Chlorhexidine for COVID-19: real-time meta analysis of 3 studies

@CovidAnalysis, April 2024, Version 3
https://c19early.org/chxmeta.html

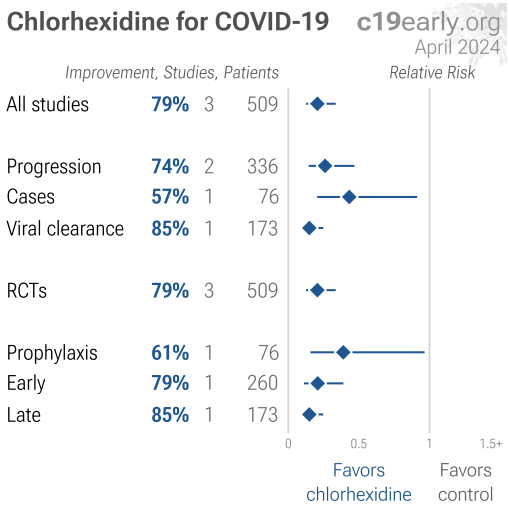
Abstract

Statistically significant lower risk is seen for progression, cases, and viral clearance. 3 studies from 3 independent teams in 3 countries show statistically significant improvements.

Meta analysis using the most serious outcome reported shows 79% [66-87%] lower risk. Currently all studies are RCTs.

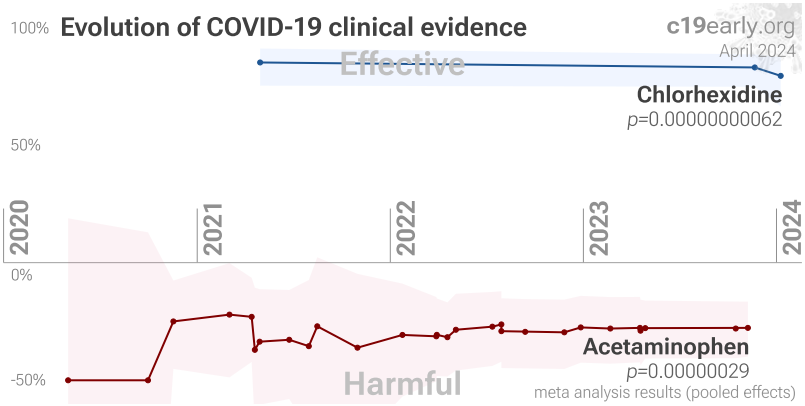
Currently there is limited data, with only 509 patients in trials to date. Studies to date are from only 3 different groups.

4 RCTs with 512 patients have not reported results (up to 2 years late).



No treatment or intervention is 100% effective. All practical, effective, and safe means should be used based on risk/benefit analysis. Multiple treatments are typically used in combination, and other treatments may be more effective. Chlorhexidine may be detrimental to the natural microbiome, raising concern for side effects, especially with prolonged or excessive use.

All data to reproduce this paper and sources are in the appendix.



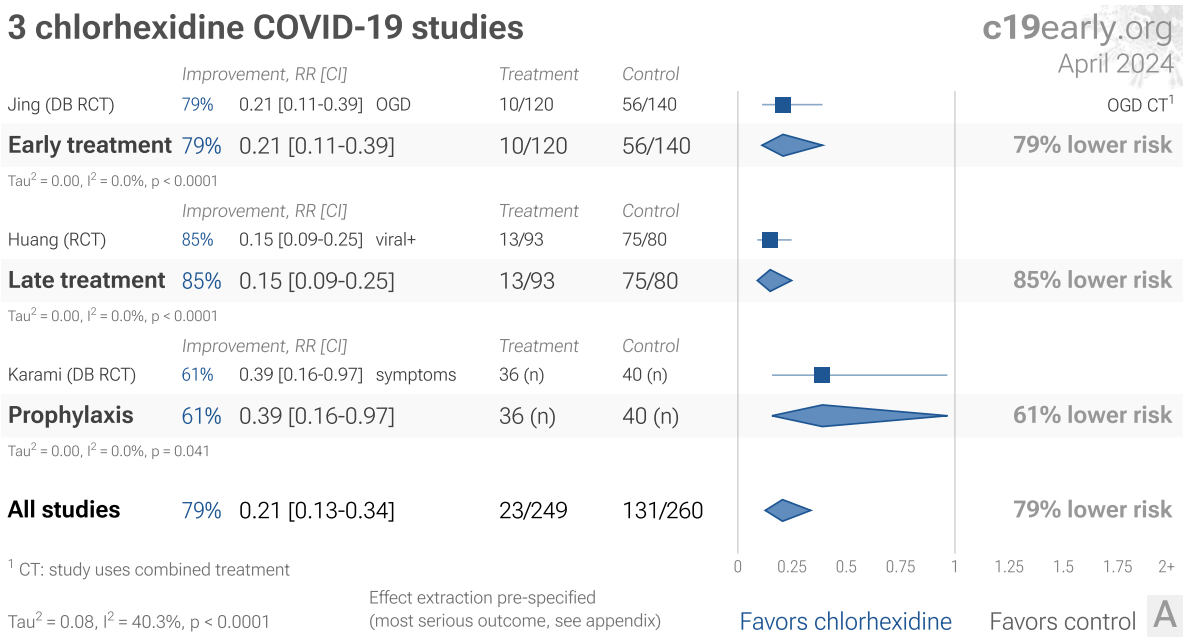
HIGHLIGHTS

Chlorhexidine reduces risk for COVID-19 with very high confidence for pooled analysis and low confidence for progression, cases, and viral clearance.

43rd treatment shown effective with ≥3 clinical studies in January 2024, now with p = 0.00000000062 from 3 studies.

We show outcome specific analyses and combined evidence from all studies, incorporating treatment delay, a primary confounding factor for COVID-19.

Real-time updates and corrections, transparent analysis with all results in the same format, consistent protocol for 69 treatments.



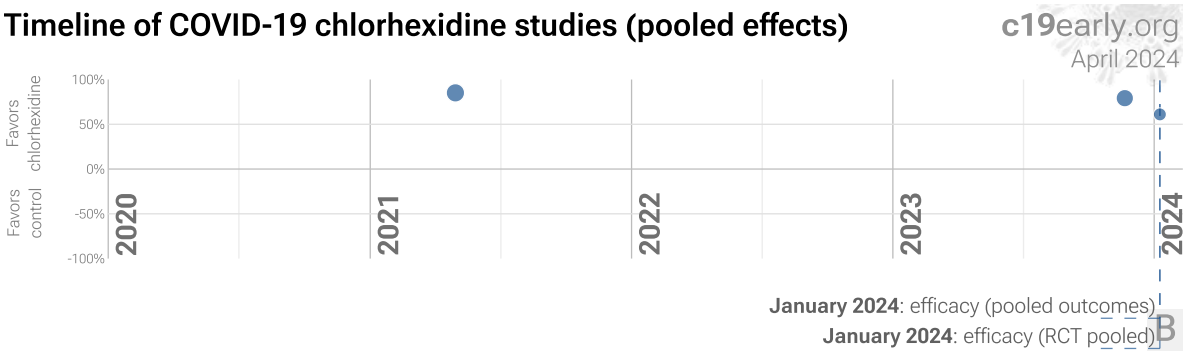


Figure 1. **A. Random effects meta-analysis.** This plot shows pooled effects, see the specific outcome analyses for individual outcomes. Analysis validating pooled outcomes for COVID-19 can be found below. Effect extraction is pre-specified, using the most serious outcome reported. For details see the appendix. **B. Timeline of results in chlorhexidine studies.** The marked dates indicate the time when efficacy was known with a statistically significant improvement of ≥10% from ≥3 studies for pooled outcomes and pooled outcomes in RCTs.

Introduction

Immediate treatment recommended. SARS-CoV-2 infection typically starts in the upper respiratory tract, and specifically the nasal respiratory epithelium. Entry via the eyes and gastrointestinal tract is possible, but less common, and entry via other routes is rare. Infection may progress to the lower respiratory tract, other tissues, and the nervous and cardiovascular systems. The primary initial route for entry into the central nervous system is thought to be the olfactory nerve in the nasal cavity *Dai*. Progression may lead to cytokine storm, pneumonia, ARDS, neurological issues *Duloquin, Hampshire, Scardua-Silva, Yang*, cardiovascular complications *Eberhardt*, organ failure, and death. Minimizing replication as early as possible is recommended. Logically, stopping replication in the upper respiratory tract should be simpler and more effective. Early or prophylactic nasopharyngeal/oropharyngeal treatment can avoid the consequences of viral replication in other tissues, and avoid the requirement for systemic treatments with greater potential for side effects.

Many treatments are expected to modulate infection. SARS-CoV-2 infection and replication involves the complex interplay of 50+ host and viral proteins and other factors *Note A, Malone, Murigneux, Lv, Lui, Niarakis*, providing many therapeutic targets for which many existing compounds have known activity. Scientists have predicted that over 7,000 compounds may reduce COVID-19 risk *c19early.org*, either by directly minimizing infection or replication, by supporting immune system function, or by minimizing secondary complications.

Other infections. Preclinical studies have shown efficacy with chlorhexidine for influenza A virus [Rius-Salvador] and respiratory syncytial virus [Rius-Salvador].

Analysis. We analyze all significant controlled studies of chlorhexidine for COVID-19. Search methods, inclusion criteria, effect extraction criteria (more serious outcomes have priority), all individual study data, PRISMA answers, and statistical methods are detailed in Appendix 1. We present random effects meta-analysis results for all studies, studies within each treatment stage, individual outcomes, and Randomized Controlled Trials (RCTs).

Treatment timing. Figure 2 shows stages of possible treatment for COVID-19. Prophylaxis refers to regularly taking medication before becoming sick, in order to prevent or minimize infection. Early Treatment refers to treatment immediately or soon after symptoms appear, while Late Treatment refers to more delayed treatment.

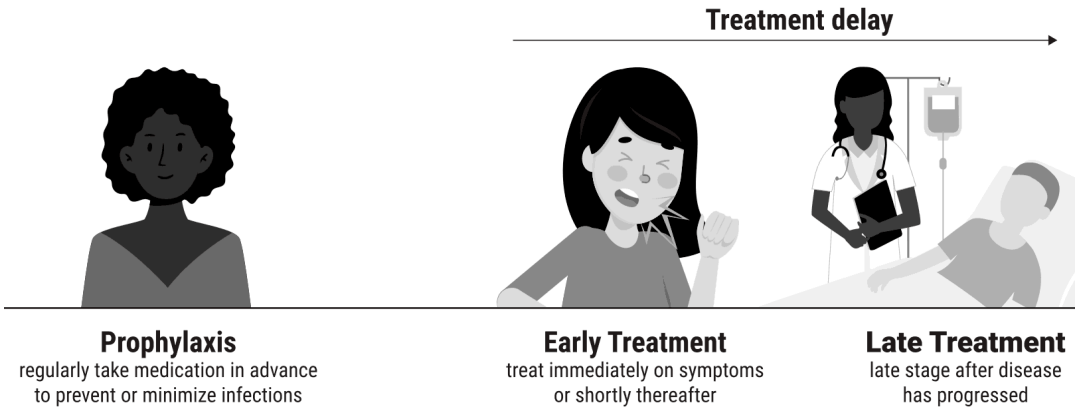


Figure 2. Treatment stages.

Results

Table 1 summarizes the results for all stages combined and for Randomized Controlled Trials. Table 2 shows results by treatment stage. Figure 3 plots individual results by treatment stage. Figure 4, 5, 6, and 7 show forest plots for random effects meta-analysis of all studies with pooled effects, progression, cases, and viral clearance.

	Improvement	*Studies*	*Patients*	*Authors*
All studies	**79%** [66-87%] ****	3	509	13
Randomized Controlled Trials	**79%** [66-87%] ****	3	509	13

Table 1. **Random effects meta-analysis for all stages combined and for Randomized Controlled Trials.** Results show the percentage improvement with treatment and the 95% confidence interval. * $p<0.05$ **** $p<0.0001$.

	Early treatment	*Late treatment*	*Prophylaxis*
All studies	**79%** [61-89%] ****	**85%** [75-91%] ****	**61%** [3-84%] *
Randomized Controlled Trials	**79%** [61-89%] ****	**85%** [75-91%] ****	**61%** [3-84%] *

Table 2. **Random effects meta-analysis results by treatment stage.** Results show the percentage improvement with treatment, the 95% confidence interval, and the number of studies for the stage. * $p<0.05$ **** $p<0.0001$.

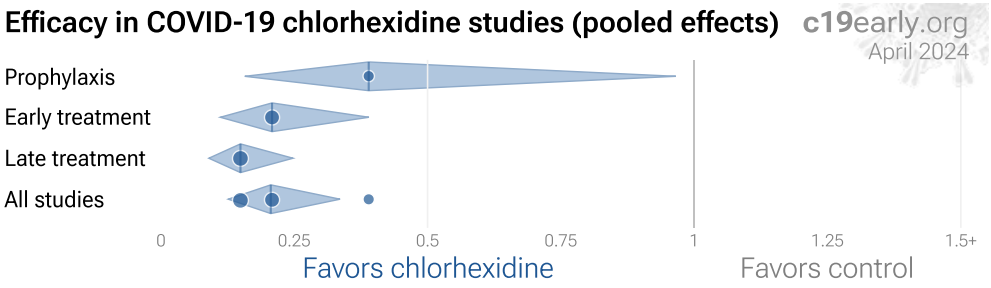


Figure 3. **Scatter plot showing the most serious outcome in all studies, and for studies within each stage.** Diamonds shows the results of random effects meta-analysis.

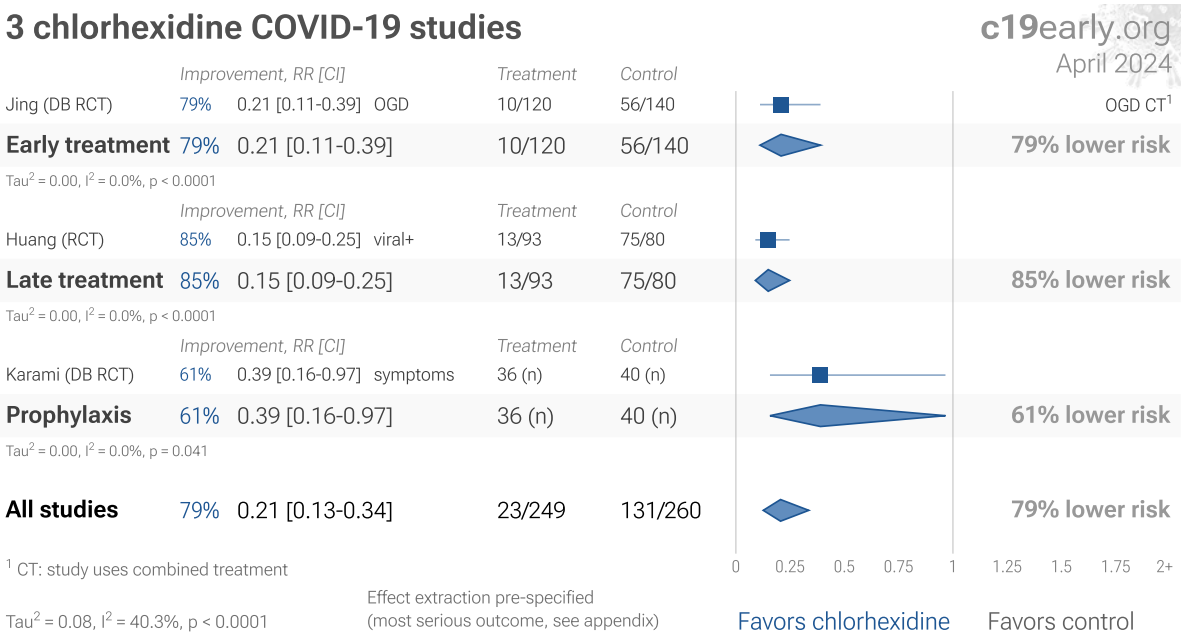


Figure 4. **Random effects meta-analysis for all studies.** This plot shows pooled effects, see the specific outcome analyses for individual outcomes. Analysis validating pooled outcomes for COVID-19 can be found below. Effect extraction is pre-specified, using the most serious outcome reported. For details see the appendix.

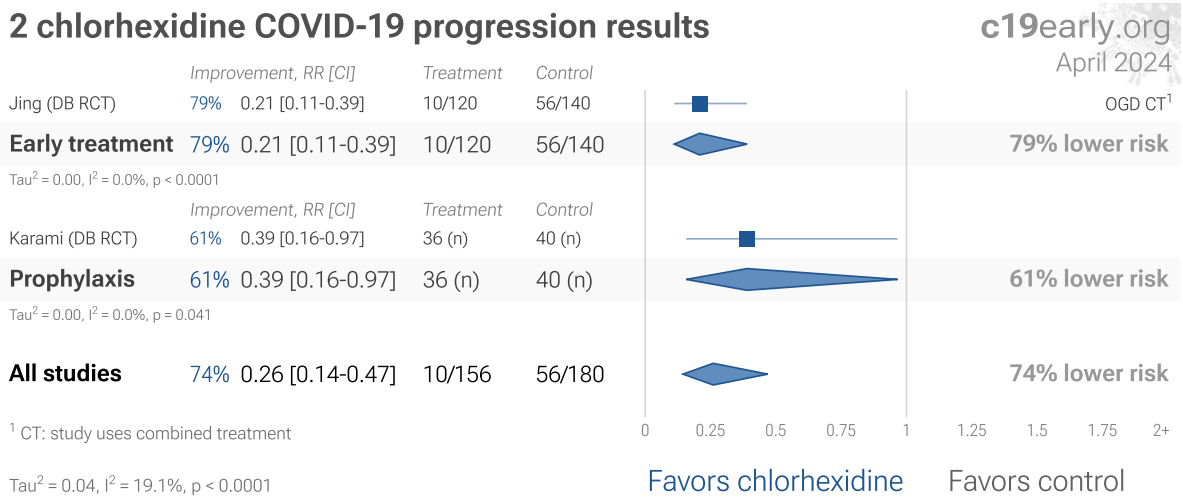


Figure 5. **Random effects meta-analysis for progression.**

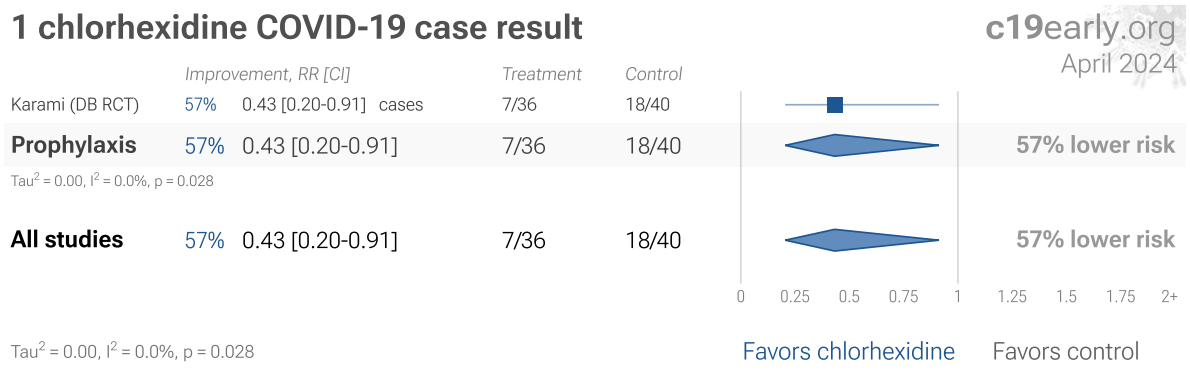


Figure 6. Random effects meta-analysis for cases.

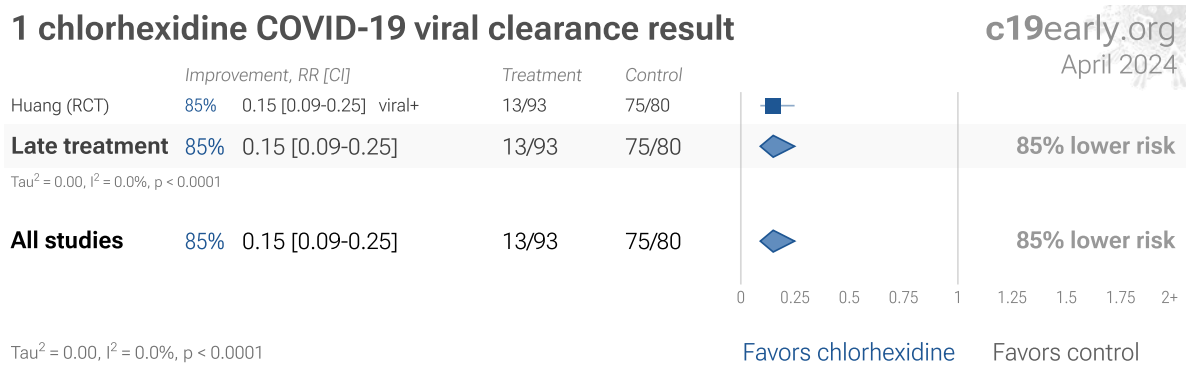


Figure 7. Random effects meta-analysis for viral clearance.

Randomized Controlled Trials (RCTs)

Currently all studies are RCTs.

Unreported RCTs

4 chlorhexidine RCTs have not reported results *Jacox, Keating, Mira, Xie*. The trials report a total of 512 patients, with 3 trials having actual enrollment of 422, and the other estimated. The results are delayed from 1.5 years to over 2 years.

Heterogeneity

Heterogeneity in COVID-19 studies arises from many factors including:

Treatment delay. The time between infection or the onset of symptoms and treatment may critically affect how well a treatment works. For example an antiviral may be very effective when used early but may not be effective in late stage disease, and may even be harmful. Oseltamivir, for example, is generally only considered effective for influenza when used within 0-36 or 0-48 hours *McLean, Treanor*. Baloxavir studies for influenza also show that treatment delay is critical — *Ikematsu et al.* report an 86% reduction in cases for post-exposure prophylaxis, *Hayden et al.* show a 33 hour reduction in the time to alleviation of symptoms for treatment within 24 hours and a reduction of 13 hours for treatment within 24-48 hours, and *Kumar et al.* report only 2.5 hours improvement for inpatient treatment.

Treatment delay	*Result*
Post-exposure prophylaxis	86% fewer cases *Ikematsu*
<24 hours	-33 hours symptoms *Hayden*
24-48 hours	-13 hours symptoms *Hayden*
Inpatients	-2.5 hours to improvement *Kumar*

Table 3. **Studies of baloxavir for influenza show that early treatment is more effective.**

Figure 8 shows a mixed-effects meta-regression for efficacy as a function of treatment delay in COVID-19 studies from 69 treatments, showing that efficacy declines rapidly with treatment delay. Early treatment is critical for COVID-19.

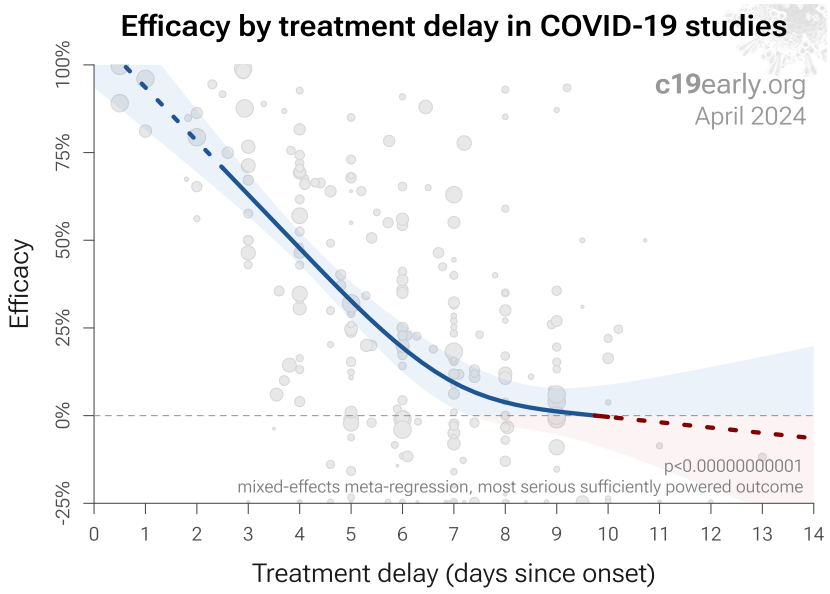


Figure 8. **Early treatment is more effective.** Meta-regression showing efficacy as a function of treatment delay in COVID-19 studies from 69 treatments.

Patient demographics. Details of the patient population including age and comorbidities may critically affect how well a treatment works. For example, many COVID-19 studies with relatively young low-comorbidity patients show all patients recovering quickly with or without treatment. In such cases, there is little room for an effective treatment to improve results, for example as in *López-Medina et al.*

Variants. Efficacy may depend critically on the distribution of SARS-CoV-2 variants encountered by patients. Risk varies significantly across variants *Korves*, for example the Gamma variant shows significantly different characteristics *Faria, Karita, Nonaka, Zavascki*. Different mechanisms of action may be more or less effective depending on variants, for example the degree to which TMPRSS2 contributes to viral entry can differ across variants *Peacock, Willett*.

Regimen. Effectiveness may depend strongly on the dosage and treatment regimen.

Other treatments. The use of other treatments may significantly affect outcomes, including supplements, other medications, or other interventions such as prone positioning. Treatments may be synergistic *Alsaidi, Andreani, De Forni, Fiaschi, Jeffreys, Jitobaom, Jitobaom (B), Ostrov, Said, Thairu, Wan*, therefore efficacy may depend strongly on combined treatments.

Medication quality. The quality of medications may vary significantly between manufacturers and production batches, which may significantly affect efficacy and safety. *Williams et al.* analyze ivermectin from 11 different sources, showing highly variable antiparasitic efficacy across different manufacturers. *Xu et al.* analyze a treatment from two different manufacturers, showing 9 different impurities, with significantly different concentrations for each manufacturer.

Effect measured. Across all studies there is a strong association between different outcomes, for example improved recovery is strongly associated with lower mortality. However, efficacy may differ depending on the effect measured, for example a treatment may be more effective against secondary complications and have minimal effect on viral clearance.

Meta analysis. The distribution of studies will alter the outcome of a meta analysis. Consider a simplified example where everything is equal except for the treatment delay, and effectiveness decreases to zero or below with increasing delay. If there are many studies using very late treatment, the outcome may be negative, even though early treatment is very effective. All meta analyses combine heterogeneous studies, varying in population, variants, and potentially all factors above, and therefore may obscure efficacy by including studies where treatment is less effective. Generally, we expect the estimated effect size from meta analysis to be less than that for the optimal case. Looking at all studies is valuable for providing an overview of all research, important to avoid cherry-picking, and informative when a positive result is found despite combining less-optimal situations. However, the resulting estimate does not apply to specific cases such as early treatment in high-risk populations. While we present results for all studies, we also present treatment time and individual outcome analyses, which may be more informative for specific use cases.

Pooled Effects

Combining studies is required. For COVID-19, delay in clinical results translates into additional death and morbidity, as well as additional economic and societal damage. Combining the results of studies reporting different outcomes is required. There may be no mortality in a trial with low-risk patients, however a reduction in severity or improved viral clearance may translate into lower mortality in a high-risk population. Different studies may report lower severity, improved recovery, and lower mortality, and the significance may be very high when combining the results. *"The studies reported different outcomes"* is not a good reason for disregarding results.

Specific outcome and pooled analyses. We present both specific outcome and pooled analyses. In order to combine the results of studies reporting different outcomes we use the most serious outcome reported in each study, based on the thesis that improvement in the most serious outcome provides comparable measures of efficacy for a treatment. A critical advantage of this approach is simplicity and transparency. There are many other ways to combine evidence for different outcomes, along with additional evidence such as dose-response relationships, however these increase complexity.

Using more information. Another way to view pooled analysis is that we are using more of the available information. Logically we should, and do, use additional information. For example dose-response and treatment delay-response relationships provide significant additional evidence of efficacy that is considered when reviewing the evidence for a treatment.

Ethical and practical issues limit high-risk trials. Trials with high-risk patients may be restricted due to ethics for treatments that are known or expected to be effective, and they increase difficulty for recruiting. Using less severe outcomes as a proxy for more serious outcomes allows faster collection of evidence.

Improvement across outcomes. For many COVID-19 treatments, a reduction in mortality logically follows from a reduction in hospitalization, which follows from a reduction in symptomatic cases, which follows from a reduction in PCR positivity. We can directly test this for COVID-19.

Validating pooled outcome analysis for COVID-19. Analysis of the the association between different outcomes across studies from all 69 treatments we cover confirms the validity of pooled outcome analysis for COVID-19. Figure 9 shows that lower hospitalization is very strongly associated with lower mortality ($p < 0.000000000001$). Similarly,

Figure 10 shows that improved recovery is very strongly associated with lower mortality (*p < 0.000000000001*). Considering the extremes, *Singh et al.* show an association between viral clearance and hospitalization or death, with *p = 0.003* after excluding one large outlier from a mutagenic treatment, and based on 44 RCTs including 52,384 patients. Figure 11 shows that improved viral clearance is strongly associated with fewer serious outcomes. The association is very similar to *Singh et al.*, with higher confidence due to the larger number of studies. As with *Singh et al.*, the confidence increases when excluding the outlier treatment, from *p = 0.0000045* to *p = 0.0000000067*.

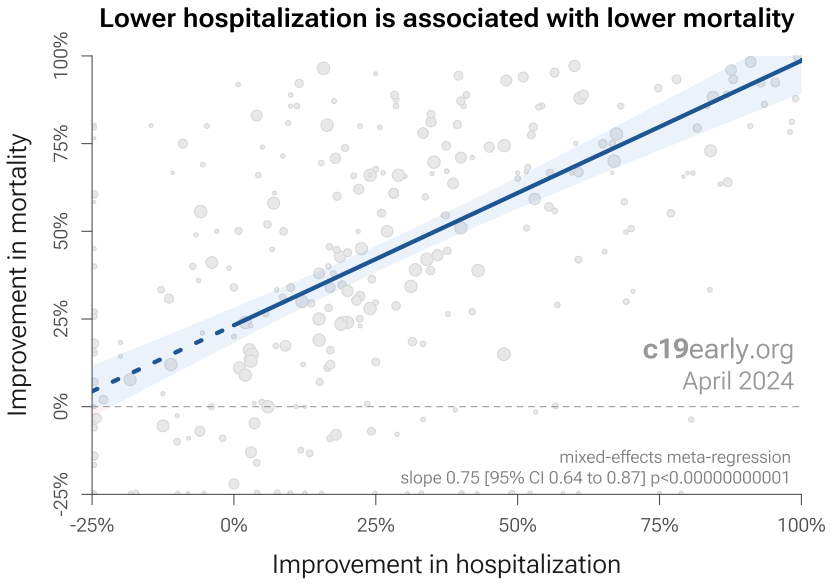


Figure 9. Lower hospitalization is associated with lower mortality, supporting pooled outcome analysis.

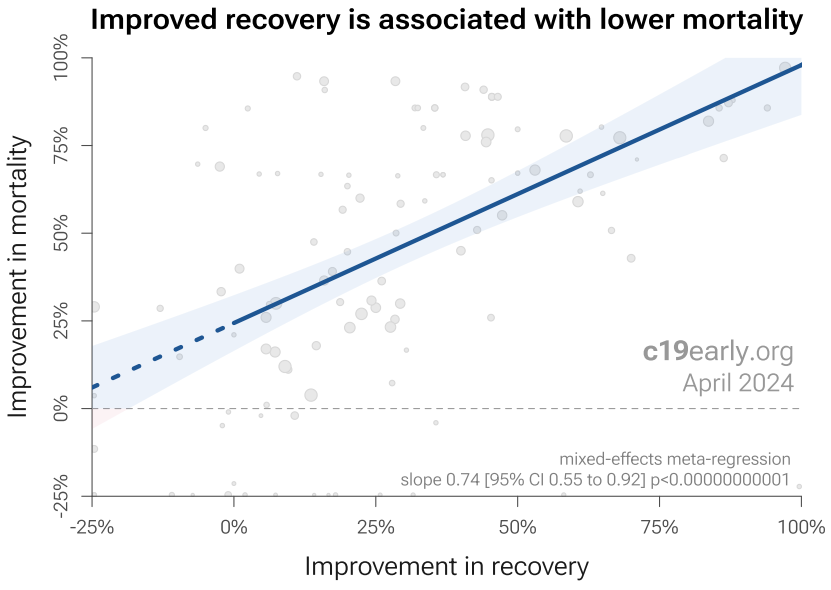


Figure 10. Improved recovery is associated with lower mortality, supporting pooled outcome analysis.

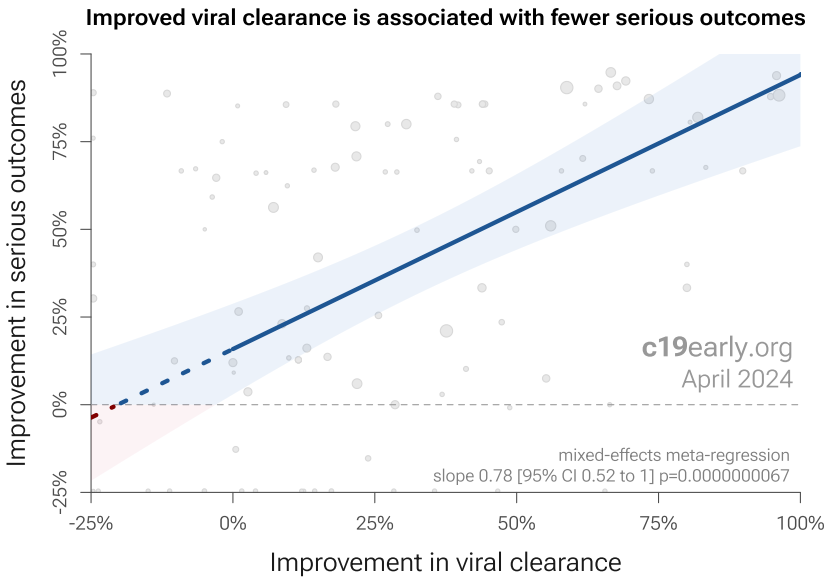


Figure 9. **Improved viral clearance is associated with fewer serious outcomes, supporting pooled outcome analysis.**

Pooled outcomes identify efficacy 4 months faster (6 months for RCTs). Currently, 44 of the treatments we analyze show statistically significant efficacy or harm, defined as ≥10% decreased risk or >0% increased risk from ≥3 studies. 85% of these have been confirmed with one or more specific outcomes, with a mean delay of 3.7 months. When restricting to RCTs only, 54% of treatments showing statistically significant efficacy/harm with pooled effects have been confirmed with one or more specific outcomes, with a mean delay of 5.8 months. Figure 12 shows when treatments were found effective during the pandemic. Pooled outcomes often resulted in earlier detection of efficacy.

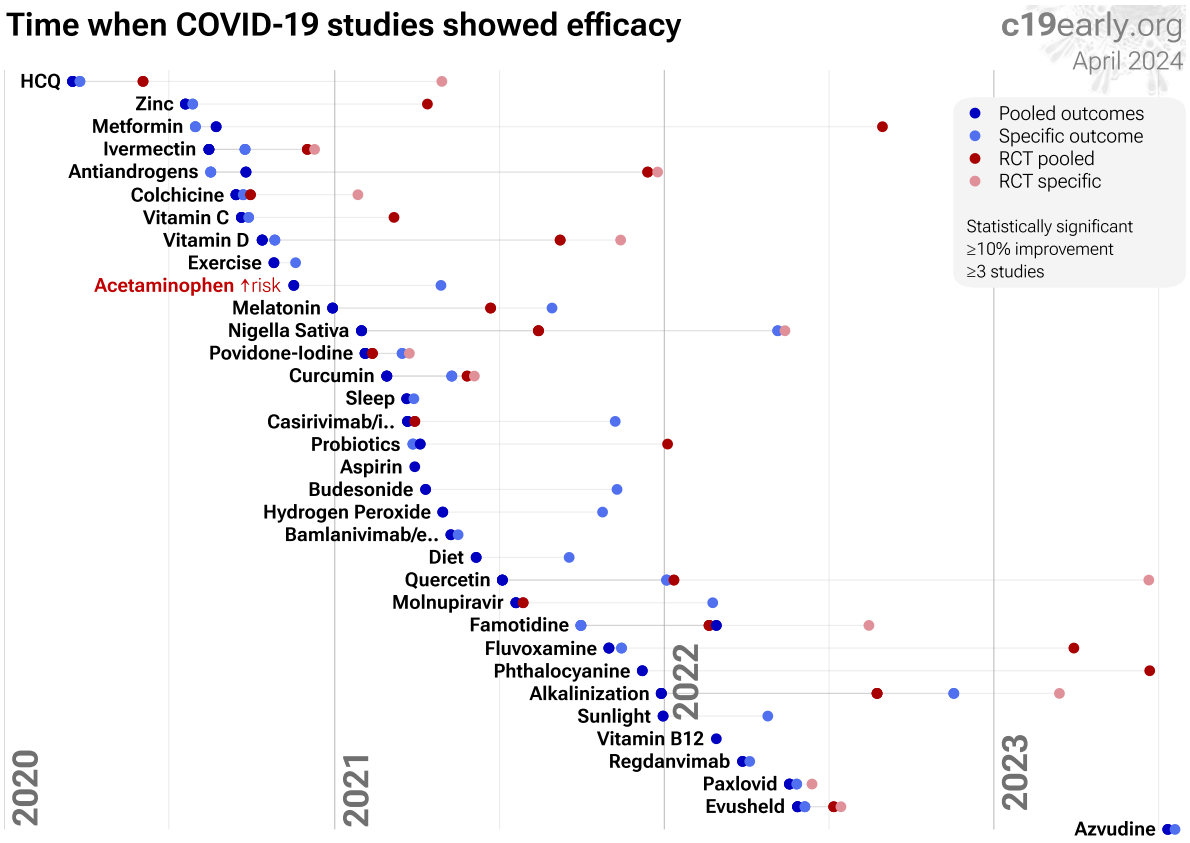


Figure 12. **The time when studies showed that treatments were effective, defined as statistically significant improvement of ≥10% from ≥3 studies.** Pooled results typically show efficacy earlier than specific outcome results. Results from all studies often shows efficacy much earlier than when restricting to RCTs. Results reflect conditions as used in trials to date, these depend on the population treated, treatment delay, and treatment regimen.

Limitations. Pooled analysis could hide efficacy, for example a treatment that is beneficial for late stage patients but has no effect on viral clearance may show no efficacy if most studies only examine viral clearance. In practice, it is rare for a non-antiviral treatment to report viral clearance and to not report clinical outcomes; and in practice other sources of heterogeneity such as difference in treatment delay is more likely to hide efficacy.

Summary. Analysis validates the use of pooled effects and shows significantly faster detection of efficacy on average. However, as with all meta analyses, it is important to review the different studies included. We also present individual outcome analyses, which may be more informative for specific use cases.

Discussion

Results for other viruses. Preclinical studies have also shown efficacy with chlorhexidine for influenza A virus *Rius-Salvador* and respiratory syncytial virus *Rius-Salvador*.

PCR viral load. Analysis of short-term changes in viral load using PCR may not detect effective treatments because PCR is unable to differentiate between intact infectious virus and non-infectious or destroyed virus particles. For example *Alemany, Tarragó-Gil* perform RCTs with cetylpyridinium chloride (CPC) mouthwash that show no difference in PCR viral load, however there was significantly increased detection of SARS-CoV-2 nucleocapsid protein, indicating viral lysis. CPC inactivates SARS-CoV-2 by degrading its membrane, exposing the nucleocapsid of the virus. To better estimate changes in viral load and infectivity, methods like viral culture or antigen detection that can differentiate intact vs. degraded virus are preferred.

Nasal/oral administration. Studies to date use a variety of administration methods to the respiratory tract, including nasal and oral sprays, nasal irrigation, oral rinses, and inhalation. Table 4 shows the relative efficacy for nasal, oral, and combined administration. Combined administration shows the best results, and nasal administration is more effective than oral. Precise efficacy depends on the details of administration, e.g., mucoadhesion and sprayability for sprays.

Nasal/oral administration to the respiratory tract	*Improvement*	*Studies*
Oral spray/rinse	38% [25-49%]	8
Nasal spray/rinse	54% [42-63%]	11
Nasal & oral	94% [74-99%]	6

Table 4. **Respiratory tract administration efficacy. Relative efficacy of nasal, oral, and combined nasal/oral administration for treatments administered directly to the respiratory tract, based on studies for povidone-iodine, iota-carrageenan, alkalinization, hydrogen peroxide, nitric oxide, chlorhexidine, cetylpyridinium chloride, and phthalocyanine. Results show random effects meta analysis for the most serious outcome reported for all prophylaxis and early treatment studies.**

Impact on the microbiome. Nasopharyngeal/oropharyngeal treatments may not be highly selective. In addition to inhibiting or disabling SARS-CoV-2, they may also be harmful to beneficial microbes, disrupting the natural microbiome in the oral cavity and nasal passages that have important protective and metabolic roles. This may be especially important for prolonged use or overuse. Table 5 summarizes the potential for common nasopharyngeal/oropharyngeal treatments to affect the natural microbiome.

Treatment	*Microbiome disruption potential*	*Notes*
Iota-carrageenan	Low	Primarily antiviral, however extended use may mildly affect the microbiome
Nitric Oxide	Low to moderate	More selective towards pathogens, however excessive concentrations or prolonged use may disrupt the balance of bacteria
Alkalinization	Moderate	Increases pH, negatively impacting beneficial microbes that thrive in a slightly acidic environment
Cetylpyridinium Chloride	Moderate	Quaternary ammonium broad-spectrum antiseptic that can disrupt beneficial and harmful bacteria
Phthalocyanine	Moderate to high	Photodynamic compound with antimicrobial activity, likely to affect the microbiome
Chlorhexidine	High	Potent antiseptic with broad activity, significantly disrupts the microbiome
Hydrogen Peroxide	High	Strong oxidizer, harming both beneficial and harmful microbes
Povidone-Iodine	High	Potent broad-spectrum antiseptic harmful to beneficial microbes

Table 5. **Potential effect of treatments on the nasophyrngeal/oropharyngeal microbiome.**

Publication bias. Publishing is often biased towards positive results, however evidence suggests that there may be a negative bias for inexpensive treatments for COVID-19. Both negative and positive results are very important for COVID-19, media in many countries prioritizes negative results for inexpensive treatments (inverting the typical incentive for scientists that value media recognition), and there are many reports of difficulty publishing positive results *Boulware, Meeus, Meneguesso, twitter.com*. For chlorhexidine, there is currently not enough data to evaluate publication bias with high confidence.

Conflicts of interest. Pharmaceutical drug trials often have conflicts of interest whereby sponsors or trial staff have a financial interest in the outcome being positive. Chlorhexidine for COVID-19 lacks this because it is off-patent, has multiple manufacturers, and is very low cost. In contrast, most COVID-19 chlorhexidine trials have been run by physicians on the front lines with the primary goal of finding the best methods to save human lives and minimize the collateral damage caused by COVID-19. While pharmaceutical companies are careful to run trials under optimal conditions (for example, restricting patients to those most likely to benefit, only including patients that can be treated soon after onset when necessary, and ensuring accurate dosing), not all chlorhexidine trials represent the optimal conditions for efficacy.

Limitations. Summary statistics from meta analysis necessarily lose information. As with all meta analyses, studies are heterogeneous, with differences in treatment delay, treatment regimen, patient demographics, variants, conflicts of interest, standard of care, and other factors. We provide analyses for specific outcomes and by treatment delay, and we aim to identify key characteristics in the forest plots and summaries. Results should be viewed in the context of study characteristics.

Some analyses classify treatment based on early or late administration, as done here, while others distinguish between mild, moderate, and severe cases. Viral load does not indicate degree of symptoms — for example patients may have a high viral load while being asymptomatic. With regard to treatments that have antiviral properties, timing of treatment is critical — late administration may be less helpful regardless of severity.

Details of treatment delay per patient is often not available. For example, a study may treat 90% of patients relatively early, but the events driving the outcome may come from 10% of patients treated very late. Our 5 day cutoff for early treatment may be too conservative, 5 days may be too late in many cases.

Comparison across treatments is confounded by differences in the studies performed, for example dose, variants, and conflicts of interest. Trials with conflicts of interest may use designs better suited to the preferred outcome.

In some cases, the most serious outcome has very few events, resulting in lower confidence results being used in pooled analysis, however the method is simpler and more transparent. This is less critical as the number of studies increases. Restriction to outcomes with sufficient power may be beneficial in pooled analysis and improve accuracy when there are few studies, however we maintain our pre-specified method to avoid any retrospective changes.

Studies show that combinations of treatments can be highly synergistic and may result in many times greater efficacy than individual treatments alone *Alsaidi, Andreani, De Forni, Fiaschi, Jeffreys, Jitobaom, Jitobaom (B), Ostrov, Said, Thairu, Wan*. Therefore standard of care may be critical and benefits may diminish or disappear if standard of care does not include certain treatments.

This real-time analysis is constantly updated based on submissions. Accuracy benefits from widespread review and submission of updates and corrections from reviewers. Less popular treatments may receive fewer reviews.

No treatment or intervention is 100% available and effective for all current and future variants. Efficacy may vary significantly with different variants and within different populations. All treatments have potential side effects. Propensity to experience side effects may be predicted in advance by qualified physicians. We do not provide medical advice. Before taking any medication, consult a qualified physician who can compare all options, provide personalized advice, and provide details of risks and benefits based on individual medical history and situations.

Notes. 1 of 3 studies combine treatments. The results of chlorhexidine alone may differ. 1 of 3 RCTs use combined treatment. Currently all studies are peer-reviewed.

Reviews. *Ting et al.* present a review covering chlorhexidine for COVID-19.

Perspective

Results compared with other treatments. SARS-CoV-2 infection and replication involves a complex interplay of 50+ host and viral proteins and other factors [Lui, Lv, Malone, Murigneux, Niarakis], providing many therapeutic targets. Over 7,000 compounds have been predicted to reduce COVID-19 risk [c19early.org], either by directly minimizing infection or replication, by supporting immune system function, or by minimizing secondary complications. Figure 13 shows an overview of the results for chlorhexidine in the context of multiple COVID-19 treatments, and Figure 14 shows a plot of efficacy vs. cost for COVID-19 treatments.

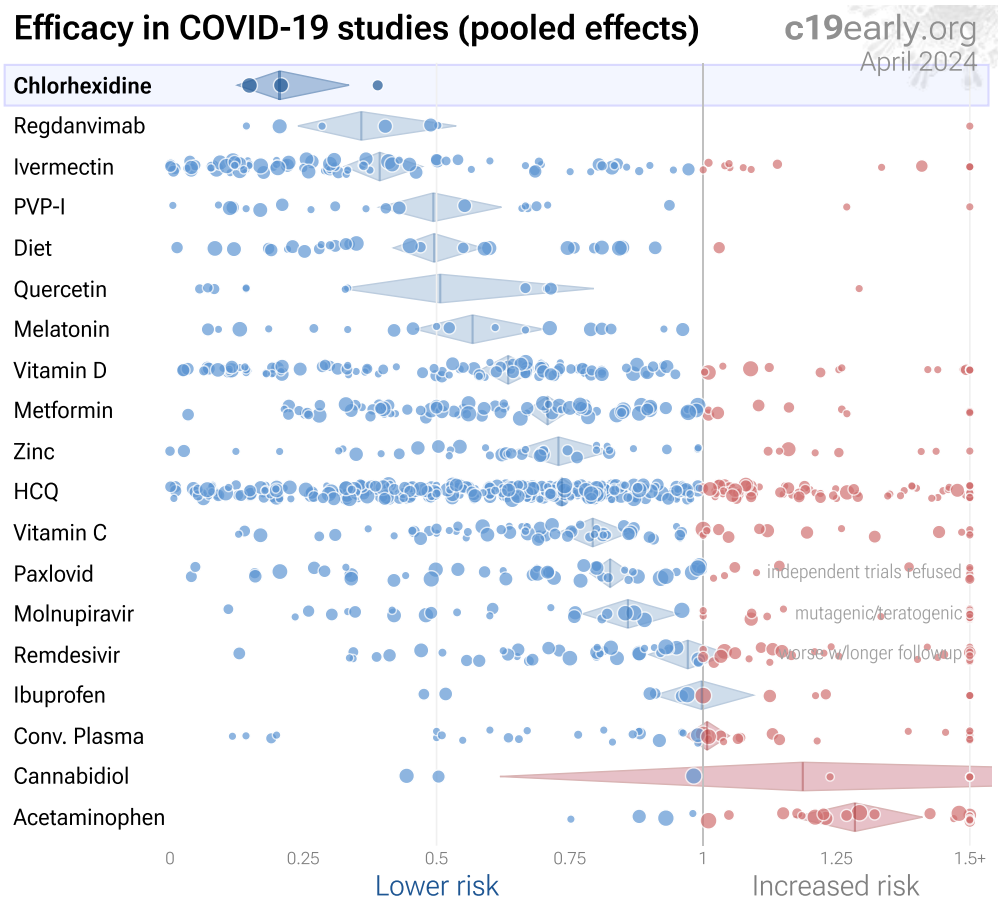


Figure 13. **Scatter plot showing results within the context of multiple COVID-19 treatments.** Diamonds shows the results of random effects meta-analysis. 0.6% of 7,400 proposed treatments show efficacy [c19early.org (B)].

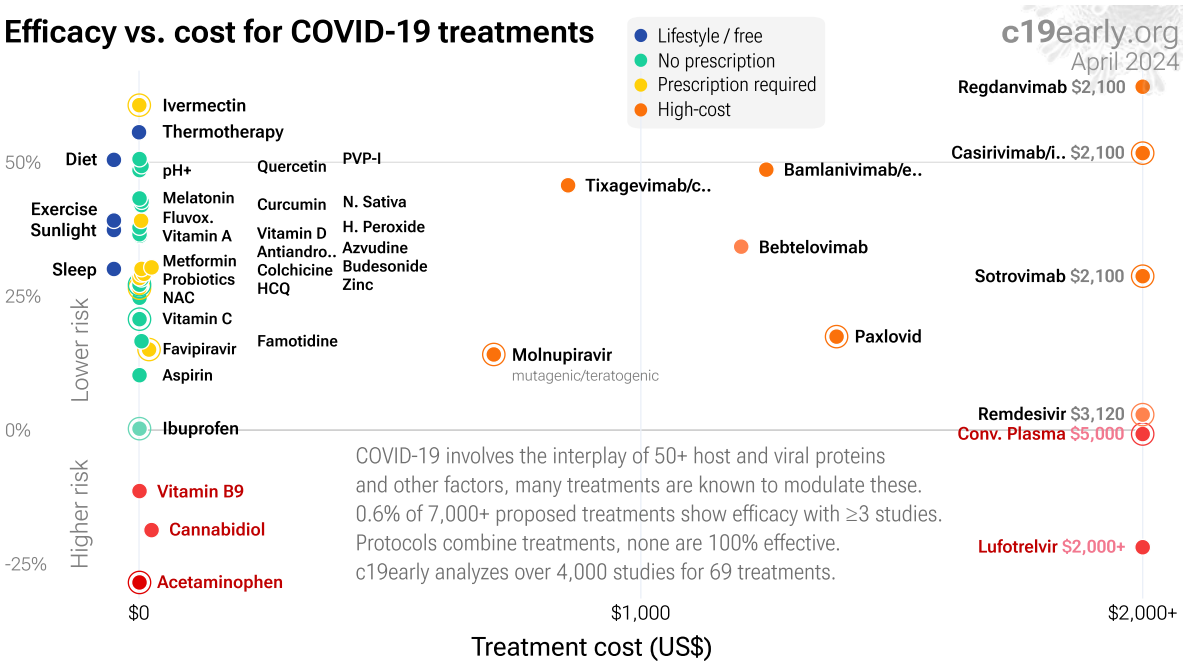


Figure 14. Efficacy vs. cost for COVID-19 treatments.

Conclusion

SARS-CoV-2 infection typically starts in the upper respiratory tract. Progression may lead to cytokine storm, pneumonia, ARDS, neurological issues, organ failure, and death. Stopping replication in the upper respiratory tract, via early or prophylactic nasopharyngeal/oropharyngeal treatment, can avoid the consequences of progression to other tissues, and avoid the requirement for systemic treatments with greater potential for side effects.

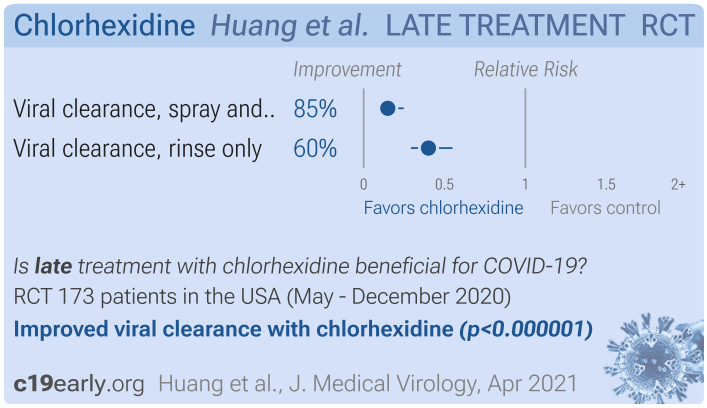
Studies to date show that chlorhexidine is an effective treatment for COVID-19. Statistically significant lower risk is seen for progression, cases, and viral clearance. 3 studies from 3 independent teams in 3 countries show statistically significant improvements. Meta analysis using the most serious outcome reported shows 79% [66-87%] lower risk. Currently all studies are RCTs.

Currently there is limited data, with only 509 patients in trials to date. Studies to date are from only 3 different groups.

Chlorhexidine may be detrimental to the natural microbiome, raising concern for side effects, especially with prolonged or excessive use.

Study Notes

Huang

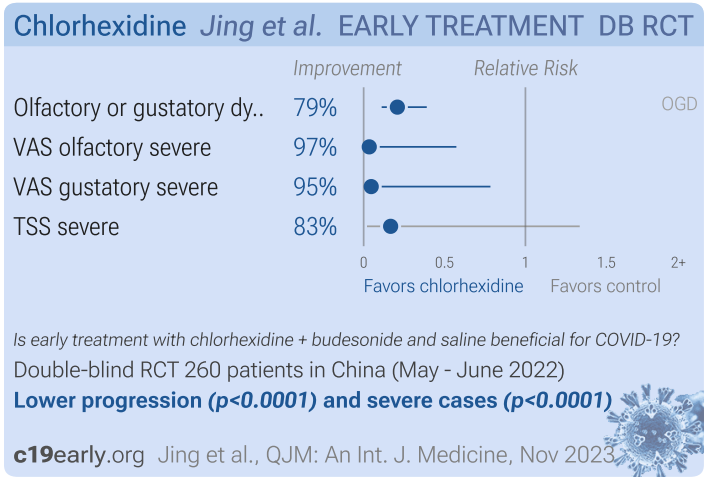


Huang: RCT 294 hospitalized patients in the USA, showing faster oropharyngeal viral clearance with chlorhexidine. Results were better with a combination of oropharyngeal rinse and posterior oropharyngeal spray compared with the rinse alone.

Jacox

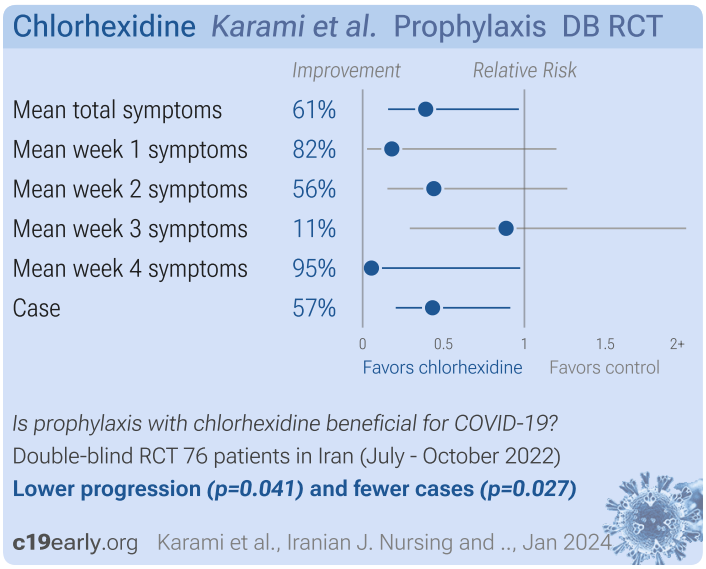
Jacox: 129 patient chlorhexidine early treatment RCT with results not reported over 2 years after completion.

Jing



Jing: RCT 379 mild COVID-19 cases showing significantly lower prevalence and severity of olfactory and gustatory dysfunction with budesonide nasal spray, chlorhexidine mouthwash, and saline nasal irrigation. The control group received no intervention, the saline group received saline nasal irrigation plus saline nasal spray and mouthwash, and the drug group received saline nasal irrigation plus budesonide nasal spray and chlorhexidine mouthwash. Saline nasal irrigation plus nasal spray and mouthwash were administered once and four times daily, respectively. Both treatment groups had significantly lower prevalence and severity olfactory and gustatory dysfunction. Prevalence was lower for the drug vs. saline group, without statistical significance.

Karami



Karami: RCT 116 healthcare workers comparing 0.2% chlorhexidine mouthwash (n=36), 7.5% sodium bicarbonate mouthwash (n=40), and placebo (n=40) twice daily for 2 weeks, with symptoms followed for 4 weeks. There were lower symtoms and cases in both treatment groups, with statistical significance for chlorhexidine only. The treatments were stopped after two weeks, results may be better with continued use, more frequent use, and with the addition of nasal use.

Keating

Keating: 245 participant chlorhexidine + PVP-I prophylaxis RCT with results not reported over 1.5 years after completion.

Mira

Mira: 48 patient chlorhexidine early treatment RCT with results not reported over 2 years after completion.

Xie

Xie: Estimated 90 patient chlorhexidine early treatment RCT with results not reported over 2 years after estimated completion.

Appendix 1. Methods and Data

We perform ongoing searches of PubMed, medRxiv, Europe PMC, ClinicalTrials.gov, The Cochrane Library, Google Scholar, Research Square, ScienceDirect, Oxford University Press, the reference lists of other studies and meta-analyses, and submissions to the site c19early.org. Search terms are chlorhexidine and COVID-19 or SARS-CoV-2. Automated searches are performed twice daily, with all matches reviewed for inclusion. All studies regarding the use of chlorhexidine for COVID-19 that report a comparison with a control group are included in the main analysis. This is a living analysis and is updated regularly.

We extracted effect sizes and associated data from all studies. If studies report multiple kinds of effects then the most serious outcome is used in pooled analysis, while other outcomes are included in the outcome specific analyses. For example, if effects for mortality and cases are both reported, the effect for mortality is used, this may be different to the effect that a study focused on. If symptomatic results are reported at multiple times, we used the latest time, for example if mortality results are provided at 14 days and 28 days, the results at 28 days have preference. Mortality

alone is preferred over combined outcomes. Outcomes with zero events in both arms are not used, the next most serious outcome with one or more events is used. For example, in low-risk populations with no mortality, a reduction in mortality with treatment is not possible, however a reduction in hospitalization, for example, is still valuable. Clinical outcomes are considered more important than viral test status. When basically all patients recover in both treatment and control groups, preference for viral clearance and recovery is given to results mid-recovery where available. After most or all patients have recovered there is little or no room for an effective treatment to do better, however faster recovery is valuable. If only individual symptom data is available, the most serious symptom has priority, for example difficulty breathing or low SpO_2 is more important than cough. When results provide an odds ratio, we compute the relative risk when possible, or convert to a relative risk according to *Zhang*. Reported confidence intervals and *p*-values were used when available, using adjusted values when provided. If multiple types of adjustments are reported propensity score matching and multivariable regression has preference over propensity score matching or weighting, which has preference over multivariable regression. Adjusted results have preference over unadjusted results for a more serious outcome when the adjustments significantly alter results. When needed, conversion between reported *p*-values and confidence intervals followed *Altman, Altman (B)*, and Fisher's exact test was used to calculate *p*-values for event data. If continuity correction for zero values is required, we use the reciprocal of the opposite arm with the sum of the correction factors equal to 1 *Sweeting*. Results are expressed with RR < 1.0 favoring treatment, and using the risk of a negative outcome when applicable (for example, the risk of death rather than the risk of survival). If studies only report relative continuous values such as relative times, the ratio of the time for the treatment group versus the time for the control group is used. Calculations are done in Python (3.12.2) with scipy (1.12.0), pythonmeta (1.26), numpy (1.26.4), statsmodels (0.14.1), and plotly (5.20.0).

Forest plots are computed using PythonMeta *Deng* with the DerSimonian and Laird random effects model (the fixed effect assumption is not plausible in this case) and inverse variance weighting. Results are presented with 95% confidence intervals. Heterogeneity among studies was assessed using the I^2 statistic. Mixed-effects meta-regression results are computed with R (4.1.2) using the metafor (3.0-2) and rms (6.2-0) packages, and using the most serious sufficiently powered outcome. For all statistical tests, a *p*-value less than 0.05 was considered statistically significant. Grobid 0.8.0 is used to parse PDF documents.

We have classified studies as early treatment if most patients are not already at a severe stage at the time of treatment (for example based on oxygen status or lung involvement), and treatment started within 5 days of the onset of symptoms. If studies contain a mix of early treatment and late treatment patients, we consider the treatment time of patients contributing most to the events (for example, consider a study where most patients are treated early but late treatment patients are included, and all mortality events were observed with late treatment patients). We note that a shorter time may be preferable. Antivirals are typically only considered effective when used within a shorter timeframe, for example 0-36 or 0-48 hours for oseltamivir, with longer delays not being effective *McLean, Treanor*.

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A summary of study results is below. Please submit updates and corrections at https://c19early.org/chxmeta.html.

Early treatment

Effect extraction follows pre-specified rules as detailed above and gives priority to more serious outcomes. For pooled analyses, the first (most serious) outcome is used, which may differ from the effect a paper focuses on. Other outcomes are used in outcome specific analyses.

Jacox, 10/20/2021, Double Blind Randomized Controlled Trial, USA, trial NCT04584684 (history) (MOR).	129 patient RCT with results unknown and over 2 years late.
Jing, 11/21/2023, Double Blind Randomized Controlled Trial, China, peer-reviewed, 7 authors, study period 5 May, 2022 - 16 June, 2022, this trial uses multiple treatments in the treatment arm	olfactory or gustatory dysfunction, 79.2% lower, RR 0.21, $p < 0.001$, treatment 10 of 120 (8.3%), control 56 of 140 (40.0%), NNT 3.2, OGD.

(combined with budesonide and saline) - results of individual treatments may vary, trial ChiCTR2200059651.	VAS olfactory severe, 96.5% lower, RR 0.03, $p < 0.001$, treatment 0 of 120 (0.0%), control 15 of 140 (10.7%), NNT 9.3, relative risk is not 0 because of continuity correction due to zero events (with reciprocal of the contrasting arm).
	VAS gustatory severe, 95.3% lower, RR 0.05, $p = 0.001$, treatment 0 of 120 (0.0%), control 11 of 140 (7.9%), NNT 13, relative risk is not 0 because of continuity correction due to zero events (with reciprocal of the contrasting arm).
	TSS severe, 83.3% lower, RR 0.17, $p = 0.07$, treatment 1 of 120 (0.8%), control 7 of 140 (5.0%), NNT 24.
Mira, 1/8/2022, Double Blind Randomized Controlled Trial, placebo-controlled, trial NCT05543603 (history), excluded in exclusion analyses: study only provides short-term viral load results.	48 patient RCT with results unknown and over 2 years late.
Xie, 2/28/2022, Double Blind Randomized Controlled Trial, placebo-controlled, trial NCT04931004 (history).	Estimated 90 patient RCT with results unknown and over 2 years late.

Late treatment

Effect extraction follows pre-specified rules as detailed above and gives priority to more serious outcomes. For pooled analyses, the first (most serious) outcome is used, which may differ from the effect a paper focuses on. Other outcomes are used in outcome specific analyses.

Huang, 4/30/2021, Randomized Controlled Trial, USA, peer-reviewed, median age 62.0, 2 authors, study period 20 May, 2020 - 15 December, 2020.	risk of no viral clearance, 85.1% lower, RR 0.15, $p < 0.001$, treatment 13 of 93 (14.0%), control 75 of 80 (93.8%), NNT 1.3, oropharyngeal rinse and spray, day 4.
	risk of no viral clearance, 59.9% lower, RR 0.40, $p < 0.001$, treatment 25 of 66 (37.9%), control 52 of 55 (94.5%), NNT 1.8, oropharyngeal rinse only, day 4.

Prophylaxis

Effect extraction follows pre-specified rules as detailed above and gives priority to more serious outcomes. For pooled analyses, the first (most serious) outcome is used, which may differ from the effect a paper focuses on. Other outcomes are used in outcome specific analyses.

Karami, 1/9/2024, Double Blind Randomized Controlled Trial, Iran, peer-reviewed, 4 authors, study period July 2022 - October 2022, trial IRCT20220328054364N1.	relative mean total symptoms, 61.0% better, RR 0.39, $p = 0.04$, treatment mean 1.8 (±3.67) n=36, control mean 4.62 (±7.37) n=40.
	relative mean week 1 symptoms, 82.0% better, RR 0.18, $p = 0.08$, treatment mean 0.22 (±1.17) n=36, control mean 1.22 (±3.14) n=40.
	relative mean week 2 symptoms, 56.0% better, RR 0.44, $p = 0.13$, treatment mean 0.66 (±2.05) n=36, control mean 1.5

	(±2.63) n=40.
	relative mean week 3 symptoms, 11.3% better, RR 0.89, *p* = 0.84, treatment mean 0.86 (±2.66) n=36, control mean 0.97 (±2.16) n=40.
	relative mean week 4 symptoms, 94.6% better, RR 0.05, *p* = 0.048, treatment mean 0.05 (±0.23) n=36, control mean 0.92 (±2.58) n=40.
	risk of case, 56.8% lower, RR 0.43, *p* = 0.03, treatment 7 of 36 (19.4%), control 18 of 40 (45.0%), NNT 3.9.
Keating, 6/30/2022, Randomized Controlled Trial, USA, this trial uses multiple treatments in the treatment arm (combined with PVP-I) - results of individual treatments may vary, trial NCT04478019 (history) (SHIELD).	245 patient RCT with results unknown and over 1.5 years late.

Supplementary Data

Supplementary Data

Footnotes

a. Viral infection and replication involves attachment, entry, uncoating and release, genome replication and transcription, translation and protein processing, assembly and budding, and release. Each step can be disrupted by therapeutics.

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