefit. Most studies EMA reviewed were small and had additional limitations, including different dosing regimens and use of concomitant medications. EMA therefore concluded that the currently available evidence is not sufficient to support the use of ivermectin in COVID-19 outside clinical trials.

Although ivermectin is generally well tolerated at doses authorised for other indications, side effects could increase with the much higher doses that would be needed to obtain concentrations of ivermectin in the lungs that are effective against the virus. Toxicity when ivermectin is used at higher than approved doses therefore cannot be excluded.

EMA therefore concluded that use of ivermectin for prevention or treatment of COVID-19 cannot currently be recommended outside controlled clinical trials. Further well-designed, randomised studies are needed to draw conclusions as to whether the product is effective and safe in the prevention and treatment of COVID-19.

This EMA public health statement has been endorsed by the COVID-19 EMA pandemic Task Force (COVID-ETF), in light of the ongoing discussions on the use of ivermectin in the prevention and treatment of COVID-19.

Bromhexine (TMPRSS2 blocker)

In a single-center randomized open-label study with HCWs managing patients with suspected and confirmed COVID-19, bromhexine hydrochloride was administered (8 mg 3 times a day for 8 weeks) to 25 HCWs, while 25 HCWs were controls; 88 % were physicians, 12 % nurses (MIKHAYLOV et al.; NCT04405999). Mean age: 41.7 vs. 39.5 years (controls).

- PCR positivity (nasopharyngeal swab) or signs of infection: 2/25 vs. 7/25 (8 % vs. 28 %, p = 0.07)
- PCR positivity: 2/25 vs. 7/25
- Asymptomatic infection: 2/25 vs. 2/25
  (one of the asymptomatic infected from the bromhexine group had missed 2 days to take the drugs)
- Symptomatic infection: 0/25 vs. 5/25, p = 0.02
- Moderate disease: 0/25 vs. 3/25
- Severe disease with hospitalization: 0/25 vs. 2/25 (but no ICU/no death).

The 2 asymptomatic infections among HCWs who took bromhexine occurred in week 2 and 3 after start of bromhexine prophylaxis.

It is important to note that the infections occurred in spite of proper PPE (FFP2 or FFP3 respirators, full skin covering, protective eye glasses). But participants had direct contact with staff outside the “red zones” without PPE. None reported unprotected contact with COVID-19 patients outside the hospital.

Bromhexine prophylaxis started the day before the first contact with COVID patients (= first day of work in an infection department). Nasopharyngeal swabs were performed every 7 days and additional tests in case of infection. No losses/no dropouts after randomization; no treatment termination or interruption because of adverse effects.

With regard to VoCs, LEE J et al. demonstrated that bromhexine is not statistically significantly less effective against B.1.1.7 and B.1.351 compared to wild type SARS-CoV-2. However, there was an insignificant trend that it may be a little less effective against B.1.351, whereas no difference was found between wild type and B.1.1.7. In general, viral inhibition on Calu-3 cells was much less pronounced by bromhexine compared to other TMPRSS2 inhibitors like camostat, nafamostat or aprotinin (50 – 60 % vs. 80 – 100 % at highest tested concentration).

Surprisingly, bromhexine hydrochloride was found to to be completely ineffective in a TMPRSS2 peptidase or HexaPro210 cleavage assay; contrary to nafamostat and camostat, it showed no inhibition and was ineffective to block SARS-CoV-2 pseudovirus entry (FRASER et al.). This study raises now a lot of doubts whether bromhexine has any anti-SARS-CoV-2 effect at all.

Based on *in vitro* results, it is probable that Ambroxol is more effective than Bromhexine. Both drugs are TMPRSS2 inhibitors, but Bromhexine was found to enhance cell-cell fusion in the presence of TMPRSS2, while ambroxol (a metabolite of bromhexine) showed inhibitory activity on cell fusion under some conditions. Virus can spread by cell fusion from infected to uninfected cells even in the absence of free virus (HÖRNICH et al.). Unfortunately, there are so far no results from prophylactic trials with Ambroxol available. The favorable results of the MIKHAYLOV study were achieved despite this paradoxical effect of Bromhexine. This rises the hope that Ambroxol may be more effective than Bromhexine, both in prophylaxis and in treatment.

CARPINTEIRO et al. showed that **ambroxol** prevents SARS-CoV-2 entry into epithelial cells by inhibiting acid sphingomyelinase. Entry of the spike of the virus requires activation of acid sphingomyelinase and release of ceramide; all of these events were prevented by pretreatment of the cells with ambroxol. This mechanism was also recapitulated in nasal epithelial cells from human volunteers prior to and after inhalation of ambroxol, inoculated with pseudotyped SARS-CoV-2 *ex vivo*. The volunteers inhaled an approximately 20 mM solution of ambroxol. Since 20-25 µM ambroxol were sufficient to block infection with the pseudotyped SARS-CoV-2, CARPINTEIRO et al. expect a very broad therapeutic window.

Moreover, escape mutants would still be inhibited by ambroxol treatment, since all mutants require ACE2 for infection. *“Thus, targeting the acid sphingomyelinase/ceramide system might be a very interesting approach to prevent infection with SARS-CoV-2 mutants”.* Taking into account the study of OLALEYE et al., ambroxol may prevent infections at different levels (blocking the binding of the S protein to its human receptor and inhibition of acid sphingomyelinase/ceramide system). *“This suggests that inhalation of ambroxol may prevent SARS-CoV-2 infection and spread in the human respiratory epithelium.”* (CARPINTEIRO et al.).

Antidepressants like fluoxetine, amitriptyline and others also inhibit the acid sphingomyelinase and act very similar to ambroxol, but have to be given systemically and have a variety of side effects and contraindications. As CARPINTEIRO et al. pointed out, in contrast to antidepressants, ambroxol is very safe, well tolerated *"and can in principle applied with no temporal limitation" … " for example, prophylactically in persons at risk to develop severe Covid-19 infections, such as elderly individuals, or after contact with an infected individual or after testing positive for SARS-CoV-2 without symptoms of COVID-19. However, ambroxol is very likely not effective at later stages when the viral infection becomes systemic"* (CARPINTEIRO et al.).

An ongoing trial combines low-dose HCQ (200 mg/day) with 8 mg TID bromhexine for PREP in HCWs (GRANADOS-MONTIEL et al.) (ELEVATE trial).

BCG booster immunization

In the first half of August, **AMIRLAK et al.** were the first who reported results of a retrospective BCG trial in HCWs. All staff of the Emirates International Hospital in the United Arab Emirates were offered a booster BCG vaccine in early March 2020. In April, May und June, all the hospital staff were routinely tested (PCR, nasopharyngeal swabs; government-mandated testing). Additional tests were performed in settings of contact with positive patients or in the case of symptoms of staff members.

Among 71 participants who received the booster BCG, no one was positive for COVID-19 until the end of June, compared to 8.6 % (18/209) who did not receive the vaccine (p = 0.004). 13 of the 18 positive cases were symptomatic. No local or systemic complications were noted following BCG vaccination. All participants had received BCG vaccination after birth.

In Greece, older adults who were hospitalized for any reason were offered to participate in a RCT to receive either a single dose of BCG vaccine or placebo at the day of their discharge **(ACTIVATE trial**; NCT03296423). Every patient is followed up for 12 months. An interim analysis (April 29th 2020) found a 53 % decrease of the incidence of new infections of any origin and location in the BCG group compared to the placebo group. Among them, the reduction of all respiratory tract infections was 80 %, and patients with coronary heart disease and COPD profited at most. However, this trial started before COVID-19 epidemic and is not specifically about the prevention of COVID-19. Nevertheless, *"this interim analysis clearly enhances the concept that BCG can be protective against COVID-19"* (https://clinicaltrials.gov/ct2/show/NCT04414267), and, as a consequence, a new trial was started (ACTIVATE II, NCT04414267) to examine the effects of a single dose of BCG vaccine in older people (> 50 years) with regard to the prevention of COVID-19 *(see below).*

A retrospective study from Rhode Islands (US) found that among 120 COVID-19 infected patients, those with BCG vaccination in their life history were less likely to require hospital admission (3.7 % vs. 15.8 %, p = 0.019), though the median age was 10 years higher in the BCG group (41 instead of 31 years). After adjusting for demographics and comorbidities, the significance of this result improved even a little more (p = 0.017). One patient in the non-

BCG group died (1/38 = 2.6 %), whereas there was no death in the BCG group (WENG C et al.). However, in Israel, BCG vaccination in childhood had no influence on the risk of COVID infection in the age group between 35 and 41 years (HAMIEL et al.). Since there were only 2 cases of severe disease and no death among the 660 infected people of that age group (one case in each group), the results don't allow suggestions with regard to the influence of childhood BCG immunization on the severity of the disease. The cohort was probably too young to analyse such effects. In contrast, in WENG et al. 25 % of all patients were > 50 years and thus differences in the severity of the disease may have become visible more clearly. WENG et al. conclude that prior BCG immunization may decrease the severity of the disease, but not the risk of infection or of symptomatic disease because there were no significant differences with regard to the frequency of COVID-specific symptoms or a summarized unweighted or weighted symptom score between BCG- and non-BCG patients.

However, more data are needed with regard to BCG vaccination. First, there may be a difference between the effect on COVID-19 in the case of a recent primary vaccination, of a recent booster vaccination (like in the study from AMIRLAK et al.), or if one looks at long-term effects of childhood vaccinations. Second, there are several types (strains) of the vaccine which may differ in their anti-COVID effects, including the new type VPM1002. Third, there is at least a theoretical possibility that BCG vaccination, especially a more recent one, may have deleterious effects in some COVID patients if it further enhances hyperactivity of the immune system and cytokine storms. Thus results from larger trials are needed in order to exclude such a deleterious effect with certainty, and results may be strain-dependent (e.g., whether the BCG strains favors Th-1- or Th-2-skewed immunity).

In their epidemiological study, DATTA and DATTA found that "early" BCG strains were most effective, whereas "late" strains seem to be ineffective to induce trained immunity because late strains "*are deficient in their ability to produce cell wall methoxymycolic acid which constitute a key group of ligands proficient in inducing trained immunity".* The event rate (a sort of mortality rate) in countries with "late strain" vaccination programs was similar to countries with no BCG program at all (0.031 vs. 0.034), while it was only 0.018 in countries with "early strain" vaccination. Altogether, DATTA and DATTA found a reduction of the event rate by about 40 %, and this applied also to high-risk subgroups like people $\geq$65 years (-36.1 %).

Anyway, the detailed study of DATTA and DATTA, which addressed many confounders (like age structure of different populations and the prevalence of relevant comorbities), may put an end to the endless discussions and douzens of papers pro or contra any effect of BCG vaccination in the past. It showed clearly that there *is* a protective effect, however, it is restricted to "early strains" and to a lesser extent to "mixed strains", but not to "late strains", and the effect is of moderate extent and by far not as large as would be required for the approval of a COVID-19 vaccine.

WHO doesn't recommend BCG vaccination for COVID prevention so far; however, the WHO statement dates from April 12th 2020 and the favorable results (ACTIVATE interim analysis; AMIRLAK et al.) were published later. Nevertheless, for the three reasons mentioned above, there is an urgent need for many more data. Interim analyses of larger trials like BRACE and BCG-CORONA would be very helpful.

There is some controversary evidence that early childhood BCG vaccination may generate some degree of protection with regard to COVID severity/mortality (e.g., see DOLGIKH S or the narrative review of BERIC-STOJSIC et al.), but it is a separate matter whether a primary vaccination of so far unvaccinated people in adulthood or older ages (risk groups) offers protection against severe COVID disease.

In a multicenter study from Turkey, infected health care workers with a history of BCG vaccination didn't have a smaller risk of hospitalization than those without a BCG scar (in fact, the risk was even higher in the BCG scar group), and the authors suggest a hyper-triggered immune system as explanation (TORUN et al.). On the other hand, based (i) on data from 20 European countries and (ii) a comparison between West and East Germany, SINGH et al. observed a significant negative correlation between COVID incidence and tuberculin immunoreactivity (as a measure of cell-mediated immunity persistence) as a result of an exposure to *Mycobacterium spp.,* which may be a BCG vaccine but also a natural infection, and there was also a trend for a negative correlation with COVID mortality.

However, though SINGH et al. reported higher latent TB infection rates in East Germany, based on a single study from 2006 (9.2 % in West Germany compared to 22.5 % in East Germany, when calculated for the year 2020 based on the original data from 2006), and this may correlate well with the lower COVID incidence and mortality in East Germany, an own analysis based on the RKI report for October 31st found no significant difference in the case fatality rates (CFR) of western and eastern German federal countries (mean 2.06 % for 10 western countries; range: 1.35 – 3.0 %; mean 1.93 % for 5 eastern countries; range: 0.81 – 2.98 %; without Berlin). And during the last days of December 2020 and in early January 2021, four out of five east German countries (+Berlin) hold the top 5 positions of recent 7-day COVID-19 incidence in Germany (compared to all 16 German countries). On January 11th, two eastern countries had the highest cumulative mortality rates (calculated since the beginning of the epidemic), and in four out of five eastern countries, the cumulative CFR was higher than in all of Germany (2.3 %, 2.6 %, 2.6 % and 2.7 % instead of 2.1 %). The new data put an end to all hypotheses about differences between western and eastern Germany in favor of the latter which were supposed to be associated with different immunization strategies in the past (e.g. higher uptake rates of vaccinations) and differences in the TB history.

In a study with 6201 HCWs from Los Angeles (among them 29.6 % with a history of BCG vaccination and 68.9 % without; rest: BCG status unknown), seroprevalence of anti-SARS-CoV-2-IgG and clinical symptoms associated with COVID-19 were significantly decreased in those with BCG vaccination, whereas no such associations were found with meningococcal, pneumococcal and influenza vaccination (but only 1.2 % of all participants were sure to have no influenza vaccination, so the influenza results have to be regarded as irrelevant) (NOVAL RIVAS et al.). The BCG-vaccinated cohort was older (43.6 years vs. 40.6 years). COVID-19 related symptoms (last 6 months): 24.4 vs. 27.3 % ($p = 0.017$, BCG vs. no-BCG). The aOR for anti-SARS-CoV-2-IgG (adjusted for age and sex) was 0.76 (0.57 – 0.99) and thus marginally significant ($p = 0.048$) (IgG index value > 0.4). Thus the results hint to a BCG signal even decades after vaccination (if occurred in childhood), but it doesn't seem to be very strong. There was no information about the timeline of BCG vaccination in that cohort (only childhood vaccine) and no subanalysis of different age groups. If BCG was given only at childhood, it would have been interesting to see whether there is an age effect.

There is a wealth of ecological studies meanwhile *pro* and *contra* any role of early childhood BCG vaccinations with regard to COVID incidence or mortality. Meanwhile, there are even data that support a disadvantageous effect of childhood BCG vaccination on the risk of

COVID infection, but also on hospitalization and thus severity of the disease (DE LA FUENTE et al.), and it is suggested that this may be related to the activation of certain innate immunity mechanisms with inflammatory reactions. If so, childhood BCG would be a risk factor for COVID-19 and particularly the cytokine storm syndrome. Whereas this study has a lot of limitations and is prone to bias (e.g., participation bias), it is a warning that the situation is by far not as clear as seems to be evidenced by a lot of other studies.

It is outside the scope of this paper to discuss the subject of childhood BCG, and there are three reasons not to do so. First, it is very questionable that a strong protective effect of early childhood BCG vaccination lasts for several decades; it seems to be limited to about 15 years (MOHAPATRA et al.). Even if there is a weak protective signal following childhood BCG vaccination, it would be a statistical signal that would be too weak that anyone can feel protected by that.

Moreover, ecological studies about BCG vaccination may overlook nonspecific immunological responses due to *environmental non-tuberculous mycobacteria* which may account for positive tuberculin tests in non-BCG-vaccinated people in some countries like India (MOHAPATRA et al.). Third, the question whether a new (recent) BCG vaccination offers some degree of protection from COVID-19 is very different from the question whether BCG vaccination decades ago has still some residual effect.

It is also important to note that BCG vaccination, as primary vaccination or as booster, is problematic or contraindicated in many risk groups for severe COVID-19 (see KLEEN et al.).

Though there are a lot of theoretical papers about the prospects of BCG vaccination for the prevention of COVID-19, or at least severe COVID-19/mortality, and the underlying mechanisms which make BCG look so promising, they will not be discussed here because one has to be realistic about the limitations to use BCG in the classical risk groups of severe COVID-19. Vaccines with inactivated mycobacteria are better tolerable and less risky for these groups.

However, interestingly, there is already a report that BCG vaccination may also be helpful for the treatment of COVID patients, both in those who already got BCG in childhood and those who didn't (PADMANABHAN et al.). In a trial from Mumbai, 60 hospitalized COVID patients with pneumonia and requirement for oxygen therapy were randomized 1 : 1 to receive a single adult dose of intradermal BCG or normal saline (beside standard of care in both groups). Compared to the control group, there was a reduction in oxygen requirements from day 3-4 and improved radiological resolution from day 7-15 in the BCG group. There were 4 ICU admissions and 2 deaths in the control group (affecting altogether 5 patients), but only 1 ICU admission and no death in the BCG group. Whereas specific IgG levels increased in the BCG group, there was no evidence that BCG induced cytokine storms. Only four patients showed localized inflammatory response at the BCG injection site. Of note, a third of the patients were naive for childhood BCG vaccination.

Median age was higher in the BCG group (49 vs. 41.5 years), and there were more obese patients (11 vs. 3) and more males (20 vs. 16) in the BCG group, thus basic risk for worse outcomes was a little higher in the BCG group.

PADMANABHAN et al. concluded, *"that BCG is a safe, cost-effective treatment that can be introduced as a standard of care in patients with moderate Covid-19 that can reduce requirement of oxygen supplemented beds and disease burden in low resource countries…"*

Though this prospective trial was about treatment of already ill patients with pneumonia and need for oxygen support, it is another hint that recent BCG vaccination may have preventive effects, as demonstrated in the retrospective preventive trial of AMIRLAK et al.

Eventually, at the end of May 2021, the results of the **ACTIVATE II** trial, a double-blind RCT from Greece, were published (TSILIKA M et al.). In contrast to the larger BCG trials with HCWs, this trial was dedicated to elder Greek patients who were randomized (1:1) to receive either BCG revaccination or placebo at hospital discharge (from hospitalization for COVID-unrelated reasons), followed by 6 months for COVID-19 infection. BCG: n = 148, mean age: 68.6 years; controls: n = 152; mean age: 68.7 years. Enrollment started on June 6th 2021; 6 months follow-up of the last patient was April 19th, 2021. For inclusion, patients had to be at least 50 years old and had to have comorbidities, defining them as high-risk patients,

The low number of treatment-associated adverse effects didn't differ between BCG and control group except for erythema at the injection site. No tuberculosis/systemic BCGitis.

BCG revaccination reduced the risk for "total COVID19 clinical and microbiological diagnoses" by 68 % (OR 0.32; CI 0.13-0.79). Five patients from the placebo group and one patient from the BCG group had severe COVID-19 and needed hospitalization; 3 patients from the placebo group died for COVID-unrelated reasons. Surprisingly, 1.3 % (2/153) of placebo participants and 4.7 % (7/148) of BCG participants were seropositive at 3-months follow-up (p = 0.099),

In detail, based on a composite endpoint of symptoms compatible with COVID-19 or microbiological diagnosis of COVID-19 ("possible/probable/definite COVID-19"), the incidence of that endpoint was 10 : 2 (placebo : BCG; p = 0.086) after 3 months. After six months, no absolute numbers are given, but the univariate OR for the composite endpoint was 0.33 (CI: 0.14 – 0.81, p = 0.015) and in the multivariate analysis, OR was 0.32 (0.13 – 0.79, p = 0.014). Calculated fron the figures, "possible/probable/definitive COVID-19" was about 7,5 % in the BCG group and about 21 % in the control group until 180 days.

One problem of this study is that many participants were lost to follow-up after 3 months. From 301 patients, 153 received placebo vaccination and 148 received BCG; all were included in the 3-months analysis. After that, 55 participants were lost to follow-up in the placebo group and 56 in the BCG group. Thus, after 6 months, the final analysis was performed in 98 (from 153) placebo-vaccinated individuals and 92 (from 148) BCG-vaccinated individuals. Moreover, the endpoint "possible / probable / definitive" COVID-19 is quite imprecise and inclusive.

TSILIKA et al. also discussed the antibody results after three months. Though there is the possibility of a chance finding (p was 0.099), it is more likely that this is a consequence of the BCG intervention. BCG is known to improve serological responses to other vaccines (references in TSILIKA et al.), "and it may induce similar effects in asymptomatic COVID-19 infections" (TSILIKA et al.) that are usually associated with low or even missing IgG responses. A previous BCG vaccination may improve the antibody response in

asymptomatically infected individuals and thus improve protection against future (re-) infections. The antibody results further indicate that BCG revaccination may reduce the severity of the disease once infected; many infections in BCG-revaccinated people may have been undetected and asymptomatic. Unfortunately, the interaction between seropositivity and history of COVID-compatible symptoms after 3 months was not reported in that study. This might have helped to understand more about the antibody data.

Of note, the study didn't investigate whether the elderly participants had gotten BCG as a child, but this is highly probable because of the vaccination strategy in Greece at the time of their birth. The authors regard their trial therefore as dealing with revaccinations. This is an important matter:

As TSILIKA et al. point out in their discussion, there seems to be different effects if BCG in adulthood or elderly is given as first vaccination or as re-vaccination (as supposed in the case of ACTIVATE-II). The effect of BCG on COVID-19 seems to be less strong if BCG is administered to naïve populations. This differential effect of BCG vaccination was first reported for other infections (before COVID-19) (references see TSILIKA et al.), but seems now to recapitulate also in COVID-19.

In the **BCG-PRIME study,** a double-blind RCT, 6132 vulnerable patients aged 60 and over were vaccinated in 20 Dutch hospitals (started in September 2020). Initial results reported by the UMC Utrecht showed that BCG vaccine **offered no protection against symptomatic COVID-19 in vulnerable elderly,** but the study is continued in order to find out whether BCG protects against severe forms of COVID-19 or against other respiratory infections." (UMC Utrecht, January 18th, 2021).

Altogether, there is a need to wait for the results of the large prophylactic studies like BRACE or BCG-COVID to understand more about favorable or unfavorable effects of BCG (re-)-vaccination in adulthood. Moreover, whereas BCG vaccination (as a first vaccination) seems to be ineffective with regard to COVID-19 infection or symptomatic disease, it is still unclear whether it affects the severity of the disease, and the results from the BCG-PRIME study about that subject are still not available.

However, in the time of COVID vaccinations of HCWs and the whole population, it is no longer important to understand the effect of BCG vaccination on COVID-19 on its own, but it is much more important to understand its interaction with COVID vaccinations that occurred before or after administration of BCG. In other words, it is no longer important to study whether BCG can be a substitute for "true" COVID vaccinations, but how BCG interacts with COVID vaccinations, e.g. if there are priming or boosting effects that may eventually yield a "double protection strategy". This is subject of a separate paper:

http://freepdfhosting.com/437d9e1634.pdf

It must be also noted that new variants that evade the early innate immune reponse may influence and attenuate the efficacy of interventions like BCG vaccinations (and others) that are intended to train the innate immune response in a Th1-skewed manner. Every study that deals with BCG vaccination or adults or other interventions for the same purpose should be seen in the context of the SARS-CoV-2 variants that circulated at the time and in the geographic area where the study was done.

Mycobacterium w (Mycobacterium Indicus pranii) injection

In a prospective trial (open label cohort study) from India with 96 front line health care workers, 32 HCWs received **heat killed Mycobacterium w (= Mw group)** (syn. Mycobacterium Indicus pranii) as a TLR2 agonist to modulate innate immune response, while 64 HCWs were controls (JAISWAL et al.). The trial was performed during the first peak of the pandemic in New Delhi in 2020.

After 100 days of follow-up for all participants, there was one symptomatic RT-PCR confirmed COVID-19 in the Mw group, and 30 in the control group (3.1 % vs. 46.8 %) (p = 0.0001; protection efficacy: 93.33 %; CI: 53.3 – 99.1 %). Hazard Ratio for developing COVID-19 in the control group was 19.025 (p=0.0038) compared to the Mw group.

Among the 30 cases in the control group, there were 4 hospitalizations. The only infection in the Mw group was mild and the symptoms were present only for 3 days, compared to a median of 12 days (range: 3 – 36 days) in the control group.

The only side effect of the intervention was a self-limiting local injection site reaction in 14 HCWs.

Mycobacterium w is an approved immunomodulatory in India, used for example in leprosy. 0.1 ml Mw (Sepsivac, Cadila Pharmaceuticals, India) is given intradermally in each arm.

Subjects in the Mw group underwent two additional random SARS-CoV-2 specific RT-PCR evaluations 4 weeks apart what means that SARS-CoV-2 detection was biased in favor of a higher chance of detection in the Mw group compared to the control group. Moreover, it was reported that the Mw group had a greater number of exposures to COVID-19.

Long-term protective efficacy is still unknown since the study reported about the first 100 days. The authors note that there are differences in the innate immune response generated by mycobacterium w and BCG vaccine, e.g. with regard to the upregulation of natural killer cells. Thus the effects of BCG vaccine on COVID prevalence and outcomes have to be investigated separately.

Moreover, the median age in both groups was 28 years (range: 22 – 56 years), so it remains to be studied whether Mw injection has the same efficacy in older people.

There is an urgent need for larger trials with that vaccine. If the results of JAISWAL et al. can be replicated in larger trials, mycobacterium w may offer a benchmark that is hard to beat by “true” COVID vaccines. Besides the high protective effect, there is only one vaccination date (in contrast to two separates dates, a few weeks apart, in most COVID vaccines), and side effects were only local reactions at the injection sites in contrast to the mild systemic illness which has to be expected preferentially on days 1 – 3 after COVID vaccination. Follow-up was 100 days, about 67 % more than the two months demanded by the FDA for COVID vaccines.

And comparing Mw with BCG instead of COVID 19 vaccines, it has to be noted that mycobacterium w is no live vaccine, but inactivated by heat, resulting in a more favorable risk profile compared to BCG.

Beside of prophylaxis, a small case series about the treatment of severe COVID patients with mycobacterium w vaccine (0.3 ml/day Immuvac for three consecutive days) is presented by SINGH SEHGAL et al., and the treatment was successful and safe. And there are preliminary reports that Sepsivac reduced death in critically ill patients by more than 50 %:

https://science.thewire.in/health/covid-19-csir-gram-negative-sepsis-sepsivac-clinical-trials/

There is a much larger ongoing trial about Mw in India (placebo-controlled RCT) with two doses of Sepsivac, 15 days apart: NCT04353518 (=CTRI/2020/05/025277)

No interim results have been published so far.

Besides *Mycobacterium indicus pranii,* there is another interesting candidate: ***Mycobacterium obuense*** **(IMM-101)** (KLEEN et al.). The rationale behind its use in COVID-19 is identical to that for *Mycobacterium indicus pranii;* it is also a TLR2 agonist, and it elicits a type-1 biased immune response. It is already subject of a prophylactic trial in cancer patients with increased risk of COVID exposure in Canada (NCT04442048, COV-IMMUNO):

„The treatment regimen with IMM-101 will be one 1.0 mg (= 0.1 mL) dose given on Day 0, followed by a second dose of 0.5 mg (= 0.05 mL) on Day 14 (-2/+5 days), and a third Dose of 0.5 mg (= 0.05 mL) on Day 45 (+/-14 days)“

Estimated study completion is planned for March 31st, 2021, and primary completion date is December 31st, 2020.

In contrast to BCG, such inactivated mycobacterial vaccines can be even given to cancer patients and other risk groups who cannot be infected by a live-attenuated mycobacterial vaccine. Compared to BCG, this is a low-threshold prophylactic measure.

KLEEN et al. propose three different ways how mycobacterial vaccines may help in the current pandemic: *„(i) as prophylaxis, with enhanced innate memory and increased basal systemic type 1 immunity preventing viral establishment; (ii) as a treatment for patients in early stages of disease, with increased local and systemic type 1 inflammation enhancing killing of virally infected host cells; (iii) as an adjuvant for future COVID-19 vaccines.”*

Fig. 3 in KLEEN et al. depicts the four different pathways how such a vaccine may act against COVID-19.

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7498663/#B210

As mentioned above in the context of BCG vaccinations, MOHAPATRA et al. suggest that contact and infections with environmental non-tuberculous mycobacteria in countries like India may offer sustained nonspecific immunological response in a similar way like supposed for BCG vaccination that may be correlated with reduced disease burden and severity of COVID-19: *"It is possible that people of the TB endemic countries like India despite vast population and growing numbers of COVID19 infection, have acquired some protections from severity and deaths from COVID-19 in comparison to TB non-endemic countries (like Europe and USA). Although it appears that such nonspecific immunity may not able to stop COVID 19 infections, but is likely to diminish its impact on severity of COVID-19".*

In fact, COVID deaths per 1.000.000 population are much less in countries like India, Pakistan and Bangladesh (February 1st, 2021: India: 111; Bangladesh: 49; Pakistan: 53) compared to world (288.6) or many countries in Europe, North, Central and South America where the burden of TB is much smaller and COVID deaths/1 Mill. about one order of magnitude higher than in India and its neighbor countries. These data support the hypothesis of MOHAPATRA et al. that environmental mycobacteria may elicit some degree of nonspecific immune response which may reduce the risk of critical outcomes. These differences of the death rates are even more surprising since about 30 % of all people in South Asia (and 63 % in Bangladesh) are carriers of a special haplotype which is associated with severe disease and inherited from Neanderthals (ZEBERG and PÄÄBO). Now deleterious in the context of COVID-19, it must have presented some advantage to people in South Asia in the past so that it was positively selected, possibly in the context of other infectious diseases. The Neanderthal haplotype is absent from Central Africa and only very rarely found in South America, whereas COVID mortality in South America is high. The MOHAPATRA paper may offer an explanation for this conundrum.

Moreover, there is an ongoing prophylactic trial in Spain with the food supplement **Manremyc** in health care workers (also known as Nyaditum resae). Manremyc contains heat-inactivated ***Mycobacterium setense manresensis*** and mannitol, and one capsule is administered orally per day for 14 days, followed by an interval of at least 6 months without Manremyc (NCT04452773). The original intention of Manremyc is to stop progression towards active tuberculosis, or the risk of relapse of active tuberculosis. Since the bacterial strain is occurring naturally in water, including tap water, it is classified as a food supplement (EFSA Panel). Thus regulatory restrictions are much lower compared to Sepsivac *(Mycobacterium indicus pranii)* or *Mycobacterium obuense* injections.

However, each Mycobacterium species or subspecies and each BCG vaccine has to been investigated separately with regard to their effects on COVID-19. For protection against COVID-19, it is essential to elicit a type-1-biased immune response. The opposite, a type-2-biased response, can be dangerous and may result in an enhancement of disease severity. As KLEEN et al. pointed out, not all BCG vaccines favor a type-1-biased response. This variability may also apply to single mycobacterium species or subspecies, whether inactivated or not. Thus one cannot assume that any BCG vaccine or mycobacterial strain is preventive and favorable with regard to COVID-19 infection or outcome.

Prolectin-M (food supplement, galectin antagonist)

In a very small pivotal trial (5 participants who got Prolectin-M and 5 controls; hospitalized, symptoms < 72 hours) from Bangalore/India (SIGAMANI et al., NCT04512027), Prolectin-M, an orally administered polysaccharide [(1-6) -alpha-D-Mannopyranose], reduced viral replication by blocking N terminal domain (NTD) of S1 subunit, resulting in significantly lowered viral gene expression. All five participants on verum were found to show a rapid drop by day 3 in copies/µL of Nucleocapsid protein gene. The absence of glycosylation sites on the N protein as a consequence of Prolectin-M administration resulted in an early production of N-specific neutralizing antibodies. Without any side effects, this trial showed that Prolectin-M (i) lowered viral infectiousness, (ii) had an ability to block viral replication and (iii) has a „potential role as a **Post Infection Immunisation**" (SIGAMANI et al.).

Of course larger trials are needed to study the effects on clinical outcomes and also its possible role in PEP.

The SIGAMANI trial studied high doses of Prolectin-M; it *„was administered orally once every hour up to a maximum dose through the day of 40 gram or 10 tablets a day. The intention was to mimic the viral replication cycle of 8 –10 hours and also to ensure the participant is consuming the tablets during the day …"* The tablet had to be kept in the mouth for 1- 2 minutes before it dissolved and was swallowed, and after meal, subjects had to wait for 30 minutes before taking the next tablet in order to avoid any potential drop in blood glucose because tablets could block absorption of carbohydrates from the meal.

Whereas the SIGMANI trial involved symptomatic „early" patients (< 72 hours following onset of symptoms), the rationale behind Prolectin-M suggests that it is very well suited for PEP, thanks to its direct antiviral effects, reduction of infectiousness and the early induction of antibody production, in combination with its simple oral administration and very good tolerability.

Neem capsules (Azadirachta indica)

In a placebo-controlled double-blind RCT fom India, the effect of NEEM prophylaxis was studies in 190 HCWs in a hospital or relatives of patients with COVID-19 infection (NESARI et al.). 95 recieved neem-leaf extract and 95 received placebo in capsules, twice a day for 28 days. Outcome was COVID infection (PCR-positivity) between baseline and day 56. Mean age was 37 years. Age of the participants was 18 – 60 years (older participants were not allowed). Participants were not allowed to take other prophylactic medication, including HCQ and antivirals.

13 participants tested positive; all were asymptomatic. But only 154 participants completed the study per-protocol; among them, 11 tested positive (3 in the Neem group and 8 in the control group. The relativ risk of SARS-CoV-2 infection in the intervention group was 0.45.

Adverse effects were minimal, there was no influence on biomarkers and quality of life.

However, it must be noted thet long-term intake of Neem is regarded as critical. While it seems to be safe for short-term use, long-term use may harm kidneys and liver; Neem may cause miscarriages, infertility and low blood sugar. Neem oil is toxic for young children und may lead to their death. In the NESARI trial, Neem was only administrated in low dose (100 mg/day) for 28 days, giving a total of 2.8 g.

Withania somnifera (Ashwagandha) (interim results)

CHOPRA et al. compared Withania somnifera (WS), an immunomodulator, directly to HCQ in a 16 week randomized prospective open label, parallel effiacacy, two arm multi-centre trial, involvong altogether 400 participants. Endpoint was COVID-19 confirmed by PCR during the 16 week study period. An interim analysis was performed based on 160 participants after 8 weeks. Mean age: about 37 years.

Participants received either 2 tablets of 250 mg standardized extract of WS twice daily or 400 mg HCQ weekly. The trial was initiated in September 2020, i.e. at a time when vaccinations were not available.

COVID-19 was found in 3.7% of participants in the HCQ group (3 infections) and 1.3% in the WS group (one infection). (Risk reduction: 65 %). 40 participants in the HCQ group and 26 participants in the WS group reported about mild adverse effects.

For more details an more robust data, one has to wait for the final report of the study that is expected by end of August 2021.

Cannabidiol

Cannabidiol *(not psychoactive! – should not be confused with THC)* was found to block SARS-CoV-2 replication in lung epithelial cells *in vitro*. It inhibits viral gene expression and reverses many effects of SARS-CoV-2 on host gene transcription. It induces interferon expression and up-regulates the antiviral signaling pathway of interferon, thereby promoting the host's innate immune response (NGUYEN et al.). This way of action suggests that cannabidiol and its active metabolite, 7-OH-cannabidiol, may work as preventive agent and also as treatment at early stages of infection (NGUYEN et al.). Since cannabidiol inhibits the life cycle of the virus after its entry into the cells, it is likely effective against variants with mutants in the spike protein, and it is also amenable to inhalation or nasal delivery.

An analysis of 93000 patients that were tested for SARS-CoV-2 at the University of Chicago Medical Center found an overall positive rate of 10 %, but only 1.2 % in 85 patients who had had cannabidiol formulations in their medical history compared to 7.1 % in 113 patients who took other cannabinoids ($p = 0.08$). This difference was in accordance with *in vitro* studies that showed

that only cannabidiol and its active metabolite are effective against SARS-CoV-2, but not other cannabinoids. To reduce confounding, 82 patients who were prescribed oral cannabidiol (Epidiolex) were matched to patients with similar characteristics who didn't take that drug. 1.2 % of the Epidiolex group had tested positive, compared to 12.2 % of the controls (p = 0.009).

Nevertheless, the authors warn that freely available formulations of hemp oil may contain too low concentrations of cannabidiol (~ 0.3 % according to their own studies), or they may also contain other cannabinoids that counter the antiviral effects of cannabidiol. "*This essentially eliminates the feasibility of marijuana serving as an effective source of antiviral CBD ….*" (NGUYEN et al.).

Supplemental: **No protective effect of HIV PREP?**

Early hopes in favor of chemoprophylactic effects of HIV drugs, based on *in vitro* data (e.g. JOCKUSCH et al.), very good experience with lopinavir/ritonavir for PEP in a hospital with highly exposed HCWs to MERS without adequate protection (PARK SY et al.) and a report from Thailand (ZHU F et al.), could not get confirmed since it is evident now that HIV-infected people who take these drugs for treatment of their HIV infection, can also get infected and seriously ill with COVID-19. This applies to reverse transcriptase inhibitors like Tenofovir/Emtricitabine (GUO W et al., HÄRTER et al., KARMEN-TUOHY et al., VIZCARRA et al.), but also to protease inhibitors like Lopinavir/Ritonavir (HÄRTER et al., KARMEN-TUOHY et al.) or Darunavir (VIZCARRA et al.).

However, in an animal experiment (ferrets) with early therapy (starting 1 day after virus inoculation), Tenofovir/Emtricitabine was evidently superior to lopinavir/ritonavir (L/R), which was only a little superior to HCQ, and both L/R and HCQ were of comparatively small effect compared to placebo, whereas Tenofovir/Emtricitabine was most successful (PARK SJ et al.).

Finally, contrary to former reports from other countries, DEL AMO et al. showed in a large cohort study from Spain (encompassing about 75% of all HIV-infected people in Spain who receive ART), that tenofovir as disoproxil fumarate (TDF) combined with emtricitabine, but not (!) tenofovir alafenamide (TAF), was superior to reduce COVID-related risks. Whereas the standardized risks of COVID infection, hospitalization, ICU care and death among all HIV-infected people under ART (standardized to age and sex of the general population in Spain between 20 and 79 years) were 30, 17.8, 2.5 and 3.7 per 10.000 until April 15th 2020, the risks for people taking TDF/Emtricitabine were 16.9, 10.5, 0 and 0 per 10.000. Because only symptomatic people got COVID tests during that time interval in Spain, *reported* COVID incidence is essentially identical to incidence of symptomatic disease. The results are based on 12.395 HIV-infected people who took TDF/Emtricitabine (21 infections, 13 hospitalizations, no ICU, no death) (among 77.590 who took any form of ART). In contrast, the combination TAF/Emtricitabine (n = 25.570) resulted in 100 infections, 52 hospitalizations, 7 ICU cases and 10 deaths (DEL AMO et al.). With this large study, the question of HIV treatments for PREP is opened again; however, it is evident that TDF/Emtricitabine offers no full protection, and much larger datasets are necessary to find out whether this combination is really able to avoid ICU admission and death.

However, in the German study (HÄRTER et al.), there were 2 seriously and 6 critically ill cases (including 3 deaths) among 33 HIV-infected people under HAART who acquired COVID-19. One of the serious cases and two among the 6 critical cases were treated with a regimen including TDF/Emtricitabine (one of them died; 59 years, hypertonia, COPD, diabetes mellitus type 2). Thus, TDF offers no full protection against critical or fatal disease.

In South Africa, HIV infection was associated with an increased risk of death compared to all COVID infected people (aHR 1.70; $p < 0.001$) and among all hospitalized COVID-19 cases (aHR 1.45; $p = 0.002$), and death was associated with CD4 cell number < 200. However, compared to HIV infected people who took abacavir, zidovudine or efavirenz (aHR defined as 1.00), those who took tenofovir had an adjusted HR of death of 0.42 (CI: 0.22 – 0.78) ($p = 0.006$), those who took lopinavir of 0.89 (CI: 0.36 – 2.16, $p = 0.79$). Azanavir (aHR 0.36) and doletugravir (aHR 0.59) also showed favorable results comparable to tenofovir, but far from statistical significance (DAVIES MA et al.). The results may be influenced and biased because tenofovir is the first-line therapy in South Africa; however, since the results rely on prescriptions and not on actual intake of ART, the protective effect may be higher in those who take their ART exactly as prescribed.

Since this study was about mortality, there is no information about the influence of different ART regimes on the risk of COVID diagnosis in South Africa. It is stated that 16 % of the people in that area are HIV positive. The standardized mortality ratio (SMR) for COVID-19 in HIV-infected people was 2.39 (CI: 1.96 – 2.86) compared to people without HIV. So even if the HR of dying is only 0.42 in HIV infected people taking tenofovir (compared to HIV infected people taking other drugs), their COVID incidence would then be similar to the incidence in people without HIV, estimated on the base of SMR or aHR for death. As a consequence, the results of DAVIES et al. are incompatible with the hypothesis of a much lower COVID incidence in people taking tenofovir compared to HIV-uninfected people.

What about HIV PREP, a study from France (Rhone department) found no protective effect (CHARRE C et al.). HIV infected people and PREP users had attack rates similar like the general population. The proportion of positive COVID PCR tests was 15.6 % among HIV infected, 14.8 % among PREP users and 19.1 % among other patients. COVID tests were usually performed only for symptomatic people, so all results are supposed to apply to symptomatic disease (serious enough to get a COVID test). Among 4755 HIV-infected persons living and registered in that area, 77 got a COVID test and 12 were found to be COVID positive. Among 1867 PREP users, 27 were tested and 4 were found to be positive. The crude attack rates were calculated as 0.24 % for the general population, 0.31 % for HIV infected people, and 0.38 % for PREP users. Correcting for differences in the positivity rates in different laboratories, the attack rates might be as high as 0.38 % in HIV infected people and 0.42 % for PREP users, but the authors state that this is a sort of worst case scenario and may overestimate the true attack rates.

However, this study has serious limitations. The authors didn't report about the severity of the disease in HIV infected or PREP user cases. As DEL AMO et al. showed, there may still be a chance that anti-HIV drugs, especially TDF, might be able to reduce the severity of the disease. However, with 12 cases among HIV-infected people and 4 among PREP users, any results would lack statistical robustness. Furthermore, the authors stated that about 10 of the 12 COVID cases among HIV infected people and all of the 4 COVID cases among the PREP users took regimens which included tenofovir disoproxyl (TDF) or tenofovir alafenamide

(TAF). According to DEL AMO et al., only TDF seems to have some protective effect with regard to COVID-19, not TAF. But the authors didn't analyse the HIV medication in their 16 COVID-infected HIV or PREP cases. One might suspect that all PREP users take TDF, but this interpretation is not clear from the original text. A third limitation is that one cannot exclude that PREP users had a more risky lifestyle even under lockdown conditions, so their higher attack rate (compared to the general population and also to HIV infected people) may result from higher exposure risks.

Taken together, the French data are not indicative of any protective effect of tenofovir (including TDF), though many questions remain open.

In a small retrospective study with 108 HIV PREP users (Tenofovir/Emtricitabine) from Brazil, among them predominantly young (mean: 33.7 years), highly educated white gay men, *regular intake* of oral HIV PREP was associated with lower self-reporting of COVID-related symptoms (OR 0.26; CI: 0.07 – 0.96). The association remained significant following different degrees of adjustment (aORs between 0.24 – 0.26). Social distancing did not moderate PrEP protective effect. However, there were no PCR tests or serology testing performed in this study. The study was based only on phone call interviews, WhatsApp or emails, and participants were asked about symptoms during the last month (rhinorrhea, cough, asthenia, headache, sore throat, fever, decreased taste, dyspnea, loss of smell, diarrhea). The control group was quite small, since 82 of the 108 participants of the PREP cohort continued regular PREP (since at least 6 months) during the study period. Nothing is said about the remaining 26 persons (whether they stopped PREP completely, or took it on an irregular base?). Thus this study has serious limitations, but gives a hint that it may be interesting to conduct well-designed studies about that sort of HIV PREP.

But in a second study from Spain (AYERDI et al.), there were no hints that MSM or transgender women who use PREP with tenofovir have a lower risk of COVID-19 infection. In fact, seroprevalence was even higher in MSM and TGW who used PREP (TDF/FTC: 14.7 %; TAF/FTC: 16.5 %) compared to those who didn't use PREP (9.2 %), but this seems to be due to differences in risk exposure (higher risk exposure in PREP users). 78.3 % of seropositive non-users had had symptomatic disease, compared to 53.3 % of TDF/FTC users and 73.3 % of TAF/FTC users. Duration of symptoms was 11.5 days in non-users (n = 23) vs. 7.0 days in TDF/FTC users (n = 60) and 13 days in TAF/FTCs users (n = 15). There were 5 hospitalizations among these 98 seropositive patients (non-user: 1/23; TDF/FTC: 3/60; TAF/FTC: 1/60); among them was 1 ICU admission (TAF/FTC). Though the differences were not statistically significant, they point to a reduced risk of symptomatic disease and faster recovery from symptoms in the TDF/FTC group.

The results are in accordance with suggestions mentioned above that only TDF but not TAF has some effect against COVID-19. But even if TDF has some favorable effect on COVID outcomes, this effect doesn't seem to be so strong that it can be recommended for COVID-19 PREP in people who have no primary (HIV-based) indication for a TDF/FTC-based PREP. There is an ongoing PREP trial with HCWs that investigates TDF/FTC alone vs. HCQ alone vs. TDF/FTC+HCQ vs. placebo (NCT04334928). Moreover, there is an ongoing RCT with lopinavir/ritonavir for pragmatic ring-prophylaxis in Switzerland (SMIT et al.).

In a therapeutic setting, Tenofovir DF (TDF) proved to be surprisingly successful in a study from Peru (CORNEJO-GIRALDO et al.). This observational study compared TDF to HCQ in

hospitalized patients with evidence of pulmonary compromise (the vast majority requiring supplemental oxygen).

Comparators: HCQ 400 mg 12 hourly at day 1, then 200 mg every 8 – 12 hours for 5 – 10 days (n = 36 patients); or TDF 300 mg per day for 7 – 10 days (n = 68 patients).

Unadjusted outcomes (HCQ vs. TDF): length of hospital stay 16.6 vs. 12.2 days (p = 0.0102), ICU admission or mechanical ventilation: 61.1 % vs. 11.8 % (p = 0.000); mortality: 50.0 % vs. 8.8 % (p = 0.000). However, there were differences in baseline characteristics at admission, and the HCQ group had more risks and worse prognosis markers on average.

After adjusting for these confounders and multiple regression, TDF decreased hospital stay by 6.10 days (p = 0.042); OR for ICU/mechanical ventilation was 0.15 (CI: 0.03 – 0.76, p = 0.022), and HR for mortality was 0.16 (CI: 0.0.3 – 0.96; p = 0.041). In another model ("estimation model of the treatment effects by regression adjustment"), the decreased stay in hospital was calculated to – 6.38 days, the decreased need for ICU/MV at 41.74 % and decreased mortality at -35.22 % (p = 0.001).

Supplemental: **various common nutritional supplements**

Based on a questionnaire in the context of a COVID Symptom Study App, a large study analysed the association between supplement intake (since the beginning of the epidemic) and occurrence of a positive PCR or antibody test result **(LOUCA et al.).**

Based on data from 327.720 participants of the App from UK (from altogether 4.544.666 users) who reported having been tested positive for SARS-CoV-2 by either PCR or serology, the following risk reductions (adjusted for potential confounders: age, sex, BMI, sign-up health status and multiple testing) were calculated (men and women together):

probiotics: 14 % (CI: 8 – 19 %)
omega-3 fatty acids: 12 % (8 – 16 %)
multivitamins: 13 % (10 – 16 %)
vitamin D: 9 % (6 – 12 %)
vitamin C, zinc, garlic: no significant effects

After further sensitivity analyzes, the results were confirmed, but the association with probiotics became weaker.

The questions for supplement use in the App considered use of probiotics, garlic, omega-3 fatty acids ("fish oils"), multivitamins, vitamin D, vitamin C or zinc and was recorded as "yes" or "no". 66.8 % of participants were female, more than 50 % overweight. *"175,652 self-reported using supplements regularly since the beginning of the pandemic, while 197,068 self-reported they were not."* (LOUCA et al.).

The protective associations for probiotics, omega-3 fatty acids, multivitamins and vitamin D were observed in females across all ages and BMI groups, but were not seen in men (except

for slight effects of multivitamins in men < 40 years and for omega-3 fatty acids in men aged 40 – 60 years).

The same overall patterns were observed in US and Swedish cohorts from the App (45757 US and 27373 Swedish users who reported about their SARS-CoV-2 test results; 67.8 % and 68.6 % female).

The overall results "*were further confirmed in a sub-analysis of 993,365 regular app users who were not tested for SARS-CoV-2 with cases (n= 126,556) defined as those with new onset anosmia (the strongest COVID-19 predictor).*" This substudy *"found a small but significant protective effect of around 5% for omega-3 fatty acids, multivitamins, vitamin D and to a lesser extent for probiotics overall and by gender"* (LOUCA et al.).

In the UK cohort, men > 60 years from UK who used zinc supplements (1.4; CI: 1.16-1.69) or vitamin C supplements (1.22; CI: 1.05 – 1.41) had a higher risk of testing positive for SARS-CoV-2.

In US men, use of probiotics or vitamin D was associated with decreased risk, and in Swedish men, probiotics, omega-3 fatty acids, multivitamins or vitamin D were associated with decreased risk.

The reasons for the sex differences, which were most pronounced in the UK cohort and could not be replicated in the other cohorts, are unknown. They may be due to residual confounding because of behavioral differences between users and non-users of supplements (e.g., lesser use of masks was documented for men compared to women; thus women who buy supplements may be more health conscious than men who take supplements), but it may also be that the immune systems responds differently to supplements. Women possess a more resilient immune system with higher numbers of circulating B cells and a slower age related decline in circulating T- and B-cells, thus supplements may support the immune system of females better than in males (LOUCA et al.).

However, the sex differences could not be replicated in the largest substudy, the untested anosmia study group.

Altogether (men + women), vitamin D was associated with 9 % risk reduction in the UK cohort, 24 % in the US cohort and 19 % in the Swedish cohort.

Multivitamin supplements were associated with 13 % risk reduction in the UK cohort, 12 % in the US cohort and 22 % in the Swedish cohort, omega-3 fatty acids with reductions of 12 %, 21 % and 16 %, probiotics with reductions of 14 %, 18 % and 37 %.

LOUCA et al. concluded: "*our data find a correlation between use of multivitamins, omega-3 fatty acids, vitamin D and probiotics and slightly lower risk of SARS-CoV-2 infection in women in the UK, US and SE, but no effect of zinc, vitamin C or garlic. The larger anosmia data confirmed a more modest effect."*

However, there remain lots of questions following a more critical look upon the presented data.

What about garlic, the null results are not surprising. Whereas allicin was found to be highly effective against SARS-CoV-2 replication in cell cultures including Calu-3 lung cells, it is very instable, quickly decomposes during cooking and is degraded in the acidic stomach. As MÖSBAUER et al. pointed out, *"biocompatible and therapeutically relevant concentrations of allicin for the treatment of respiratory tract infections might not be possible with the consumption of garlic-containing food";* they propose the development of formulations to administrate biocompatible doses of garlic organosulfur compounds *"via the pulmonary route to efficiently reach the virus, without damaging host"* (MÖSBAUER et al.).

Unfortunately, LOUCA et al. presented no results for zinc, vitamin C and garlic from the US, Swedish and anosmia study cohort. Since it is surprising that both zinc and vitamin C had no effect (or even deleterious effects in older men), it would have been interesting to see whether these results could have been replicated in the other three cohorts, but the other three cohorts were only analyzed with regard to outcomes in association with the four supplements which were found to be favorable in the UK cohort. Nevertheless, since the App was the same, data for zinc and vitamin C must have been available also for the other three cohorts.

This is a serious limitation because it would be important to know whether vitamin C and zinc supplementation are truly ineffective or even harmful (for older men).

Nevertheless, the overall and subgroup results for zinc, vitamin C and garlic in the UK cohort are in obvious contrast to the favorable results for the four other supplements in the same cohort.

Second, with regard to sex differences, significant protective effects were found for men for omega-3 in the Swedish cohort (OR ca. 0.74), for probiotics in the US cohort (OR ca. 0.66) and Swedish cohort (OR ca. 0.40), for multivitamins in the Swedish cohort (OR ca. 0.70) and for vitamin D in the US cohort (OR ca. 0.74) and Swedish cohort (OR ca. 0.74) (all sign.). Moreover, for all four supplements small, but significant risk reductions were observed in men of the large anosmia cohort (but all ORs > 0.90).

Taking the 16 items (4 supplements x 4 independent substudies) into account, there were 15 significant risk reductions (upper CI < 1.0) for females, but also 10 for men. Looking at the point estimates (independent from the upper bound of the CI), the point estimate was lower in women than in men for six items, but lower in men than in women in 7 items, and equal in 3 items (the latter 3 > OR 0.9 and from the anosmia study cohort).

Thus it remains unclear whether men really don't profit from the four supplements in contrast to women as suggested by the UK cohort.

One big problem of any study of this type is residual confounding because of behavioral differences between the subgroups, including women vs. men. Those who take supplements during the epidemic may be more afraid of COVID-19 and thus more careful to avoid contacts and prevent infection. Moreover, the daily dose may play a role. Men may need higher doses than women to achieve the same degree of protection. For many vitamins and minerals, daily intake recommendations (by food and/or supplements) are higher in men than in women.

As a consequence, this study is not very helpful but one can conclude that one can do no harm to take probiotics, vitamin D, multivitamins or omega-3 fatty acids. Unfortunately, it remains unclear whether zinc and vitamin C intake may be risky for older men?

Moreover, this study was only about PCR-confirmed infection or seropositivity, thus it is not clear whether these supplements had any effect on the severity of the disease (e.g. symptomatic vs. asymptomatic). In real life, it is more plausible that such supplements may attenuate symptoms or the severity of the disease, than that they are able to prevent infection at all. This may result in seropositivity in a person who was not so ill that he/she got a COVID PCR test. Thus it would have been more informative if the study had also looked at symptomatic disease or the severity of the disease, especially in the large UK cohort because of better statistical power there. However, the anosmia study group represents a symptom-based setting, and in that setting, the effects of the four supplements were very small, but still significant, and similar in men and women, indicating against sex differences. Unfortunately, this large cohort was not analysed with regard to the important question whether vitamin C and/or zinc supplementation are needless or even harmful.

In a population-based longitudinal study from UK (COVIDENCE) from 1st May 2020 to 5th February 2021 with test-confirmed COVID as outcome (by PCR or antigen testing), intake of vitamin D supplements was associated with a reduced risk of a positive test in crude (OR 0.80; 0.65 – 0.98) and minimally adjusted analyses (aOR 0.80, 0.65 – 0.99), but not in the fully adjusted model (aOR 0.93; 0.75 – 1.16) **(HOLT et al.).** Nevertheless, the 7 % reduction is well in accordance with the confidence interval in the study from LOUCA et al. (6 – 12 %).

In COVIDENCE, vitamin C, zinc, fish oil/omega-3 showed no effect in the minimally adjusted model (OR 1.03, 0.93 and 0.98), and probiotics showed a favorable trend with an insignificant OR of 0.69 (0.44 – 1.10), but there were no significant results in the fully adjusted model for any supplement, and thus the results after full adjustment were not shown. However, in contrast to vitamin D, only few participants took the other supplements and the study seems to be underpowered to study their effects.

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 only an insignificant protective effect of vitamin C + zinc prophylaxis (80 mg zinc* as zinc oxide and 500 mg vitamin C per day for 42 days) **(SEET et al.).** SARS-CoV-2 infection was confirmed by PCR (at any time) or antibody test on day 42.

*not given whether 80 mg as zinc oxide or zinc oxide with 80 mg elemental zinc ; however, zinc oxide contains 80 % elemental zinc so that even 80 mg zinc oxide would provide 64 mg of elemental zinc

Controls (n = 619) got 500 mg vitamin C per day (for 42 days) (Zinc + vitamin C: n = 634). Confirmed SARS-CoV-2 by PCR or serology: 47.3 % (Zinc + vitamin C) vs. 70.0 % (Vit. C). Relative risk ratio 0.67 (CI: 0.38 – 1.08), absolute risk reduction in case of the use of zinc in addition to vitamin C was 23 % (CI: -5 to + 41 %).

Point estimates for adjusted ORs (depending on model, 6 different models were taken into account: between 0.42 and 0.45, but all of them n.s.).

Symptomatic COVID-19: 5.2 % (Zinc + Vit.C) vs. 10.3 % (Vit. C) (- 49.5 %). Symptomatic disease among those diagnosed with SARS-CoV-2: 11.0 % vs. 15.0 % (- 26.7 %). No hospitalization, no death in any study arm (young age!).

The favorable results from the Singapore RCT are in contrast to the disappointing results mentioned above (e.g. LOUCA et al.). Maybe the high dose of zinc (at least 64 mg/day of elemental zinc), the combination with vitamin C, and/or the young age of the men in that study may account for this difference. Adjusted ORs between 0.42 and 0.45 for infection with SARS-CoV-2 and a relative risk reduction of 49.5 % for symptomatic disease are impressive results for such a simple and universally available intervention. In LOUCA et al., the effects of both vitamin C and zinc were neutral in young (< 40) and middle-aged (40 – 60 years) men, but unfavorable in older men (> 60 years). However, one has to consider the possibility of residual confounding for that group because also probiotics and omega-3 fatty acids were found to be unfavorable in older men in the LOUCA study, in contrast to their neutral effects in other groups of men and favorable effects in women.

Vitamin C was found to inhibit 3CLpro in vitro at mmol/concentrations (MALLA et al.); however, these comparatively high concentrations may indicate that common oral doses of Vitamin C (even a few grams per day) are much too low.

A second study (MARGOLIN L et al.), this time from US and only of retrospective design, analysed the effect of a complex OTC formulation, consisting of:

● zinc 25 mg (not given what sort of zinc formulation)
● quina (10 drops, quina-bark extract, maybe started with 1 drop and then increasing up to 8-16 drops daily) - a zinc ionophore
● quercetin 400 mg - another zinc ionophore
● vitamin C 1000 mg
● vitamin D 1000 IU (25 microgram)
● Vitamin E 400 mg
● L-lysine 500 mg (faciliating zinc absorption in the gut)

These were the prophylactic doses; in case of flu-like illness, the same dose should be doubled (either taken separately or together). However, from day 2 of symptoms, zinc should be increased for 2 – 3 days up to 200 mg/day, if tolerated. Depending on the individual situation, azithromycin or doxycline may have been given to prevent secondary infections.

However, study participants were also recommended to add some additional components like copper or *Bupleurum falcatum.*

In the multiply exposed population (n = 113), about half of the participants were compliant (n = 53) and the other half was non-compliant (n = 60). These represented the two groups that were compared to one another (however, “non-compliance” didn’t mean that they took absolutely nothing of the recommended supplements; some took vitamin C or vitamin D or stopped the complex regimen but continued with vitamin C or D).

Within 20 weeks of observation, 4 % (2 / 53) of the compliant group reported flu-like symptoms, but there was no positive COVID test. In contrast, 20 % of the non-compliant group presented flu-like symptoms (12 / 60) and 15 % (9 / 60) had a positive SARS-CoV2-test.

In subgroup analyses of non-compliant participants, it was found that supplementation with vitamin C and D alone without zinc and zinc ionophores had no effect. Zinc and zinc ionophores seem to play a central role in this concept, whereas the importance of the other ingredients is less clear.

But it must be noted that this study was done between March and July 2020 in a population that was not yet exposed to VoCs. It is unclear whether this simple concept will work as well in the presence of aggressive VoCs like Delta.

Supplemental: **Influenza or MMR vaccination?**

Whereas **seasonal influenza immunization** was already strongly recommended at least for elderly and people with comorbidities in order to avoid overload of the health care systems, hospitals and ICUs, both *in vitro* results and an animal experiment in a mouse model showed that influenza immunization may have a direct preventive effect with regard to COVID-19 outcomes (BAI L et al.). Contrary to other viruses like human rhinovirus, human parainfluenza virus, respiratory syncytial virus or enterovirus 71, influenza A virus (IAV) infection induced elevated expression of ACE2, resulting in enhancement of SARS-CoV-2 infectivity. In the mouse model (humanACE2-transgenic mice), pre-infection with IAV resulted in increased SARS-CoV-2 viral load and more lung damage compared to mice that were not infected by IAV at the time of SARS-CoV-2 inoculation. Coinfection of IAV and SARS-CoV-2 aggravates SARS-CoV-2 infection and severity.

Moreover, several ecological studies suggested some degree of a protective effect of influenza immunization on COVID infection or outcomes (described in detail in the "discussion" section of DEBISARUN et al.). However, the evidence level of ecological studies has to be regarded as comparatively low. Attenuated live vaccines (like BCG, measles) instead of inactivated vaccines (like the common influenza vaccines) are supposed to boost trained immunity. But **DEBISARUN et al.** demonstrated *in vitro* that the inactivated quadrivalent influenza vaccine used in the Netherlands during the 2019-2020 influenza season actually induced trained immunity. Among other effects, an improvement of the cytokine responses following stimulation of human immune cells with SARS-CoV-2 was noted. Dutch hospital employees who had received the vaccine had a lower risk of COVID-19 infection (RR 0.61; CI: 0.4585 – 0.8195, p = 0.001).

In a large study from Russia with 541.377 hospitalized COVID-19 patients (76.5 % with laboratory-verified COVID-19 and 23.5 % with clinical verification), influenza vaccination within the last six months reduced the risk of ICU transfer (OR 0.76, p = 0.031), mechanical ventilation requirement (OR 0.74, p = 0.061) and death (HR 0.775, p = 0.014) **(DEMKINA et al.).**

In a cohort study from the UK Biobank **(XIANG et al.),** influenza vaccination (- 1 year) was associated with reduced risk of infection compared to population controls (OR 0.73; CI: 0.65 – 0.83) and compared to test-negative individuals (OR 0.60; CI: 0.53 – 0.68). The same applied to severe cases (OR 0.74 or 0.61, depending on the model; all sign.) and mortality (OR 0.28, CI: 0.13-0.63; and OR 0.23, CI: 0.11-0.52 in both models; all ORs for vaccination within the last year). Influenza vaccination seems to protect stronger from mortality than it protects from infection.

Effect sizes became smaller with longer time windows. Significant protective signals were also found for pneumococcal vaccines (OR 0.50, CI: 0.31-0.82) in one model in the one-year window,(and to a lesser extent in longer time windows up to 10 years, still significant), tetanus vaccines (5 years, 10 years) and, to a lesser extent, typhoid vaccines (5 years, 10 years).

A retrospective cohort study of 6921 COVID-19 patients registered to a General Practioner practice in South West England during the first wave of the pandemic found an adjusted OR for a combined endpoint of hospitalization or all-cause mortality of 0.85 (CI: 0.75 – 0.97, p = 0.02) and an aOR of 0.76 (CI: 0.64 – 0.90) for all-cause mortality **(WILCOX et al.).** Influenza vaccinations since January 1st, 2019 and COVID-19 diagnosis were taken into account.

In a study from the Michigan Health System, the adjusted odds ratio for testing positive for COVID-19 was 0.76 (CI: 0.68 – 0.86, p < 0.001; multivariable logistic regression) among those who had got influenza vaccination between August 1st, 2019 until July 15th, 2020, compared to those without influenza vaccination in that time interval (based on 27201 patients who were tested for COVID-19). The tests were performed between February 27th and July 15th, 2020. In case of influenza vaccination, aOR for hospitalization was 0.58 (CI: 0.46 – 0.73), aOR for mechanical ventilation was 0.45 (CI: 0.27 – 0.78). Hospital stay was shorter (RR 0.76; CI: 0.65 – 0.89). But there were no significant differences in mortality (0.84; CI: 0.51 – 1.36) and marginally significant differences in the need for ICU (OR 0.64; CI: 0,41 – 1.00, p = 0.5). Median time between influenza vaccination and COVID-19 testing was 225 days **(CONLON et** al.).

In a smaller retrospective study from Florida with 2005 adult patients who tested positive for COVID-19 **(WANG MJ et al.),** the adjusted odds for hospitalization was 2.44 (CI: 1.68 – 3.51) and the adjusted odds for ICU admission was 3.29 (CI. 1.18 – 13.77) when the patients had not received the influenza vaccination within the last year. Unadjusted OR for hospitalization were 2.84 (CI: 2.03 – 4.07), uOR for ICU admission was 5.64 (CI: 2.11- 23.01). Only 10.7 % of the 2005 COVID patients had recent (up-to-date) influenza vaccination.

In their ecological study from Italy, **MARIN-HERNANDEZ et al.** found a negative correlation between (i) the percentage of vaccinated adults greater than 65 years old and (ii) the percentage of COVID-19 death from each region in Italy up o May 2nd, 2020. The correlation was found to be moderate to strong (r = - 0.5874; n = 21 regions; p = 0.0051). The percentage of COVID-19 deaths for each region decreased by 0.345 for each additional percent of adults > 65 years vaccinated against influenza.

A study from Spain (South Catalonia) found no protective effect of influenza vaccination during prior autumn **(VILA-CORCOLES et al.).** In a cohort of 77669 community-dwelling individuals, HR of COVID 19 death was 1.12 (CI: 0.66 – 1.89) in those who got the vaccine

after adjustment for age and sex. Multivariable adjustment reduced the HR to 0.90 (0.49 – 1.64). In nursing home residents (n = 1414), influenza vaccination was even associated with an increased risk of COVID death (HR 2.16; 1.02 – 4.56 adjusted for age and sex; HR 2.34; 1.08 – 5.07 in the multivariable model).

A study from Brazil reportet about 472,688 severe cases and 177,640 death, i.e. a lethality of 37.58 % in severe case **(SARDINHA et al.).** The study was a cross-sectional study about all severe cases and deaths in Brazil between March 1st and December 12th, 2020. Among 62,711 severe cases with trivalent inactivated influenza vaccination (received during the influenza vaccination campaign 2020), mortality was 33.2 %. Among 409,897 severe cases without influenza vaccination, mortality was 38.3 % (risk difference: 5.1 %; relative risk reduction: 13.3 %. Unvaccinated severe cases had ORs of 1.45 for mechanicl ventilation, 1.33 for ICU admission and 1.25 for death (all sign., $p < 0.001$). The interaction with BCG vaccination could not be analysed in that study because the underlying database didn't encompass BCG status. Moreover, it has to be noted that the study was about a time interval before the arrival and dominance of VoCs in Brazil.

An earlier retrospective observational study from Brazil among hospitalized patients with COVID-19 between January 1st and June 23rd, 2020, reported an adjusted OR of 0.93 (CI: 0.87 – 0.98) for ICU, an aOR of 0.83 (CI: 0.77 – 0.88) for invasive ventilation and an aOR of 0.84 (CI: 0.78 – 0.90) for death in hospitalized patients who had recent inactivated trivalent influenza vaccination compared to those who had not **(FINK et al.).** The study was based on 53752 clinically confirmed hospitalized COVID-19 cases (median age 56 years). In the whole patient population, 29 % needed ICU care, 16 % got invasive ventilation, and 46.5 % died. Interestingly, FINK et al. noted that the protective effects were larger when the vaccine was administered after onset of COVID symptoms and also among younger patients (10 – 19 years), whereas the protective effect against mortality became negligible at ages > 80 years.

The protective effect of influenza vaccination against death from COVID-19 was 17 % if given prior to onset of symptoms, but 36 % if given after the onset (n.s.). 5.6 % of all vaccinated patients had gotten the vaccine after onset of symptoms. Moreover, both young and older patients (</> 60 years) profited to about the same extent from the vaccine If given after the onset of symptoms, whereas if given before, the risk reduction was 31 % in people under the age of 60, but only 12 % in people of 60 years or beyond. Influenza vaccinations before the 2020 vaccination campaign had no protective effect.

A study presented at the European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) 2021 (SINGH D et al.) found a reduced risk of stroke, sepsis and deep vein thrombosis in patients with COVID-19 who had been vaccinated against flu; including a reduced risk of visiting an emergency department or to be admitted to ICU. Based on TriNetX, two groups of 37377 patients each were matched for factors that affect the risk of severe COVID-19; one group had received flu vaccination between two weeks and six months ago, the other group was unvaccinated. The patient population was international and included US, UK, Germany, Italy, Israel and Singapore.

Unvaccinated patients were up to 20 % more likely to be admitted to ICU and had a higher risk of sepsis (up to + 45 %), stroke (up to 58 %) and deep vein thrombosis (up to + 40 %). Surprisingly, the risk of death was not reduced [1].

There are also suggestions that **MMR vaccination** may have some effects on COVID 19 incidence and severity. Whereas it is supposed that this may be due to the measles component of the vaccine, GOLD et al. showed an inverse correlation ($r = -0.71$, $p < 0.001$) between mumps antibody titres (from MMR vaccination = MMR II group) in recovered patients and the severity of their former COVID-19 disease.* This association did not apply to patients with mumps titres due to former mumps disease. No associations were found with measles und rubella antibody titres, both following vaccination or natural infection.

*"within the MMR II group, mumps titers of 134 to 300 arbitrary units (AU)/ml (*n* = 8) were found only in those who were functionally immune or asymptomatic; all with mild symptoms had mumps titers below 134 AU/ml (*n* = 17); all with moderate symptoms had mumps titers below 75 AU/ml (*n* = 11); all who had been hospitalized and had required oxygen had mumps titers below 32 AU/ml (*n* = 5)." (GOLD et al.).

However, a possible protective effect of vaccines like MMR is not directly antibody-mediated, since the antibodies generated by these vaccines (and many others: BCG, pneumococcal, Rotavirus, Diphtheria, Tetanus, Pertussis, Hepatitis B, Haemophilus influenzae, Hepatitis B, meningococcal vaccines) are not cross-reactive and unable to neutralize SARS-CoV-2 (KANDEIL A et al.). Thus the mumps antibody titres in the study from GOLD et al. have to be regarded only as a proxy for other pathways and capabilities of the immune system (like trained immunity) which were elicited by MMR vaccination, but not by natural disease. ANBARASU et al. suggest induction of interferons and activation of killer cells as innate immune responses following MMR vaccination.

ASHFORD et al. asume that the low infection rates and mild disease in children > 1 years in US may be due to childhood vaccinations, especially MMR, since the first MMR dose is recommended at 12-15 months by the CDC. However, data from Germany (where the first MMR dose is recommended in a similar age and vaccination rates are very high) put this into question, as far as the infection rates are concerned:

Incidence per 100.000, Germany, RKI, age groups: 0 – 6 years, November 25th, 2020 (survstat@rki 2.0):

Age: 0 years	576,00
1 year	443,13
2 years	466,62
3 years	500,86
4 years	534,69
5 years	597,88
6 years	591,28

Incidence per 100.000, Germany, RKI, age groups: 0 – 6 years, April 1st, 2021; cumulated incidence 2020 – March 2021 (source: survstat@rki 2.0):

Age: 0 years	1345
1 year	1418
2 years	1482

3 years	1643
4 years	1839
5 years	2032
6 years	2087

Until the end of November, the incidence was only about 21 % lower in children aged 1 compared to aged 0, and in children aged 2 years, incidence is only 19 % lower. At the age of 2, one can be sure that most of all children have got both MMR doses. So if there is an effect of MMR on COVID incidence at all, it cannot be large. A very low incidence in pre-school and primary school children as suggested in early papers about possible effects of MMR like SIDIQ et al. (see Fig.1 in SIDIQ et al. for Italy, China, South Korea) cannot be upheld any more.

However later data from Germany (April 1st, 2021) showed that the effect was completely lost when new variants like B.1.1.7 arrived that seem to escape from the trained innate immune response. If MMR vaccination had a small protective effect on COVID incidence in children, it would have been completely lost with the rise of B.1.1.7.

Nevertheless, even the new data from Germany don't exclude the possibility that the high proportion of asymptomatic or mild disease in children is a consequence of MMR vaccination as suggested by ASHFORD et al., and it would be interesting to analyse the vaccination history of the rare cases of more severe grades of disease in preschool or school children. Nevertheless, the effects of MMR vaccination on COVID incidence and outcomes may be different in young children compared to (re-)vaccination of adults; thus there is a need to study that subject separately in adults (like in the CROWN CORONATION trial).

So far, **LARENAS-LINNEMANN and RODRIGUEZ-MONROY** reported about 36 COVID patients (6 > 55 years) who got infected within a few months after MMR vaccination, and all of them had a mild course of COVID-19. But the evidence from that small study is very weak. It may be a starting point for large studies of that kind.

ZIMMERMANN and CURTIS report that measles vaccines influence the ratio between CD4 and CD8 cells in favor of CD 8 cells. The latter play an important role in the defense against COVID-19.

In a study from the Mayo Clinic, based on 137037 individuals who received a PCR test between February 15th and July 14th of 2020, 7 (out of 18) different vaccines, given during the last 1, 2 or 5 years, were associated with a reduced risk of a positive test **(PAWLOWSKI et al.).** However, 6 of the 7 vaccines were given preferentially to children, or there was at least a bias towards children when comparing vaccinated and unvaccinated participants. *"We note that for some vaccines, differences in age between the vaccinated and unvaccinated (matched) cohorts may have influenced the results"* (PAWLOWSKI et al.).

Only geriatric flu vaccination is not at all affected by that problem and allows assumptions regarding the role of adult vaccinations on COVID risk.

Vaccine	Age group	Inactivated vs. live vaccine	RR 1 year	RR 2 years	RR 5 years
HIB	most <9	inact.	0.53	0.51	0.61
MMR	most <9	live	0.56	0.69	0.76
Polio	most <9	inact.	0.57	0.51	0.62
Varicella	most <9	live	0.62	0.63	0.80
Pneumococcal PCV13	mixed, vaccinated biased towards <9 and 60-69y	inact.	0.72	0.67	0.68
Flu 65y+	>= 65	inact.	0.74	0.81	0.75
Hep.A-Hep.B	mixed, vaccinated biased <9 y	inact.	0.80	0.70	0.86
any influenza		mostly inactivated	0.85	(0.92 ns.)	(1.03 ns.)
RZV		live or recombinant	(0.91 ns.)	0.81	0.81
Pneumococcal PPSV23		inact.	(1.06 ns.)	0.79	(0.93 ns.)

Point estimates of RRs (relativ risks) from PAWLOWSKI et al. (all significant if not given in brackets).

Once tested positive, there was no significant association between recent vaccinations and hospitalization or ICU admission (within the 1 year horizon; 2 and 5 year horizon not given). However, the study was underpowered for that purpose because the bias towards young participants makes hospitalization and ICU admission unlikely. Most CIs around point estimates for hospitalization or ICU admission are extremely large so that it makes no sense to look for signals or insignificant trends.

The most robust results (due to their preference for adults or elderly and a higher number of cases) were obtained for “geriatric flu (65+)” and “any influenza”. For geriatric flu, the RR of hospitalization was 1.0 (CI: 0.82 – 1.3), and for any influenza, the RR was 1.1 (0.83 – 1.5) for vaccinated individuals. For ICU, the RR was 0.97 (0.57- 1.7) for geriatric flu and 1.1 (0.56 – 2.2) for any influenza vaccination.

"These results suggest that vaccination status is associated with differential rates of SARS-CoV-2 infection, but there is not enough evidence to determine if vaccination status is associated with COVID-19 disease severity." (PAWLOWSKI et al.).

There is a need for a similar study based on a much larger data set that allows to explore specifically the effects of *adult* (re-)vaccination on COVID infection, hospitalization or ICU admission. The main question, that remains largely unanswered by that study, is whether adults, including elderly, profit from a recent vaccination with regard to the risk of COVID-19 infection and outcome? Despite matching, supplementary table 1 shows that there is a lot of imbalance with regard to age. Nevertheless, the "geriatric flu"/"influenza" results that are based mainly on adults or elderly already suggest that influenza vaccination may result in a moderately lower risk of catching a COVID-19 infection, but once infected, it doesn't seem to reduce the severity of the disease.

MAYADAS et al. found that prior MMR or Tetanus-Diphtheria-pertussis (Tdap) vaccination elicits cross-reactive T-cells that mitigate COVID-19. They described an overlapping T cell population with effector memory T cells including cross-reactive clones recognizing SARS-CoV-2, MMR and Tdap epitopes. *"A propensity-weighted analysis of 73,582 COVID-19 patients revealed that severe disease outcomes (hospitalization and transfer to intensive care unit or death) were reduced in MMR or Tdap vaccinated individuals by 38-32% and 23-20% respectively"* in that study from US. *"In summary, SARS-CoV-2 re-activates memory T cells generated by Tdap and MMR vaccines, which may reduce disease severity."* (MAYADAS et al.).

The study included all patients tested positive for COVID-19 between March 8, 2020, and March 31, 2021 within the Cleveland Clinics health system (n = 73,582 COVID positive patients; 11,483 were vaccinated with MMR and 36,893 with Tdap). COVID-related hospitalization was reduced by 38 % by MMR and by 23 % by Tdap, admission to ICU or death by 32 % vs. 20 % (after adjustment for 44 patient characteristics). (ORs between 0.62 and 0.68 for MMR and 0.77 and 0.80 for Tdap, all significant).

However, as MAYADAS et al. point out: *"The time interval from vaccination (either MMR or Tdap) to positive COVID-19 test was not significantly associated with outcome, possibly because this cohort is dominated by individuals who had MMR or Tdap vaccines within the past 20 years. Thus, this may not be the ideal dataset to test the effect of interval from vaccination to disease."*

A study based on the data from the UK Biobank **(MONEREO-SANCHEZ et al.)** found a protective effect of diphtheria (OR 0.46) and tetanus (0R 0.50) vaccination within the last ten years (mean age of participants: 71.5 years; 103049 participants) on the risk of severe disease, compared to those participants who had received only other vaccinations. Also the risk of testing positive was slightly, but significantly reduced (diphtheria: OR 0.81; tetanus: 0.83), whereas no effects were seen for pertussis vaccination (neither for positive test nor for severity).

In the time of COVID vaccinations, there is an urgent need to study the interaction between COVID and non-COVID vaccinations: are there heterologous effects that recent non-COVID-vaccinations increase the efficacy of COVID vaccinations? Before long-term clinical studies may offer definitive answers, laboratory data on titers of Abs and nAbs titers or T cell immunity may provide preliminary evidence whether heterologous vaccinations may prime or boost the immunological response to COVID-specific vaccinations.

Of note, CHUMAKOV et al. (co-authored by R. GALLO) discuss the possibility that live-attenuated vaccines like BCG, OPV or MMR may even work in ring prophylaxis or PEP due to their *immediate* action on trained innate immunity, strengthening the earlymost and unspecific immune response following infection.

IPV

Whereas live-(attenuated) vaccines are suggested to train innate immunity, the study from PAWLOWSKI et al. also found protective effects of inactivated vaccines, with strongest effects for HIB and polio (IPV). Such effects cannot be explained by the model of trained innate immunity. With regard to IPV, **COMUNALE et al.** showed that poliovirus vaccination raises antibodies that cross-react with SARS-CoV-2, and the primary target of these antibodies is the RdRp of poliovirus and coronavirus, thereby preventing viral replication that may cause disease progression in infected individuals.

COMUNALE et al.: *"An IPV-induced adaptive humoral immune response suggests poliovirus immunization in infants and children, as part of national vaccination efforts, provides protection from SARS-CoV-2 infection until young adulthood. The association between national median age and COVID-19 prevalence and mortality rates across countries suggests a lack of immunity to SARS-CoV-2 in older adults, compared to younger individuals, who may still possess immunity from childhood vaccinations, including poliovirus inoculations."*

Importantly, *"adults re-immunized with IPV exhibited similar antibody responses to both poliovirus and SARS-CoV-2 RdRp, compared to children who received IPV as part of their childhood vaccinations."* COMUNALE et al. suggest that *"IPV immunization may induce adaptive, generally long-term, and specific immunity to poliovirus and SARS-CoV-2 infection. The similarities in structure and function between proteins of SARS-CoV-2 and poliovirus, including RdRp, support this contention."* COMUNALE et al. are currently conducting a larger clinical trial (NCT04639375) about the anti-COVID potential of IPV.

Finally, NCT04523246 is an ongoing trial about the protective effects of the **Varicella-zoster vaccine** (2 doses, 90 days apart) in nursing home residents with the intention to train the innate immune system in a BCG-like manner.

Informational: **Bamlanivimab**

In the BLAZE 2 prevention trial, Bamlanivimab (LY-CoV555) significantly reduced the risk of symptomatic COVID-19 among residents and staff of long-term care facilities. The RCT (with a dose of 4200 mg of bamlanivimab or placebo) included 965 participants who tested SARS-CoV-2 negative at baseline (299 residents, 666 staff). Odds ratio for symptomatic COVID-19 among participants who had received LY-CoV555 was 0.43 (p = 0.00021) after 8 weeks of follow-up. For nursing home residents, OR was 0.20 (p = 0.00026), thus OR must be > 0.43 for staff. All 4 COVID-attributed deaths among the 299 residents occurred in the placebo arm.

132 persons (41 residents and 91 staff) who tested positive at baseline (not included in the 965 participants mentioned above) were also randomized to LY-CoV555 or placebo. There were 4 deaths among the 41 SARS-CoV-2+ residents, but all occurred in the placebo arm. There was no death among staff.

https://investor.lilly.com/news-releases/news-release-details/lillys-neutralizing-antibody-bamlanivimab-ly-cov555-prevented

However, the results haven't been published so far in a journal, and some questions remain, e.g. about differences in the efficacy between residents and staff, severity of symptoms, other unfavorable outcomes (e.g. ICU admission).

Though PREP, PEP or early treatment of infected people with increased risks for severe COVID with mABs seems very promising (for short overview, see COHEN MS), and its potential may increase if different antibody formulations are combined (see NCT04427501 for combination of bamlanivimab and etesevimab), one may ask whether *health systems that were unable so far to recommend or deliver simple and cheap oral or inhalative prophylactics or treatments to quarantined contacts or early outpatients,* will soon be able to deliver expensive antibody infusions to those people who were affected by prophylactic or therapeutic nihilism until now?

Moreover, new variants of SARS-CoV-2 may evade from therapeutic mABs, thus the efficacy of antibody-based therapies or prophylactics will become increasingly uncertain. Since mABs PEP or treatment must start quickly, there may be no time for sequencing the individual or locally circulating virus variant in order to determine the mABs preparation that would still work in that special case. The south-african variant (501Y.V2 / B.1.351) already evaded from current mABs treatments (see WIBMER et al., HU J et al.), and the same applies to the Brazilian variant (501Y.V3 / P.1) (see LIU H et al.), whereas mABs are expected to work against the British variant 501Y.V1 (ZHANG G et al.).

With regard to the Indian variant B.1.617, Bamlanivimab was found to be completely ineffective and Casirivimab showed reduced efficacy. Etesevimab alone, Imdevimab alone and the combination of Imdevimab and Casirivimab were found to be effective, whereas the combination of Etesevimab and Bamlanivimab showed reduced effectiveness. In summary, monotherapies with Casirivimab and particularly Bamlanivimab are not suited for patients infected with B.1.617 (HOFFMANN et al.), or in a PEP setting where B.1.617 is circulating.

The effectiveness against B.1.351 was similar to the effectiveness against B.1.617, except for the important difference that both Etesevimab alone and the combination of Etesevimab and Bamlanivimab are ineffective against 1.351 (HOFFMANN et al.).

In the future, bispecific or multispecific antibodies will become necessary to be effective against different mutants of the virus (see DE GASPARO et al). However, they will be more expensive, more difficult to produce and thus even more difficult to become available for early treatment, PREP or PEP.

However, as long as escape mutations from antibody treatment are rare, antibody infusions like LY-CoV555 (Bamlanivimab) or Casirivimab/Imdevimab (Regeneron) may be an ideal solution for COVID outbreaks in home care facilities and similar settings.

As soon as there is the first COVID case or positive PCR test, all residents and staff (at least staff at risk) may get an antibody infusion, independent of whether PCR+, PCR- or not yet tested. According to the BLAZE trial, antibodies work both in prevention and (very) early therapy of COVID+ people and reduce the risk of symptomatic disease, and, most of all, death.

Thus antibody therapies with currently available antibody formulations like LY-CoV555 or the Regeneron product may be very valuable to reduce the death toll in home care facilities and similar settings during the time window from now

- **until all residents and staff are protected by two doses of COVID vaccines**
- **until viral strains become dominant that evaded from currently available antibody regimens.**
- **until bi- or multispecific antibodies are available and approved,**

but it may be useful also in the case of breakthrough infections that may occur because of immunosenescence. Even in fully vaccinated people, breakthrough infections are not always asymptomatic or mild; they may result in death.*

***Breakthrough infection site of the CDC (US):**

https://www.cdc.gov/vaccines/covid-19/health-departments/breakthrough-cases.html

In the future, multispecific DARPIns (=designed ankyrin repeat proteins; i.e. genetically engineered antibody mimetic proteins) seem to be better suited to cope with a large number of different critical mutants of SARS-CoV-2 (ROTHENBERGER et al.). Unfortunately, none of these antivirals is available so far.

Effectiveness of four different monoclonal antibodies according to PLANAS et al.:

	Bamlanivimab	Etesivimab	Casirivimab	Imdevimab
D614G	+	+	+	+
B.1.1.7	+	-	+	+
B.1.351	-	-	+	+
B.167.2	-	+	+	+

Informational: **intravenous ozonized saline therapy**

Not accessible for private persons and thus mentioned here only shortly for reasons of completeness, SHARMA et al. reported about a quite successful procedure for COVID prophylaxis in HCWs from India in their retrospective controlled cohort study. Based on the knowledge that medical ozone therapy has immunomodulatory, antioxidant and antiviral effects, ozonized saline was administered i.v. once a day for a total of 4 days in one month in addition to standard prophylaxis for COVID-19 to HCWs in a hospital setting that cared for COVID patients. Fresh ozonized saline was prepared for every administration and was given over 1 hour. The exact time sequence when ozone was given is not mentioned in the paper (“*once a day for 4 days in a month”).* Of note, all HCWs (including the control group and irrespective of the risk of exposure) were prescribed chemoprophylaxis including HCQW (400 mg twice a day on day 1, then 400 mg once weekly), multivitamin, vitamin C (1 g once a day) and zinc (50 mg once a day) and they were given a steam inhaler and advised to take steam inhalation twice a day. It was not decribed in the study whether and how well the HCWs complied to that regimen.

Among the 235 hospital HCWs of that non-randomized retrospective, 64 had received the ozone prophylaxis (mean age: 31.8 years), 171 did not (mean age: 29.1 years). The incidence of COVID-19 (PCR- or chest-CT-confirmed) was 4.6 % in the ozon group compared to 14.03 % in the control group (p = 0.04; risk reduction: 67 %). For HCWs working in the “red zone” (n = 22 vs. 117), the rates were 8.7 % vs. 15.3 %. In the “orange zone” (less risks; n = 23 vs. 25), the rates were 4.34 % vs. 20 %, and in the “green zone” (minimal risk of exposure; n = 19 vs. 29), they were 0 % vs. 3.4 %. There were no major adverse events of the ozon therapy.

Though the results are impressive with its risk reduction of 67 %, a lot of limitations have to be considered. First, ozone was offered on top of a complex chemoprophylactic regimen. But

it was not studied whether those who opted to take ozone differed in their compliance with the standard prophylactic regimen. This may introduce a lot of bias. For example, HCWs who are most fearful to catch COVID-19 might have opted for the ozone regimen, but they are also more probable to comply more exactly to the standard prophylactic regimen. Eventually, it is not clear when ozone was administered (once weekly?) or whether different time intervals (</> one week) between ozone administrations influenced its prophylactic effectiveness.

***Informational:* Ramipril (RAAS inhibitor) (no effect)**

Whereas there are a lot of retrospective studies with controversary results with regard to the preventive effect of RAAS inhibitors, data from prospective trials are scarce.

Results from the RASTAVI trial (NCT03201185) showed no effect of Ramipril on COVID-19 incidence (AMAT-SANTOS et al.). The RASTAVI trial is not primarily about COVID 19; instead, it randomly allocates patients to Ramipril or control group after successful transcatheter aortic valve replacement at 14 centers in Spain. But a non-pre-specified interim analysis was performed to study the impact of rampiril on COVID-19 risk.

There were 50 patients in the Ramipril group and 52 in the control group (median age 82.3 years, 56.9 % male), and median time of Ramipril treatment was 6 months (IQR: 2.9 – 11.4 months). 5 patients in the Ramipril group and 6 patients in the control group were diagnosed with COVID-19. 2/5 patients from the Ramipril group and 3/6 patients from the control group needed hospital admission and oxygen supply, and 2 of each group died. Thus Ramipril had no effect on COVID incidence, severity or mortality.

***Informational:* preexisting aspirin prescription**

OSBORN et al. reported about a retrospective analysis from the US Veterans Health Administration, including 28,350 patients for whom 14-day mortality data are available, and 26,346 patients with 30-day mortality data (89 % men, mean age 58.4 years, both for aspirin users and non-users). 6842 and 6342 patients from both cohorts had taken aspirin. Following propensity score matching, preexisting aspirin prescription was associated with an OR of 0.38 (CI: 0.32 – 0.46) for 14-day mortality and 0.38 (CI: 0.33 – 0.45) for 30-day mortality. The unadjusted OR was 0.68 (0.57 – 0.80) for 14-day mortality and 0.68 (0.59 – 0.77) for 30-day mortality. OSBORN et al. suggest that aspirin mitigates thrombotic and inflammatory pathways that contribute to the severity of COVID-19 (*see also* MOHAMED-HUSSEIN et al. with regard to aspirin for prophylaxis of COVID-19-induced coagulopathy).

In a retrospective population-based cross-sectional study with 10477 people from Israel (tested for COVID-19 between February 1st and June 30th, 2020, all regular consumers of low-dose aspirin for cardiovascular prevention), compared to tested people who were not aspirin consums, low-dose aspirin reduced the risk of COVID-19 infection (PCR +) by 29 % (aOR 0.71; CI: 0.52 – 0.99; p = 0.041) **(MERZON et al. (2)).**

In case of infection, duration of the disease (calculated as time between first positive and second negative PCR test) was shortened by 2 days (19.8 vs. 21.9 days).

In contrast to these favorable results, **REESE et al.** reported about increased risk of severe disease, hospitalization and mortality in pre-COVID aspirin users ("indication prior to contracting COVID-19"), based on a large data set of 250.533 COVID patients from US; mean age 41.6 years, mean BMI 29.5. The results for aspirin were consistent for different indications of aspirin use like angina pectoris, fever, migraine, myocardial infarction, osteoarthritis, pain and rheumatoid arthritis. Thus they seem also to apply to a broad range of dosages. ORs (after propensity matching 1 : 1, aspirin intake vs. no intake for the same indication in COVID-19 patients) for increased severity ranged from 2.4 (indication: myocardial infarction) and 2.7 (angina pectoris) to 3.5 for fever and 4.6 for pain, suggesting a dose-effect relationship: Even low dose aspirin (like in the case of patients with a history of myocardial infarction or angina pectoris) seems to have a deleterious effect, and this effect becomes more pronounced for aspirin use for indications that are commonly associated with higher doses.

Similar associations were found for other COX inhibitors except for those that inhibit exclusively COX2 (like diclofenac, celecoxib). Moreover, both aspirin and paracetamol use were associated with significantly increased mortality (overall mortality rate of the 250.553 COVID patients: 1.9 %). The aspirin results are based on 5930 COVID patients who were aspirin users (all indications together) and 5930 matched controls (COVID patients with no aspirin use at the time of index encounter).

REESE et al. point out that some NSAIDs have been associated with abnormalities of immune function. They may also mask warning signs of severe infection, e.g. fever, resulting in delayed diagnosis*. "NSAIDs have multiple effects on the immune system, including inhibition of neutrophil adherence, decreased neutrophil degranulation and oxidant production, inhibition of neutrophil elastase activity and induction of neutrophil apoptosis, and inhibition of antibody production"* (REESE et al.). However, they did not observe an association of NSAID use with neutrophil counts in COVID-19 patients. There were insufficient data available for REESE et al. to assess other potential associations with immune cell function. Thus the reason for the deleterious effects of COX inhibitors (except for "pure" COX2-inhibitors) remains unclear.

However, this study is based on COVID patients (with confirmed infection and at least mild disease; outpatients and inpatients). It doesn't allow any conclusion whether aspirin intake may reduce the risk to acquire SARS-CoV-2 infection (PCR+) or symptomatic COVID-19 as such. Nevertheless, once diagnosed with COVID-19, pre-COVID aspirin intake (and its continuation) has a deleterious effect, including mortality, in contrast to the OSBORN study that showed reduced mortality. In OSBORN et al., participants were on average 17 years older. However, age cannot explain the discrepancy in that case. For example, the "aspirin for osteoarthritis" subgroup from REESE et al. had a mean age of nearly 68 years – but the

OR for more severe disease was 3.25. Thus it is not so simple that one may argue that aspirin may be more deleterious in younger and middle-aged adults and favorable in older adults.

DREW et al. reported about the risk (i) of a positive COVID test and (ii) visit of a clinic or hospital (with or without hospitalization) because of COVID-19 related symptoms in users of the COVID Symptom Study smartphone App from US, UK and Sweden dependent on the use or not-use of aspirin and other NSAIDs (the same app as was utilized for the LOUCA study on supplement consumption). The study encompassed the time interval between March 24 and May 8, 2020 and 2,736,091 individuals (US, UK, Sweden) with 60,817,043 person-days of follow-up and 8966 positive COVID-19 tests. After adjustment for lifestyle factors, comorbidities and symptoms at baseline, there was no association between any NSAID use (including aspirin) and a positive test (HR 1.02; CI: 0.94 – 1.10). The authors concluded that their *"results do not support an association of NSAID use, including aspirin, with COVID-19 infection."*

Looking separately at aspirin use, there was no association with a positive test in the comorbidity-adjusted (HR 1.07, n.s.) and comorbidity-and-symptoms-ad-baseline-adjusted model (HR 1.03, n.s.). Crude 30-day-indicence of a positive test was 0.49 % in non-users of any NSAID (incl. aspirin) vs. 0.30 % in aspirin users (without other NSAIDs), but this advantage of aspirin users was lost following multivariable stratification (HR 1.13, n.s.) and the adjustments mentioned above. Aspirin users were much older than non-users of any NSAIDs (68 vs. 47 years).

0.07 % of aspirin users and 0.08 % of no-users of any NSAID visited a clinic or hospital; what must not necessarily mean hospital admission; but it may be an indicator for more severe symptoms. HR for aspirin users was 1.06 (n.s.) following multivariable stratification, 0.82 (0.52 – 1.29) after adjustment for comorbidities and 0.79 (0.49 – 1.24) after adjustment for comorbidities and symptoms at baseline. The small insignificant trend for a favorable effect of aspirin-only use (HR 0.79) was not seen in users of other NSAIDs (without aspirin) (HR 1.06, n.s.) and users of aspirin + other NSAIDs (HR 0.99, n.s.) in the most adjusted model.

In summary, the role of pre-COVID aspirin use (continued after diagnosis) remains unclear. Since both the OSBORN and the REESE study are based on several thousand participants with pre-COVID-19 aspirin use and as many controls, one cannot explain the strong discrepancy with arguments of statistical robustness.

Informational: **Vitamin D prophylaxis? (Calcifediol superior to other "forms of vitamin D"?)**

Vitamin D supplementation is suggested as a sort of PREP in people with low vitamin D levels in blood or, if unmeasured, who have a lifestyle which is consistent with having low vitamin D levels. There are also self-tests available on the market which offer a rough estimation of vitamin D status. It looks meanwhile very plausible that people with vitamin D deficiency and, maybe to a smaller degree, vitamin D insufficiency, have a higher risk of severe disease and complications like ICU need or ventilation than people with vitamin D status in the normal range. On the other hand, it was also found that low vitamin D levels in

hospitalized patients are a consequence of COVID-19 disease, since 25(OH)D levels decrease quickly during the acute phase of immunological reactions. Moreover, low vitamin D levels in severe patients may be a consequence of their comorbidities. There is so far no evidence that people with vitamin D levels in the normal range can profit from vitamin D prophylaxis with regard to COVID 19 infections or outcomes. On the other hand, for people with low vitamin D levels or a lifestyle which is suggestive of vitamin D deficiency or insufficiency, it makes sense to improve their vitamin D status anyway, whether it may help with regard to COVID-19 or not.

Since it takes a long time to overcome vitamin D deficiency or insufficiency with normal supplemental doses (except for an excessive vitamin D bolus as a consequence of "bad results" of a vitamin D blood test, controlled by a doctor), people with suspected risk of (or proven) vitamin D insufficiency should start as quickly as possible with vitamin D supplementation as a sort of PREP; vitamin D intake (in normal supplemental doses) will probably come too late if it starts as PEP or early therapy since these doses are too small to quickly improve vitamin D serum levels. Thus vitamin D supplementation (in the ideal case, following a vitamin D blood test) is another potential measure of COVID chemoprophylaxis for some groups of people (but probably not for those with vitamin D levels in the normal range). However, in the absence of trials which examined vitamin D chemoprophylaxis of COVID-19, vitamin D could not be included in the main section of this narrative review, in spite of the high plausibility that vitamin D supplementation may help people with vitamin D insufficiency/deficiency to decrease the risks of bad outcomes in the case of COVID infection (DARLING et al., DANESHKHAH A et al., DAVIES et al., DE SMET et al., JAIN A et al., LAU FH et al., MELTZER DO et al., MOK CK et al., NOTARI and TORRIERI, PANGIOTOU et al., PUGACH and PUGACH, GRANT WB et al., MERZON E et al.). For example, MERZON et al. showed that low 25(OH) D level is significantly associated with COVID-19 infection (aOR 1.45), and the association is even stronger with hospitalization (aOR 1.95). Thus, suboptimal vitamin D level (defined as < 30 ng/ml) is associated with both increased risk of infection at all and increased risk of severe disease in case of infection, but reverse causality has to be considered too:

In a small case control study from California, GUPTA D et al. found no association between pre-diagnosis serum 25-hydroxy-vitamin D levels (within 180 days of diagnosis; OR 1.00; CI: 0.98 - 1.02; n = 107), whereas serum vitamin D levels were found to be lower by 2.70 ng/ml (n = 203; p = 0.034) in positive individuals, compared to controls. However, this study was only about positive COVID-tests and not severity of the disease.

JAIN et al. found highly significant associations between vitamin D deficiency, high IL-6, ferritin and TNFalpha levels, severe disease (with demand for ICU admission) and mortality (21.0 vs. 3.1 % in vitamin D deficient patients vs. non-deficient patients). In a study with 551 patients from Mexico, vitamin D deficiency (<= 12ng/ml or <= 30 nmol/l) was not associated with increased critical disease (aOR 0.97), but mortality (aHR 2.11; CI: 1.24 – 3.58, p = 0.006), and this association remained stable after adjustment for visceral fat (VANEGAS-CEDILLO et al.). The effect of vitamin D was partly mediated by its effect on D-dimer and cardiac ultrasensitive troponins (VANEGAS-CEDILLO et al.).

Whereas GRANT et al. propose high dose supplementation (10.000 IU/d for a few weeks, followed by 5000 IU/day) to raise 25(OH)D concentrations above 40-60 ng/ml (100-150 nmol/L), PUGACH and PUGACH found in a country-based study from Europe, that 25(OH)D less than 25 nmol/L (10 ng/ml) is strongly correlated with increased mortality (unadjusted

and adjusted for age). There was also a trend for vitamin deficiency below 50 nmol/l, but it missed significance (p = 0.12). In the range between 25 and 50 nmol/l, the correlation was only weak (r = 0.38 instead of r = 0.76 for < 25 nmol/l). Thus, the concentration-effect relationship doesn't seem to be linear, and concentrations around 50 nmol/l don't seem to be unfavorable (i.e. not too low) with regard to COVID-19 outcomes.

Daily doses of 2000 or 4000 IU are regarded as too high by the German BfR (Bundesinstitut für Risikobewertung; 31.07.2020). A detailed discussion of the dosage question is presented by SIMONSON, including the question of a high loading dose. There is no consensus, but most of the recommendations seem to converge between 800 and 2000 IU/day, but not more than 4000 IE/day (SMOLLICH M), and vitamin D3 ist regarded as more effective than vitamin D2. The optimal 25(OH)D concentration in blood with regard to combating COVID-19 is still unknown, but may be in the range of 40 – 60 ng/ml or even higher (SIMONSON). In contrast to these conservative suggestions, LIU G et al. suggested a single dose of 300.000 IU for both prevention and treatment of COVID-19. They regard 100 ng/ml as a safety margin to reduce the risk of hypercalcemia, and the blood level should not exceed 150 ng/ml. Toxic symptoms were reported in association with serum 25(OH)D levels of 213 ng/ml or more (LIU G et al.). However, SMOLLICH warns that there are hints that high serum levels of vitamin D may be immunosuppressive. Immunosuppressive effects might be welcome in later stages of the disease, but not for the purpose of prophylaxis and early treatment.

A meta-analysis restricted to double-blind RCTs (39 trials, 29.841 participants) found that daily supplementation of 400 – 1000 IU vitamin D reduced the risk of acute respiratory tract infection (OR 0.70; CI: 0.55 – 0.89) (JOLLIFFE et al.). However, there are so far no such trial results in the context of COVID-19.

In a very detailed and large study from Israel, encompassing 4.6 million members of the Clalit Health Services and additionally 52.405 infected patients and 524.050 matched controls without COVID-19 infections of whom (i) baseline vitamin D levels (2010 – 2019) and (ii) acquisition of vitamin D supplements during the last 4 months were known, ISRAEL et al. found a strong relationship between vitamin D deficiency and COVID-19 risk, and even people with vitamin D levels between 50 and 75 nmol/l were found to have a slightly increased risk, but the risk was much more pronounced in those < 30 nmol/l.

However, a protective effect of vitamin D supplementation was only found for vitamin D drops (risk reduktion ~ 10 %; OR 0.905), not tablets/capsules. The authors hypothesize that in the case of drops, part of the vitamin D is directly absorbed by the oropharyngeal mucosal membranes, and higher local vitamin D concentrations in that area may provide some direct protection. In the case of tablets or capsules, vitamin D is resorbed in the intestine and then carried to the body by the blood, but the amounts of vitamin D which arrive in the oropharyngeal area by that way may be too low. The higher risk of COVID-19 infection for those who took tablets/capsules (OR ~ 1.25) may be explained by selection bias: it is more probable that people who know about their low vitamin D status may take such tablets/capsules, thus they might be of higher risk for COVID infection even if they take the supplement. The same may apply to drops, but if drops are much more effective with regard to COVID-19 infection than tablets/capsules, drops may overcompensate that disadvantage of the selection bias.

If one assumes (in a pessimistic manner) (i) a null effect for vitamin D tablets/capsules and (ii) an increased risk for people who take these tablets/capsules (OR 1.25) as a consequence of that selection bias (preferentially people with known low vitamin status select themselves to take supplements), the ~ 10 % risk reduction in drop users (who may have the same underlying selection bias like users of tablets/capsules) may effectively reflect a risk reduction of roughly 30 % for people with low vitamin D status who use drops compared to those who use tablets or capsules. Interestingly, all people who took vitamin D drops profited from them, independent of their vitamin D level, and the group with the highest vitamin D levels (>75 nmol/l) profited most (OR 0.81). This is another hint that local absorption of vitamin D in the oropharynx may be more relevant than gastrointestinal resorption and distribution by the blood.

Again, it is important to note that this study is only about COVID infection risk and not about severity of the disease, and there are suggestions from other studies that vitamin D status may have a more pronounced effect on the severity of the disease compared to simple diagnosis of infection.

Though the evidence for a role of vitamin D for PREP of COVID-19 became stronger than it was suggested before since the study from ISRAEL et al., vitamin D drops weren't included in the trial section above because that was not a prospective or retrospective prophylactic trial in the narrower sense, and there are no hints that the people took vitamin D supplements for the purpose of COVID prophylaxis. There was also no exact temporal matching of intake of vitamin D supplements and the occurrence of COVID infection except for a large time window of four months.

Very surprisingly, a large study based not on vitamin D levels themselves but on genetic variants which are associated with vitamin D levels found no evidence of a protective effect (BUTLER-LAPORTE G et al.). The study was based on 443.734 participants of a genome-wide association study (GWAS) of European ancestry. Genetically increased 25OH-D-levels showed no clear association with COVID susceptibility (insignificant reduction with an OR of 0.88), but increased the OR of hospitalization (OR = 2.34; CI: 1.33 – 4.11) and severe disease (OR 2.21; CI: 0.87 – 5.55). In an extended analysis with up to 960.000 persons as controls, 6232 COVID infections, 2900 hospitalizations and 620 infected people who needed ventilation or died, the insignificant trend for a slightly reduced susceptibility persisted (ORs between 0.69 and 0.89, depending on the genetical methods, i.e. inclusion or exclusion of special polymorphisms; but all ORs were insignificant), whereas the effect on hospitalization disappeared (ORs 1.08 – 1.14, all of them insignif.), maybe because of different strategies for hospitalization in different countries, introducing a lot of regional bias. However, the risk of severe outcomes persisted (ORs 1.80 – 3.07, all of them significant).

The results are restricted to people of European ancestry and may not apply to other populations. Moreover, the authors emphasize that their study doesn't consider frank vitamin D deficiency, *"and it remains possible that vitamin D supplementation may remain beneficial in this population".* They recommend *not* to use vitamin D supplements for protection against COVID 19, and ongoing supplemental trials (n > 15) should closely monitor for signals of harm, e.g. by frequent interim analyses.

In a similar study based on the UK biobank and the SUNLIGHT consortium, encompassing 17965 COVID-19 cases (including 11085 laboratory or physician confirmed cases, 7885

hospitalized cases and 4336 severe respiratory cases) and 1,370,547 controls, primarily of European ancestry, PATCHEN et al. found that *"genetically predicted differences in long-term vitamin D nutritional status do not causally affect susceptibility to and severity of COVID-19 infection",* but *"these results do not exclude the possibility of low-magnitude causal effects, nor do they preclude potential causal effects of acute responses to therapeutic doses of vitamin D."* In contrast, a study from Portugal found an association between genetic susceptibility to vitamin D deficiency and severity of COVID-19 (FREITAS et al.).

On the other hand, a meta-analysis of 11 studies that measured plasma vitamin D levels on admission (ten cohort studies and one case control study) found no significant association of low plasma vitamin D levels with regard to mortality (BIGNARDI et al.) (RR 1.35; CI: 0.84 – 1.86), independent of the cut-off value (<20 or 25 ng/ml and < 10 or 12 ng/ml). However, there is still a trend that vitamin deficiency seems to have a small effect on mortality: the RR for mortality was 1.65 (CI: - 0.45 to + 3.75; 3 studies) if 25(OH)D was < 10 or 12 ng/ml, but 1.34 (0.79 - 1.89) if it was < 20 or 25 ng/ml (9 studies). Of note, the vitamin D level on admission must not necessarily represent the vitamin D level at the time of infection or before infection, since COVID-19 disease may influence calcium metabolism and 25(OH)-D levels. BIGNARDI et al. compared studies that adjusted for age and several confounding factors to those that did not mention (!) adjustment to confounders. RR was 1.49 (0.44 – 2.55) for the adjusted studies and 1.43 (1.18 – 1.69) for the non-adjusted. The differences in statistical significance let BIGNARDI et al. conclude that the positive results (association) in former studies are caused by confounders.

Taking together, the question of vitamin D supplementation is reopened again, and the urgent question is no longer whether vitamin D supplementation is protective or needless, but whether it is protective or deleterious. The only possibility to resolve this question are interim analyses of ongoing trials which focus especially on the endpoint "severe disease" or similar bad outcomes and not only on COVID infection or symptomatic disease. Since PREP trials with health care workers are dominated by younger participants with low risk of severe disease, they may be unable to detect a deleterious signal of vitamin D supplementation. The more it is necessary to look at trials which include preferentially elder people.

On the other hand, the paper of BUTLER-LAPORTE et al. should not be taken too seriously. In a large study from the UK Biobank with 341.484 participants, 656 inpatients with confirmed COVID-19 and 203 deaths (a study size similar to the original, non-extended data set from BUTLER-LAPORTE et al.), COVID susceptibility and outcome were directly compared with data from former vitamin D level measurements (HASTIE CE et al.). 25(OH)D was associated with a reduced risk of mortality (per +10 nmol/l: HR 0.92; CI: 0.86 – 0.98) in univariable analysis, which became attenuated to a small insignificant trend after adjustment for confounders (per + 10 nmol/l.: HR 0.98; CI: 0.91 – 1.06). In this more direct approach than the indirect genomic approach of BUTLER-LAPORTE et al., there was no hint for any increase of mortality risk due to higher vitamin D levels, but still a chance of a small reduction of mortality. The conflicting results may be due to the possibility that one or some of the genetic polymorphisms which are associated with increased vitamin D levels have other effects (independent of vitamin D) which influence COVID mortality. Moreover, the GWAS study is about genome, not methylome. Since COVID mortality is a problem of the elderly, the expression of genes that were subject to the study of BUTLER-LAPORTE et al. may be different in that age group. Respecting the possibility of age-dependent changes in the

expression of many genes, including vitaminD-relevant genes, genome analysis is a quite distant approach to examine the association between vitamin D status and COVID mortality.

BAKALOUDI and CHOURDAKIS reported a strong positive association between vitamin D deficiency (range: 6.9 – 75.1 %) among European countries and COVID-19 infections ($r = 0.82$; $p < 0.01$) and COVID-19 mortality ($r = 0.53$; $p = 0.05$) per million population. The countries that could be included in the study represented 64 % of the entire population of Europe (not all countries could be included due to the lack of representative data on vitamin D deficiency in some countries). Compared to other (earlier) epidemiological studies on vitamin D, this study is based on the cumulative incidence on mortality up to December 23rd what presents a much more robust data base than earlier studies which may be influenced much more by the individual stage of the pandemic within a given country (that's why early epidemiological/ecological studies about that subject are not mentioned here). As expected, Italy is an outlier on the "bad side" of the regression line for obvious reasons based in the early history of the pandemic, and excluding Italy from the analysis would strengthen the association with vitamin D deficiency ($r = 0.90$ for infection and $r = 0.70$ for mortality).

However, in an update with incidence and mortality data up to February 4th, and now with 24 European countries instead of 14 in the former study and more recent data on vitamin D deficiency for some countries, the correlations among vitamin D deficiency (6.0 – 75.5 %) and COVID-19 infections ($r = 0.190$; $p = 0.374$) and mortality ($r = 0.129$, $p = 0.549$) became insignificant (BAKALOUDI and CHOURDAKIS (2)). Taking mean vitamin D level (instead of % of vitamin D deficient people) into account, there was no associaton with COVID incidence ($r = -0.001$) and only an insignificant trend that higher mean vitamin D levels are associated with reduced COVID mortality/1 million inhabitants (-0.115; $p = 0.619$). The authors don't discuss the reasons for the large differences to their former study that they don't mention at all. Nevertheless, also the new study offers a slight signal that it should be wise to avoid vitamin D deficiency during COVID 19 pandemics.

Another study from Europe, based on vitamin D levels from 19 countries and mortality data from January 22nd, 2021, defined a cutoff for vitamin D deficient countries of 50 nmol/l (AHMAD et al.). In that study, a mean vitD level of <= 50 nmol/l (for a country) was associated with a relative risk of death of 2.155 (CI: 1.038 – 4.347, $p = 0.032$) compared to countries with vitD levels > 50 nmol/l.

An own re-analysis of the BAKALOUDI and CHOURDAKIS data about country-level mean vitamin D levels and mortality rates (their Suppl. Fig. 2B), based on more recent mortality data (Worldometer, March 12th) and some additional countries with vitD values taken from the AHMAD paper, confirmed the very weak association that was found by BAKALOUDI and CHOURDAKIS (2); in the own re-analysis, r was – 0.107 for 28 countries compared to – 0.115 for 21 countries in BAKALOUDI and CHOURDAKIS (2). However, after exclusion of five very problematic countries (two because of very divergent vitD values in AHMAD versus BAKALOUDI/CHOURDAKIS, two because of very highly contested mortality data and one because of doubts on the representativeness of the very low vitD level), the association became a little stronger ($r=-0.21$ instead of -0.107), but still insignificant ($p = 0.336$) (see **Supplement 2** at the end of this paper).

A Chinese study found a potential threshold of 41 nmol/l 25(OH)D for "protection" against COVID-19, and vitamin D deficiency was significantly associated with severe/critical disease

after controlling for demographics and comorbidities (but this cannot exclude the possibility of reverse causation, as mentioned above, since Vitamin D levels were measured after disease onset) (YE K et al.).

A placebo-controlled RCT is planned now with 1500 newly diagnosed people and 1200 of their household contacts to explore the effects of vitamin D3 supplementation (VIVID trial, WANG R et al.), representing an early/preemptive treatment and a ring prophylaxis setting. In both groups, participants on verum will receive a loading dose of 9600 IU at day 1 and again on day 2 and then continue with a daily dose of 3200 IU for four weeks. The authors avoid a massive bolus because it is not physiological and showed no benefits with regard to respiratory infections and other adverse outcomes in previous trials. Blood samples will be taken at baseline and after 4 weeks.

WALK et al. found no protective effect of vitamin D in a study from the Netherlands. Based on their study about vitamin K, they recommend to combine vitamin D with **vitamin K** supplementation in order to avoid deleterious effects of calcium on elastic fibers. Elastic fibers have a high affinity for calcium, but calcification of elastic fibers promotes degeneration of the fibers. This may be no problem in healthy subjects, but in case of COVID 19 infection, inflammation and proteases damage pulmonary elastic fibers. The partially degraded fibers become more sensitive to calcium ions, resulting in further degradation. Whereas vitamin D has anti-inflammatory effects and dampens cytokine storms, it increases proteolysis through calcification by increased availability of calcium. Vitamin K is suggested to counteract this deleterious effect of vitamin D on damaged elastic fibers (DOFFERHOFF et al.). LINNEBERG et al. demonstrated significantly increased mortality in hospitalized patients from Denmark with low vitamin K status.

Eventually, DAN et al. reported in the beginning of December about their systematic review which included 11 studies, all of them are based on individual patient data (no ecological studies). 6 trials studied the effect of vitamin D levels on COVID infection risk, 3 on severity of the disease in infected people, and 2 the risk of death in infected people. 10 of the 11 studies demonstrated more favorable outcomes in those without vitamin D level deficiency (though vitamin D deficiency was defined differently in the included studies, ranging from 10 to 30 ng/ml). 1 study (HASTIE et al., UK) found that the protective effect of vitamin D sufficiency in the crude data became insignificant after adjusting for confounders (see above), but even in that study, there was still a small trend in favor of vitamin D.

LOUCA et al. found small protective effects of vitamin D supplementation on COVID infection (PCR+ or seropositivity) as well as symptomatic disease (anosmia). The preventive effect was more pronounced in women (OR 0.88) than in men (OR 0.97), and older men didn't profit at all (men > 60: OR 0.99) in the UK cohorts. However, two other cohorts (US and Sweden) showed stronger effects for both men and women (for details, *see above*). Most important, there was no subgroup for whom vitamin D supplementation had a deleterious effect on COVID incidence, thus it can be regarded as safe.

For further reading, LORDAN et al. gave a detailed overview about the evidence for and against the role of vitamin D in prevention and treatment. They were unable to draw final conclusions about its efficacy and summarized *"Until such time that sufficient evidence emerges, individuals should follow their national guidelines surrounding vitamin D intake to achieve vitamin D sufficiency."*

A well designed and highly recognized study from US found that recent vitamin D insufficiency or deficiency is *not* associated with an increased risk of SARS-CoV-2 IgG seropositivity (LI Y et al. (3)). The study is based on 18148 working-age individuals (employees and their spouses) who where annually tested for total vitamin D (in 2019 or 2020) and screened for SARS-CoV-2 IgG between August and November 2020. Median age was 47 years. 900 participants were found to be seropositive.

In the crude data, both vitamin D less than 20 mg/ml and less than 30 mg/ml, measured in 2019 or 2020 (analysed separately), was associated with a significantly increased risk of seropositivity (uORs between 1.28 and 1.44, all p <= 0.001). However, after adjusting for age, sex, race/ethnicity, education, BMI, blood pressure, smoking status and geographical location, the effect vanished completely. Adjusted ORs for IgG seropositivity ranged from 1.05 to 1.12 (all n.s.) for < 30 ng/ml vs. >= 30 ng/ml and from 0.93 – 1.04 (all n.s.) for < 20 mg/ml vs. >= 20 ng/ml. This study is of high importance because the vitamin D measurements were performed quite recently, whereas other studies were based on measurements many years ago.

However, the null result is not really surprising. Vitamin D is no strong antiviral and it seems to be far too optimistic to expect that vitamin D sufficiency can prevent an infection at all. If vitamin D sufficiency (instead of deficiency or insufficiency) has a prophylactic effect, it is much more likely that is affects the severity of the disease. Unfortunately, LI Y et al. (3) didn't ask the participants for a history of symptoms compatible with COVID-19, and their severity. They didn't use the opportunity to correlate vitamin D status with a history of presence or absence (and, if present, severity) of symptoms in those tested seropositive. However, this might have been methodically impossible because of ist retrospective design.

In a study from Andalusia encompassing all COVID-19 patients hospitalized between January and November 2020, prescription of vitamin D within 15 or 30 days prior to hospitalization (for any reason) was associated with reduced mortality due to COVID infection in hospitalized patients (LOUCERA et al.). The protective effect was more pronounded when vitamin D was prescribed within 15 days instead of 30 days, and much more pronounced for calcifediol (25-Hydroxy-Vitamin D3) compared to cholecalciferol (vitamin D3).

Log Hazard Ratio: -1.27 vs. –0.56 (<=15 days; calcifediol vs. cholecalciferol)
Log Hazard Ratio: -1.01 vs. –0.27 (<=30 days) (all associations are significant)

In that study, 358 (15 days) or 416 (30 days) of the hospitalized patients had cholecalciferol and 193 (or 210) calcifediol prescriptions among the total retrospective cohort of hospitalized patients. Log Hazard Ratios were calculated following propensity score matching. Because of their large database from the Andalusian health care system, *"confounding effects between the compared groups due to the known variables associated to the outcomes considered can be ruled out."* (LOUCERA et al.).

Reasons for the stronger effect of calcifediol are (i) its more reliable intestinal absorption (close to 100 %) and (ii) its ability to rapidly restore serum concentrations because it doesn't require hepatic hydroxylation.

Whereas this study doesn't allow any conclusions whether vitamin D supplementation influences the risk of infection or hospitalization, it indicates that vitamin D supplementation seems to reduce the severity of the disease once a patient is hospitalized. Moreover, the large difference in the effect size between calcifediol and cholecalciferol may offer an explanation why studies about "vitamin D" yield controversary or week results (e.g. LOUCA et al.). It seems to matter a lot what exact form of "vitamin D" is consumed. Unfortunately, nearly all of the commercially available formulations contain cholecalciferol.

Of note, there are suggestions that vitamin D deficiency may be associated with poor response to vaccinations (AHMAD et al.).

Discussion

Except for mycobacterium w vaccine (like Sepsivac) and Ivermectin in combination with iota-carrageenan nasal spray, **umifenovir (Arbidol)** was the most successful agent for chemoprophylaxis in the sense of PEP or periexposure prophylaxis, both in HCWs (two trials) and household contacts of infected people (one trial), based on results which reached high statistical significance. Umifenovir is evidently superior to HCQ in the PEP situation. There is a plausible dose-effect relationship of umifenovir, and if people get infected in spite of umifenovir PEP (which may happen preferentially in the low dose regimen of 200 mg per day), the probability of hospitalization is smaller than in infected people who didn't take umifenovir as PEP. There was no case of severe disease among those who took Arbidol for PEP in the low dose trial.

With regard to **HCQ,** umifenovir outcompetes HCQ by far. The first HCQ prophylaxis trial from which there is a report was stopped because of its ineffectiveness. However, HCQ seems to reduce the risk of COVID disease by nearly 50 % if the loading dose was taken within the first 24 hours after exposure and by nearly 30 % if it was taken between 24 and 48 hours after exposure. After that time interval, the protective effect fades away, but these results are not statistically significant, maybe as a consequence of underpowering and early stop of the trial. However, preliminary reports from India may indicate that HCQ has some effects with regard to PREP (BHATTACHARYA R et al., CHATTERJEE et al., KHURANA et al. and the paper from the Ministry), which is in line with the backward calculation of the time trend observed in the data from BOULWARE et al./WISEMAN et al.. However, the RAJASINGHAM PREP trial found only a small prophylactic effect with an insignificant HR of 0.66 only under the most favorable conditions. The protective effects were so small that only meta-analyses of HCQ trials in different situations (PREP+PEP or PREP+PEP+early therapy) could eventually generate statistically significant results (GARCIA-ALBENIZ et al., LAPADO et al.), but the protection was rather small (22 – 24 %).

In summary, in contrast to umifenovir, HCQ failed so far in PEP except for a possible partial effect in cases when PEP started very early after exposure (< 24 hours, at latest < 48 hours), but even in these cases it is evidently inferior to umifenovir. Moreover, in contrast to umifenovir 200 mg/day, there are also no hints that COVID 19 is milder in those who took HCQ for prophylaxis (but got the disease in spite of HCQ), compared to those who didn't take HCQ prophylaxis (MITJA et al.). BARNABAS et al. found that HCQ PEP (started within 4

days after exposure) is absolutely ineffective, and may even increase the risk of COVID-19 infection a little (~ 20 %), though the study was underpowered to show this with certainty. Data from BOULWARE et al./WISEMAN et al. point to an increased risk for COVID infection in older people who took HCQ for PEP, and the age limit for a harmful effect seems to be around 45 years. Older people should *never* take HCQ for prophylaxis, whereas in younger people, it may help possibly to a moderate extent if taken at the day of exposure or the next day.

However, HCQ holds still some promise with regard to PREP in younger people (for PREP, see RAJASINGHAM et al.). Though the knowledge that HCQ prophylaxis is harmful in older people is largely based on PEP trials (like BOULWARE/WISEMAN et al., MITJA et al.), the same seems to apply to PREP trials (see CHATTERJEE et al.), but it is not established so well in PREP compared to PEP because most PREP trials are from India and involve very young HCWs. However, the age signal in PEP trials is so strong now that it would be irresponsible to recommend HCQ for prophylaxis to people beyond ~45 years.

Of note, an age signal was also found in studies that compared COVID-19 risks in patients with autoimmune disorders (like RA or SLE) who took HCQ with those who didn't *(see above)*. Only few of these studies performed subgroup analyses with different age groups. But in accordance with the PEP/PREP trials, JUNG SY et al. found a trend for a reduced risk of COVID-19 infection in younger HCQ users (< 60 years) compared to nonusers (uOR 0.66, aOR 0.69; n.s.) and an increased risk in older HCQ users (> 59 years) (uOR 1.61; aOR 1.37, n.s.). A similar insignificant age signal was reported by LAPLANA et al. (COVID-19 incidence in HCQ-treated patients: 4.7 % < 51 years, 6.25 % > 50 years; no HCQ: 3.5 % and 3.3 %). These results support the hypothesis that HCQ prophylaxis in middle-aged/older people increases the risk of COVID-19 infection or disease.

The favorable results and comparatively safe profile of HCQ in the PREP trial from BHATTACHARYA et al. have to be seen in the context of the very young age of the participants (mean age of HCQ and control group: 26.5 and 27.7 years), and the protective effect was not found in participants beyond 50 years; instead, the risk of infection was (insignificantly) increased in that age group. The same applies to the promising results from CHATTERJEE et al. and KHURANA et al.; both encompass comparatively young HCWs (mean age far below 40 years), and CHATTERJEE et al. also found that HCQ prophylaxis was more successful in young people, so one actually has to consider an age-dependent prophylactic effect of HCQ which could be explained possibly by the ageing of mitochondria, but also its negative effect on the expression of interferon-stimulated genes, its suppression of innate immunity and lymphocyte counts. Older people may be more prone to these immunosuppressive effects of HCQ which are unwanted in the earliest stages of a viral infection and may overcompensate the purported antiviral activity of HCQ in older people.

But chemoprophylaxis is needed at most for elderly and those with comorbidities. PEP/PREP in young HCWs is to a lesser degree for their own sake (since they will probably suffer only mild disease) than for the sake of others like employers (to avoid absentism) and colleagues and patients (to prevent infectiousness). However, meanwhile new knowledge about long-term sequelae of COVID-19, including mild infections in outpatients, like Long-COVID and hints for increased biological ageing (e.g. effects on telomere length and other indicators of biological age) warn that even mild or moderate disease in young or middle-aged persons should not be regarded as harmless.

Even if HCQ PREP proves to be successful in young HCWs, one may ask very critically whether HCQ PREP can be recommended for long term prophylaxis in elder or comorbid people? Certainly *not*. Not to forget the cardiological risks of HCQ especially for older people and those with cardiac diseases and the need for ECG monitoring (at least once) because of the possibility of QTc prolongation and risk of arrhythmias (for recommendation of ECG regimes, see OFFERHAUS et al.). Since umifenovir is much more tolerable than HCQ, one may even consider umifenovir for short-term PREP. There is no experience with long-term intake of umifenovir (for several months or years), thus it can be considered so far only for PEP or for short-term PREP.

Nevertheless, a possible role of HCQ as a combination partner in prophylactic situations is still open, and there are first hints from Bulgaria (SIMOVA et al.) that a combination of HCQ and zinc may work in prophylaxis. The most obvious combination partner for HCQ is zinc, for which HCQ acts as an ionophore, but also other combinations may be promising, e.g. low-dose doxycycline. There is an ongoing trial about HCQ + zinc (15 mg/day) for prophylaxis in military HCWs from Tunisia (NCT04377646). Again, age may be a problem. As pointed out above (see LOUCA et al.), there are first hints from an UK cohort study that simple zinc supplementation may increase COVID-19 infection risks in older men. Since HCQ prophylaxis seems to be harmful on its own in older people, its combination with zinc may even increase the deleterious effect. It should become mandatory that any study with HCQ and/or zinc should be controlled for age- and sex-dependent effects and signals, with a very critical look on the results for older men. Taken together (and taking into account LOUCA et al.), it may happen that zinc strengthens the age-dependent effect of HCQ prophylaxis: it may strengthen its preventive effect in young people, and it may increase its deleterious effect in older people.

Ivermectin seems to become increasingly interesting with regard to prophylaxis. Due to its short half-life, it was considered at first to be ineffective (because of too low plasma levels following usual oral doses compared to those that would be needed for effective antiviral activity *in vivo,* compared to *in vitro* antiviral activity against SARS-CoV-2). Concentrations that were found to be highly effective *in vitro* could be toxic for humans. Based on these laboratory data, IVM was suggested to be at best a candidate for a combination partner with other compounds in order to generate some sort of synergism with another suboptimal agent (e.g. BRAY et al., MOLENTO, CHACCOUR et al., PENA-SILVA et al.). However, animal models showed that IVM may achieve up to 3-fold higher levels in pulmonary tissue than in plasma 1 week after oral dosing (CHACCOUR et al.). PENA-SILVA et al. proposed the development of an inhaled formulation to deliver a high local concentration to the lungs.

Meanwhile, the results from more than 10 prospective and retrospective clinical trials and 2 ecological studies (like HELLWIG and MAIA, TANIOKA et al.) are much more favorable than thoretically expected based on the *in vitro* data. This discrepancy is still unexplained so that HELLWIG and MAIA had no alternative to propose that “unknown inhibitory effects have to be considered after serum levels of IVM declined.” This discrepancy between the favorable clinical data and the unfavorable *in vitro* data (that suggest that IVM has to be ineffective at the doses that were used in the prophylactic or therapeutic trials) seems to contribute to the decision of the EMA not to approve IVM for prophylaxis and treatment of COVID-19 (according to their statement from March 2021 where they mention the laboratory results).

Apart from the negative statement from the EMA, the history of IVM seems to be opposite to the history of HCQ: whereas there were high expectations for HCQ prophylaxis based on theoretical and *in vitro* evidence (initially only based on Vero cells), the expectations for IVM were very low. Meanwhile, IVM has to be considered more seriously as a prophylactic agent than HCQ. In both cases, the initial suggestions were based on *in vitro* data. In HCQ, a "wrong" cell line was used for the experiments since Vero cells don't express TMPRSS2 and are very unlike from human respiratory epithelium. Later, the supposed strong anti-SARS-CoV-2 effect could not be replicated on cell lines that are similar to human respiratory epithelium, but at that point of time, the hype about HCQ had already manifested, and many trials had been started. Moreover, HCQ impairs innate immunity, suppresses the expression of interferon-stimulated genes and may cause lymphopenia – unwanted features in a PREP, PEP or early treatment setting.

In contrast, early *in vitro* data for IVM were disappointing. As mentioned above, the concentrations that are needed for a direct antiviral effect are regarded as much too high for human use, and possibly toxic.

Eventually, there is a need to explain the discrepancy between the surprisingly good prophylactic (and also therapeutic) effects of IVM and the pharmacological parameters like short half-life. Like in the case of HCQ, immunomodulatory effects on innate immunity may be the answer, but this time in the opposite direction:

The protective effect of IVM was observed without reduction of the effect size if IVM is administered only once per month (ALAM et al.). This effectiveness cannot be explained by the direct antiviral effects, since the half life of IVM is only 16-18 hours in plasma, followed by wide tissue distribution, but even this ranges only from 4 up to 12 days (ALAM et al.). This makes it very hard to explain prophylactic antiviral activity of a single dose of IVM for a full month. ALAM et al. noted that IVM has also immunomodulatory effects.

In fact, it was found to be very successful in the treatment of rosacea. However, rosacea is supposed to be caused by a dysregulation of the innate immune system (ALI ST et al., GUPTA et al., STEIN GOLD et al.). Successful treatment of rosacea by IVM may suggest that IVM helps to restore the regular function of the innate immune system. If so, this may also explain its prophylactic effect against COVID-19 far beyond its presence in the body that may be associated with direct antiviral action.

Eventually, it was found that IVM is able to inhibit SARS-CoV-2 in Vero cells, but **not** in human-derived airway epithelial cells (DINESH KUMAR et al). Thus any prophylactic or therapeutic effect that was seen in studies with IVM cannot be attributed to direct antiviral activity, supporting the immunomodulatory hypothesis.

In that sense, one may ask whether it acts on the innate immune system in a similar way like mycobacterium w or other mycobacterial antigens, like some BCG strains or beta-glucans?

However, though immunomodulatory effects may explain the contradiction between clinical experience and *in vitro* antiviral activity, a lot of questions remain about IVM.

The trials with the best results (up to 100 % protection) were from Argentinia and included the local administration of iota-carrageenan besides IVM use. Since the carrageenan nasal

spray was found to be effective on its own (~ 80 % risk reduction; FIGUEROA et al.), it may have well contributed to these favorable results. If one excludes trials with this combined procedure, the effect size of IVM is attenuated a little bit from the remaining studies. Thus meta-analyses like that from the IVMMETA site may overestimate the effect size of IVM alone due to the inclusion of studies with the combined regimen.

Moreover, most reports about favorable results from IVM prophylaxis are from countries with young populations. HCQ is a warning that one needs to look at different age groups separately. This is not gone or shown (published) in many studies because the subgroup analysis often fails to reach statistical significance. Journals and peer reviewers don't like subgroup analyses with insignificant results and they are regarded to waste space in journals. But HCQ prophylaxis taught us that it is mandatory to present subgroup analyses with special reference to older subgroups, even if they are small and results are (very) insignificant. But such data may help to avoid that people from special subgroups (like elderly) are offered prophylactic regimens that could be harmful to them. Even insignificant on their own, these data may be used for systematic reviews or meta-analyses and thus contribute to the generation of significant results.

That said, IVM prophylaxis looks now much more promising than HCQ prophylaxis, including elderly (see BERNIGAUD et al. for people ~ 90 years old), but more data and subgroup analyses are necessary, as well as better evidence for PREP and PEP dosing regimens. It is also an open question whether oral IVM drops are more effective than IVM tablets, and whether local carrageenan administration has an additive or synergistic effect, as suggested by the favorable results (80 % risk reduction) of iota-carrageenen nasal spray alone, in the absence of IVM (FIGUEROA et al.).

But there are a lot of uncertainties about the prophylactic dose. Three trials (BEHERA et al. 1, BEHERA et al. 2, SEET et al.) indicate that a single dose of 0.2 mg/kg is not enough for prophylaxis. A second dose should be given 72 hours later, particularly in a PEP or early treatment situation. Otherwise, it is unclear whether the next doses should be given one week, two weeks or one month apart. ALAM et al. showed that even one monthly dose might be sufficient for PREP, and this may be in accordance with an immunomodulary effect. Nevertheless, the experience from BEHERA et al. and SEET et al. indicate that one should start prophylaxis with a 2-dose-regimen 72 hours apart.

Bromhexine proved to be very successful in a small RCT from Russia, yielding 71 % prevention from infection and 100 % from symptomatic disease (MIKHAYLOV et al.). Unfortunately, the trial was quite small (n = 50). Bromhexine was taken for 8 weeks in that trial, thus it seems to be suited for PREP over a longer time interval.

However, HÖRNICH et al. warned to be careful: in cell culture, bromhexine acted paradoxically. Though bromhexine is an inhibitor of TMPRSS2 like camostat or nafamostat, it activated the fusion of infected and uninfected cells to promote transfection and may thus contribute to infection, in contrast to camostat/nafamostat (for nafamostat for intranasal prophylaxis in a Syrian hamster model, see NEARY et al.).

Since **Ambroxol** doesn't show that paradoxical effect, it might be better suited for prophylaxis and therapy of COVID-19 (e.g. CARPINTEIRO et al). Based on laboratory data, but also nasal epithelial cells exposed to ambroxol *in vivo* and subsequently infected with SARS-CoV-2 (pseudovirus) *ex vivo,* ambroxol had the potential to be a top candidate for prophylaxis; particularly because it is comparatively safe and can be used for an unlimited time. It is a pity and a missed chance that there are so far no trial results with ambroxol for prophylaxis or early treatment.

Lactoferrin is another promising agent; however, more research is needed (independent of the producer of liposomal lactoferrin; see SERRANO et al.), and it is an important question whether simple oral non-liposomal lactoferrin is effective too since the access to liposomal lactoferrin is difficult.

Finally, more data are needed with regard to **interferon spray** and/or nose drops and **thymosin alpha 1** in chemoprophylaxis. Thymosin alpha 1 seems to be a promising agent for early treatment because it can avoid or dampen hyperinflammation and cytokine storms. Since the trial of MENG et al. was without true control group, the evidence for interferon nasal drops in combination with thymosin alpha 1 is much weaker than the evidence for Umifenovir. And the trial of LIU X et al. found no advantage of thymosin in a prophylactic setting, thus the theoretical expectations could not be replicated in a real world setting.

With regard to **BCG booster immunization,** the results from a single hospital in the United Arab Emirates are very promising, but there are larger trials ongoing and one has to wait for their results. Moreover, there are other limitations. First, all participants in that small trial had gotten the BCG vaccine after birth, so the vaccination in March 2020 was a booster. The authors discuss evidence that a boostered immunization may be more effective than a single vaccination. Thus it is not clear whether people who get the BCG vaccine for the first time (for the purpose of COVID prevention) may profit to the same extent. Second, there are many countries where BCG vaccine is no longer available, and there are already warnings that BCG medications which are prescribed for the treatment of urinary bladder cancer should not be misused as vaccine since they are not qualified for that purpose and the concentration of immunostimulants in these preparations would be much too high [1]. So even if BCG vaccine proves to be successful not only as a booster but also in the case of primary vaccination, many developed countries which abolished BCG vaccination won't profit from it. The same applies to the improved BCG vaccine VPM1002 that is also subject of ongoing trials. Nevertheless, BCG vaccination of older people (> 50 years) may be an interesting preventive measure with regard to any infection risk, independent of its effect on COVID-19, as demonstrated in the ACTIVATE trial. But KLEEN et al. warn to use BCG in older people, immunosenescent people, people with cancer and other comorbidities. Those who need protection from severe COVID-19 and COVID-19 death at most, seem to be least suitable for BCG prophylaxis.

But since it cannot be expected that BCG vaccination avoids infection or symptomatic disease as such (see WANG et al. and HAMIEL et al.), it is of high importance that one can be sure that it doesn't enhance immune hyperreactivity and cytokine storms in some of the

infected people. This needs large trials in high-risk settings in high-risk countries where many infections occur. Moreover, there may be differences between different BCG strains. We need a type-1-biased immune response; a type-2-biased immune response may result in more severe disease and higher risk of mortality. But not all BCG strains generate a type-1-biased response, and some BCG vaccines may be disadvantageous (KLEEN et al.).

Whereas the need for a type-1-biased immune response is inevitable, the risk of an attenuated mycobacterial live vaccine in elder or immunosenescent people can be overcome simply by **inactivated mycobacterial strains,** either as an injectable vaccine (Mycobacterium indicus pranii = M. w., Mycobacterium obuense = IMM-101) or as oral capsules (Mycobacterium setense manresensis = Manremyc/Nyaditum resae).

Very impressive are the results for **mycobacterium w** injection (JAISWAL et al.; formulation: Sepsivac) since this trial was prospective instead of retrospective, and the bias was in favor of (i) a higher risk of infection (because of higher exposure) and (ii) a higher risk of detection of the infection in the vaccinated group (due to more PCR testing), compared to the control group. And since nearly half of the control group was infected within 100 days, it is evident that this study was performed in an extremely risky setting, what makes the results even more reliable.

One may speculate that the future of COVID prevention may be a combination of (i) stimulation of a type-1-biased immune response by inactivated mycobacteria and (ii) a suboptimal and (at first) non-(fully)-sterilizing COVID vaccine of the first generation, followed later (months or years) by better and sterilizing COVID vaccines of a second generation, probably by the nasal route or a combination of intranasal administration and i.m. injection. Even then, inactivated mycobacteria of special strains may play a role as a booster or adjuvant to ensure a strong type-1-biased immune response and to improve the efficacy and protection by the „true“ COVID vaccines.

Moreover, the risk of escape mutations from the immune response, particularly from the binding and inactivation by neutralizing antibodies from the COVID-specific vaccines, demonstrates the urgent need for a second component of the vaccine protection strategy that is independent from SARS-CoV-2-specific antibodies.

This subject is discussed in more detail in a separate paper:

A potential strategy to overcome COVID-19: combination of COVID vaccines with type-1-biased immunomodulation, e.g. by inactivated mycobacteria – a strategy of “double protection”

URL for download: http://freepdfhosting.com/437d9e1634.pdf

More research in the field of chemo- and immunoprophylaxis is needed urgently, and as discussed above, animal models seem to be well suited to investigate PREP/PEP and early therapeutic effects of selected agents before time-consuming and expensive clinical trials are started which may end up in disappointing results; however, these animal models are less suited to study later and more severe stages of COVID-19 disease (e.g. effects on cytokine storms) which are more specific to humans (though there are meanwhile some genetically engineered or immunosuppressed mouse and hamster models available that are able to recapitulate severe disease).

Whereas the limited availability and high costs of rhesus and cynomolgus macaques suggest serious limitations for PREP, PEP and early treatment research, hamsters and ferrets are well suited for that purpose (MONCHATRE-LEROY et al.; see ROSENKE et al. for Syrian hamsters of any age and sex). Thus one can take hamsters or ferrets as the primary animal models for studies of potential prophylactic antivirals (and their combinations), and only the most successful candidates or combinations would be selected for investigation in the expensive and hardly available primate models.

To avoid disappointments, one can follow this pathway:

in silico, in vitro (Calu-3 cells if possible; Vero cells are not well suited for that purpose,e.g. because of missing TMPRSS2 expression)

↓

(if not on Calu-3 cells in the step before):
in vitro, primary human nasal epithelial cell line (hNEC) (with TMPRSS2 expression)

↓

hamster or ferret model

↓ selection of the most promising candidates/combinations

Rhesus or cynomolgus macaques (PREP, PEP or early treatment)

↓

clinical trial

Limitations

This paper is limited to published results of prospective or retrospective trials for PEP or PREP of COVID-19. But this approach is very wide and inclusive; it is not restricted to RCTs like the living review of BARTOSZKO et al. that excludes all trials that are not RCTs.

Nevertheless, the very strict approach of BARTOSZKO et al. is very interesting because it presents the opposite pole compared to this paper here, and that makes it so interesting to see the differences in the conclusions between both approaches.

There are plenty of other agents which look very promising with regard to chemoprophylaxis; however, because this paper will focus primarily on results of clinical trials in the direct context of COVID-19 prevention, other agents with prophylactic potential based only on *in silico, in vitro* or theoretical evidence cannot be included here.

They may be mentioned in the "chemoprophylaxis trial paper" (which isn't updated any more): **http://freepdfhosting.com/9686575098.pdf**

or the "early treatment paper" with its special focus on local prophylaxis (e.g. PVP-iodine) which is discussed in detail here: **http://freepdfhosting.com/35f285c9f2.pdf**

and in:

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.

http://freepdfhosting.com/66b45bc8c1.pdf

Moreover, some (but not all) of the candidates are also listed in the **Supplement.**

Apart from the Supplement, among the many other agents for which a prophylactic role is suggested (but the evidence is too small so that they could not be mentioned in the sections above), only a few will be mentioned here for the reasons given above:

Vitamin C is another candidate for prophylaxis, and FEYAERTS and LUYTEN recommended in their detailed paper about the potential role of vitamin C in treatment and prophylaxis a prophylactic dose of 1 – 2 g/day. In spite of a few data on high dose i.v. vitamin C in critical patients, there are so far no results from ongoing trials for prophylactic use in COVID-19. LOUCA et al. found no effect of vitamin C supplementation in their UK cohort with regard to the risk of COVID infection (PCR+ or seropositivity), and in older men (> 60 years), the risk of infection was slightly (but significantly) increased. But the study didn't report about COVID outcomes/severity of the disease. Interestingly, multivitamins (which usually contain also vitamin C) had no harmful effect in men, but their effect in older men was zero (OR 1.0). Unfortunately, vitamin C was not subject of the analysis of the three other cohorts of that study. In their large PREP RCT with young migrant workers in Singapore, SEET et al. used vitamin C (500 mg per day) for the control group, so the study design of the SEET study doesn't allow any conclusions about an own prophylactic effect of that intervention.

Zinc: In a retrospective study from Spain with 249 hospitalized patients (median age: 65 years), low zinc levels at admission (< 50 microgram/dl, = 7.6 mikroMol) "*correlated with worse clinical presentation, longer time to reach stability and higher mortality*", and a study of SARS-CoV-2 replication in Vero E6 cells by the same group showed that low zinc levels favored viral expansion in infected cells (VOGEL et al.; VOGEL-GONZALEZ et al.). Mortality was 21 % in 58 individuals with zinc levels < 50 microgram/dl at admission, and 5 % in 191 individuals with zinc levels above this threshold ($p < 0.001$). Adjusted OR for in-hospital death was 3.2 (1.01 – 10.12, $p = 0.047$) for zinc serum level < 50 microgram/dl. Median time to reach clinical stability was 25 vs. 8 days ($p < 0.001$). Patients who died had a mean zinc level of 49 microgram/dl at admission, compared to 62 microgram/dl in survivors ($p < 0.001$). Low zinc levels were also associated with higher CRP and IL-6.

The authors suggested serum zinc levels as a novel biomarker to predict COVID-19 outcome and proposed clinical trials about zinc supplementation for prophylaxis and treatment "with people at risk of zinc deficiency" like elderly or people with chronic diseases. The prevalence of zinc deficiency in older adults is 15 – 31 % in developed countries (VOGEL et al.). Morever, in their *in vitro* study, they found that chloroquine doesn't act as ionophore for zinc. VOGEL et al. didn't recommend a specific zinc dose for prophylaxis or treatment.

However, as mentioned above, zinc supplementation was found to increase the risk of COVID infection in older men (> 60 years) in an UK cohort and was neutral in women and younger men (LOUCA et al.) *(details see above*). And in hospitalized patients from Egypt, the addition of zinc to HCQ treatment had no effect (neither favorable nor unfavorable) compared to HCQ treatment alone (ABD-ELSALAM et al.).

In their RCT with young male migrant workers in Singapore, SEET et al. found a favorable effect of zinc (64 or 80 mg elemental zinc/day) + vitamin C (500 mg/day), given in divided doses per day, compared to 500 mg vitamin C once a day (controls). Though insignificant, there was a strong trend in favor of a favorable effect on the risk of infection (PCR+ or serology; relative risk ratio 0.67; CI: 0.38 – 1.08), and an even stronger effect with regard to symptomatic disease (-49.5 %). However, mean age was 33 years and thus that study doesn't participate to the question whether vitamin C, zinc or both have no effect or even an unfavorable effect for older men (as suggested by data from the LOUCA UK substudy).

Doxycycline has also been suggested for prophylaxis with a dose of only 20 mg/day (for example, see YATES et al.). However, whereas there are a few clinical trials with doxycycline for treatment (some of them in combination with ivermectin or HCQ), there seem to be no registered prophylactic trials, though a concept for such a trial (DOXY-PRO) has already been published (YATES et al. 2). For *in vitro* evidence of antiviral activity, see GENDROT et al.; they proposed to investigate doxycycline *in vivo* in animal experiments.

Melatonin is another agent which is associated with a lot of hopes with regard to chemoprophylaxis and treatment (eg., SHNEIDER; TAN and HARDELAND; ZHANG R et al.), and melatonin at a daily dose of 2 mg (Circadin tablets) is subject of a chemoprophylactic trial (MeCOVID) (GARCIA et al.). However, in spite of several theoretical papers that discuss

the mechanisms of melatonin and are quite promising, there are so far no results from prospective chemoprophylactic trials in the context of COVID-19. No reports on results of MeCOVID were found in an extensive search on April 30th 2021, though the study must have been finished since many months.

JEHI et al. reported about a cohort of 13.967 patients from all Cleveland Clinics in Ohio and Florida for whom COVID test results were available. 7.9 % of the patients had a positive test result. Among 531 patients who were melatonin users (as home medication), positivity rate was only 3.0 % (16/531). However, there are no informations about melatonin doses, and the result may be influenced by serious bias. For example, persons taking melatonin may be more health conscious and thus at lower risk to acquire COVID-19. Though the JEHI data are an interesting hint in favor of melatonin, they are no substitute for prospective trials.

CARDINALI et al. proposed **chronotherapy** for elderly during COVID pandemic with melatonin administration at a single timepoint at bedtime (noting that 50 – 100 mg per os are regarded as safe and proposed for prevention of vulnerable individuals) and bright light exposure in the morning.

Echinacea extract was also suggested with regard to both COVID-19 prophylaxis and treatment (e.g., AUCOIN et al., SIGNER et al.). A detailed description of the potential benefits from *Echinacea* is given by AUCOIN et al.

Echinacea was found to be effective against SARS-CoV-2 and other coronaviruses *in vitro* in different cell lines in concentrations of 50 microgram/ml, but not 10 microgram/ml of the formulation “Echinaforce” (SIGNER et al.). There seems to be no gradual increase of inhibitory activity if the concentration is increased, but a threshold somewhere between 10 and 50 microgram/ml, followed by a more sudden increase of inhibitory activity above that threshold.

Beside of its prophylactic potential, *Echinacea* is also known to inhibit cytokine secretions during virus infection and may limit the damage of the respiratory epithelium provoked by the immune system (AUCOIN et al.). However, there are so far no results from clinical trials with COVID-19, but there is an ongoing clinical trial in Iran (IRCT20200415047089N1) with echinacea which examines improvement of clinical symptoms and need for hospitalization in suspected COVID cases, comparing ginger and *Echinacea.*

In a randomized, double blind, placebo controlled trial with 325 persons in the Echinaforce drops group (0.9 ml 3 times a day, in case of a cold 5 times a day during acute stages of the disease) and 348 controls about the *prophylactic* effect of Echinaforce for four months, only a small effect was found with regard to any cold episodes (on average, 0.46 vs. 0.54 per participant), cumulative cold disease days (2.07 vs. 2.44 per participant), but not disease days per sick person (4.5 vs. 4.5 days). If one looks only at confirmed viral infections, there were on average 0.166 vs. 0.213 episodes per participant. If one restricts analysis to common human coronaviruses, the incidence was 0.065 vs. 0.095 per person (JAWAD et al., SIGNER et al.), suggesting a risk reduction of about 30 % for symptomatic coronavirus infections. Echinaforce seems to act more specifically against coronaviruses than against

other viruses or other causes of common cold. However, it remains unknown whether this small to moderate efficacy may also apply to COVID-19.

In summary, if one looks at the RCT results on common coronaviruses on one side and *in vitro* data for SARS-CoV-2 and other coronaviruses on the other, usual doses of Echinaforce may have a potential for a small or moderate protective effect against COVID 19 infection or disease, but only a RCT can prove that. Moreover, the effects seem to be quite limited and the paper of SIGNER et al. seems to be very optimistic with regard to that potential. However, even a product with a limited or moderate effect may be interesting as a combination partner with other prophylactic agents with limited and moderate protective effectiveness if they act in an additive or even in a synergistic manner.

Based on *in vitro* results, BAJRAI et al. proposed the mixing of ***Hypericum perforatum*** (containing pseudohypericin, hypericin, hyperforin, adhyperforin, quercetin, quercitrin) and *Echinacea.* This mixture *"may empower the inhibition of the virus by upregulating the mRNA expression process, lower the viral load, and neutralizing the virus envelop receptor as anti-viral or/and virucidal activities, respectively; and definitely it is related to the pro-inflammatory cytokines such as: IL-6, TNF-α, INF-β as anti-inflammatory therapy."* (BAJRAI et al). They also suggest that such a combination may protect people who contact infected patients, or as early treatment for asymptomatic people who have a positive COVID test. In single use regimens, *Hypericum perforatum* should be preferred to *Echinacea* (according to the *in vitro* results); however, when used in combination for synergistic effects, both agents should be administered at different times.

Another important limitation is that this paper discusses preferentially systemic chemoprophylaxis. **Local prophylaxis in the uppermost airways,** especially in the nasal tract and also in the oropharynx may also be important as pre- and postexposure prophylaxis. There are several agents which are very promising (povidone-iodine, iota-carrageenan, hypertonic saline solution, liposomal lactoferrin, beta-chitosan, xylitol, hydrogen peroxide) as nose drops, nose spray or aerosol (nebulization), and the antiviral activity of these agents was already shown at least *in vitro,* in part also *in vivo* in therapeutic situations (e.g., liposomal lactoferrin) or PREP (iota-carrageenan).

For example, if a possible exposure is expected, one may consider iota-carrageenan (e.g., VEGA et al., FIGUEROA et al.) as nasal and throat spray as preexposure prophylaxis and povidone-iodine (~0.5 – 1.25 %) as nasal spray and throat gargle/throat spray after the event in addition to adequate masking in order to reduce residual risks which may persist even in the presence of a mask since it is generally accepted that masks don't offer 100 % protection.

However, this is only a theoretical concept and not yet based on clinical trials for chemoprophylaxis. For this reason, these agents weren't included in this paper, but they are discussed in more detail in:

Early unspecific systemic and local therapeutic options in COVID-19 disease

available from: **http://freepdfhosting.com/35f285c9f2.pdf** **and**

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.

http://freepdfhosting.com/66b45bc8c1.pdf

DE VRIES et al. 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.

Subjective ranking

Rank 1:

Mycobacterium w injection (JAISWAL et al.) (setting: PREP)

HR 0.067 (significant, p = 0.0001)

Highly exposed front line health care workers at the first peak of the pandemic in India.

(prospective design, highly significant result p = 0.0001, bias in favor of "worse results" in the study group: more COVID exposure, more PCR testing; very high exposure risk).

Mycobacterium w injection may be become part of the "double protection strategy" in case that variants from SARS-CoV-2 evade immune control from COVID vaccines. It could offer a second line of defence in addition to antibody-generating COVID vaccines, stimulating trained innate immunity in a Th1-biased manner.

Effect of VoCs: Not studied. Based on theoretical considerations, it might be that Mycobacterium w injections might be less effective against VoCs because some VoCs were found to escape from innate immunity. However, the effectiveness of Sepsivac is based on strengthening trained innate immunity. This makes it questionable whether Sepsivac is as effective against VoCs as it is against wildtype SARS-CoV-2.

Rank 2:

Ivermectin for PREP or PEP (multi-dose regimen) (+ carrageenan nasal spray)

based on:

PEP, prospective RCT in family contacts, HR 0.13 (NCT04422561; SHOUMAN W)

PREP, retrospective observational case-control trial: HR 0.27 (BEHERA et al.) (sign.)

PREP, prospective cohort study (aRR 0.17; HR 0.15 for the 2-dose regimen; p < 0.001) (BEHERA et al. (2)). Very large study!

PEP, prospective uncontrolled trial, 0 symptomatic infection (AGUIRRE-CHANG et al) (very low evidence from that trial).

PEP, prospective placebo-controlled trial, 0 % vs. 11.2 % positive PCR results within 28 days (p < 0.0001); NCT04425850 – however, the intervention included also carrageenan administration. HR 0.00 Ivermectin (buccal drops) + carrageenan nasal spray in HCWs 5 times a day (sign., p < 0.0001)

Intervention: „1 drop IVM buccal drops (6mg/ml) + 5 sprays carrageenan nasal spray (0·17mg/spray) (buccal – nasal) both repeated 5 times per day + PPE“ vs. „PPE only“ in controls

Meta-analysis from the IVMMETA site: 11 studies (PREP/PEP) with a protective effect of 89%. Confined to 3 RCTs, the protective effect was still 89 % (February 2021). Later, the addition of the SEET RCT with a single dose of IVM reduced the combined results to 85 %.

Effect of VoCs: Not studied. Since the mechanism of the prophylactic effect of IVM is not well understood, it is difficult to predict whether the prophylactic effectiveness will be affected by VoCs.

Serious limitation: the best results were obtained in studies where IVM was combined with local administration of iota-carrageenan as nasal spray. However, iota-carrageenan nasal spray was found to have an own effect on the risk of symptomatic COVID-19 (~ 80% risk reduction; range: 37 – 95 %). Excluding the studies with this combined regimen, the risk reduction by IVM alone seems to be a little attenuated and in the range of 70 – 80 %, but not 85 % and beyond as suggested by IVMMETA. Moreover, a single-dose regimen of IVM was found to be fully ineffective (BEHERA et al. 1, BEHERA et al. 2, SEET et al.).

That said, the rank 2 is given to the combination of IVM (multi-dose) and iota-carrageenan nasal spray. It is less clear whether IVM (multi-dose) alone would be effective enough to merit rank 2. There is no clear evidence that IVM (multi-dose) alone is more effective than umifenovir, bromhexine, iota-carrageenan nasal spray or inosine-glutathione inhalations. There seems to be no effectiveness (and thus no rank) for a single dose of IVM.

The anti-COVID effect of IVM seems to be purely immunomodulatory; IVM showed no antiviral effects in an assay with human respiratory epithelium (DINESH KUMAR et al.).

Rank 3:

Umifenovir (ZHANG et al., YANG et al.) (setting: PEP).

(disadvantage: retrospective design);

but highly significant results and plausible dose-effect relationship between the high-dose trial of ZHANG et al. and the low dose trial of YANG et al.;

ZHANG et al.:

Household contacts: HR 0.025 (point estimate for 3 x 200 mg Arbidol*), highly significant, retrospective non-randomized controlled study

HCWs: HR 0.056 (point estimate for 3 x 200 mg Arbidol*), highly significant, retrospective non-randomized controlled study

*in most cases; a few participants took 400 mg/day.

Note: no experience with long-term use over several months. For that reason, it doesn't seem to be suited for long-term PREP. This restricts its potential use to short-term PREP, peri-exposure prophylaxis, PEP

Effect of VoCs: Since Umifenovir is an entry inhibitor, there is uncertainty whether the efficacy of Umifenovir can be attenuated by mutations in the RBD of the spike protein. The same applies with regard to its function to increase the innate immune response by stimulating the interferon response.

Rank 4:

Bromhexine hydrochloride (8 mg 3 times daily) (MIKHAYLOV et al.) (setting: PREP)

(prospective randomized trial; only high exposed HCWs)

71 % risk reduction for PCR positivity (2 vs. 7 cases)

100 % risk reduction for symptomatic infection (0 vs. 5 cases)

No interruption or termination because of adverse effects; middle-aged (not young-biased) study population.

Note: with 100 % protection from symptomatic disease, bromhexine may possibly deserve a higher ranking and compete with ranks 1 or 2. However, the trial was very small (only 25 participants in the bromhexine group and 25 participants in the control group). If the effect can be replicated in other prophylactic studies, a higher rank may become feasible. Moreover, it is contraindicated for persons with a history of gastric ulcer.

Though this trial was about PREP, there are first favorable experiences with bromhexine in treatment of infected people (see the "early treatment paper" http://freepdfhosting.com/35f285c9f2.pdf). This may indicate that it can also be used in a PEP situation.

Effect of VoCs: According to *in vitro* results in the context of TMPRSS2 expressing cells, B.1.1.7 doesn't affect the efficacy of bromhexine compared to wildtype SARS-CoV-2 at all. There was a small, but insignificant reduction of efficacy against B.1.351 in the figures that seemed to be so irrelevant to the authors that they didn't mention it in the study (LEE J et al.).

In vitro **data suggest that Ambroxol has to be expected to have an ever stronger prophylactic effect compared to bromhexine (OLALEYE et al., CARPINTEIRO et al.). Unfortunately, there are so far no results from a clinical trial of ambroxol prophylaxis. As CARPINTEIRO et al. pointed out, it can in principle be applied with no temperal limitation, and it may be administered as inhalation. Its efficacy would not be affected by VoCs. It has the potential for a top candidate for prophylaxis.**

Rank 5

Iota-carrageenan nasal spray (FIGUEROA et al.) (setting: PREP)

NCT04521322, CARR-COV-02

80 % risk reduction of symptomatic PCR-confirmed infection

Commercially available formulation with 1.7 microgram/ml iota-carrageenan.

However, a critical reevaluation of the data indicates that the effect size may range from 37 % to 95 % reduction due to limitations of the study design. (Symptoms compatible with COVID-19 were only reduced by 37 % and PCR testing was done only once, 48 – 72 hours after symptom onset). On the other hand, if one excludes infections that were PCR diagnosed within the first 6 days and thus probably acquired before the start of the carrageenan intervention, the risk reduction for PCR-confirmed symptomatic disease rises to 95 %. Thus the range between 37 and 95 % encompasses a worst case and a best case scenario.

Effect of VoCs: Not studied. Since the mechanisms of the prophylactic effect of carrageenan are not directly related to the interaction with the RBD of the spike protein and more in the sense of a physical barrier, it is highly improbable that VoCs may impact the efficacy of iota-carrageenan.

Rank 6:

Inosine-glutathione inhalation (DUBINA et al.) (setting: PREP)

(prospective design, significant results; only highly exposed HCWs)

HR 0.23 (inosine-glutathione inhalation) (time-consuming!) (significant, p = 0.02)

Effect of VoCs: unclear because the mechanism behind the prophylactic effect of this inhaled formulation is unclear. If the hypothesis of DUBINA et al. about the underlying mechanism is correct, it is unlikely that the effectiveness of that inhalation is reduced by VoCs.

Rank 7:

Bamlanivimab (LY-CoV555) (setting: PEP, early therapy)

Well powered RCT

OR 0.43 for symptomatic disease (highly significant; nursing home residents and staff);

OR 0.20 for residents, but OR > 0.43 for staff

Expensive; difficult to access in an acute situation for prophylaxis.

Effect of VoCs: Very great concern. Mutant variants may evade from antibody treatment; the south-african and the brazilian variants (5011Y.V2 and V3) were already found to have done so (WIBMER et al., HU J et al., LIU H et al.), but it still seems to work against the British variant B.1.1.7 (501Y.V1) (ZHANG G et al.).

However, as long as escape mutations from antibody treatment are rare, antibody infusions like LY-CoV555 or the combined formulation from REGENERON with two different mABs may be an ideal solution for COVID outbreaks in home care facilities. As soon as there is the first COVID case or positive PCR test, all residents and staff (at least staff at risk) may get an antibody infusion, independent of whether PCR+, PCR- or not yet tested. According to the BLAZE trial, antibodies work both in prevention and (very) early therapy of COVID+ people and reduce the risk of symptomatic disease, and, most of all, death.

Thus antibody therapies may be very valuable to reduce the death toll in home care facilities and similar settings during the time window from now

- **until all residents and staff are protected by two doses of COVID vaccines with proven efficacy in that (very old) population**

- **in case of breakthrough infections that may occur in aged people because of immunosenescence, low or non-existing (or vaned) neutralizing antibody titers**

However, there is a need to adapt the antibody treatment to new variants and one can only hope that mABs like Ly-CoV1404 become available quickly that was found to neutralize many variants including B.1.1.7, B.1.351, B.1.427/B.1.429, P.1, and B.1.526 (WESTENDORF K et al.).

Rank 8:

Neem capsules (Azadirachta indica) (setting: PREP/PEP) (NESARI et al.)

Placebo-controlled RCT from India (HCWs, contacts of COVID patients)

RR 0.45 for SARS-CoV-2 infection (positivity)

No information about the effects on symptomatic disease because all 13 infections in the NEEM and control group were asymptomatic (young study population, ~ 37 years).

Not suited for long-term use because it may harm kidneys, liver and fertility. Toxic in small children. Short-term use seems to be safe (no effect on blood biomarkers in that study).

Effect of VoCs: need for concern; the study was performed in 2020 in the absence of VoCs in India. Because of the mechanism of Neem against COVID-19 is not well understood, it is difficult to forecast whether VoCs will attenuate the prophylactic efficacy.

Comment:

Liposomal lactoferrin from SERRANO et al. cannot be considered here since it was not a controlled trial. Moreover, it is not clear whether the index patients were still infectious when PEP started.

The **ARGOVIT™ RCT** (ALMANZA-REYES et al.) is not respected here despite its favorable results because (i) the formulation is only available and approved for oral and nasal use in Russia; (ii) the internal (e.g. mucosal) use of colloidal silver is highly contested due to a lack of safety data; (iii) the Russian ARGOVIT product differs a lot from common water with colloidal silver. Moreover, whereas the reduction of confirmed COVID-19 ranged from 84.8 to 99 % depending on the mathematical model and way of use of ARGOVIT (99 % in the case of most extensive use), the risk reduction of any symptomatic respiratory infection was only 48.6 %.

No ranking and a WARNING:

Hydroxychloroquine

PEP:

Though early administration of HCQ was able to demonstrate a risk reduction of up to 65 % (point estimate) in a placebo-controlled RCT (WISEMAN et al.) if administered very early (1-2 days – elapsed time – after exposure), and the effectiveness may possibly be even higher in situations when several favorable factors are combined with one another (start 1-2 days after exposure, young adults, household contacts, no comorbidities), there is

● no effect if started > 3 (elapsed time) days after exposure in any subgroup

● increased risk of infection in elderly beyond an age limit somewhere between 45 and 50 years (WARNING)

● thus not suited for prophylaxis in those who need prophylaxis at most, i.e. the elderly and those with certain comorbidities.

BARNABAS et al. found in their PEP RCT trial (< 96 hours, median 2 days, IQR 1 – 3 days) no protective effect of HCQ at all. Instead, the risk of infection or symptomatic disease was slightly (but insignificantly) increased. Because of daily PCR testing, the method of BARNABAS et al. was much more rigorous than BOULWARE et al. and MITJA et al.

PREP:

There are some studies (preferentially from India) that suggest that HCQ PREP may have a weak to moderate effect in PREP in young adults. However, its effect seems to be neutral or even harmful in middle-aged and older adults, i.e. for those populations who need prophylaxis at most. Moreover, also the results for young people (young HCWs) are ambiguous and may be dose-dependent, with better results for low doses.

If so, the preventive effect of HCQ in young people, if present at all, would be purely immunomodulatory and not associated with a direct antiviral activity, in accordance with results from cell cultures that showed no antiviral effect of HCQ in human respiratory epithelium cells (in contrast to some antiviral activity on Vero cells) – a phenomenon that seems to apply to both HCQ and IVM.

References

[1] https://medicalxpress.com/news/2021-07-flu-jab-severe-effects-covid-.html

ABD-ELSALAM S et al., Do Zinc Supplements Enhance the Clinical Efficacy of Hydroxychloroquine?: a Randomized, Multicenter Trial. Biol Trace Elem Res 2020 Nov 27;1-5. doi: 10.1007/s12011-020-02512-1.

ABELLA BS et al., Efficacy and Safety of Hydroxychloroquine vs Placebo for Pre-exposure SARS-CoV-2 Prophylaxis Among Health Care Workers: A Randomized Clinical Trial. JAMA Intern Med 2020 Sep 30. doi: 10.1001/jamainternmed.2020.6319.

ACOSTA-ELIAS J, ESPINOSA-TANGUMA R, The Folate Concentration and/or Folic Acid Metabolites in Plasma as Factor for COVID-19 Infection. Front Pharmacol 2020 Jul 16;11:1062. doi: 10.3389/fphar.2020.01062. eCollection 2020.

AGARWAL M et al., Hydroxychloroquine as a Chemoprophylactic Agent for COVID-19: A Clinico-Pharmacological Review. Front Pharmacol 2020 Dec 17;11:593099. doi: 10.3389/fphar.2020.593099.

AGOSTINIS P et al., Response to a massive SARS-CoV-2 infection in a nursing home transformed into a caring center. Aging Clin Exp Res 2021 Jan 28. doi: 10.1007/s40520-020-01784-w.

AGUIRRE-CHANG C, TRUJILLO FIGUEREDO A, COVID-19: IVERMECTIN PROPHYLAXIS IN ADULT CONTACTS. First Report on Health Personnel and Post-Exposure Prophylaxis. July 2020. DOI: 10.13140/RG.2.2.11985.35680/3

AHMAD A et al., Mean Vitamin D levels in 19 European Countries & COVID-19 Mortality over 10 months. https://www.medrxiv.org/content/10.1101/2021.03.11.21253361v1.full.pdf

ALAM MT et al., Ivermectin as Pre-exposure Prophylaxis for COVID-19 among Healthcare Providers in a Selected Tertiary Hospital in Dhaka – An Observational Study. European Journal ofMedical and Health Sciences, doi:10.24018/ejmed.2020.2.6.599

ALI ST et al., The treatment of rosacea with topical ivermectin. Drugs Today (Barc). 2015 Apr;51(4):243-50. doi: 10.1358/dot.2015.51.4.2318439.Drugs Today (Barc). 2015. Review.

AMANI B et al., Efficacy and safety of arbidol (umifenovir) in patients with COVID-19: A systematic review and meta-analysis. Immun Inflamm Dis 2021 Aug 4. doi: 10.1002/iid3.502.

AMAT-SANTOS IJ et al., Ramipril in High-Risk Patients With COVID-19. J Am Coll Cardiol 2020 Jul 21;76(3):268-276. doi: 10.1016/j.jacc.2020.05.040.

AMIRLAK I et al., Effectiveness of booster BCG vaccination in preventing Covid-19 infection. https://www.medrxiv.org/content/10.1101/2020.08.10.20172288v1.full.pdf

ANBARASU A et al., Vaccine repurposing approach for preventing COVID 19: can MMR vaccines reduce morbidity and mortality? Hum Vaccin Immunother 2020 Sep 1;16(9):2217-2218. doi: 10.1080/21645515.2020.1773141.

AREFIN MK, Povidone Iodine (PVP-I) Oro-Nasal Spray: An Effective Shield for COVID-19 Protection for Health Care Worker (HCW), for all. Indian J Otolaryngol Head Neck Surg 2021 Apr 8;1-6. doi: 10.1007/s12070-021-02525-9.

ASHFORD JW et al., MMR Vaccination: A Potential Strategy to Reduce Severity and Mortality of COVID-19 Illness. Am J Med 2020 Oct 23;S0002-9343(20)30902-5. doi: 10.1016/j.amjmed.2020.10.003.

AUCOIN M et al., The effect of *Echinacea* spp. on the prevention or treatment of COVID-19 and other respiratory tract infections in humans: A rapid review. Adv Integr Med 2020; doi: 10.1016/j.aimed.2020.07.004. Online ahead of print.

AYERDI O et al., Preventive Efficacy of Tenofovir/Emtricitabine Against Severe Acute Respiratory Syndrome Coronavirus 2 Among Pre-Exposure Prophylaxis Users. Open Forum Infect Dis 2020 Sep 25;7(11):ofaa455. doi: 10.1093/ofid/ofaa455.

BAE S et al., Recent Hydroxychloroquine Use Is Not Significantly Associated with Positive PCR Results for SARS-CoV-2: A Nationwide Observational Study in South Korea. Viruses 2021 Feb 20;13(2):329. doi: 10.3390/v13020329.

BAJRAI LH et al., In vitro screening of anti-viral and virucidal effects against SARS-CoV-2 by Hypericum perforatum and Echinacea. https://www.biorxiv.org/content/10.1101/2021.01.11.426295v1.full.pdf

BAI L et al., Co-infection of influenza A virus enhances SARS-CoV-2 infectivity. https://www.biorxiv.org/content/10.1101/2020.10.14.335893v1.full.pdf

BAKALOUDI DR, CHOURDAKIS M, Is vitamin D deficiency associated with the COVID-19 epidemic in Europe?
https://www.medrxiv.org/content/10.1101/2021.01.28.21250673v1.full.pdf

BAKALOUDI DR, CHOURDAKIS M (2), Prevalence of vitamin D is not associated with the COVID-19 epidemic in Europe. A judicial update of the existing evidence.
https://www.medrxiv.org/content/10.1101/2021.03.04.21252885v1.full.pdf

BARNABAS RV et al., Hydroxychloroquine as Postexposure Prophylaxis to Prevent Severe Acute Respiratory Syndrome Coronavirus 2 Infection : A Randomized Trial. Ann Intern Med 2020 Dec 8. doi: 10.7326/M20-6519.

BARTOSZKO JJ et al., Prophylaxis for covid-19: living systematic review and network meta-analysis. https://www.medrxiv.org/content/10.1101/2021.02.24.21250469v1.full.pdf Final publication: BMJ 2021 Apr 26;373:n949. doi: 10.1136/bmj.n949.

BAVDEKAR SB et al., Physician Health in the Times of COVID-19. J Assoc Physicians India 2020 Dec;68(12):21-27.

BEHERA P et al., Role of ivermectin in the prevention of COVID-19 infection among healthcare workers in India: A matched case-control study.
https://www.medrxiv.org/content/10.1101/2020.10.29.20222661v1.full.pdf
Final publication: PLoS One 2021 Feb 16;16(2):e0247163. doi: 10.1371/journal.pone.0247163. eCollection 2021.

BEHERA P et al. (2), Prophylactic role of ivermectin in SARS-CoV-2 infection among healthcare workers. Research Square, doi:10.21203/rs.3.rs-208785/v1 (Preprint)

BEN-ZUK N et al., Potential Prophylactic Treatments for COVID-19. Viruses 2021 Jul 2;13(7):1292. doi: 10.3390/v13071292.

BERIC-STOJSIC B et al., Childhood Immunization and COVID-19: An Early Narrative Review. Front Public Health. 2020; 8: 587007. doi: 10.3389/fpubh.2020.587007

BERNIGAUD C et al., Ivermectin benefit: from scabies to COVID-19, an example of serendipity Annals of Dermatology and Venereology, doi:10.1016/j.annder.2020.09.231

BERSANELLI M et al., The right immune-modulation at the right time: thymosin α1 for prevention of severe COVID-19 in cancer patients. Future Oncol 2021 Feb 4. doi: 10.2217/fon-2020-0754.

BESSIERE P et al., Intranasal type I interferon treatment is beneficial only when administered before clinical signs onset in the SARS-CoV-2 hamster model. https://www.biorxiv.org/content/10.1101/2021.02.09.430458v1.full.pdf

BIGNARDI PR et al., Is the vitamin D status of patients with COVID-19 associated with reduced mortality? https://www.medrxiv.org/content/10.1101/2021.03.25.21254310v1.full.pdf

BITA A et al., Natural and semisynthetic candidate molecules for COVID-19 prophylaxis and treatment. Rom J Morphol Embryol 2020;61(2):321-334. doi: 10.47162/RJME.61.2.02.

BHATT P et al., Hydroxychloroquine Prophylaxis against Coronavirus Disease-19: Practice Outcomes among Health-Care Workers. https://www.medrxiv.org/content/10.1101/2021.08.02.21260750v1.full.pdf

BHATTACHARYA R et al., Pre exposure Hydroxychloroquine use is associated with reduced COVID19 risk in healthcare workers https://www.medrxiv.org/content/10.1101/2020.06.09.20116806v1

BMJ India correspondent: Covid-19: Doctors criticise Indian research agency for recommending hydroxychloroquine prophylaxis. BMJ 2020; 369:m2170; doi: https://doi.org/10.1136/bmj.m2170.

BOULWARE DR et al., A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. N Engl J Med 2020 Juni; doi: 10.1056/NEJMoa2016638.

BRAY M et al., Ivermectin and COVID-19: A report in *Antiviral Research*, widespread interest, an FDA warning, two letters to the editor and the authors' responses. Antiviral Res. 2020 Jun; 178: 104805. doi: 10.1016/j.antiviral.2020.104805

BUTLER-LAPORTE G et al., Vitamin D and Covid-19 Susceptibility and Severity: a Mendelian Randomization Study.
https://www.medrxiv.org/content/10.1101/2020.09.08.20190975v1.full.pdf

CARDINALI DP et al., Elderly as a High-risk Group during COVID-19 Pandemic: Effect of Circadian Misalignment, Sleep Dysregulation and Melatonin Administration. Sleep Vigil 2020; doi: 10.1007/s41782-020-00111-7.

CARPINTEIRO A et al., Inhibition of acid sphingomyelinase by ambroxol prevents SARS-CoV-2 entry into epithelial cells. J Biol Chem 2021 Apr 22;100701. doi: 10.1016/j.jbc.2021.100701.

CARVALLO H et al., Study of the Efficacy and Safety of Topical Ivermectin + Iota-Carrageenan in the Prophylaxis against COVID-19 in Health Personnel. Journal of Biomedical Research and Clinical Investigation, doi:10.31546/2633-8653.1007

CHACCOUR C et al., Ivermectin and COVID-19: Keeping Rigor in Times of Urgency. Am J Trop Med Hyg. 2020 Jun; 102(6): 1156–1157. doi: 10.4269/ajtmh.20-0271

CHARRE C et al., Coronavirus disease 2019 attack rate in HIV-infected patients and in preexposure prophylaxis users. AIDS 2020 Oct 1;34(12):1765-1770. doi: 10.1097/QAD.0000000000002639.

CHATTERJEE P et al., Healthcare workers & SARS-CoV-2 infection in India: A case-control investigation in the time of COVID-19. Indian J Med Res. 2020; 151(5):459-467. doi: 10.4103/ijmr.IJMR_2234_20.

CHEN W et al., A Study on Clinical Effect of Arbidol Combined With Adjuvant Therapy on COVID-19. J Med Virol. 2020, 8.6.2020. doi: 10.1002/jmv.26142.

CHOPRA A et al., Withania somnifera as a Safer Option to Hydroxychloroquine in the Chemoprophylaxis of COVID-19: Results of Interim Analysis. Complement Ther Med 2021 Aug 18;102768. doi: 10.1016/j.ctim.2021.102768.

CHUMAKOV et al., Old vaccines for new infections: Exploiting innate immunity to control COVID-19 and prevent future pandemics. Proc Natl Acad Sci U S A. 2021 May 25;118(21):e2101718118. doi: 10.1073/pnas.2101718118.

COHEN MS, Monoclonal Antibodies to Disrupt Progression of Early Covid-19 Infection. N Engl J Med 2021 Jan 21;384(3):289-291. doi: 10.1056/NEJMe2034495.

COMUNALE BA et al., Poliovirus Vaccination Induces a Humoral Immune Response that Cross Reacts with SARS-CoV-2.
https://www.medrxiv.org/content/10.1101/2021.06.19.21257191v1.full.pdf

CONLON A et al., Impact of the influenza vaccine on COVID-19 infection rates and severity. Am J Infect Control 2021 Feb 22;S0196-6553(21)00089-4. doi: 10.1016/j.ajic.2021.02.012.

CORBETT KS et al., Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N Engl J Med 2020; 383:1544-1555. DOI: 10.1056/NEJMoa2024671

CORNEJO-GIRALDO M et al., Tenofovir-DF versus Hydroxychloroquine in the Treatment of Hospitalized Patients with COVID-19: An Observational Study (THEDICOV).
https://www.medrxiv.org/content/10.1101/2021.03.24.21254238v1.full.pdf

DADRAS A et al., An Urgent Industrial Scheme both for Total Synthesis, and for Pharmaceutical Analytical Analysis of Umifenovir as an Anti-Viral API for Treatment of COVID-19. Comb Chem High Throughput Screen 2021 Feb 3.
doi: 10.2174/1386207324666210203175631.

DAN P et al., Effect of Vitamin D deficiency on COVID-19 status: A systematic review.
https://www.medrxiv.org/content/10.1101/2020.12.01.20242313v1.full.pdf

DANESHKHAH A et al., The Role of Vitamin D in Suppressing Cytokine Storm in COVID-19 Patients and Associated Mortality. https://www.medrxiv.org/content/10.1101/2020.04.08.20058578v1

DARLING AL et al., Vitamin D status, body mass index, ethnicity and COVID-19: Initial analysis of the first-reported UK Biobank COVID-19 positive cases (n 580) compared with negative controls (n 723).
https://www.medrxiv.org/content/10.1101/2020.04.29.20084277v1.full.pdf

DATTA NR, DATTA S., Comprehensive analysis of the key epidemiological parameters to evaluate the impact of BCG vaccination on COVID-19 pandemic. https://www.medrxiv.org/content/10.1101/2020.08.12.20173617v1

DAVIES G et al., Evidence Supports a Causal Model for Vitamin D in COVID-19 Outcomes. https://www.medrxiv.org/content/10.1101/2020.05.01.20087965v1.full.pdf

DAVIES MA et al., HIV and risk of COVID-19 death: a population cohort study from the Western Cape Province, South Africa. https://www.medrxiv.org/content/10.1101/2020.07.02.20145185v1.full.pdf

DAVOUDI-MONFARED E et al. Efficacy and safety of interferon beta-1a in treatment of severe COVID-19: A randomized clinical trial. https://www.medrxiv.org/content/10.1101/2020.05.28.20116467v1

DEBISARUN PA et al., The effect of influenza vaccination on trained immunity: impact on COVID-19. https://www.medrxiv.org/content/10.1101/2020.10.14.20212498v1.full.pdf

DEL AMO J et al., Incidence and Severity of COVID-19 in HIV-Positive Persons Receiving Antiretroviral Therapy: A Cohort Study. Ann Intern Med 2020: doi: 10.7326/M20-3689.

DE GASPARO R et al., Bispecific antibody prevents SARS-CoV-2 escape and protects mice from disease. https://www.biorxiv.org/content/10.1101/2021.01.22.427567v1.full.pdf

DE LA FUENTE J et al., Citizen science initiative points at childhood BCG vaccination as a risk factor for COVID-19. Transbound Emerg Dis 2021 Apr 7. doi: 10.1111/tbed.14097.

DE MELO GD et al., Anti-COVID-19 efficacy of ivermectin in the golden hamster. https://www.biorxiv.org/content/10.1101/2020.11.21.392639v1.full.pdf

DEMKINA AE et al., Risk factors for outcomes of COVID-19 patients: an observational study of 795 572 patients in Russia.
https://www.medrxiv.org/content/10.1101/2020.11.02.20224253v1.full.pdf

DENG L et al., Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: A retrospective cohort study. J Infect. 2020; pii: S0163-4453(20)30113-4. doi: 10.1016/j.jinf.2020.03.002

DE SMET D et al., Vitamin D deficiency as risk factor for severe COVID-19: a convergence of two pandemics. https://www.medrxiv.org/content/10.1101/2020.05.01.20079376v1

DE VRIES RD et al., Intranasal fusion inhibitory lipopeptide prevents direct-contact SARS-CoV-2 transmission in ferrets. Science *Science* 17 Feb 2021: eabf4896
DOI: 10.1126/science.abf4896

DEV N et al., Risk factors and frequency of COVID-19 among healthcare workers at a tertiary care centre in India: a case–control study. Transactions of The Royal Society of Tropical Medicine and Hygiene, trab047, https://doi.org/10.1093/trstmh/trab047

DHIBAR DP et al., Post Exposure Prophylaxis with Hydroxychloroquine (HCQ) for the Prevention of COVID-19, a Myth or a Reality? The PEP-CQ Study. Int J Antimicrob Agents 2020 Nov 6;106224. doi: 10.1016/j.ijantimicag.2020.106224.

DHILLON RA et al., A The mystery of COVID-19 reinfections: A global systematic review and meta-analysis of 577 cases.
https://www.medrxiv.org/content/10.1101/2021.07.22.21260972v1.full.pdf

DI CASTELNUOVO A et al., Low dose hydroxychloroquine is associated with lower mortality in COVID-19: a meta-analysis of 26 studies and 44,521 patients.
https://www.medrxiv.org/content/10.1101/2020.11.01.20223958v1.full.pdf

DINESH KUMAR N et al., Moxidectin and ivermectin inhibit SARS-CoV-2 replication in Vero E6 cells but not in human primary airway epithelium cells.
https://www.biorxiv.org/content/10.1101/2021.05.17.444467v1.full.pdf

DOFFERHOFF ASM et al., Reduced vitamin K status as a potentially modifiable risk factor of severe COVID-19. Clin Infect Dis 2020 Aug 27;ciaa1258. doi: 10.1093/cid/ciaa1258.

DOLGIKH S et al., Covid-19 vs BCG Universal Immunization: Statistical Significance at Six Months of Exposure.
https://www.medrxiv.org/content/10.1101/2020.09.06.20189423v1.full.pdf

DREW DA et al., Aspirin and NSAID use and the risk of COVID-19.
https://www.medrxiv.org/content/10.1101/2021.04.28.21256261v1.full.pdf

DUBINA MV et al., Pathogenesis-based pre-exposure prophylaxis associated with low risk of SARS-CoV-2 infection in healthcare workers at a designated Covid-19 hospital.
https://www.medrxiv.org/content/10.1101/2020.09.25.20199562v1.full.pdf;
Final publication:
DUBINA MV et al., Pathogenesis-based preexposure prophylaxis associated with a low risk of SARS-CoV-2 infection in healthcare workers at a designated COVID-19 hospital: a pilot study. BMC Infect Dis 2021 Jun 7;21(1):536. doi: 10.1186/s12879-021-06241-1.

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Safety of heat-killed *Mycobacterium setense manresensis* as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 2019 Nov; 17(11): e05824. Published online 2019 Nov 11.
doi: 10.2903/j.efsa.2019.5824

ELGAZZAR A et al., Efficacy and Safety of Ivermectin for Treatment and prophylaxis of COVID-19 Pandemic. Research Square, doi:10.21203/rs.3.rs-100956/v2

EMMERICH FG, Comparisons between the Neighboring States of Amazonas and Para in Brazil in the Second Wave of COVID-19 Outbreak and a Possible Role of Early Ambulatory Treatment. Int J Environ Res Public Health. 2021 Mar 24;18(7):3371. doi: 10.3390/ijerph18073371.

FAN L et al., COVID-19 Drug Treatment in China. Curr Pharmacol Rep 2020 Jun 19;1-9. doi: 10.1007/s40495-020-00218-5.

FANG J et al., Efficacy of Early Combination Therapy With Lianhuaqingwen and Arbidol in Moderate and Severe COVID-19 Patients: A Retrospective Cohort Study. Front Pharmacol. 2020; 11: 560209. doi: 10.3389/fphar.2020.560209

FERNANDES DE et al., Pre-exposure prophylaxis during the SARS-CoV-2 pandemic: can PrEP prevent COVID-19-related symptoms? Epidemiol Infect 2020 Sep 28;1-16. doi: 10.1017/S0950268820002253. Online ahead of print.

FERNANDEZ-GUTIERREZ B et al., Hospital admissions in inflammatory rheumatic diseases during the COVID-19 pandemic: incidence and role of disease modifying agents. https://www.medrxiv.org/content/10.1101/2020.05.21.20108696v1.full.pdf

FERREIRA A et al., Chronic treatment with hydroxychloroquine and SARS-CoV-2 infection. https://www.medrxiv.org/content/10.1101/2020.06.26.20056507v1.full.pdf

FESSLER MC, STRICKER RB, Pre-Exposure Prophylaxis for COVID-19 in Pregnant Women. Int J Gen Med 2021; 14: 279 – 284

FEYAERTS AF, LUYTEN W., Vitamin C as prophylaxis and adjunctive medical treatment for COVID-19? Nutrition 2020; 79-80: 110948; https://doi.org/10.1016/j.nut.2020.110948

FIGUEROA JM et al., Efficacy of a nasal spray containing Iota-Carrageenan in the prophylaxis of COVID-19 in hospital personnel dedicated to patients care with COVID-19 disease. A pragmatic multicenter, randomized, double-blind, placebo-controlled trial (CARR-COV-02). https://www.medrxiv.org/content/10.1101/2021.04.13.21255409v1.full.pdf

FINK G et al., Inactivated trivalent influenza vaccination is associated with lower mortality among patients with COVID-19 in Brazil. BMJ Evid Based Med 2020 Dec 11;bmjebm-2020-111549. doi: 10.1136/bmjebm-2020-111549.

FRASER B et al., Structure, activity and inhibition of human TMPRSS2, a protease implicated in SARS-CoV-2 activation.
https://www.biorxiv.org/content/10.1101/2021.06.23.449282v1.full.pdf

FREITAS AT et al., Vitamin D-related polymorphisms and vitamin D levels as risk biomarkers of COVID-19 infection severity.
https://www.medrxiv.org/content/10.1101/2021.03.22.21254032v1.full.pdf

GARCIA IG et al., A Randomized Multicenter Clinical Trial to Evaluate the Efficacy of Melatonin in the Prophylaxis of SARS-CoV-2 Infection in High-Risk Contacts (MeCOVID Trial): A Structured Summary of a Study Protocol for a Randomised Controlled Trial Trials 2020 Jun 3;21(1):466. doi: 10.1186/s13063-020-04436-6.

GARCIA-ALBENIZ et al., Brief communication: A meta-analysis of randomized trials of hydroxychloroquine for the prevention of COVID-19. https://www.medrxiv.org/content/10.1101/2020.09.29.20203869v1

GENDELMAN O et al., Continuous Hydroxychloroquine or Colchicine Therapy Does Not Prevent Infection With SARS-CoV-2: Insights From a Large Healthcare Database Analysis. Autoimmun Rev. 2020; 19(7):102566. doi: 10.1016/j.autrev.2020.102566

GEBRECHERKOS T et al., Effect of co-infection with parasites on severity of COVID-19. https://www.medrxiv.org/content/10.1101/2021.02.02.21250995v1.full.pdf

GENTRY CA et al., Long-term hydroxychloroquine use in patients with rheumatic conditions and development of SARS-CoV-2 infection: a retrospective cohort study. Lancet Rheumatol. 2020.doi:10.1016/S2665-9913(20)30305-2.

GIES V et al., Beyond Anti-viral Effects of Chloroquine/Hydroxychloroquine. Front Immunol 2020 Jul 2;11:1409. doi: 10.3389/fimmu.2020.01409. eCollection 2020.

GIRI A et al., Mutagenic, Genotoxic and Immunomodulatory effects of Hydroxychloroquine and Chloroquine: a review to evaluate its potential to use as a prophylactic drug against COVID-19. Genes Environ 2020 Sep 2;42:25. doi: 10.1186/s41021-020-00164-0. eCollection 2020.

GLANS H et al., Shedding of infectious SARS-CoV-2 from airways in hospitalized COVID-19 patients in relation to serum antibody responses. https://www.medrxiv.org/content/10.1101/2020.09.11.20191940v1.full.pdf

GOLD JE et al., Analysis of Measles-Mumps-Rubella (MMR) Titers of Recovered COVID-19 Patients. mBio 2020 Nov 20;11(6):e02628-20. doi: 10.1128/mBio.02628-20.

GRANADOS-MONTIEL J et al., New prophylaxis regimen for SARS-CoV-2 infection in health professionals with low doses of hydroxychloroquine and bromhexine: a randomised, double-blind placebo clinical trial (ELEVATE Trial). BMJ Open 2021 Aug 3;11(8):e045190. doi: 10.1136/bmjopen-2020-045190.

GRANT WB et al., Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. Nutrients. 2020 Apr 2;12(4):988. doi: 10.3390/nu12040988.

GRAU-PUJOL B et al., Pre-exposure prophylaxis with hydroxychloroquine for COVID- 19: initial results of a double-blind, placebo-controlled randomized clinical trial. BMC Infect Dis 2020:pre-print. doi:10.21203/rs.3.rs-72132/v1

GRENDROT M et al., In vitro Antiviral Activity of Doxycycline against SARS-CoV-2. Molecules 2020 Oct 31;25(21):E5064. doi: 10.3390/molecules25215064.

GROSSEGESSE M et al., Deep time course proteomics of SARS-CoV and SARS-CoV-2-infected human lung epithelial cells (Calu-3) reveals strong induction of interferon-stimulated gene (ISG) expression by SARS-CoV-2 in contrast to SARS-CoV. https://www.biorxiv.org/content/10.1101/2021.04.21.440783v1.full.pdf

GUO K et al., Interferon Resistance of Emerging SARS-CoV-2 Variants. https://www.biorxiv.org/content/10.1101/2021.03.20.436257v1.full.pdf

GUO W et al., A survey for COVID-19 among HIV/AIDS patients in two Districts of Wuhan, China. Lancet. 2020; https://ssrn.com/abstract=3550029

GUPTA D et al., Temporal association of reduced serum vitamin D with COVID-19 infection: A single-institution case-control and historical cohort study. https://www.medrxiv.org/content/10.1101/2021.06.03.21258330v1.full.pdf

GUPTA G et al., Ivermectin 1% cream for rosacea. Skin Therapy Lett. 2015 Jul-Aug;20(4):9-11.

GÜRSOY C et al. COVID-19 Pneumonia during Hydroxychloroquine Treatment of Rheumatoid Arthritis. J Coll Physicians Surg Pak 2020 Oct;30(10):168-170. doi: 10.29271/jcpsp.2020.supp2.S168.

HADJADJ J et al., Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020; 369: 718 - 724

HAMIEL U et al., SARS-CoV-2 Rates in BCG-Vaccinated and Unvaccinated Young Adults. JAMA 2020 May 13;323(22):2340-2341. doi: 10.1001/jama.2020.8189.

HÄRTER G et al., COVID-19 in people living with human immunodeficiency virus: A case series of 33 patients. https://www.medrxiv.org/content/10.1101/2020.04.28.20073767v1

HASTIE CE et al., Vitamin D and COVID-19 infection and mortality in UK Biobank. Eur J Nutr 2020 Aug 26;1-4. doi: 10.1007/s00394-020-02372-4.

HE Y, DONG YC, A perspective on re-detectable positive SARS-CoV-2 nucleic acid results in recovered COVID-19 patients. Disaster Med Public Health Prep 2020 Oct 22;1-19 (epub)

HELLWIG MD, MAIA A, A COVID-19 Prophylaxis? Lower incidence associated with prophylactic administration of Ivermectin. Int J Antimicrob Agents 2020 Nov 28;106248. doi: 10.1016/j.ijantimicag.2020.106248.

HERNANDEZ AV ez al., Impact of Prophylactic Hydroxychloroquine on People at High Risk of COVID-19: A Systematic Review and Meta-Analysis. J Clin Med 2021 Jun 13;10(12):2609. doi: 10.3390/jcm10122609.

HOANG T et al., Systematic review and meta-analysis of factors associated with repositive viral RNA after recovery from COVID-19. J Med Virol 2020 Nov 2. doi: 10.1002/jmv.26648.

HOFFMANN M et al., SARS-CoV-2 variant B.1.617 is resistant to Bamlanivimab and evades antibodies induced by infection and vaccination.
https://www.biorxiv.org/content/10.1101/2021.05.04.442663v1.full.pdf

HOLT H et al., Risk factors for developing COVID-19: a population-based longitudinal study (COVIDENCE UK).
https://www.medrxiv.org/content/10.1101/2021.03.27.21254452v1.full.pdf

HÖRNICH BF et al., SARS-CoV-2 differs from SARS-CoV in the requirements for receptor expression and proteolytic activation to trigger cell-cell fusion and is not inhibited by Bromhexine. https://www.biorxiv.org/content/10.1101/2020.07.25.221135v1.full.pdf
Final publication:
HÖRNICH BF et al., SARS-CoV-2 and SARS-CoV Spike-Mediated Cell-Cell Fusion Differ in Their Requirements for Receptor Expression and Proteolytic Activation.
J Virol. 2021 Apr 12;95(9):e00002-21. doi: 10.1128/JVI.00002-21. Print 2021 Apr 12.

HU J et al., Emerging SARS-CoV-2 variants reduce neutralization sensitivity to convalescent sera and monoclonal antibodies.
https://www.biorxiv.org/content/10.1101/2021.01.22.427749v1.full.pdf

HUANG D et al., Efficacy and safety of umifenovir for coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. J Med Virol 2020; doi: 10.1002/jmv.26256.

HUH K et al., Association of prescribed medications with the risk of COVID-19 infection and severity among adults in South Korea. Int J Infect Dis 2020 Dec 19;S1201-9712(20)32565-0. doi: 10.1016/j.ijid.2020.12.041.

HUI KK, Povidone-iodine and carrageenan are candidates for SARS-CoV-2 infection control. Hong Kong Med J 2020 Oct;26(5):464. doi: 10.12809/hkmj208889.

IBRAHIM H et al., Therapeutic blockade of inflammation in severe COVID-19 infection with intravenous n-acetylcysteine. *Clin Immunol*. 2020;219:108544. doi:10.1016/j.clim.2020.108544

ISHIKADO A et al., Liposomalization of lactoferrin enhanced it's anti-inflammatory effects via oral administration. Biol Pharm Bull 2005; 28 (9): 1717- 1721

ISRAEL A et al., The link between vitamin D deficiency and Covid-19 in a large population. https://www.medrxiv.org/content/10.1101/2020.09.04.20188268v1.full.pdf

JAIN A et al., Analysis of vitamin D level among asymptomatic and critically ill COVID-19 patients ad its correlation with inflammatory markers. Scientific reports 2020: 10: 20191

JAISWAL SR et al., Innate Immune Response Modulation and Resistance to SARS-CoV-2 infection: A Prospective Comparative Cohort Study in High Risk Healthcare Workers. https://www.medrxiv.org/content/10.1101/2020.10.20.20214965v1.full.pdf

JAWAD M et al., Safety and efficacy profile of Echinacea purpurea to prevent common cold episodes: a randomized, double-blind. Placebo-Controlled Trial Evid-Based Complement Alternative Med. 2012;2012:841315.

JEHI R et al., Individualizing Risk Prediction for Positive Coronavirus Disease 2019 Testing: Results from 11,672 Patients. Chest 2020; S0012-3692(20)31654-8. doi: 10.1016/j.chest.2020.05.580.

JI SM et al., Immunoregulation of Thymosin Alpha 1 Treatment of Cytomegalovirus Infection Accompanied With Acute Respiratory Distress Syndrome After Renal Transplantation. Transplant Proc 2007_ 39 (1): 115-119; doi: 10.1016/j.transproceed.2006.10.005.

JOCKUSCH S et al., Triphosphates of the Two Components in DESCOVY and TRUVADA are Inhibitors of the SARS-CoV-2 Polymerase . https://www.biorxiv.org/content/10.1101/2020.04.03.022939v1.full.pdf

JOLLIFFE D et al., Vitamin D supplementation to prevent acute respiratory infections: systematic review and meta-analysis of aggregate data from randomised controlled trials. https://www.medrxiv.org/content/10.1101/2020.07.14.20152728v1

JOMAH S et al., Clinical efficacy of antivirals against novel coronavirus (COVID-19): A review. J Infect Public Health 2020 Aug 3;S1876-0341(20)30593-1. doi: 10.1016/j.jiph.2020.07.013.

JUNG SY et al., Hydroxychloroquine pre-exposure on infection with SARS-CoV-2 in rheumatic disease patients: A population-based cohort study. Clin Microbiol Infect . 2020 Dec 11;S1198-743X(20)30752-7. doi: 10.1016/j.cmi.2020.12.003.

KAMSTRUP P et al., Hydroxychloroquine as a primary prophylactic agent against sars-cov-2 infection: a cohort study. Int J Infect Dis 2021 Jun 1;S1201-9712(21)00478-1. doi: 10.1016/j.ijid.2021.05.076.

KANDEIL A et al., Common childhood vaccines do not elicit a cross-reactive antibody response against SARS-CoV-2. PLoS One 2020 Oct 28;15(10):e0241471. doi: 10.1371/journal.pone.0241471.

KARMEN-TUOHY S et al., Outcomes among HIV-positive patients hospitalized with COVID-19. https://www.medrxiv.org/content/10.1101/2020.05.07.20094797v1

KASHOUR T, TLEYHEJ IM, It is time to drop hydroxychloroquine from our COVID-19 armamentarium. Med Hypotheses 2020 Nov;144:110198.
doi: 10.1016/j.mehy.2020.110198. Epub 2020 Aug 17.

KASHOUR Z et al., Mortality, viral clearance, and other clinical outcomes of hydroxychloroquine in COVID-19 Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Clin Transl Sci 2021 Feb 19. doi: 10.1111/cts.13001.

KAUR H et al., Folic acid as placebo in controlled clinical trials of hydroxychloroquine prophylaxis in COVID-19: Is it scientifically justifiable? Med Hypotheses 2021 Feb 20;149:110539. doi: 10.1016/j.mehy.2021.110539.

KELLENI MT, Early use of non-steroidal anti-inflammatory drugs in COVID-19 might reverse pathogenesis, prevent complications and improve clinical outcomes. Biomed Pharmacother 2020 Nov 4;133:110982. doi: 10.1016/j.biopha.2020.110982.

KHURANA A et al., Prevalence and clinical correlates of COVID-19 outbreak among healthcare workers in a tertiary level hospital.
https://www.medrxiv.org/content/10.1101/2020.07.21.20159301v1.full.pdf

KLEEN TO et al., Mitigating Coronavirus Induced Dysfunctional Immunity for At-Risk Populations in COVID-19: Trained Immunity, BCG and "New Old Friends". Front Immunol 2020 Sep 4;11:2059. doi: 10.3389/fimmu.2020.02059. eCollection 2020.

KOENIGHOFER M et al., Carrageenan Nasal Spray in Virus Confirmed Common Cold: Individual Patient Data Analysis of Two Randomized Controlled Trials. Multidiscip Respir Med. 2014 Nov 12;9(1):57. doi: 10.1186/2049-6958-9-57. eCollection 2014.

KONIG M et al.,Baseline use of hydroxychloroquine in systemic lupus erythematosus does not preclude SARS- CoV-2 infection and severe COVID-19. Ann Rheum Dis. http://dx.doi.org/10.1136/annrheumdis-2020-217690

KORMAN TM, Favipiravir and the Need for Early Ambulatory Treatment of COVID-19. Antimicrob Agents Chemother 2020 Dec 7;AAC.02489-20. doi: 10.1128/AAC.02489-20.

KORY P et al., Review of the Emerging Evidence Demonstrating the Efficacy of Ivermectin in the Prophylaxis and Treatment of COVID-19. Doi: 10.31219/osf.io/wx3zn

Final publication:

KORY P et al., Review of the Emerging Evidence Demonstrating the Efficacy of Ivermectin in the Prophylaxis and Treatment of COVID-19. Am J Ther 2021 Apr 22;28(3):e299-e318. doi: 10.1097/MJT.0000000000001377.

KUMAR J et al., Efficacy and safety of hydroxychloroquine/chloroquine against SARS-CoV-2 infection: A systematic review and meta-analysis. J Infect Chemother 021 Feb 22;S1341-321X(21)00061-1. doi: 10.1016/j.jiac.2021.02.021.

KUMAR GOENKA M et al., COVID-19 prevalence among health-care workers of Gastroenterology department: An audit from a tertiary-care hospital in India. JGH Open. 2020 Nov 9;5(1):56-63. doi: 10.1002/jgh3.12447. eCollection 2021 Jan.

LADAPO JA et al., Randomized Controlled Trials of Early Ambulatory Hydroxychloroquine in the Prevention of COVID-19 Infection, Hospitalization, and Death: Meta-Analysis https://www.medrxiv.org/content/10.1101/2020.09.30.20204693v1.full.pdf

LAPLANA M et al., Lack of protective effect of chloroquine derivatives on COVID-19 disease in a Spanish sample of chronically treated patients. https://www.medrxiv.org/content/10.1101/2020.09.03.20158121v1.full.pdf

LARENAS-LINNEMANN D, RODRIGUEZ-MONROY F, Thirty-six COVID-19 cases preventively vaccinated with mumps-measles-rubella vaccine: All mild course . Allergy 2020 Sep 7. doi: 10.1111/all.14584.

LAU FH et al., Vitamin D Insufficiency is Prevalent in Severe COVID-19. https://www.medrxiv.org/content/10.1101/2020.04.24.20075838v1

LEBLANC EV, COLPITTS CC, The green tea catechin EGCG provides proof-of-concept for a pan-coronavirus entry inhibitor.
https://www.biorxiv.org/content/10.1101/2021.06.21.449320v1.full.pdf

LEE AC et al., Nasopharyngeal Type-I Interferon for Immediately Available Prophylaxis Against Emerging Respiratory Viral Infections. Front Immunol 2021 May 19;12:660298. doi: 10.3389/fimmu.2021.660298. eCollection 2021.

LEE J et al., TMPRSS2 and RNA-dependent RNA polymerase are effective targets of therapeutic intervention for treatment of COVID-19 caused by SARS-CoV-2 variants (B.1.1.7 and B.1.351). https://www.biorxiv.org/content/10.1101/2021.04.06.438540v1.full.pdf

LEE SH et al., Can post-exposure prophylaxis for COVID-19 be considered as one of outbreak response strategies in long-term care hospitals? Int J Antimicrob Agents. 2020: 105988. doi: 10.1016/j.ijantimicag.2020.105988.

LEWIS K et al., The efficacy and safety of hydroxychloroquine for COVID-19 prophylaxis: A systematic review and meta-analysis of randomized trials. PLoS One 2021 Jan 6;16(1):e0244778. doi: 10.1371/journal.pone.0244778. eCollection 2021.

LI G et al., Enantiomers of Chloroquine and Hydroxychloroquine Exhibit Different Activities Against SARS-CoV-2 in vitro, Evidencing S-Hydroxychloroquine as a Potentially Superior Drug for COVID-19. https://www.biorxiv.org/content/10.1101/2020.05.26.114033v1.

LI Y et al., An exploratory randomized, controlled study on the efficacy and safety of lopinavir/ritonavir or arbidol treating adult patients hospitalized with mild/moderate COVID-19 (ELACOI). https://www.medrxiv.org/content/10.1101/2020.03.19.20038984v1

LI Y et al., Face masks to prevent transmission of COVID-19: a systematic review and meta-analysis. https://www.medrxiv.org/content/10.1101/2020.10.16.20214171v1.full.pdf

LI Y et al. (3), Assessment of the Association of Vitamin D Level With SARS-CoV-2 Seropositivity Among Working-Age Adults. JAMA Netw Open 2021; 4 (5) e2111634.

LINNEBERG A et al., Low vitamin K status predicts mortality in a cohort of 138 hospitalized patients with COVID-19.
https://www.medrxiv.org/content/10.1101/2020.12.21.20248613v1.full.pdf

LIU G et al., A Single Large Dose of Vitamin D Could be Used as a Means of Coronavirus Disease 2019 Prevention and Treatment.
Drug Des Devel Ther 2020 Aug 21;14:3429-3434. doi: 10.2147/DDDT.S271754. eCollection 2020.

LIU H et al., 501Y.V2 and 501Y.V3 variants of SARS-CoV-2 lose binding to Bamlanivimab in vitro. https://www.biorxiv.org/content/10.1101/2021.02.16.431305v1.full.pdf

LIU Q et al., The effect of Arbidol Hydrochloride on reducing mortality of Covid-19 patients: a retrospective study of real world date from three hospitals in Wuhan.
https://www.medrxiv.org/content/10.1101/2020.04.11.20056523v1.full.pdf

LIU X et al., Analysis of the prophylactic effect of thymosin drugs on COVID-19 for 435 medical staff: A hospital-based retrospective study. J Med Virol 2020; doi: 10.1002/jmv.26492

LIU Y et al., Thymosin alpha 1 (Tα1) reduces the mortality of severe COVID-19 by restoration of lymphocytopenia and reversion of exhausted T cells. Clin Infect Dis. 2020; ciaa630. doi: 10.1093/cid/ciaa630.

LOPEZ DE LA IGLESIA J et al., Hydroxichloroquine for pre-exposure prophylaxis for SARS-CoV-2. https://www.medrxiv.org/content/10.1101/2020.08.31.20185314v1.full.pdf

LORDAN R et al., Dietary Supplements and Nutraceuticals Under Investigation for COVID-19 Prevention and Treatment. ArXiv 2021 Feb 3;arXiv:2102.02250v1.

LOUCA P et al., Dietary supplements during the COVID-19 pandemic: insights from 1.4M users of the COVID Symptom Study app - a longitudinal app-based community survey. https://www.medrxiv.org/content/10.1101/2020.11.27.20239087v1.full.pdf

LOUCERA C et al., Real world evidence of calcifediol use and mortality rate of COVID-19 hospitalized in a large cohort of 16,401 Andalusian patients. https://www.medrxiv.org/content/10.1101/2021.04.27.21255937v1.full.pdf

MACIAS J et al., Similar incidence of Coronavirus Disease 2019 (COVID-19) in patients with rheumatic diseases with and without hydroxychloroquine therapy. https://www.medrxiv.org/content/10.1101/2020.05.16.20104141v1

MACIAS J et al. (2), Similar incidence of coronavirus disease 2019 (COVID-19) in patients with rheumatic diseases with and without hydroxychloroquine therapy. PLoS One 2021 Apr 8;16(4):e0249036. doi: 10.1371/journal.pone.0249036. eCollection 2021.

MADHI SA et al., Safety and efficacy of the ChAdOx1 nCoV-19 (AZD1222) Covid-19 vaccine against the B.1.351 variant in South Africa. https://www.medrxiv.org/content/10.1101/2021.02.10.21251247v1.full.pdf

MALLA TN et al., Vitamin C inhibits SARS coronavirus-2 main protease essential for viral replication. https://www.biorxiv.org/content/10.1101/2021.05.02.442358v1.full.pdf

MANOHARAN L et ak,m Chemoprophylaxis trial designs in epidemics: insights from a systematic review of COVID-19 study registrations. Trials 2021 May 29;22(1):370. doi: 10.1186/s13063-021-05323-4.

MAISONNASSE P et al., Hydroxychloroquine use against SARS-CoV-2 infection in non-human primates. Nature 2020; 585: 584 – 587

MARIN-HERNANDEZ D et al., Epidemiological evidence for association between higher influenza vaccine uptake in the elderly and lower COVID-19 deaths in Italy. J Med Virol 2021 Jan;93(1):64-65. doi: 10.1002/jmv.26120.

MARGOLIN L et al., 20-Week Study of Clinical Outcomes of Over-the-Counter COVID-19 Prophylaxis and Treatment. J Evid Based Integr Med Jan-Dec 2021;26:2515690X211026193. doi: 10.1177/2515690X211026193.

MAYADAS T et al., Protective heterologous T cell immunity in COVID-19 induced by MMR and Tdap vaccine antigens.
https://www.biorxiv.org/content/10.1101/2021.05.03.441323v1.full.pdf

MELTZER DO et al., Association of Vitamin D Deficiency and Treatment with COVID-19 Incidence .
https://www.medrxiv.org/content/10.1101/2020.05.08.20095893v1

MENG Z et al., An experimental trial of recombinant human interferon alpha nasal drops to prevent coronavirus disease 2019 in medical staff in an epidemic area.
https://www.medrxiv.org/content/10.1101/2020.04.11.20061473v1
Final publication:
MENG Z et al., The effect of recombinant human interferon alpha nasal drops to prevent COVID-19 pneumonia for medical staff in an epidemic area. Curr Top Med Chem 2021 Apr 28. doi: 10.2174/1568026621666210429083050.

MERZON E et al., Low plasma 25(OH) vitamin D3 level is associated with increased risk of COVID-19 infection: an Israeli population-based study.
https://www.medrxiv.org/content/10.1101/2020.07.01.20144329v1

MERZON E et al. (2) The use of aspirin for primary prevention of cardiovascular disease is associated with a lower likelihood of COVID-19 infection. The FEBS Journal (February23rd, 2021). https://doi.org/10.1111/febs.15784

MIKHAYLOV EN et al., Bromhexine Hydrochloride Prophylaxis of COVID-19 for Medical Personnel: A Randomized Open-Label Study.
https://www.medrxiv.org/content/10.1101/2021.03.03.21252855v1.full.pdf

Ministry of Health and Family Welfare, Government of India. Revised advisory on the use of Hydroxychloroquine (HCQ) as prophylaxis for COVID-19 infection (in supersession of previous advisory dated 23 Mar 2020):

https://www.mohfw.gov.in/pdf/RevisedadvisoryontheuseofhydroxychloroquineasprophylaxisforSARSCOVID19infection.pdf

MITJA O et al., A Cluster-Randomized Trial of Hydroxychloroquine as Prevention of Covid-19 Transmission and Disease.
https://www.medrxiv.org/content/10.1101/2020.07.20.20157651v1

MOHAMED-HUSSEIN AAR et al., Should aspirin be used for prophylaxis of COVID-19-induced coagulopathy?,
Med Hypotheses. 2020; 144:109975. doi: 10.1016/j.mehy.2020.109975.

MOHAPATRA PR et al., Immunity and protection from COVID-19-Environmental mycobacteria play a role. J Med Virol 2020 Jun 24;10.1002/jmv.26214.
doi: 10.1002/jmv.26214.

MOK CK et al. Calcitriol, the active form of vitamin D, is a promising candidate for COVID-19 prophylaxis. https://www.biorxiv.org/content/10.1101/2020.06.21.162396v1.full.pdf

MOLENTO MB, COVID-19 and the rush for self-medication and self-dosing with ivermectin: A word of caution. One Health 2020 Jun 24;10:100148.
doi: 10.1016/j.onehlt.2020.100148.

MONCHATRE-LEROY E et al., Hamster and ferret experimental infection with intranasal low dose of a single strain of SARS-CoV-2.
https://www.biorxiv.org/content/10.1101/2020.09.24.311977v1.full.pdf

MONEREO-SANCHEZ J et al., Vaccination history for diphtheria and tetanus is associated with less severe COVID-19.
https://www.medrxiv.org/content/10.1101/2021.06.09.21257809v1.full.pdf

MONK PD et al., Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Respir Med 2020 Nov 12;S2213-2600(20)30511-7.

MONTI M et al., Hydroxychloroquine as Prophylaxis for COVID-19: A Review. Front Pharmacol 2020 Dec 3;11:605185. doi: 10.3389/fphar.2020.605185. eCollection 2020.

MORGENSTERN J et al., Retrospective cohort study of Ivermectin as a SARS-CoV-2 pre-exposure prophylaxis method in Healthcare Workers.
https://www.medrxiv.org/content/10.1101/2021.04.10.21255248v1.full.pdf

MÖSBAUER K et al., Allicin inhibits SARS-CoV-2 replication and abrogates the antiviral host response in the Calu-3 proteome.
https://www.biorxiv.org/content/10.1101/2021.05.15.444275v1.full.pdf

MUELLER L et al., Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. doi: https://doi.org/10.1101/2021.03.03.21251066

NESARI TM et al., Neem (Azadirachta Indica A. Juss) Capsules for Prophylaxis of COVID-19 Infection: A Pilot, Double-Blind, Randomized Controlled Trial. Altern Ther Health Med 2021 Apr 23;AT6831.

NGUYEN LC et al., Cannabidiol Inhibits SARS-CoV-2 Replication and Promotes the Host Innate Immune Response. https://www.biorxiv.org/content/10.1101/2021.03.10.432967v1.full.pdf

NOJOMI M et al., Effect of Arbidol (Umifenovir) on COVID-19: a randomized controlled trial. BMC Infect Dis 2020 Dec 14;20(1):954. doi: 10.1186/s12879-020-05698-w.

NOTARI A, TORRIERI G, COVID-19 transmission risk factors.
https://www.medrxiv.org/content/10.1101/2020.05.08.20095083v1

NOVAL RIVAS M et al., BCG vaccination history associates with decreased SARS-CoV-2 seroprevalence across a diverse cohort of healthcare workers. J Clin Invest 2020 Nov 19;145157. doi: 10.1172/JCI145157.

OFFERHAUS R et al., Prophylactic (Hydroxy)chloroquine in COVID-19: Potential Relevance for Cardiac Arrhythmia Risk. Heart Rhythm 2020 Jul 2;S1547-5271(20)30634-2. doi: 10.1016/j.hrthm.2020.07.001.

ONABAJO OO et al., Interferons and viruses induce a novel truncated ACE2 isoform and not the full-length SARS-CoV-2 receptor. Nat Genet
2020 Oct 19. doi: 10.1038/s41588-020-00731-9.

OSBORN TF et al., Association of Mortality and Aspirin Prescription for COVID-19 Patients at the Veterans Health Administration.
https://www.medrxiv.org/content/10.1101/2020.12.13.20248147v1.full.pdf

PADHI AK et al., Unraveling the mechanism of arbidol binding and inhibition of SARS-CoV-2: Insights from atomistic simulations. Eur J Pharmacol 2020 Dec 30;173836. doi: 10.1016/j.ejphar.2020.173836.

PADMANABHAN U et al., Phase II Clinical trial for Evaluation of BCG as potential therapy for COVID-19. https://www.medrxiv.org/content/10.1101/2020.10.28.20221630v1.full.pdf

PANAGIOTOU G et al., Low serum 25-hydroxyvitamin D (25[OH]D) levels in patients hospitalized with COVID-19 are associated with greater disease severity: results of a local audit of practice. https://www.medrxiv.org/content/10.1101/2020.06.21.20136903v1

PARK SJ et al., Antiviral Efficacies of FDA-Approved Drugs Against SARS-CoV-2 Infection in Ferrets. mBio 2020; 11(3):e01114-20. doi: 10.1128/mBio.01114-20.

PARK SY et al., Post-exposure prophylaxis for Middle East respiratory syndrome in healthcare workers. J Hosp Infect. 2019 Jan;101(1):42-46. doi: 10.1016/j.jhin.2018.09.005

PATCHEN BK et al., Genetically predicted serum vitamin D and COVID-19: a Mendelian randomization study.
https://www.medrxiv.org/content/10.1101/2021.01.29.21250759v1.full.pdf

PAWLOWSKI C et al., Exploratory analysis of immunization records highlights decreased SARS-CoV-2 rates in individuals with recent non-COVID-19 vaccinations. *Scientific Reports* 2021: 11; Article number: 4741 (2021)

PENA-SILVA R e al., Pharmacokinetic considerations on the repurposing of ivermectin for treatment of COVID-19. Br J Clin Pharmacol . 2020 Jul 17;10.1111/bcp.14476.

PHAM K et al., Failure of chronic hydroxychloroquine in preventing severe complications of COVID-19 in patients with rheumatic diseases. Rheumatol Adv Pract 2021 Mar 2;5(1):rkab014. doi: 10.1093/rap/rkab014. eCollection 2021.

PLANAS D et al., Reduced sensitivity of infectious SARS-CoV-2 variant B.1.617.2 to monoclonal antibodies and sera from convalescent and vaccinated individuals. https://www.biorxiv.org/content/10.1101/2021.05.26.445838v1.full.pdf

PRUIYSSERS AJ et al., Remdesivir potently inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. https://www.biorxiv.org/content/10.1101/2020.04.27.064279v1.full.pdf

PUGACH IZ, PUGACH S, Strong Correlation Between Prevalence of Severe Vitamin D Deficiency and Population Mortality Rate from COVID-19 in Europe. https://www.medrxiv.org/content/10.1101/2020.06.24.20138644v1.full.pdf

RAJASINGHAM R et al., Hydroxychloroquine as pre-exposure prophylaxis for COVID-19 in healthcare workers: a randomized trial. https://www.medrxiv.org/content/10.1101/2020.09.18.20197327v1.full.pdf

RAKEDZON et al., Hydroxychloroquine and Coronavirus Disease 2019: A Systematic Review of a Scientific Failure. Rambam Maimonides Med J 2020; 11 (3): doi: 10.5041/RMMJ.10416.

RAMEZANPOUR M et al. (2017): In Vitro Safety Evaluation of Human Nasal Epithelial Cell Monolayers Exposed to Carrageenan Sinus Wash. Int Forum Allergy Rhinol 2017 Dec;7(12):1170-1177. doi: 10.1002/alr.22021.

REESE J et al., Cyclooxygenase inhibitor use is associated with increased COVID-19 severity. https://www.medrxiv.org/content/10.1101/2021.04.13.21255438v1.full.pdf

RENTSCH CT et al., Hydroxychloroquine for prevention of COVID-19 mortality: a population-based cohort study. https://www.medrxiv.org/content/10.1101/2020.09.04.20187781v1.full.pdf

REVOLLO B et al., Hydroxychloroquine pre-exposure prophylaxis for COVID-19 in healthcare workers. J Antimicrob Chemother. 2020 Nov 21:dkaa477. doi: 10.1093/jac/dkaa477.

ROBINOT R et al., SARS-CoV-2 infection damages airway motile cilia and impairs mucociliary clearance. https://www.biorxiv.org/content/10.1101/2020.10.06.328369v1.full.pdf

ROJAS-SERRANO J et al., Hydroxychloroquine For Prophylaxis Of COVID-19 In Health Workers: A Randomized Clinical Trial.
https://www.medrxiv.org/content/10.1101/2021.05.14.21257059v1.full.pdf

ROSENKE K et al., Hydroxychloroquine Proves Ineffective in Hamsters and Macaques Infected With SARS-CoV-2. bioRxiv 2020 Jun 11;2020.06.10.145144; doi: 10.1101/2020.06.10.145144

ROSENKE K et al., Hydroxychloroquine prophylaxis and treatment is ineffective in macaque and hamster SARS-CoV-2 disease models. JCI Insight. 2020 Oct 22:143174. doi: 10.1172/jci.insight.143174.

ROSENKE K et al., Defining the Syrian hamster as a highly susceptible preclinical model for SARS-CoV-2 infection.
https://www.biorxiv.org/content/10.1101/2020.09.25.314070v1.full.pdf

ROTHENBERGER S et al., Multi-specific DARPin(R) therapeutics demonstrate very high potency against mutated SARS-CoV-2 variants in vitro.
https://www.biorxiv.org/content/10.1101/2021.02.03.429164v1.full.pdf

ROTHER N et al., Hydroxychloroquine inhibits trained immunity - implications for COVID-19. https://www.medrxiv.org/content/10.1101/2020.06.08.20122143v1

SALLARD E et al., Clinical trial protocols of repurposed prophylaxis for COVID-19: a review. Med Mal Infect. 2020 Oct 3:S0399-077X(20)30711-3. doi: 10.1016/j.medmal.2020.09.013.

SANTOS CS et al., Biologic agents for rheumatic diseases in the break of COVID-19: friend or foe? https://www.medrxiv.org/content/10.1101/2020.09.01.20184333v1

SARDINHA DM et al., Analysis of 472,688 severe cases of COVID-19 in Brazil showed lower mortality in those vaccinated against influenza.
https://www.medrxiv.org/content/10.1101/2021.05.11.21257053v1.full.pdf

SCHILLING WHK et al., The WHO guideline on drugs to prevent COVID-19: small numbers- big conclusions. Wellcome Open Res 2021 Mar 30;6:71.
doi: 10.12688/wellcomeopenres.16741.1. eCollection 2021.

SEET RCS et al., Positive impact of oral hydroxychloroquine and povidone-iodine throat spray for COVID-19 prophylaxis: an open-label randomized trial.
Int J Infect Dis. 2021 Apr 14:S1201-9712(21)00345-3. doi: 10.1016/j.ijid.2021.04.035.

SERRANO G et al., Liposomal Lactoferrin as Potential Preventative and Cure for COVID-19. Int J Res Health Sci. 2020;8(1):8-15

SHARMA A et al., Intravenous ozonized saline therapy as prophylaxis for healthcare workers (HCWs) in a dedicated COVID-19 hospital in India - A retrospective study. Eur Rev Med Pharmacol Sci 2021 May;25(9):3632-3639. doi: 10.26355/eurrev_202105_25847.

SHEAFF RJ et al., A New Model of SARS-CoV-2 Infection Based on (Hydroxy)Chloroquine Activity. https://www.biorxiv.org/content/10.1101/2020.08.02.232892v1

SHNEIDER A et al., Can melatonin reduce the severity of COVID-19 pandemic? Int Rev Immunol. 2020 Apr 29:1-10. doi: 10.1080/08830185.2020.1756284.

SIGAMANI A et al., Galectin antagonist use in mild cases of SARS-CoV-2 cases; pilot feasibility randomised, open label, controlled trial.
https://www.medrxiv.org/content/10.1101/2020.12.03.20238840v1.full.pdf

SIGNER J et al., In vitro virucidal activity of Echinaforce®, an Echinacea purpurea preparation, against coronaviruses, including common cold coronavirus 229E and SARS-CoV-2. Virol J 2020 Sep 9;17(1):136. doi: 10.1186/s12985-020-01401-2.

SIDIQ KR et al., Does Early Childhood Vaccination Protect Against COVID-19? Front Mol Biosci 2020 Jun 5;7:120. doi: 10.3389/fmolb.2020.00120. .

SIMONSON W, Vitamin D dosing considerations in COVID-19. Geriatr Nurs 2020 Aug 25;S0197-4572(20)30255-X. doi: 10.1016/j.gerinurse.2020.08.011.

SIMOVA I et al., Hydroxychloroquine for prophylaxis and treatment of COVID-19 in health care workers. New Microbes New Infect 2020 Nov 12;100813. doi: 10.1016/j.nmni.2020.100813.

SINGER ME et al., Hydroxychloroquin ineffective for COVID-19 prophylaxis in lupus and rheumatoid arthritis. Ann Rheum Dis. 2020; annrheumdis-2020-218500. doi: 10.1136/annrheumdis-2020-218500.

SINGH S et al., "Trained immunity" from Mycobacterium spp. exposure or BCG vaccination and COVID-19 outcomes. PLoS Pathog. 2020 Oct 29;16(10):e1008969. doi: 10.1371/journal.ppat.1008969. eCollection 2020 Oct.

SINGH SEHGAL I et al., Safety of an immunomodulator *Mycobacterium* w in COVID-19. Lung India. 2020 May-Jun; 37(3): 279–281. doi: 10.4103/lungindia.lungindia_242_20

SKIPPER CP, BOULWARE DR, Hydroxychloroquine in Nonhospitalized Adults With Early COVID-19. Ann Intern Med. 2021 Mar;174(3):434-435. doi: 10.7326/L20-1426.

SMIT M et al., Efficacy of pragmatic same-day ring prophylaxis for adult individuals exposed to SARS-CoV-2 in Switzerland (COPEP): protocol of an open-label cluster randomised trial. BMJ Open 2020 Nov 12;10(11):e040110. doi: 10.1136/bmjopen-2020-040110.

SMIT M et al. (2), Prophylaxis for COVID-19: a systematic review. Clin Microbiol Infect 2021 Jan 18;S1198-743X(21)00040-9. doi: 10.1016/j.cmi.2021.01.013.

SMOLLICH M, Vitamin D im Komtext von COVID-19. Deutsche Apotheker-Zeitung 2020; 160: 3994 – 3996.

STEIN GOLD L et al., Long-term safety of ivermectin 1% cream vs azelaic acid 15% gel in treating inflammatory lesions of rosacea: results of two 40-week controlled, investigator-blinded trials. J Drugs Dermatol. 2014 Nov;13(11):1380-6.J Drugs Dermatol. 2014.

STRICKER RB, FESTER MC, Hydroxychloroquine Pre-Exposure Prophylaxis for COVID-19 in Healthcare Workers from India: A Meta-Analysis. .J Infect Public Health. 2021 Aug 5;14(9):1161-1163. doi: 10.1016/j.jiph.2021.08.001.

SU Y et al., Efficacy of early hydroxychloroquine treatment in preventing COVID-19 pneumonia aggravation, the experience from Shanghai, China. Biosci Trends 2020 Dec 18. doi: 10.5582/bst.2020.03340.

SUN Q et al., The effect of thymosin α1 on mortality of critical COVID-19 patients: A multicenter retrospective study. Int Immunopharmacol 2020 Oct 31;107143. doi: 10.1016/j.intimp.2020.107143.

SYED F et al., Pre-Exposure Prophylaxis with Various Doses of Hdroxychloroquine among high-risk COVID 19 Healthcare Personnel: CHEER randomized controlled trial. https://www.medrxiv.org/content/10.1101/2021.05.17.21257012v1.full.pdf

TALARICO F et al., Psychiatric side effects induced by chloroquine and hydroxychloroquine: a systematic review of case reports and population studies. https://www.medrxiv.org/content/10.1101/2020.10.05.20207423v1.full.pdf

TAN DX, HARDELAND R, Potential utility of melatonin in deadly infectious diseases related to the overreaction of innate immune response and destructive inflammation: focus on Melatonin in deadly virus infection. doi: https://doi.org/10.32794/mr11250052

TAN Q, JIN Y, Oseltavimir is ineffective against COVID-19: in silico assessment, in vitro and retrospective study. https://www.medrxiv.org/content/10.1101/2020.05.15.20102392v1

TANDON T et al., Knowledge, attitude, and perception of Indian population toward coronavirus disease (COVID-19). J Family Med Prim Care. 2020 Aug; 9(8): 4265–4269. doi: 10.4103/jfmpc.jfmpc_818_20

TANIOKA H et al.,lWhy COVID-19 is not so spread in Africa: How does Ivermectin affect it? https://www.medrxiv.org/content/10.1101/2021.03.26.21254377v1.full.pdf

THAKAR A et al., Chloroquine nasal drops in asymptomatic & mild COVID-19: An exploratory randomized clinical trial. Indian J Med Res
2021 Jan & Feb;153(1 & 2):151-158. doi: 10.4103/ijmr.IJMR_3665_20.

TORUN S et al., The Characteristics of Health Care Workers with COVID-19 and Relationship Between COVID-19 Mortality and BCG/Tuberculosis History: a multi-center study. https://www.medrxiv.org/content/10.1101/2020.10.08.20209403v1.full.pdf

TSILIKA M et al., ACTIVATE-2: A double-blind randomized trial of BCG vaccination against COVID-19 in individuals at risk.
https://www.medrxiv.org/content/10.1101/2021.05.20.21257520v1.full.pdf

TURNER RB et al., Prevention of Experimental Coronavirus Colds with Intranasal α-2b Interferon. The Journal of Infectious Diseases, 154 (3): 443-447.
https://doi.org/10.1093/infdis/154.3.443

UGARTE-GIL MF et al., Response to: 'Hydroxychloroquine ineffective for COVID-19 prophylaxis in lupus and rheumatoid arthritis' by Singer et al . Ann Rheum Dis Actions 2020; annrheumdis-2020-218683. doi: 10.1136/annrheumdis-2020-218683.

VALLEJOS et al. (cited in News):
Coronavirus in Argentina: Warnings and evidence on the consumption of ivermectin against Covid-19. https://totalnewsagency.com/2020/12/07/coronavirus-en-la-argentina-advertencias-y-evidencias-sobre-el-consumo-de-ivermectina-contra-el-covid-19/

VAN DOREMALEN N et al., ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques.
https://www.biorxiv.org/content/10.1101/2020.05.13.093195v1.full.pdf

VANEGAS-CEDILLO PE et al., Serum Vitamin D levels are associated with increased COVID-19 severity markers and mortality independent of visceral adiposity.
https://www.medrxiv.org/content/10.1101/2021.03.12.21253490v1.full.pdf

VARESE A et al., Iota-carrageenan prevents the replication of SARS-CoV-2 on an in vitro respiratory epithelium model.
https://www.biorxiv.org/content/10.1101/2021.04.27.441512v1.full.pdf

VEGA JC et al., Iota carrageenan and xylitol inhibit SARS-CoV-2 in Vero cell culture
https://www.biorxiv.org/content/10.1101/2020.08.19.225854v1.full.pdf

VILA-CORCOLES A et al., COVID19-related and all-cause mortality among middle-aged and older adults across the first epidemic wave of SARS-COV-2 infection in the region of Tarragona, Spain: results from the COVID19 TARRACO Cohort Study, March-June 2020. https://www.medrxiv.org/content/10.1101/2021.02.02.21251028v1.full.pdf

VIVANCO-HIDALGO RM et al., Incidence of COVID-19 in patients exposed to chloroquine and hydroxychloroquine: results from a population-based prospective cohort in Catalonia, Spain, 2020. Euro Surveill . 2021 Mar;26(9). doi: 10.2807/1560-7917.ES.2021.26.9.2001202.

VIZCARRA P et al., Description of COVID-19 in HIV-infected Individuals: A Single-Centre, Prospective Cohort. Lancet HIV . 2020 May 28;S2352-3018(20)30164-8. doi: 10.1016/S2352-3018(20)30164-8.

VOGEL AB et al., Immunogenic BNT162b vaccines protect rhesus macaques from SARS-CoV-2. *Nature* (2021). https://doi.org/10.1038/s41586-021-03275-y

VOGEL M et al., Low zinc levels at clinical admission associates with poor outcomes in COVID-19. https://www.medrxiv.org/content/10.1101/2020.10.07.20208645v1.full.pdf

VOGEL-GONZALEZ M et al., Low Zinc Levels at Admission Associates with Poor Clinical Outcomes in SARS-CoV-2 Infection. Nutrients 2021 Feb 9;13(2):562. doi: 10.3390/nu13020562.

WANG N et al., Retrospective Multicenter Cohort Study Shows Early Interferon Therapy Is Associated with Favorable Clinical Responses in COVID-19 Patients. Cell Host Microbe 2020 Jul 18;S1931-3128(20)30401-7. doi: 10.1016/j.chom.2020.07.005.

WANG R et al., The vitamin D for COVID-19 (VIVID) trial: A pragmatic cluster-randomized design. Contemp Clin Trials 2020 Oct 9;106176. doi: 10.1016/j.cct.2020.106176.

WANG X et al., The anti-influenza virus drug, arbidol is an efficient inhibitor of SARS-CoV-2 in vitro. Cell Discov. 2020; 6: 28. doi: 10.1038/s41421-020-0169-8

WANG Y et al., Inhibition of the infectivity and inflammatory response of influenza virus by Arbidol hydrochloride in vitro and in vivo (mice and ferret). Biomed Pharmacother 2017 Jul;91:393-401. doi: 10.1016/j.biopha.2017.04.091.

WANG Y et al., Lactoferrin for the treatment of COVID-19 (Review). Exp Ther Med. 2020 Dec; 20(6): 272. doi: 10.3892/etm.2020.9402

WANG Z et al., Clinical Features of 69 Cases With Coronavirus Disease 2019 in Wuhan, China. Clin Infect Dis 2020 Jul 28;71(15):769-777. doi: 10.1093/cid/ciaa272.

WENG C-H et al.,Bacillus Calmette-Guérin vaccination and clinical characteristics and outcomes of COVID-19 in Rhode Island, United States: a cohort study. Epidemiol Infect 2020 Jul 9;148:e140. doi: 10.1017/S0950268820001569.

WESTENDORF K et al., LY-CoV1404 potently neutralizes SARS-CoV-2 variants. https://www.biorxiv.org/content/10.1101/2021.04.30.442182v1.full.pdf

WHO: Drugs to prevent COVID-19: A WHO living guideline. First version, pubslihed 2 March 2021. https://www.who.int/publications/i/item/WHO-2019-nCoV-prophylaxes-2021-1

WHO Solidarity Trial Consortium, Repurposed antiviral drugs for COVID-19; interim WHO SOLIDARITY trial results
https://www.medrxiv.org/content/10.1101/2020.10.15.20209817v1.full.pdf

WIBMER CK et al., SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. https://www.biorxiv.org/content/10.1101/2021.01.18.427166v1.full.pdf

WILCOX CR et al., Association between influenza vaccination and hospitalisation or all-cause mortality in people with COVID-19: a retrospective cohort study. BMJ Open Respir Res 2021 Mar;8(1):e000857. doi: 10.1136/bmjresp-2020-000857.

WILLYARD C, How anti-ageing drugs could boost COVID vaccines in older people. Nature 2020; 586: 352-354

WISEMAN DM et al., Preventing disease after exposure to COVID-19 using hydroxychloroquine: A summary of a protocol for exploratory re-analysis of age and time-nuanced effects.
https://www.medrxiv.org/content/10.1101/2020.08.19.20178376v1.full.pdf

WISEMAN DM et al., (2) Effective post-exposure prophylaxis of Covid-19 is associated with use of hydroxychloroquine: Prospective re-analysis of a public dataset incorporating novel data. https://www.medrxiv.org/content/10.1101/2020.11.29.20235218v1.full.pdf

XIANG Y et al., Exploring drugs and vaccines associated with altered risks and severity of COVID-19: a UK Biobank cohort study of all ATC level-4 drug categories.
https://www.medrxiv.org/content/10.1101/2020.12.05.20244426v1.full.pdf

XU K et al., Clinical Efficacy of Arbidol in Patients with 2019 Novel Coronavirus-Infected Pneumonia: A Retrospective Cohort Study.
https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3542148

YANG A et al., Use of Hydroxychloroquine and Interferon alpha-2b for the Prophylaxis of COVID-19. Med Hypotheses 2020 May 20; 144: 109802.
doi: 10.1016/j.mehy.2020.109802.

YANG A et al. (2), Hydroxychloroquine and Interferons for the Prophylaxis and Early Treatment of Covid-19 Current Clinical Advances. Journal of Clinical and Cellular Immunology. 2020;11(5).

YANG A et al. (3), Potentials of Interferons and Hydroxychloroquine for the Prophylaxis and Early Treatment of COVID-19. J Cell Immunol 2020;2(6):333-340.
doi: 10.33696/immunology.2.063.

YANG C et al., Effectiveness of Arbidol for COVID-19 Prevention in Health Professionals. Front Public Health. 2020 May 29;8:249. doi: 10.3389/fpubh.2020.00249. eCollection 2020.

YANG MJ et al., Influenza Vaccination and Hospitalizations Among COVID-19 Infected Adults. J Am Board Fam Med 2021 Feb;34(Suppl):S179-S182. doi: 10.3122/jabfm.2021.S1.200528.

YATES PA et al., Doxycycline treatment of high-risk COVID-19-positive patients with comorbid pulmonary disease. Ther Adv Respir Dis Jan-Dec 2020;14:1753466620951053. doi: 10.1177/1753466620951053.

YATES PA et al. (2), A Proposed Randomized, Double Blind, Placebo Controlled Study Evaluating Doxycycline for the Prevention of COVID-19 Infection and Disease In Healthcare Workers with Ongoing High Risk Exposure to COVID-19. https://www.medrxiv.org/content/10.1101/2020.05.11.20098525v1.full.pdf

YE K et al., Does Serum Vitamin D Level Affect COVID-19 Infection and Its Severity?-A Case-Control Study. J Am Coll Nutr 2020 Oct 13;1-8. doi: 10.1080/07315724.2020.1826005.

ZEBERG H, PÄÄBO S. The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. *Nature* (2020). https://doi.org/10.1038/s41586-020-2818-3

ZHANG G et al., The basis of a more contagious 501Y.V1 variant of SARS-COV-2. https://www.biorxiv.org/content/10.1101/2021.02.02.428884v1.full.pdf

ZHANG J et al., Potential of Arbidol for Post-exposure Prophylaxis of Covid-19 Transmission. http://www.chinaxiv.org/abs/202002.00065
Curr Med Sci 2020 May 30: doi: 10.1007/s11596-020-2203-3.

ZHANG R et al., COVID-19: Melatonin as a potential adjuvant treatment Life Sci. 2020 Jun 1; 250: 117583. doi: 10.1016/j.lfs.2020.117583

ZHONG J et al., COVID-19 in patients with rheumatic disease in Hubei province, China: a multicentre retrospective observational study. Lancet Rheumatol. 2020;2(9):e557–e564. doi:10.1016/S2665-9913(20)30227-7

ZHOU J et al., Clinical characteristics of re-positive COVID-19 patients in Huangshi, China: A retrospective cohort study. PLoS One 2020, 15(11):e0241896. doi: 10.1371/journal.pone.0241896.

ZHU F, Reply to Comments on 'Co-infection of SARS-CoV-2 and HIV in a patient in Wuhan city, China'. J Med Virol. 2020; doi: 10.1002/jmv.25838.

ZHU Z et al., Arbidol Monotherapy is Superior to Lopinavir/ritonavir in Treating COVID-19. J Infect. 2020. pii: S0163-4453(20)30188-2. doi: 10.1016/j.jinf.2020.03.060

ZIMMERMANN P, CURTIS N, Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. Arch Dis Child. 2020 Dec 1;archdischild-2020-320338. doi: 10.1136/archdischild-2020-320338.

[1]

https://www.deutsche-apotheker-zeitung.de/news/artikel/2020/05/28/deutsche-bcg-praeparate-sind-keine-impfstoffe-gegen-tuberkulose

[2]

https://www.faisafrica.com/news/are-current-putative-covid-19-vaccines-effective-against-the-b-1-351-variants/

28.06.2020 (last update August 22th , 2021) (the project was then stopped because of own non-COVID illness)

Contact:

mpg.2011@t-online.de

R. Steinmeyer

Supplement

List of potential candidates for chemoprophylaxis (PREP and/or PEP).

For reasons of clarity, only a single reference is mentioned for each agent, according to the following preference: finished clinical trial > ongoing clinical trial (register number) > any other paper which suggests directly or *indirectly* a possible prophylactic role of the agent

Ajurvedic Raksha kit

CTRI/2020/08/027316

Alpha 1 Antitrypsin inhalation

AZOUZ MP et al., Alpha 1 Antitrypsin is an Inhibitor of the SARS-CoV2-Priming Protease TMPRSS2. https://www.biorxiv.org/content/10.1101/2020.05.04.077826v1

Ambroxol

OLALEYE OA et al., Ambroxol Hydrochloride Inhibits the Interaction between Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein's Receptor Binding Domain and Recombinant Human ACE2.
https://www.biorxiv.org/content/10.1101/2020.09.13.295691v1.full.pdf

CARPINTEIRO A et al., Inhibition of acid sphingomyelinase by ambroxol prevents SARS-CoV-2 entry into epithelial cells. J Biol Chem 2021 Apr 22;100701. doi: 10.1016/j.jbc.2021.100701.

Ambroxol + Luteolin

NABAVI SF et al., Rationale for Effective Prophylaxis Against COVID-19 Through Simultaneous Blockade of Both Endosomal and Non-Endosomal SARS-CoV-2 Entry into Host Cell. Clin Transl Sci 2021 Jan 6. doi: 10.1111/cts.12949

AOIM-Z tablets (herbal)

CTRI/2020/05/025222

ARGOVIT (special formulation of colloidal silver with hydrolyzed collagen approved in Russia)

ALMANZA-REYES H et al., Evaluation of silver nanoparticles for the prevention of SARS-CoV-2 infection in health workers: in vitro and in vivo.
https://www.medrxiv.org/content/10.1101/2021.05.20.21256197v1.full.pdf

RCT with 231 COVID-exposed HCWs in a hospital in Mexiko; 114 participants used ARGOVIT for mouthwash and/or oral spray and also for nasal administration, whereas 117 controls were advised to continue to use their common mouthwash and/or nose spray (not specified). After 9 weeks of the intervention between April and June 2020, there were 1.8 % confirmed COVID-19 infections in the ARGOVIT group compared to 28.2 % in the control group ($p = 0.000$; absolute risk reduction: 26.4 %; relative risk reduction: 93.6 %). Since not all participants fulfilled the complete procedure (2 x mouthwash, 2 x gargle, 1 x nasal administration per day), a regression analysis calculated the efficacy to 84.8 %. On the other hand, another regression analysis estimated that the risk of infection is 1 : 109 if someone performs the “full” procedure.

Interestingly, the risk of any symptomatic respiratory infection was only reduced by a relative risk reduction of 48.6 % (18.4 % in the ARGOVIT group and 35.6 % in controls).

The procedure was well tolerated and there were no side effects. Before the RCT was started, the antiviral activity of ARGOVIT was studied on Vero cells and found to inhibit viral replication in a dose-dependent manner up to 80 %. But the mechanism of the antiviral action of colloidal silver is not well understood so far.

Meanwhile, Vero cells are recognized as an unsuitable assay, because many results from Vero cells could not be replicated in other cell-based assays and/or translated in clinical reality. Calu-3 cells and particularly human respiratory epithelium cells should be preferred. Nevertheless, these experiments were performed in early 2020 at a time when the use of Vero cells assay was still standard (though the study was published as a preprint one year later).

The medical use of colloidal silver is highly contested, though it is wellknown as an antiseptic before the arrival of antibiotics in the 20th century. Problems concern long-term safety, resorption from skin or mucous membranes, accumulation in the body (including discoloration of the skin), but also accumulation in internal organs; hints for a genotoxic and

a carcinogenic potential (sarcomas in rats). In the Mexican ARGOVIT RCT, the formulation was used for nine weeks.

The legal situation for medical or cosmetic use of colloidal silver differs between countries. The uncertainties seem to stem preferentially from a lack of research on safety issues than real data that point to dangers (for Germany, see BfR 10/2011). Moreover, external use on the epidermis, use on mucosal membranes (with some retention in the body) like mouthwash, throat spray or nasal administration, inhalation of solutions with colloidal silver, and drinking of water with colloidal silver may differ in their risk profiles.

Of note, the ARGOVIT™ formulation from Russia differs from common water with colloidal silver because it also contains hydrolyzed collagen that was found to reduce cytotoxity. That said, ARGOVIT may be less "risky" than conventional aqueous formulations with colloidal silver. Unfortunately, there is so far very few literature about medical use of ARGOVIT and particularly its safety. ARGOVIT is registered as an oral and nasal hygiene product since 2015.

Because of that special formulation of ARGOVIT™, the Argovit RCT doesn't allow any conclusions whether common water with colloidal silver may be also effective in the prevention of COVID-19.

Arsenicum album 30 C (homoeopathic)

CTRI/2020/06/026056

ASA-20 (ayurvedic: Ayush kwath, Samsamani vati, Anu taila)

CTRI/2020/06/026055

Ashwagandha

CTRI/2020/08/027163

Astrodimer (nasal spray or inhalation)

https://www.biorxiv.org/content/10.1101/2020.08.20.260190v1.full.pdf

AT-527 (Roche)

(in development for therapy, PREP and PEP)

Ayurveda spice mix tablet

CTRI/2020/07/026674

Ayush Khwat (ayurvedic)

CTRI/2020/06/025779

AZD7442 (antibodies)

Azithromycin

NCT04369365

Azithromycin + HCQ

NCT04344379

Baicalein

LIU H et al., *Scutellaria baicalensis* extract and baicalein inhibit replication of SARS-CoV-2 and its 3C-like protease *in vitro.* https://www.biorxiv.org/content/10.1101/2020.04.10.035824v1

Baicalin

SU H et al., Discovery of baicalin and baicalein as novel, natural product inhibitors of SARS-CoV-2 3CL protease in vitro.
https://www.biorxiv.org/content/10.1101/2020.04.13.038687v1

BCG vaccine

NCT04348370

For VPM1002: NCT04387409

For RUTI vaccine: NCT04453488

Berberine

VARGHESE FS et al., Berberine and obatoclax inhibit SARS-CoV-2 replication in primary human nasal epithelial cells in vitro.
https://www.biorxiv.org/content/10.1101/2020.12.23.424189v1.full.pdf

Beta-chitosan

ALITONGBIEKE G et al., Study on β-Chitosan against the binding of SARS-CoV-2S-RBD/ACE2.
https://www.biorxiv.org/content/10.1101/2020.07.31.229781v3

Beta-glucans

GELLER A et al. Could the Induction of Trained Immunity by β-Glucan Serve as a Defense Against COVID-19? Front Immunol. 2020 Jul 14;11:1782. doi: 10.3389/fimmu.2020.01782. eCollection 2020.

Biological response modifier glucan (BRMG) secreted by the black yeast *Aureobasidium pullulans* AFO-202

IKEWAKI N et al., Biological response modifier glucan through balancing of blood glucose may have a prophylactic potential in COVID-19 patients. *J Diabetes Metab Disord* (2020). https://doi.org/10.1007/s40200-020-00664-4

Biomodulina (a polypeptide thymic factor in InmunyVital®)

RPCEC00000310

Bromelain

SAGAR S et al., Bromelain Inhibits SARS-CoV-2 Infection in VeroE6 Cells
https://www.biorxiv.org/content/10.1101/2020.09.16.297366v1

Bromhexine

NCT04340349 (combined with HCQ)

For bromhexine alone:
HABTEMARIAM S et al., Possible use of the mucolytic drug, bromhexine hydrochloride, as a prophylactic agent against SARS-CoV-2 infection based on its action on the Transmembrane Serine Protease 2. Pharmacol Res. 2020 Jul; 157: 104853. doi: 10.1016/j.phrs.2020.104853

MIKHAYLOV et al. (see above).

Bryonia alba 30 C (homoeopathic)

CTRI/2020/06/025558

Buformin (biguanide) inhalation

LEHRER S, Inhaled biguanides and mTOR inhibition for influenza and coronavirus (Review). World Acad Sci J 2020 May;2(3):1. doi: 10.3892/wasj.2020.42. Epub 2020 Mar 29.

Calcitriol (see also vitamin D)

MOK CK et al. Calcitriol, the active form of vitamin D, is a promising candidate for COVID-19 prophylaxis. https://www.biorxiv.org/content/10.1101/2020.06.21.162396v1.full.pdf

Cannabis

NCT03944447 (OMNI-CAN)

Carrageenan

VEGA JC et al., Iota carrageenan and xylitol inhibit SARS-CoV-2 in Vero cell culture https://www.biorxiv.org/content/10.1101/2020.08.19.225854v1.full.pdf

NCT04521322 (nasal spray)

Chyawanprash (ayurvedic)

CTRI/2020/05/025275

GUPTA A et al, Chyawanprash for the prevention of COVID-19 infection among healthcare workers: A Randomized Controlled Trial. https://www.medrxiv.org/content/10.1101/2021.02.17.21251899v1.full.pdf

Comment: That RCT (with 199 front-line HCWs) was underpowered, since there were no infections both in the study group and in the control group during the 30 days of the intervention. During four months of follow-up, there were 4 symptomatic COVID cases in the control group (one of them needed hospitalization), whereas there were only 2 cases of asymptomatic PCR-confirmed infections in the intervention group. This may indicate a long term effect of the immunomodulatory and adaptogenic ayurvedic formulation, but a larger and longer lasting trial is needed to draw definite conclusions.

Clofazimine

YUAN S et al., Clofazimine broadly inhibits coronaviruses including SARS-CoV-2. Nature 2021; 593: 418 – 423 *(including the prophylactic and therapeutic efficacy in a Syrian hamster model).*

ColdZyme Mouth spray

POSCH W et al., ColdZyme Maintains Integrity in SARS-CoV-2-Infected Airway Epithelia. mBio 2021 Apr 27;12(2):e00904-21. doi: 10.1128/mBio.00904-21.

ColdZyme is composed of glycerol, water, buffer, $CaCl_2$, menthol, and trypsin from the Atlantic cod. It is available as mouth spray *"and forms a physical barrier that interferes with entry of common cold viruses, which subsequently become trapped and inactivated".* POSCH et al. studied ColdZyme on standardized three-dimensional (3D) *in vitro* models mimicking the *in vivo* human airway epithelium and nasal epithelium.

The protective effect from SARS-CoV-2 infection was observable for *"up to 2 h following application of the ColdZyme mouth spray to the apical side of fully differentiated respiratory epithelia. A clinical trial evaluation of ColdZyme in response to experimentally induced common cold applied 6 doses of the mouth spray daily, which corresponds to our time window of 1 to 2 h of effectiveness* (clinical trial: COLDPREVII, https://clinicaltrials.gov/ct2/show/NCT02479750)." (POSCH et al.).

However, calculated from their figure 1 d, viral infection was reduced only by one order of magnitude, measured by the number of infected cells.

The study found also a protective effect of ColdZyme mouth spray against SARS-CoV-2 infection in nasal epithelia by applying the ColdZyme mouth spray. However, this was associated with tissue damage of the thinner nasal epithelium; POSCH et al. assume that tissue damage could rely on the power of the spray appropriate for the oral cavity. *("Despite significantly decreasing SARS-CoV-2 viral loads in nasal epithelia, higher IC C3 production and tissue disruption were found by image analyses following ColdZyme treatment").* They gave no recommendation whether Coldzyme (in the currently available spray bottle) may be also used as nasal spray (in addition to its use as throat spray), or whether this should be avoided.

Moreover, POSCH et al. found that ColdZyme mouth spray dampens innate immune activation upon SARS-CoV-2 infection of the nasal epithelial cultures. One may ask whether dampening of the innate immune reponse in the area of the body where the virus enters is desirable, particularly in the early (viral) phase of the disease or for prophylaxis? Such a dampening effect may improve symptoms of common cold and may be welcome in case of common cold, but it might be counterproductive in case of a recent inoculation or early infection with SARS-CoV-2.

Curcumin

CTRI/2020/07/026820

DHAR S, BHATTACHARJEE P, Promising role of curcumin against viral diseases emphasizing COVID-19 management: A review on the mechanistic insights with reference to host-pathogen interaction and immunomodulation. J Funct Foods 2021 Apr 20;104503. doi: 10.1016/j.jff.2021.104503.

THIMMULAPPA RK et al., Antiviral and immunomodulatory activity of curcumin: A case for prophylactic therapy for COVID-19. Heliyon. 2021 Feb;7(2):e06350. doi: 10.1016/j.heliyon.2021.e06350.

But: possible increase of ACE2 expression, possible stimulation of IL-6 and TNF-alpha (contradictory results from different studies):

NUGRAHA RV et al., Traditional Herbal Medicine Candidates as Complementary Treatments for COVID-19: A Review of Their Mechanisms, Pros and Cons. Evid Based Complement Alternat Med. 2020; 2020: 2560645. doi: 10.1155/2020/2560645

Cyclotide complex herbal syrup

IRCT20160131026298N4

Disulfiram

FILLMORE N et al., Disulfiram associated with lower risk of Covid-19: a retrospective cohort study. https://www.medrxiv.org/content/10.1101/2021.03.10.21253331v1.full.pdf

Doxycycline

YATES PA et al. (2), A Proposed Randomized, Double Blind, Placebo Controlled Study Evaluating Doxycycline for the Prevention of COVID-19 Infection and Disease In Healthcare Workers with Ongoing High Risk Exposure to COVID-19. https://www.medrxiv.org/content/10.1101/2020.05.11.20098525v1.full.pdf

Drug-free nasal spray AM-301

FAIS F et al., Drug-free nasal spray as a barrier against SARS-CoV-2 infection: safety and efficacy in human nasal airway epithelia.
https://www.biorxiv.org/content/10.1101/2021.07.12.452021v1.full.pdf

(99 % efficient in an *in vitro* study of 3 D human primary nasal epithelial model)

Ebselen

MALLA TN et al., Ebselen Reacts with SARS Coronavirus-2 Main Protease Crystals. doi: https://doi.org/10.1101/2020.08.10.244525

Echinacea

JAWAD M et al., Safety and efficacy profile of Echinacea purpurea to prevent common cold episodes: a randomized, double-blind. Placebo-Controlled Trial Evid-Based Complement Alternative Med. 2012;2012:841315.

Echinaforce

SIGNER J et al., In vitro virucidal activity of Echinaforce®, an Echinacea purpurea preparation, against coronaviruses, including common cold coronavirus 229E and SARS-CoV-2. Virol J 2020 Sep 9;17(1):136. doi: 10.1186/s12985-020-01401-2.

EGCG, Green tea polyphenols, theaflavins

MHATRE S et al., Antiviral activity of green tea and black tea polyphenols in prophylaxis and treatment of COVID-19: A review. Phytomedicine . 2020 Jul 17;153286. doi: 10.1016/j.phymed.2020.153286.

STOROZHUK M, COVID -19: could green tea catechins reduce the risks?
https://www.medrxiv.org/content/10.1101/2020.10.23.20218479v1.full.pdf

OHGIGANI E et al., Significant inactivation of SARS-CoV-2 by a green tea catechin, a catechin-derivative and galloylated theaflavins in vitro.
https://www.biorxiv.org/content/10.1101/2020.12.04.412098v1.full.pdf

LEBLANC EV, COLPITTS CC, The green tea catechin EGCG provides proof-of-concept for a pan-coronavirus entry inhibitor.
https://www.biorxiv.org/content/10.1101/2021.06.21.449320v1.full.pdf

LEBLANC and COLPITTS showed that EGCG inhibits infectivity of murine, bat, and human CoVs by blocking cell surface binding. This suggests that EGCG inhibits *"a conserved step in CoV attachment, such as initial binding to glycans. These findings demonstrate that blocking primary attachment is a potential antiviral strategy to prevent infection by diverse CoVs"* Unfortunately, *"EGCG does not accumulate at high levels, is unstable under physiological conditions, and is rapidly metabolized".* Thus the authors suggest EGCG as the basis for the development of pharmacological entry inhibitors as an effective antiviral strategy to protect against future coronavirus infections.

As "Previfenon": NCT04446065

EGCG against new virus variants:

LIU J et al., Epigallocatechin Gallate from Green Tea Effectively Blocks Infection of SARS-CoV-2 and New Variants by Inhibiting Spike Binding to ACE2 Receptor. https://www.biorxiv.org/content/10.1101/2021.03.17.435637v1.full.pdf

EIDD-2801 (Molnupiravir: broad spectrum antiviral in phase II clinical trials for treatment)

WAHL A et al., Acute SARS-CoV-2 Infection is Highly Cytopathic, Elicits a Robust Innate Immune Response and is Efficiently Prevented by EIDD-2801. Res Sq 2020 Sep 24;rs.3.rs-80404. doi: 10.21203/rs.3.rs-80404/v1.

WAHL G et al., SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801 Nature 2021 Feb 9. doi: 10.1038/s41586-021-03312-w.

ABDELNABI R et al., Molnupiravir (EIDD-2801) inhibits SARS-CoV2 replication in Syrian hamsters model. https://www.biorxiv.org/content/10.1101/2020.12.10.419242v1.full.pdf

Favipiravir

NCT04448119 (CONTROL-COVID)

Fisetin

WILLYARD C, How anti-ageing drugs could boost COVID vaccines in older people. Nature 586**,** 352-354 (2020)

Flavonol morin

GUPTA A et al., Flavonol morin targets host ACE2, IMP-α, PARP-1 and viral proteins of SARS-CoV-2, SARS-CoV and MERS-CoV critical for infection and survival: a computational analysis. J Biomol Struct Dyn 2021 Feb 1;1-32. doi: 10.1080/07391102.2021.1871863.

Folic acid

PACTR202005599385499

see also: KAUR et al.

Fucoidan (extract from brown algae)

SONG S et al., Inhibitory activities of marine sulfated polysaccharides against SARS-CoV-2. Food Funct 2020 Sep 23;11(9):7415-7420. doi: 10.1039/d0fo02017f.

GABA (gamma-aminobutyric acid) and homotaurine

TIAN J et al., GABA administration prevents severe illness and death following coronavirus infection in mice. https://www.biorxiv.org/content/10.1101/2020.10.04.325423v1.full.pdf

In experiments with mice, gamma-aminobutyric acid (GABA) proved to be very successful if started to be administered immediately after inoculation with a mouse coronavirus (MHV-1). Whereas > 60 % of control mice succumbed to the infection, infected mice that received GABA directly after infection became only mildly ill; all of them recovered. If GABA started three days after inoculation, i.e. after appearance of illness in mice, it was still advantageous because it reduced the severity of the disease score and increased the frequency of recovery (only 11 % died).

Homotaurine is regarded as an alternative to GABA. GABA-receptor-agonists like GABA and homotaurine act anti-inflammatory, but not as antivirals. Thus the authors were surprised about the much more pronounced effect of GABA when given immediately after inoculation, much better than if GABA was started after 3 days. This is a paradoxon because anti-inflammatory instead of antiviral effects are suggested to be unwanted in the earliest phase of the coronavirus disease. TIAN et al. suppose that GABA may have an indirect antiviral effect: the activation of GABA receptors may limit the influx of calcium ions into the

epithelial cells, but many viruses (including coronaviruses) elevate intracellular calcium ion concentration to enhance the replication of the virus.

However, the authors warn:
"until clinical trials are completed and GABA and/or homotaurine are approved for use in the treatment of COVID-19 by relevant governing bodies, GABA and homotaurine should not be consumed by COVID-19 patients as they may pose health risks, such as dampening beneficial immune or physiological responses." Of note, this study was not about COVID-19 but another (murine) corona virus. Until October 5th, no clinical trials or registered with GABA or homotaurine in the context of COVID-19 were reported.

Ginseng

ADUSEI-MENSAH F et al., Prevention and Control of Acute Respiratory Viral Infections in Adult Population: A Systematic Review and Meta-Analysis on Ginseng-Based Clinical Trials. https://www.medrxiv.org/content/10.1101/2021.07.23.21260970v1.full.pdf

Based on 5 RCTs in the context of other acute respiratory illnesses (before COVID-19), the authors found that ginseng reduced the risk of ARI bei 38 % and shortened its duration by 3 days. However, it must be noted that this study is not specifically about COVID-19.

Glutathione, inosine and potassium chloride for inhalation (5 min inhalation 4 x a day)

ISRCTN34160010 (see DUBINA et al.)

Glycyrrhizin

VAN DE SAND L et al., Glycyrrhizin effectively neutralizes SARS-CoV-2 in vitro by inhibiting the viral main protease.

PASALI D et al., Glycyrrhizin for topical use and prophylaxis of COVID-19: an interesting pharmacological perspective. J Biol Regul Homeost Agents Jan-Feb 2021;35(1 Suppl. 2):15-19. doi: 10.23812/21-1supp2-4.

Grapefruit seed extract: *see* Xlear

Griffithsin

MILLET J et al., Middle East respiratory syndrome coronavirus infection is inhibited by griffithsin. Antiviral Res. 2016 Sep; 133: 1–8. doi: 10.1016/j.antiviral.2016.07.011

Hesperidine

HAGGAG YA et al., Is hesperidin essential for prophylaxis and treatment of COVID-19 Infection? Medical Hypotheses 144; 2020: 109957. https://doi.org/10.1016/j.mehy.2020.109957

BITA A et al., Natural and semisynthetic candidate molecules for COVID-19 prophylaxis and treatment. Rom J Morphol Embryol 2020;61(2):321-334. doi: 10.47162/RJME.61.2.02.

Hydroxychloroquine

RAJASINGHAM R et al., Hydroxychloroquine as pre-exposure prophylaxis for COVID-19 in healthcare workers: a randomized trial. https://www.medrxiv.org/content/10.1101/2020.09.18.20197327v1.full.pdf

Hypericum perforatum

BAJRAI LH et al., In vitro screening of anti-viral and virucidal effects against SARS-CoV-2 by Hypericum perforatum and Echinacea. https://www.biorxiv.org/content/10.1101/2021.01.11.426295v1.full.pdf

IMM-101 (see *Mycobacterium obuense)*

Influenza vaccination

See BAI L et al., DEBISARUN et al.

Ingarin (a broad-spectrum antiviral for prophylaxis and treatment of flu)

MALIK I et al., Ingavirin might be a promising agent to combat Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2). Ceska Slov Farm Summer 2020;69(3):107-111.

Inosine-glutathione inhalation

See above (DUBINA et al.)

Interferon alpha 1 b spray

CHICTR2000030013

Interferon alpha 2 b

YANG A et al., Use of Hydroxychloroquine and Interferon alpha-2b for the Prophylaxis of COVID-19. Med Hypotheses 2020 May 20; 144: 109802.
doi: 10.1016/j.mehy.2020.109802.

Isoprenosin

NCT04360122

IPV (inactivated polio vaccine)

NCT04639375

Ivermectin

NCT04384458 and others

Lablab purpureus (intranasal administration; successful *in vivo* in mice)

LIU YM, A Carbohydrate-Binding Protein from the Edible Lablab Beans Effectively Blocks the Infections of Influenza Viruses and SARS-CoV-2. Cell Rep 2020 Aug 11;32(6):108016. doi: 10.1016/j.celrep.2020.108016. Epub 2020 Jul 24.

Lactobacillus coryniformis K8

NCT04366180

Lactoferrin

NCT04526821 (bovine)

CHANG R et al., Lactoferrin as potential preventative and adjunct treatment for COVID-19. Int J Antimicrob Agents 2020 Jul 30;106118. doi: 10.1016/j.ijantimicag.2020.106118.

Levamisol

NCT04360122

Liposomal lactoferrin

SERRANO G et al., Liposomal Lactoferrin as Potential Preventative and Cure for COVID-19. Int J Res Health Sci. 2020;8(1):8-15

Lopinavir/Ritonavir

NCT04328285 and others (PREP and PEP)

Manremyc (food supplement; *Mycobacterium s. manresensis*)

NCT04452773

For safety of heat-killed *Mycobacterium setense manresensis* as a novel food, see EFSA: https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5824

Measles vaccine (or MMR)

NCT04333732
(CROWN Coronation – the largest prophylactic trial ever planned for COVID 19 was originally designed to study the effect of different doses of chloroquine and switched then from chloroquine to one dose of MMR vaccine. The size of the trial was reduced from 55.000 to 30.000 participants because MMR vaccine doesn't need several trial arms for different doses, in contrast to chloroquine).

Mefloquin

EUCTR2020-01194-69-ES

Melatonin

NCT04353128 (MeCOVID trial)

Metformin

NCT04510194

Methylene Blue (local?)

CAGNO V et al., Methylene Blue has a potent antiviral activity against SARS-CoV-2 in the absence of UV-activation in vitro.
https://www.biorxiv.org/content/10.1101/2020.08.14.251090v1

Miniprotein LCB1v1.3 (systemic or nasal administration) (PREP, PEP, treatment)

CASE JB et al., Ultrapotent miniproteins targeting the SARS-CoV-2 receptor-binding domain protect against infection and disease. Cell Host Microbe 2021 Jun 24;S1931-3128(21)00286-9. doi: 10.1016/j.chom.2021.06.008.

Mitoquinone

OUYANG L, GONG J, Mitochondrial-targeted ubiquinone: A potential treatment for COVID-19. Med Hypotheses. 2020 Nov; 144: 110161. doi: 10.1016/j.mehy.2020.110161

Monoclonal antibodies

SAJNA KV, KAMAT S, Antibodies at work in the time of severe acute respiratory syndrome coronavirus 2. Cytotherapy. 2020 Aug 31:S1465-3249(20)30846-X. doi: 10.1016/j.jcyt.2020.08.009.

COHEN MS, Monoclonal Antibodies to Disrupt Progression of Early Covid-19 Infection. N Engl J Med 2021 Jan 21;384(3):289-291. doi: 10.1056/NEJMe2034495.

Monoclonal antibody inhalation

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.

PIEPENBRINK MS et al., Therapeutic activity of an inhaled potent SARS-CoV-2 neutralizing human monoclonal antibody in hamsters.
https://www.biorxiv.org/content/10.1101/2020.10.14.339150v1.full.pdf

MP1032 (a small molecule)

SCHUMANN S et al., Immune-Modulating Drug MP1032 with SARS-CoV-2 Antiviral Activity In Vitro: A potential Multi-Target Approach for Prevention and Early Intervention Treatment of COVID-19. Int J Mol Sci 2020 Nov 20;21(22):8803. doi: 10.3390/ijms21228803.

Mucodentol gel

IRCT20090304001739N3

Mycobacterium obuense (IMM-101)

NCT04442048
see: KLEEN et al.

Mycobacterium W suspension

NCT04353518
see: JAISWAL et al.

N-Acetylcysteine

DE FLORA S et al., Rationale for the use of N-acetylcysteine in both prevention and adjuvant therapy of COVID-19. FASEB J 2020 Aug 11;10.1096/fj.202001807. doi: 10.1096/fj.202001807.

Nafamostat *(but not:* camostat), intranasal dosing twice daily

Highly effective in a Syrian hamster model:

NEARY M et al., Evaluation of intranasal nafamostat or camostat for SARS-CoV-2 chemoprophylaxis in Syrian golden hamsters.
https://www.biorxiv.org/content/10.1101/2021.07.08.451654v1.full.pdf

N-dihydrogalactochitosan

WEISS CM et al., N-dihydrogalactochitosan reduces mortality in a lethal mouse model of SARS-CoV-2. https://www.biorxiv.org/content/10.1101/2021.08.10.455872v1.full.pdf

Neem (Azadirhachta indica)

CTRI/2020/07/026560 (see NESARI et al.)

Niclosamide-lysozyme particles (for inhalation) (efficacious in lethal murine infection models)

BRUNAUGH AD et al., Broad-spectrum, patient-adaptable inhaled niclosamide-lysozyme particles are efficacious against coronaviruses in lethal murine infection models. https://www.biorxiv.org/content/10.1101/2020.09.24.310490v1.full.pdf

Nicotine

FARSALINOS K. Nicotine and SARS-CoV-2: COVID-19 may be a disease of the nicotinic cholinergic system; Toxicol Rep. 2020 Apr 30, doi: 10.1016/j.toxrep.2020.04.012. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7192087/

***Nigella sativa* seeds**

ISLAM MN et al., Revisiting pharmacological potentials of Nigella sativa seed: A promising option for COVID-19 prevention and cure. Phytother Res 2020 Oct 12. doi: 10.1002/ptr.6895.

SHIRVANI H et al., Potential role of *Nigella sativa* supplementation with physical activity in prophylaxis and treatment of COVID-19: a contemporary review. Sport Sci Health 2021 May 28;1-6. doi: 10.1007/s11332-021-00787-y.

Nitazoxanid

NCT04359680 and others

NO (nitric oxid) inhalation

GIANNI S et al., Nitric oxide gas inhalation to prevent COVID-2019 in healthcare providers. https://www.medrxiv.org/content/10.1101/2020.04.05.20054544v1

AKABERI D et al., Mitigation of the replication of SARS-CoV-2 by nitric oxide in vitro. Redox Biol 2020 Sep 21;37:101734. doi: 10.1016/j.redox.2020.101734. Online ahead of print.

NORS (NO-releasing suspension) (local)

NCT04337918

OM 85

ACTRNI2620000473965

Oral polio vaccine

NCT04540185, NCT04445428

Outer Membrane Vesicle (OMV) complex contained in VA-MENGOC-BC

RPCEC00000314

Peginterferon lambda 1a

NCT04344600

Povidone-iodine (**local prophylaxis,** mentioned in the text above)

NCT04364802

Prevengho-Vir (homoeopathic; Cuba)

RPCEC00000312

Probenecid

MURRAY J et al., Probenecid Inhibits SARS-CoV-2 Replication In Vivo and In Vitro. https://www.biorxiv.org/content/10.1101/2021.05.21.445119v1.full.pdf

(This study included the use of probenecid for PREP in hamsters; probenecid was given 24 hours before inoculation).

Probiotics

INFUSINO F et al., Diet Supplementation, Probiotics, and Nutraceuticals in SARS-CoV-2 Infection: A Scoping Review. Nutrients 2020; 12(6):E1718. doi: 10.3390/nu12061718.

GOHIL K et al., Probiotics in the prophylaxis of COVID-19: something is better than nothing. *3 Biotech* volume 11, Article number: 1 (2021)

See also: LORDAN R et al.

Lactocare: IRCT20101020004976N6

Probiotics based on commensal bacteria with α-Gal epitopes to modify the microbiota and increase the α-Gal-induced protective immune response and reduce the severity of COVID-19.

URRA JM et al., The antibody response to the glycan α-Gal correlates with COVID-19 disease symptoms. J Med Virol 2020 Oct 3. doi: 10.1002/jmv.26575.

Prolectin-M = (1-6)-Alpha-D-Mannopyranose (food supplement)

SIGAMANI A et al., Galectin antagonist use in mild cases of SARS-CoV-2 cases; pilot feasibility randomised, open label, controlled trial. https://www.medrxiv.org/content/10.1101/2020.12.03.20238840v1.full.pdf

Prunella vulgaris extract

https://www.biorxiv.org/content/10.1101/2020.08.28.270306v1.full.pdf

Pterostilbene

ter ELLEN BM et al., Resveratrol And Pterostilbene Potently Inhibit SARS-CoV-2 Infection In Vitro. https://www.biorxiv.org/content/10.1101/2020.09.24.285940v1.full.pdf

PUFAs (polyunsaturated fatty acids)

WANG H et al., The role of high cholesterol in age related COVID19 lethality. https://www.biorxiv.org/content/10.1101/2020.05.09.086249v2

see also NCT04483271 for Omega 3

PUL-042 Inhalation (local)

NCT04313023

Quercetin

COLUNGA BIANCATELLI RML et al., Quercetin and Vitamin C: An Experimental, Synergistic Therapy for the Prevention and Treatment of SARS-CoV-2 Related Disease (COVID-19). Front Immunol. 2020 Jun 19;11:1451. doi: 10.3389/fimmu.2020.01451. eCollection 2020.

Proposed doses:

	Quercetin:	Vitamin C:
Prophylaxis	250–500 mg BID	500 mg BID
Mild cases	250–500 mg BID	500 mg BID
Severe Cases	500 mg BID	3 gr q6 for 7 days

Recombinant human ACE2-Fc

ZHANG Z et al., Potent prophylactic and therapeutic efficacy of recombinant human ACE2-Fc against SARS-CoV-2 infection in vivo. Cell Discov 2021 Aug 12;7(1):65. doi: 10.1038/s41421-021-00302-0.

Remdesivir (for PEP if made available for inhalation):

WILLIAMSON B. et al., Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. https://www.biorxiv.org/content/10.1101/2020.04.15.043166v1

MOON C et al., Development of Remdesivir as a Dry Powder for Inhalation by Thin Film Freezing.https://www.biorxiv.org/content/10.1101/2020.07.26.222109v1.full.pdf

RENESSANS (from kelp algae)

"The medicinal preparation "RENESSANS" comprises components in the following ratio thereof: 0.4-2.0% by mass of iodine, 0.8-4.0% by mass of potassium iodide, 10.0-40.0% by mass of starch, 0.4-2.0% by mass of ascorbic acid, 1.2-4.8% by mass of glucose, 0.3-1.8% by mass of sodium chloride, with the remainder being purified water. A pharmaceutically effective quantity of the preparation is administered intravenously, intramuscularly or perorally." Source: https://patents.google.com/patent/WO2012158002A1/en

ALTAF I et al., An in vitro assessment of anti-SARS-CoV-2 activity of oral preparations of iodine complexes (RENESSANS).
https://www.biorxiv.org/content/10.1101/2020.06.29.171173v1

NAWAZ M et al., An assessment of efficacy of Iodine complex (Renessans) against SARS-CoV-2 in non-human primates (Rhesus macaque).
https://www.biorxiv.org/content/10.1101/2020.11.17.377432v1.full.pdf

Respiratory Detox Shot (herbal TCM liquid)

HETRICK B et al., A traditional Chinese medicine, Respiratory Detox Shot (RDS), inhibits the infection of SARS-CoV, SARS-CoV-2, and the Influenza A virus in vitro. https://www.biorxiv.org/content/10.1101/2020.12.10.420489v1.full.pdf

Resveratrol

ter ELLEN BM et al., Resveratrol And Pterostilbene Potently Inhibit SARS-CoV-2 Infection In Vitro. https://www.biorxiv.org/content/10.1101/2020.09.24.285940v1.full.pdf

Rosocyanin (a curcumin-boron-complex)

BITA A et al., Natural and semisynthetic candidate molecules for COVID-19 prophylaxis and treatment. Rom J Morphol Embryol 2020;61(2):321-334. doi: 10.47162/RJME.61.2.02.

RTB101

NCT04409327

Sea cucumber sulfated polysaccharide

SONG S et al., Inhibitory activities of marine sulfated polysaccharides against SARS-CoV-2. Food Funct 2020 Sep 23;11(9):7415-7420. doi: 10.1039/d0fo02017f.

Siddha formulations (ayurvedic)

CTRI/2020/07/026673

Silibinin/silymarin

BOSCH-BARRERA J et al., Silibinin and SARS-CoV-2: Dual Targeting of Host Cytokine Storm and Virus Replication Machinery for Clinical Management of COVID-19 Patients. J Clin Med 2020 Jun 7;9(6):1770. doi: 10.3390/jcm9061770.

Sodium chlorite solution (local)

KARNIK-HENRY MS, Acidified Sodium Chlorite Solution: A Potential Prophylaxis to Mitigate Impact of Multiple Exposures to COVID-19 in Frontline Healthcare Providers. Hosp Pract (1995). 2020 Jun 4. doi: 10.1080/21548331.2020.1778908.
https://www.tandfonline.com/doi/full/10.1080/21548331.2020.1778908

Spermidine

GASSEN NC et al., Analysis of SARS-CoV-2-controlled autophagy reveals spermidine, MK-2206, and niclosamide as putative antiviral therapeutics.
https://www.biorxiv.org/content/10.1101/2020.04.15.997254v1.full.pdf

Spirulina

JOSEPH J et al., Green tea and Spirulina extracts inhibit SARS, MERS, and SARS-2 spike pseudotyped virus entry in vitro.
https://www.biorxiv.org/content/10.1101/2020.06.20.162701v1.full.pdf

Sulforaphane

ORDONEZ AA et al., Sulforaphane exhibits in vitro and in vivo antiviral activity against pandemic SARS-CoV-2 and seasonal HCoV-OC43 coronaviruses.
https://www.biorxiv.org/content/10.1101/2021.03.25.437060v1.full.pdf

Suramin

https://www.biorxiv.org/content/10.1101/2020.08.28.270306v1.full.pdf

Tafenoquine

DOW G et al., Tafenoquine inhibits replication of SARS-Cov-2 at pharmacologically relevant concentrations in vitro. https://www.biorxiv.org/content/10.1101/2020.07.12.199059v1

TaibUVID (*Nigella sativa*, chamomile, natural honey)

EL SAYED SM et al., Promising preventive and therapeutic effects of TaibUVID nutritional supplements for COVID-19 pandemic: towards better public prophylaxis and treatment (A retrospective study). Am J Blood Res 2020 Oct 15;10(5):266-282.

Tenofovir/Emtricitabin

NCT04334928

***[Thymosin]:* no prophylactic effect?**

LIU X et al., Analysis of the prophylactic effect of thymosin drugs on COVID-19 for 435 medical staff: A hospital-based retrospective study. J Med Virol 2020; doi: 10.1002/jmv.26492

BERSANELLI M et al.,The right immune-modulation at the right time: thymosin α1 for prevention of severe COVID-19 in cancer patients. Future Oncol 2021 Feb 4. doi: 10.2217/fon-2020-0754.

Ongoing trial: NCT04428008

Tinospora cordifolia (ayurvedic: Guduchi Ghan Vati)

KUMAR A et al., A Retrospective Study on Efficacy and Safety of Guduchi Ghan Vati for Covid-19 Asymptomatic Patients.
https://www.medrxiv.org/content/10.1101/2020.07.23.20160424v1.full.pdf

CTRI/2020/08/027034

Tranexamic acid (TXA)

NCT04550338

Twakadi Tea

CTRI/2020/07/026925

Trehalose

SHETTY R et al., Potential Ocular and Systemic COVID-19 prophylaxis Approaches for Healthcare Professionals, Indian J Ophthalmol 2020; 68(7):1349-1356. doi: 10.4103/ijo.IJO_1589_20.

for systemic (oral) administration of Trehalose:

MARTINON D et al., Potential Fast COVID-19 Containment With Trehalose. Front Immunol 2020 Jul 7;11:1623. doi: 10.3389/fimmu.2020.01623. eCollection 2020.

(note: dose for prophylaxis and safety for special groups like elderly and comorbid patients is completely unclear!)

Ubiquinone

ISRAEL A et al., Systematic analysis of electronic health records identifies drugs reducing risk of COVID-19 hospitalization and severity.
https://www.medrxiv.org/content/10.1101/2020.10.13.20211953v1.full.pdf

Umifenovir

ZHANG J et al., Potential of Arbidol for Post-exposure Prophylaxis of Covid-19 Transmission.
http://www.chinaxiv.org/abs/202002.00065
Curr Med Sci 2020 May 30: doi: 10.1007/s11596-020-2203-3.

Unani formulation

CTRI/2020/08/027222

Varenicline nasal spray (varenicline is used to aid smoking cessation as oral tablet)

NAU J et al., Varenicline Prevents SARS-CoV-2 Infection In Vitro and in Rhesus Macaques. https://www.biorxiv.org/content/10.1101/2021.06.29.450426v1.full.pdf

Vitamin C

FEYAERTS AF, LUYTEN W., Vitamin C as prophylaxis and adjunctive medical treatment for COVID-19? Nutrition 2020; 79-80: 110948; https://doi.org/10.1016/j.nut.2020.110948

TCTR20200404004

Vitamin D (several ongoing trials, a lot of literature, mentioned in the text above)

NCT04483635

Vitamin D3, Vitamin K2-7, Magnesium

CTRI/2020/06/026191

Vitamin K (need for combination with vitamin D)

WALK J et al., Vitamin D - contrary to vitamin K - does not associate with clinical outcome in hospitalized COVID-19 patients,
https://www.medrxiv.org/content/10.1101/2020.11.07.20227512v1.full.pdf

LINNEBERG A et al., Low vitamin K status predicts mortality in a cohort of 138 hospitalized patients with COVID-19.
https://www.medrxiv.org/content/10.1101/2020.12.21.20248613v1.full.pdf

Withania somnifera

s. CHOPRA et al.

Xlear (nasal spray with xylitol and grapefruit seed extract)

FERRER GA et al., A Nasal Spray Solution of Grapefruit Seed Extract plus Xylitol Displays Virucidal Activity Against SARS-Cov-2 In Vitro.
https://www.biorxiv.org/content/10.1101/2020.11.23.394114v1.full.pdf

Xylitol

VEGA JC et al., Iota carrageenan and xylitol inhibit SARS-CoV-2 in Vero cell culture
https://www.biorxiv.org/content/10.1101/2020.08.19.225854v1.full.pdf

Yashtimadhu tablets

CTRI/2020/05/025093

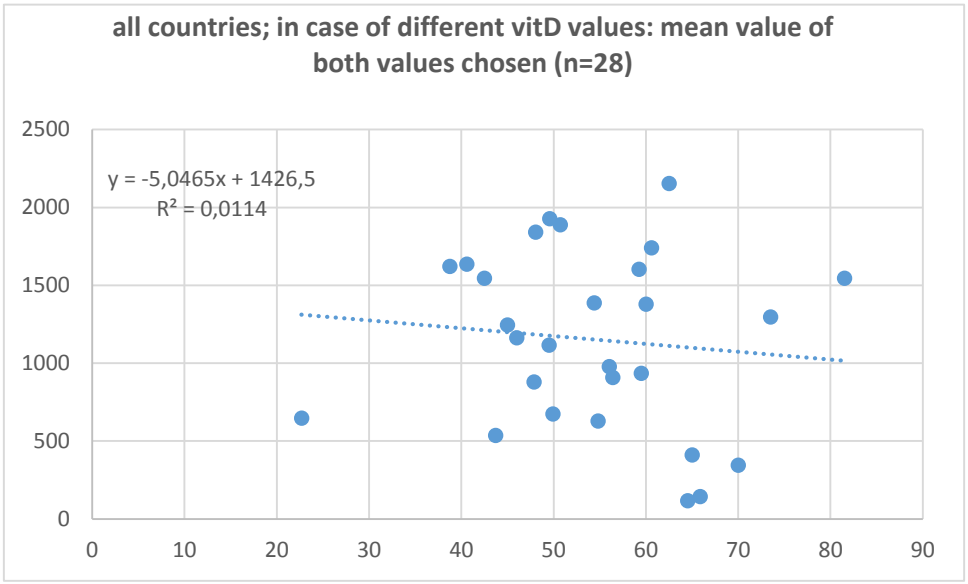
Zinc (also as partner for combinations)

ROY A et al., Can Concomitant Use of Zinc and Curcumin With Other Immunity-Boosting Nutraceuticals Be the Arsenal Against COVID-19? Phytother Res 2020; 10.1002/ptr.6766. doi: 10.1002/ptr.6766.

Supplement 2

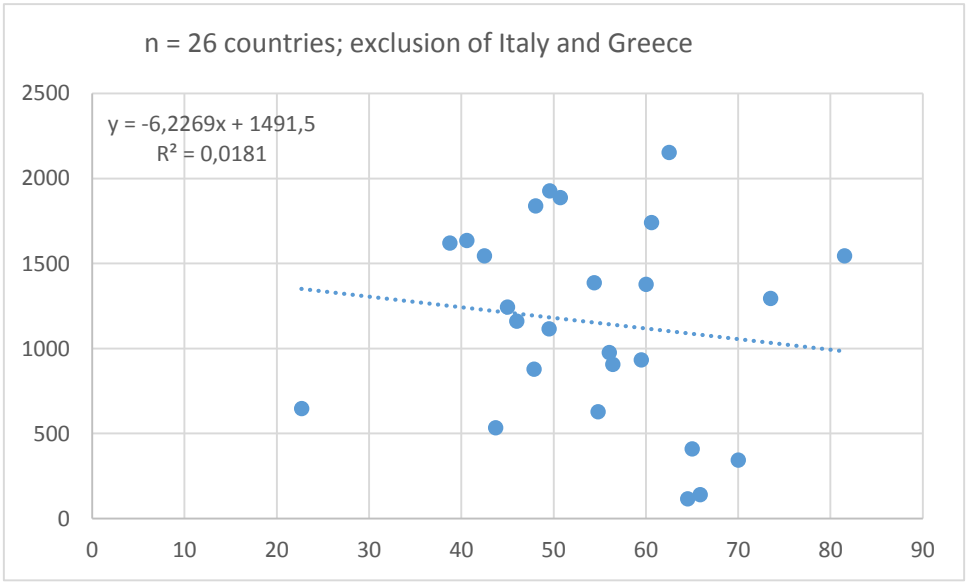
deaths/
1 M popul.

(March 12th)



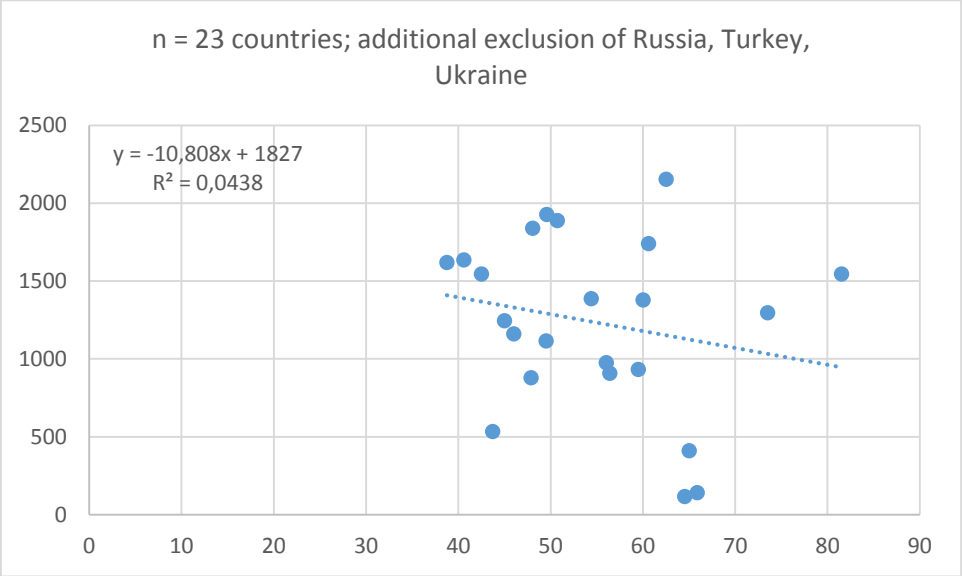
Reanalysis of Suppl. Fig. 2B in BAKALOUDI DR, CHOURDAKIS M (2), based on deaths/1 M population from Worldometer (March 12th) and inclusion of 7 additional countries from AHMAD et al. (Austria, Switzerland, Sweden, Spain, Netherlands, Hungary, Slovakia). For countries with different vitD values in BAKALOUDI/CHOURDAKIS and AHMAD (8 of 12 countries), the mean value of both values was used.

R = -0.107 (p = 0.56) and thus very similar to R=-0.115 (p = 0.619) in BAKALOUDI/CHOURDAKIS (Suppl. Fig. 2B) based on 21 countries



Same as before, but Italy and Greece were exluded because of very different vitD values in BAKALOUDI/CHOURDAKIS vs. AHMAD (Italy: 68.5 vs. 50 nmol/l; Greece: 41.8 vs. 58 nmol/l).

All other countries with different vitD values showed only minor differences and were included with the mean value from both values. R = -0.135, p = 0.511.



Same as before, but Russia, Turkey and Ukraine were also excluded. Russia and Turkey because of doubts about their mortality data; Turkey is preferentially Asia; doubts whether the very low vitD value for Ukraine in BAKALOIDU/CHOURDAKIS is representative (not compatible with vitD values from neighbour countries). R = - 0.21. p = 0.336.