Nitric Oxide for COVID-19: real-time meta analysis of 11 studies

@CovidAnalysis, March 2024, Version 11
https://c19early.org/nometa.html

Abstract

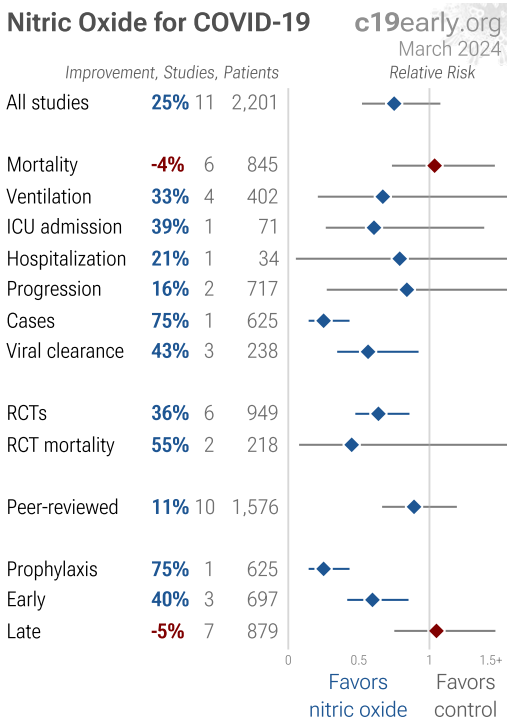
Statistically significant lower risk is seen for cases and viral clearance. 5 studies from 4 independent teams in 4 countries show statistically significant improvements.

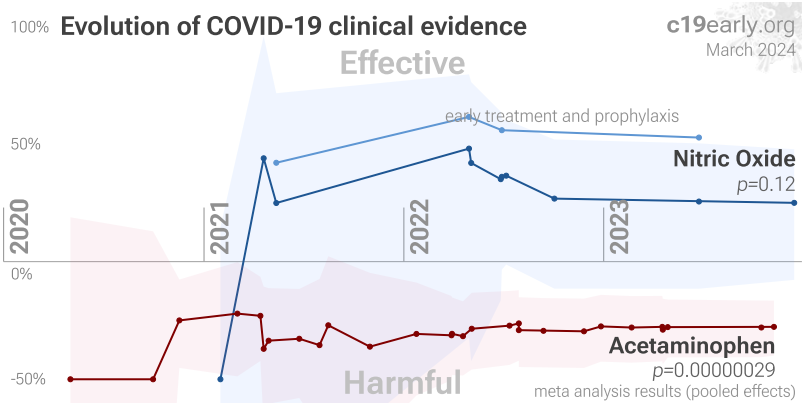
Meta analysis using the most serious outcome reported shows 25% [-8-48%] lower risk, without reaching statistical significance. Results are similar for Randomized Controlled Trials and worse for peer-reviewed studies. Early treatment shows efficacy while late treatment does not, consistent with expectations for an effective topical nasopharyngeal/oropharyngeal treatment.

Mortality results are negative, however all results to date are from late treatment trials.

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 are more effective. Nitric Oxide may affect the natural microbiome, especially with prolonged use.

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





HIGHLIGHTS

Nitric Oxide reduces risk for COVID-19 with high confidence for viral clearance, low confidence for cases and in pooled analysis, and very low confidence for ICU admission.

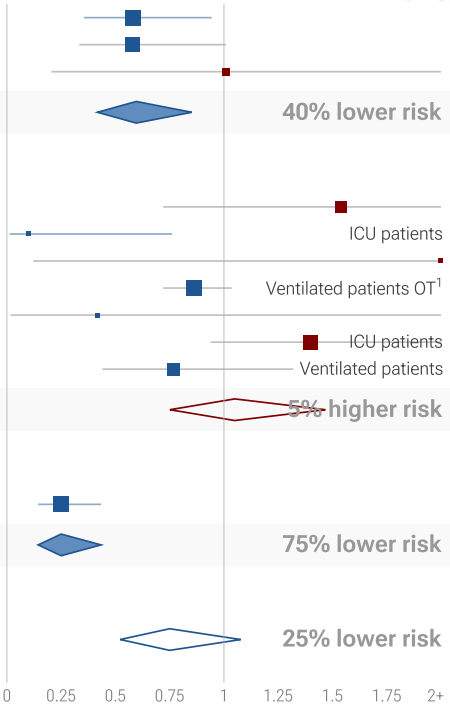
We show traditional outcome specific analyses and combined evidence from all studies, incorporating treatment delay, a primary confounding factor in COVID-19 studies.

Real-time updates and corrections, transparent analysis with all results in the same format, consistent protocol for 66 treatments.

11 nitric oxide COVID-19 studies

c19early.org
March 2024

		Improvement, RR [CI]		Treatment	Control
Winchester (DB RCT)	42%	0.58 [0.36-0.94]	no improv.	8/15	23/25
Tandon (DB RCT)	42%	0.58 [0.33-1.01]	no improv.	14/64	26/69
Bryan (DB RCT)	-1%	1.01 [0.21-4.95]	progression	3/261	3/263
Early treatment	40%	0.60 [0.42-0.85]		25/340	52/357
$Tau^2 = 0.00$, $I^2 = 0.0\%$, $p = 0.0045$					
		Improvement, RR [CI]		Treatment	Control
Chandel	-54%	1.54 [0.72-2.78]	death	12/66	36/206
Moni (RCT)	90%	0.10 [0.01-0.76]	death	0/14	4/11
Strickland (RCT)	-179%	2.79 [0.12-64.0]	ventilation	1/19	0/15
Poonam	14%	0.86 [0.72-1.04]	death	32/41	56/62
Valsecchi	58%	0.42 [0.02-9.86]	death	0/20	1/51
Al Sulaiman (ICU)	-40%	1.40 [0.94-2.11]	death	44/56	52/125
Di Fenza (SB RCT)	23%	0.77 [0.44-1.32]	death	94 (n)	99 (n)
Late treatment	-5%	1.05 [0.75-1.47]		89/310	149/569
$Tau^2 = 0.08$, $I^2 = 61.6\%$, $p = 0.79$					
		Improvement, RR [CI]		Treatment	Control
SaNOtize	75%	0.25 [0.14-0.43]	cases	13/203	108/422
Prophylaxis	75%	0.25 [0.14-0.43]		13/203	108/422
$Tau^2 = 0.00$, $I^2 = 0.0\%$, $p < 0.0001$					
All studies	25%	0.75 [0.52-1.08]		127/853	309/1,348



[1] OT: comparison with other treatment

$Tau^2 = 0.20$, $I^2 = 77.9\%$, $p = 0.12$

Effect extraction pre-specified
(most serious outcome, see appendix)

Favors nitric oxide — Favors control

A

Efficacy in COVID-19 nitric oxide studies (pooled effects)

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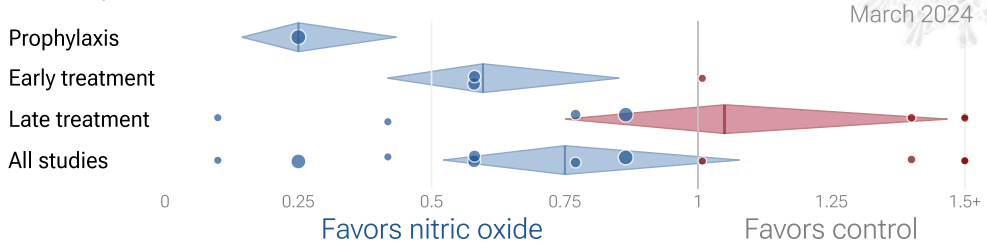
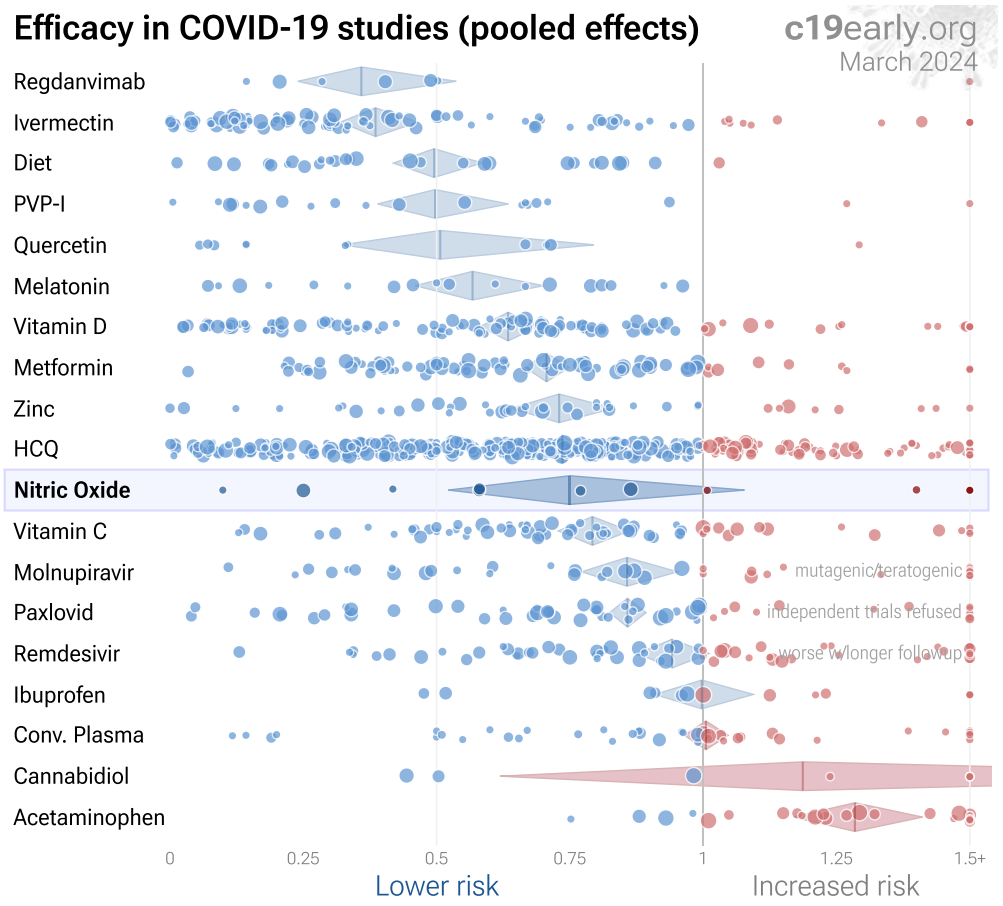


Favors nitric oxide — Favors control

B



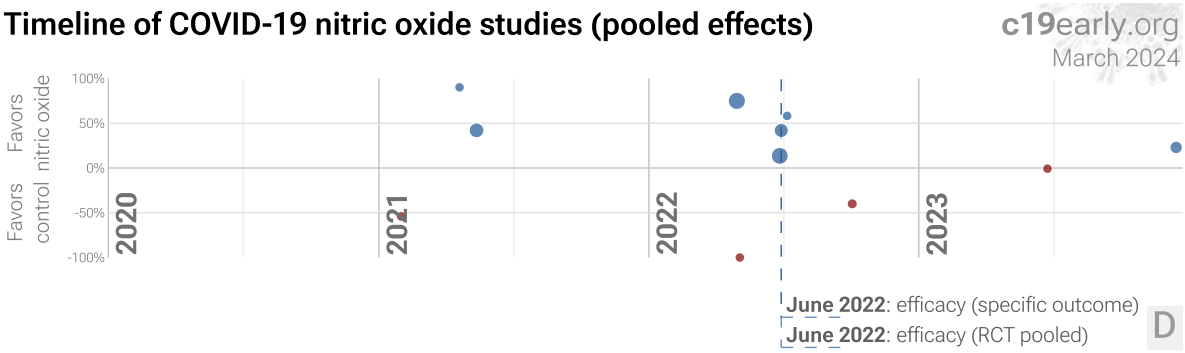


Figure 1. **A. Random effects meta-analysis.** This plot shows pooled effects, see the specific outcome analyses for individual outcomes, and the heterogeneity section for discussion. Effect extraction is pre-specified, using the most serious outcome reported. For details of effect extraction see the appendix. **B. 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. **C. Results within the context of multiple COVID-19 treatments.** 0.6% of 6,686 proposed treatments show efficacy *c19early.org*. **D. Timeline of results in nitric oxide studies.** The marked dates indicate the time when efficacy was known with a statistically significant improvement of ≥10% from ≥3 studies for one or more specific outcome 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

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, providing many therapeutic targets for which many existing compounds have known activity. Scientists have predicted that over 6,000 compounds may reduce COVID-19 risk c19early.org (B), either by directly minimizing infection or replication, by supporting immune system function, or by minimizing secondary complications.

Analysis. We analyze all significant controlled studies of nitric oxide 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, peer-reviewed studies, 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.

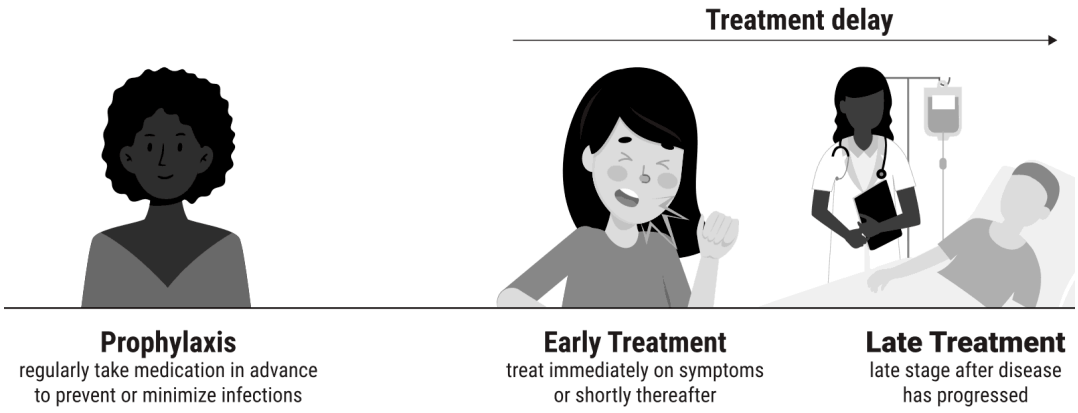


Figure 2. Treatment stages.

Preclinical Research

An *In Vitro* study supports the efficacy of nitric oxide Akaberi.

Preclinical research is an important part of the development of treatments, however results may be very different in clinical trials. Preclinical results are not used in this paper.

Results

Table 1 summarizes the results for all stages combined, for Randomized Controlled Trials, for peer-reviewed studies, with different exclusions, and for specific outcomes. Table 2 shows results by treatment stage. Figure 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 show forest plots for random effects meta-analysis of all studies with pooled effects, early treatment and prophylaxis, mortality results, ventilation, ICU admission, hospitalization, progression, recovery, cases, viral clearance, and peer reviewed studies.

	Improvement	*Studies*	*Patients*	*Authors*
All studies	25% [-8-48%]	11	2,201	164
Exc. late treatment	**53%** [22-71%] **	4	1,322	18
Peer-reviewed studies	**11%** [-19-34%]	10	1,576	163
Randomized Controlled Trials	36% [14-53%] **	6	949	95
RCTs exc. late treatment	40% [15-58%] **	3	697	17
Mortality	-4% [-46-27%]	6	845	138
Ventilation	33% [-114-79%]	4	402	58
Viral	43% [8-65%] *	3	238	30
RCT mortality	55% [-163-92%]	2	218	70

Table 1. **Random effects meta-analysis for all stages combined, for Randomized Controlled Trials, for peer-reviewed studies, with different exclusions, and for specific outcomes.** Results show the percentage improvement with treatment and the 95% confidence interval. ** $p<0.01$ **** $p<0.0001$.

	Early treatment	*Late treatment*	*Prophylaxis*
All studies	40% [15-58%] **	-5% [-47-25%]	75% [57-86%] ****
Exc. late treatment	40% [15-58%] **		75% [57-86%] ****
Peer-reviewed studies	40% [15-58%] **	-5% [-47-25%]	
Randomized Controlled Trials	40% [15-58%] **	33% [-114-79%]	
Mortality		-4% [-46-27%]	
Ventilation		33% [-114-79%]	
Viral	35% [-6-60%]	64% [26-83%] **	
RCT mortality		55% [-163-92%]	

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. ** $p<0.01$ **** $p<0.0001$.

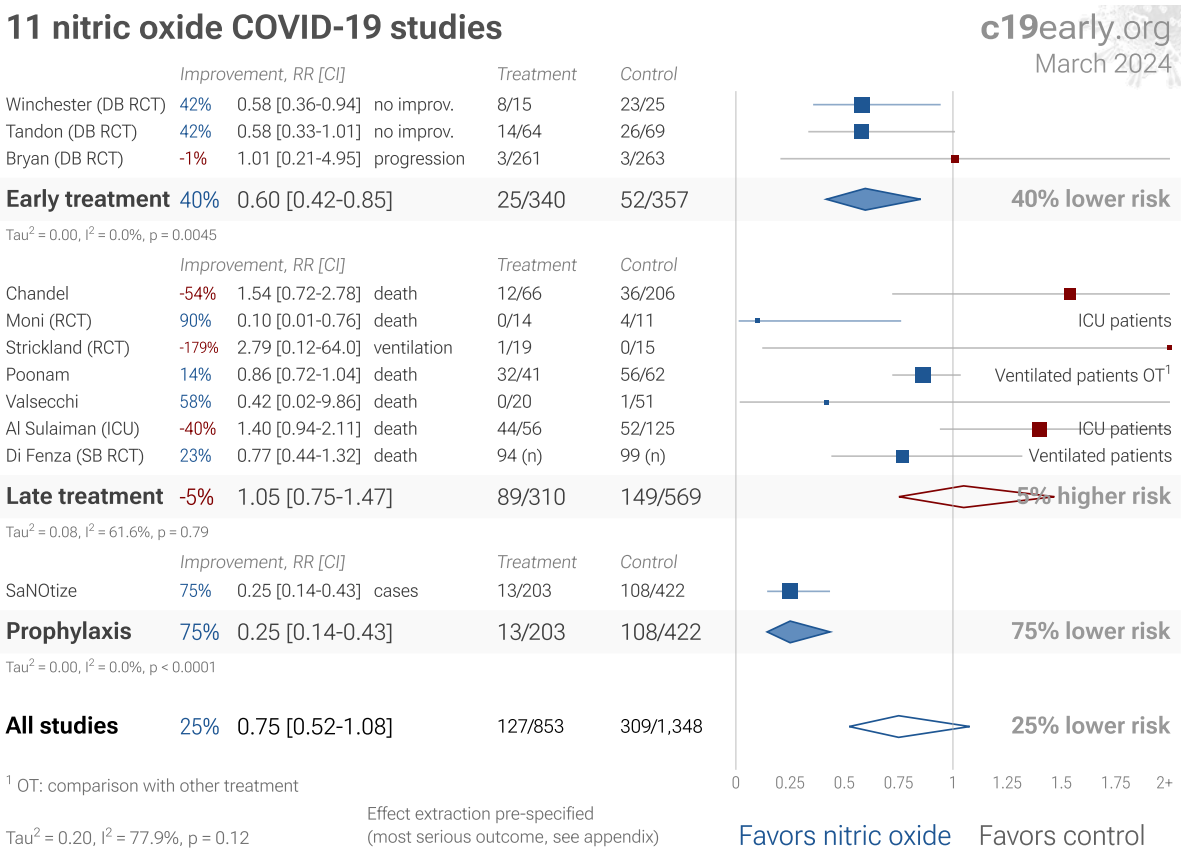


Figure 3. **Random effects meta-analysis for all studies with pooled effects.** This plot shows pooled effects, see the specific outcome analyses for individual outcomes, and the heterogeneity section for discussion. Effect extraction is pre-specified, using the most serious outcome reported. For details of effect extraction see the appendix.

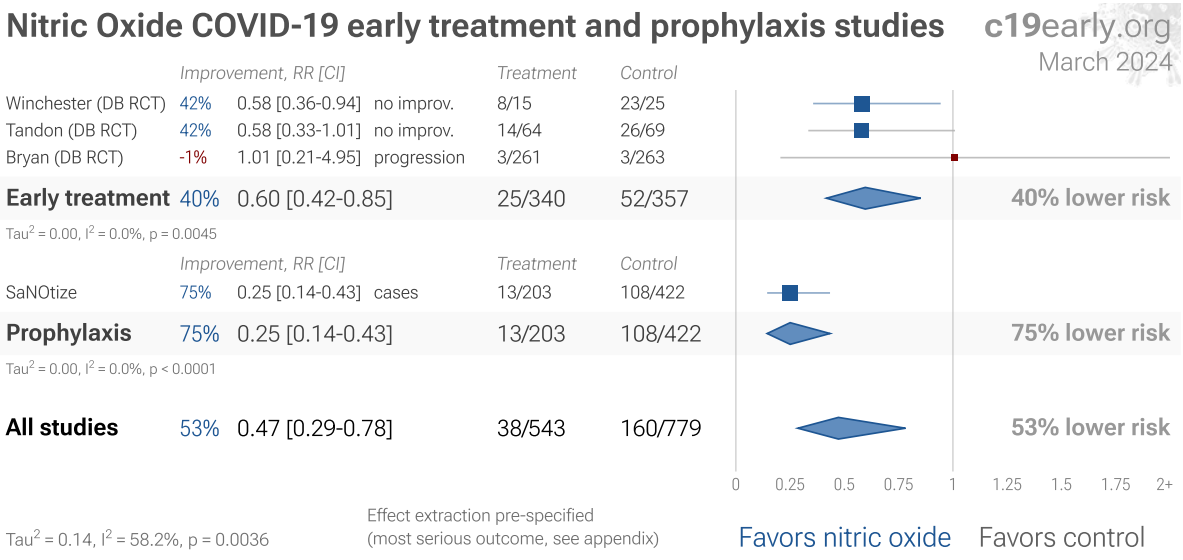


Figure 4. **Random effects meta-analysis for early treatment and prophylaxis.**

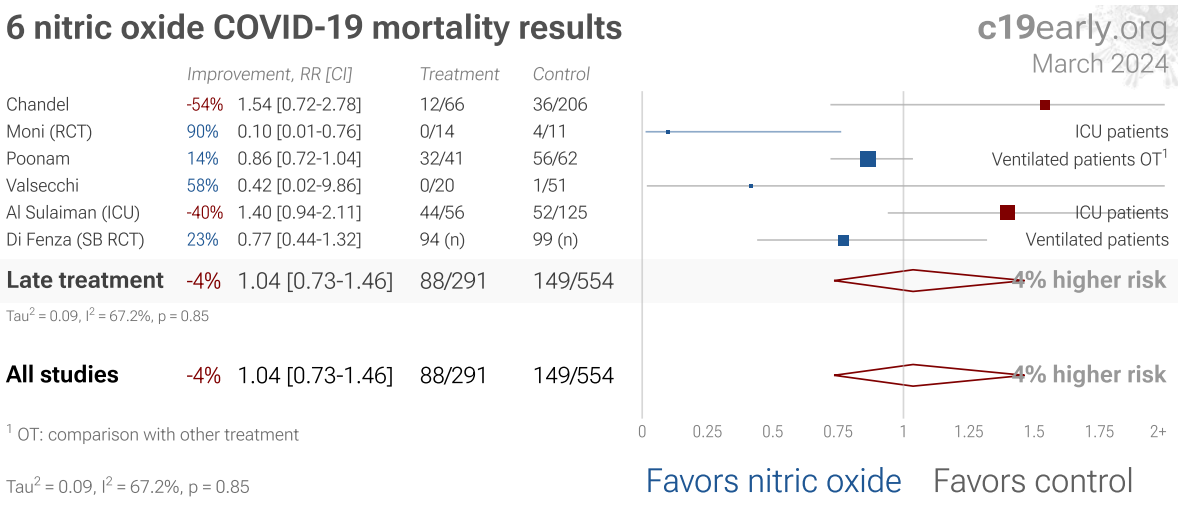


Figure 5. Random effects meta-analysis for mortality results.

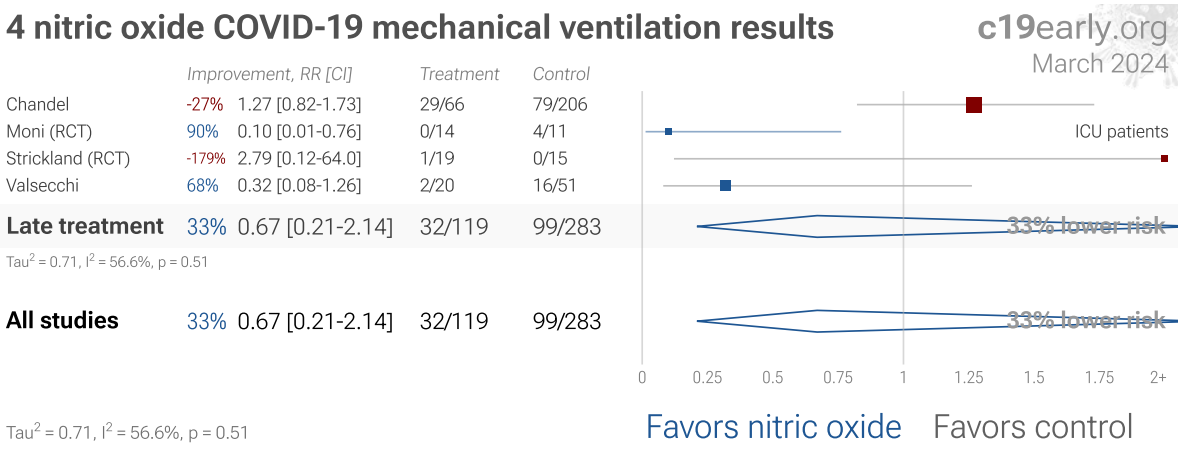


Figure 6. Random effects meta-analysis for ventilation.

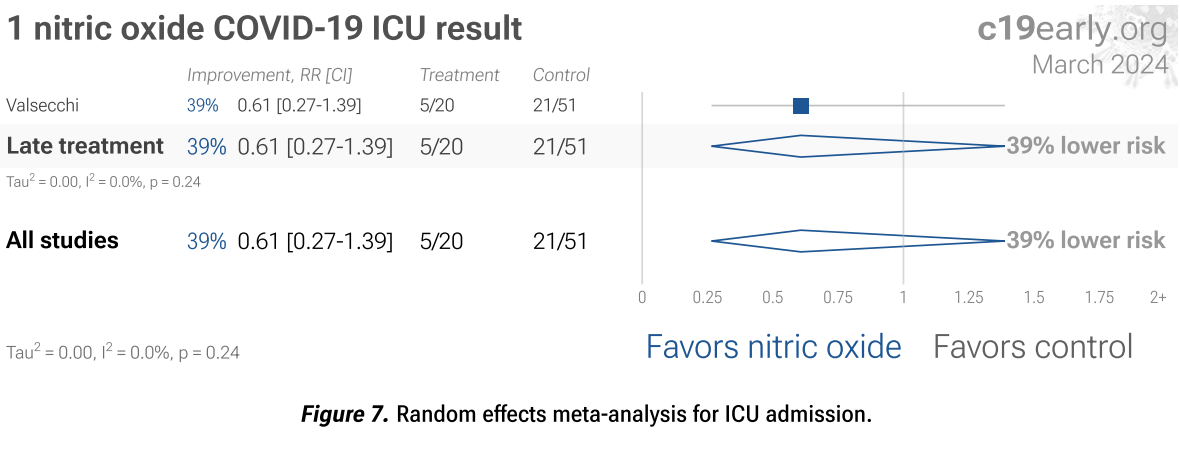


Figure 7. Random effects meta-analysis for ICU admission.

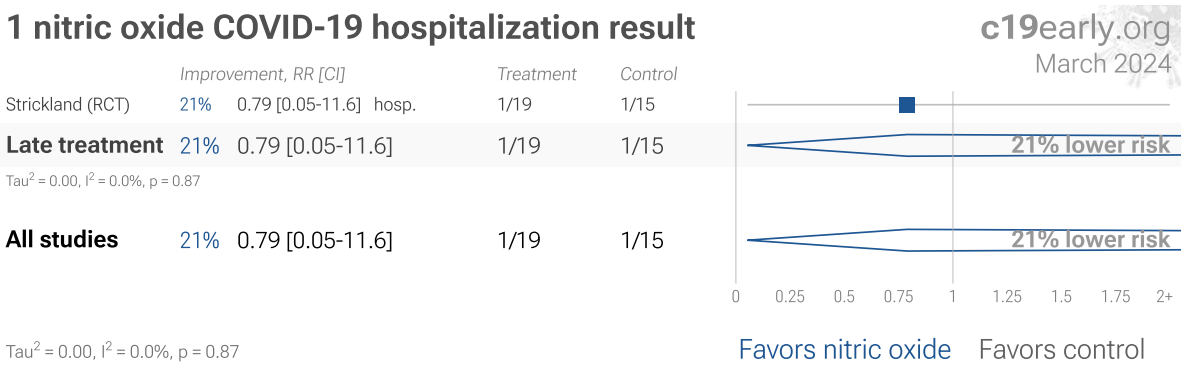


Figure 8. Random effects meta-analysis for hospitalization.

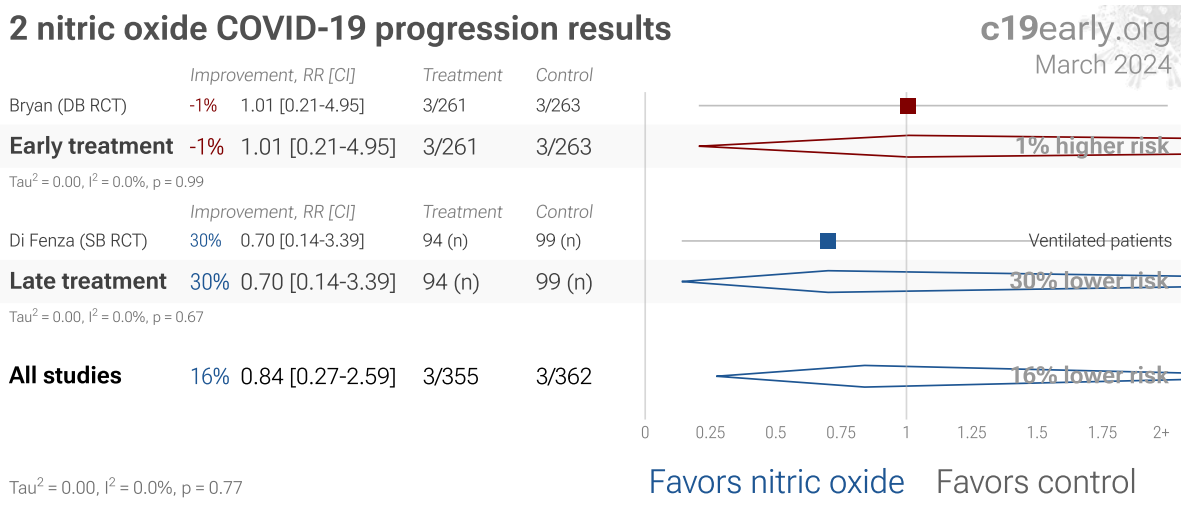


Figure 9. Random effects meta-analysis for progression.

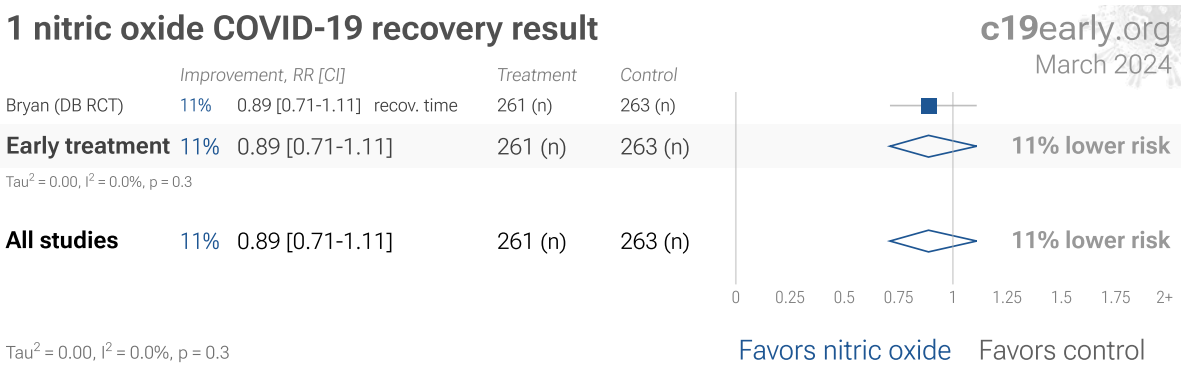


Figure 10. Random effects meta-analysis for recovery.

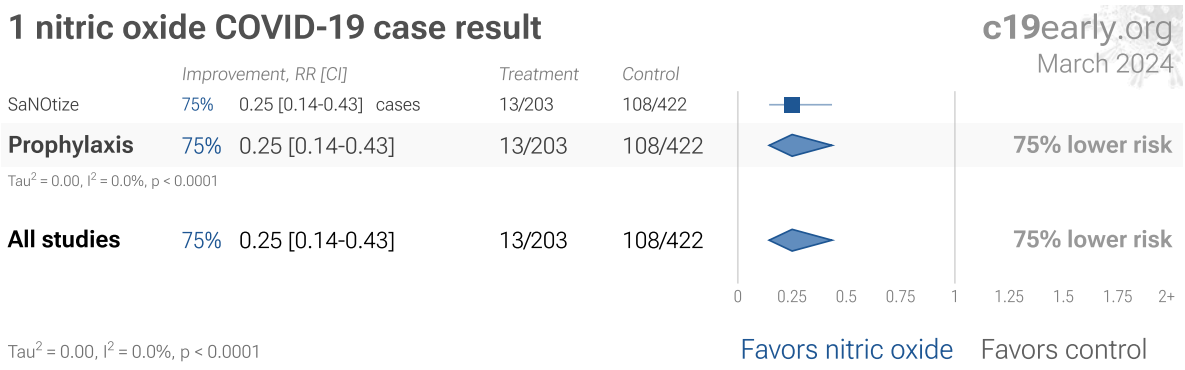


Figure 11. Random effects meta-analysis for cases.

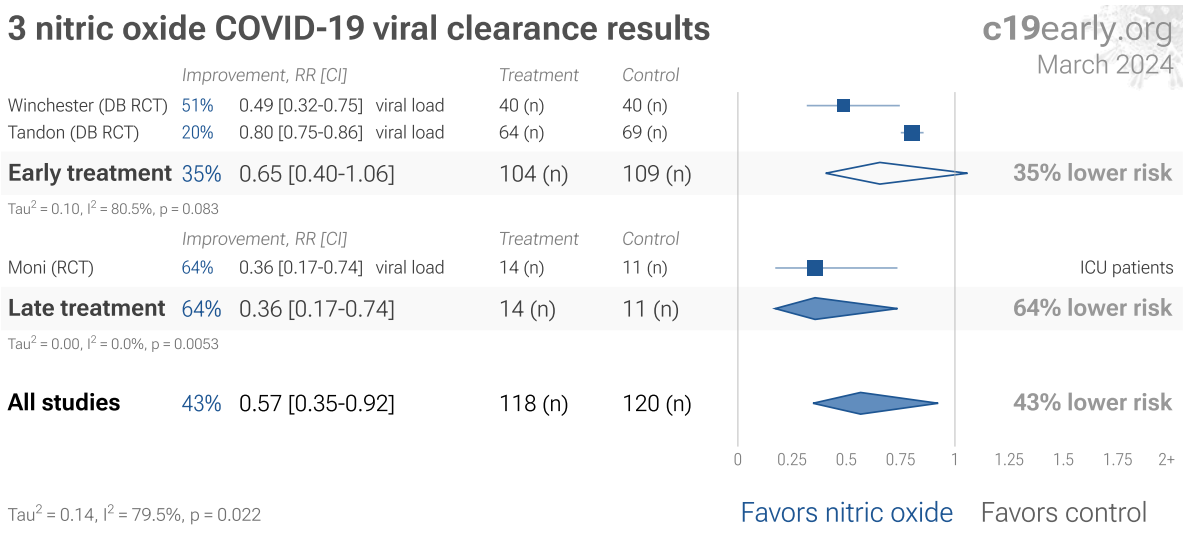


Figure 12. Random effects meta-analysis for viral clearance.

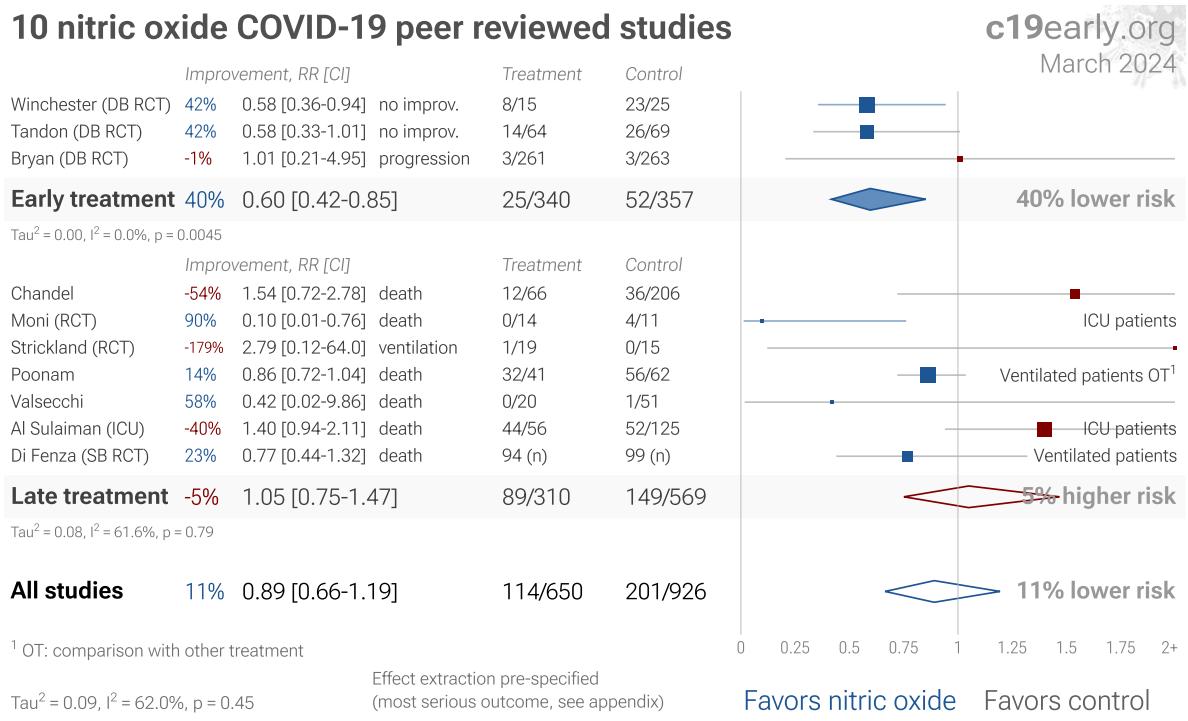


Figure 13. **Random effects meta-analysis for peer reviewed studies.** Effect extraction is pre-specified, using the most serious outcome reported, see the appendix for details. *Zeraatkar et al.* analyze 356 COVID-19 trials, finding no significant evidence that preprint results are inconsistent with peer-reviewed studies. They also show extremely long peer-review delays, with a median of 6 months to journal publication. A six month delay was equivalent to around 1.5 million deaths during the first two years of the pandemic. Authors recommend using preprint evidence, with appropriate checks for potential falsified data, which provides higher certainty much earlier. *Davidson et al.* also showed no important difference between meta analysis results of preprints and peer-reviewed publications for COVID-19, based on 37 meta analyses including 114 trials.

Randomized Controlled Trials (RCTs)

Figure 14 shows a comparison of results for RCTs and non-RCT studies. The median effect size for RCTs is 32% improvement, compared to 14% for other studies. Figure 15, 16, and 17 show forest plots for random effects meta-analysis of all Randomized Controlled Trials, all early treatment and prophylaxis RCTs, and RCT mortality results. RCT results are included in Table 1 and Table 2.

RCTs have many potential biases. Bias in clinical research may be defined as something that tends to make conclusions differ systematically from the truth. RCTs help to make study groups more similar and can provide a higher level of evidence, however they are subject to many biases *Jadad*, and analysis of double-blind RCTs has identified extreme levels of bias *Gøtzsche*. For COVID-19, the overhead may delay treatment, dramatically compromising efficacy; they may encourage monotherapy for simplicity at the cost of efficacy which may rely on combined or synergistic effects; the participants that sign up may not reflect real world usage or the population that benefits most in terms of age, comorbidities, severity of illness, or other factors; standard of care may be compromised and unable to evolve quickly based on emerging research for new diseases; errors may be made in randomization and medication delivery; and investigators may have hidden agendas or vested interests influencing design, operation, analysis, and the potential for fraud. All of these biases have been observed with COVID-19 RCTs. There is no guarantee that a specific RCT provides a higher level of evidence.

Conflicts of interest for COVID-19 RCTs. RCTs are expensive and many RCTs are funded by pharmaceutical companies or interests closely aligned with pharmaceutical companies. For COVID-19, this creates an incentive to show efficacy for patented commercial products, and an incentive to show a lack of efficacy for inexpensive treatments. The bias is expected to be significant, for example *Als-Nielsen et al.* analyzed 370 RCTs from Cochrane reviews, showing that trials funded by for-profit organizations were 5 times more likely to recommend the

experimental drug compared with those funded by nonprofit organizations. For COVID-19, some major philanthropic organizations are largely funded by investments with extreme conflicts of interest for and against specific COVID-19 interventions.

RCTs for novel acute diseases requiring rapid treatment. High quality RCTs for novel acute diseases are more challenging, with increased ethical issues due to the urgency of treatment, increased risk due to enrollment delays, and more difficult design with a rapidly evolving evidence base. For COVID-19, the most common site of initial infection is the upper respiratory tract. Immediate treatment is likely to be most successful and may prevent or slow progression to other parts of the body. For a non-prophylaxis RCT, it makes sense to provide treatment in advance and instruct patients to use it immediately on symptoms, just as some governments have done by providing medication kits in advance. Unfortunately, no RCTs have been done in this way. Every treatment RCT to date involves delayed treatment. Among the 66 treatments we have analyzed, 63% of RCTs involve very late treatment 5+ days after onset. No non-prophylaxis COVID-19 RCTs match the potential real-world use of early treatments (they may more accurately represent results for treatments that require visiting a medical facility, e.g., those requiring intravenous administration).

Non-RCT studies have been shown to be reliable. Evidence shows that non-RCT trials can also provide reliable results. *Concato et al.* found that well-designed observational studies do not systematically overestimate the magnitude of the effects of treatment compared to RCTs. *Anglemyer et al.* summarized reviews comparing RCTs to observational studies and found little evidence for significant differences in effect estimates. *Lee et al.* showed that only 14% of the guidelines of the Infectious Diseases Society of America were based on RCTs. Evaluation of studies relies on an understanding of the study and potential biases. Limitations in an RCT can outweigh the benefits, for example excessive dosages, excessive treatment delays, or Internet survey bias could have a greater effect on results. Ethical issues may also prevent running RCTs for known effective treatments. For more on issues with RCTs see *Deaton, Nichol*.

Using all studies identifies efficacy 5.7+ months faster for COVID-19. 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. Of the 44 treatments with statistically significant efficacy/harm, 28 have been confirmed in RCTs, with a mean delay of 5.7 months. When considering only low cost treatments, 23 have been confirmed with a delay of 6.9 months. For the 16 unconfirmed treatments, 3 have zero RCTs to date. The point estimates for the remaining 13 are all consistent with the overall results (benefit or harm), with 10 showing >20%. The only treatments showing >10% efficacy for all studies, but <10% for RCTs are sotrovimab and aspirin.

Summary. We need to evaluate each trial on its own merits. RCTs for a given medication and disease may be more reliable, however they may also be less reliable. For off-patent medications, very high conflict of interest trials may be more likely to be RCTs, and more likely to be large trials that dominate meta analyses.

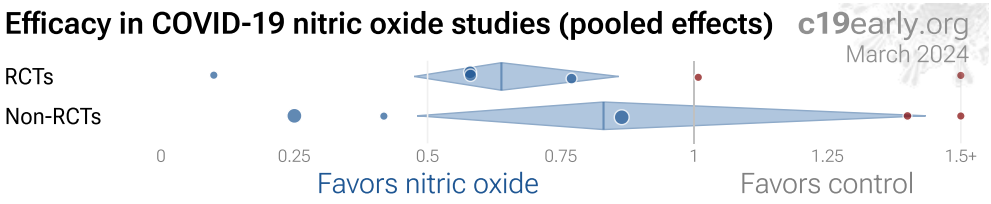


Figure 14. **Results for RCTs and non-RCT studies.**

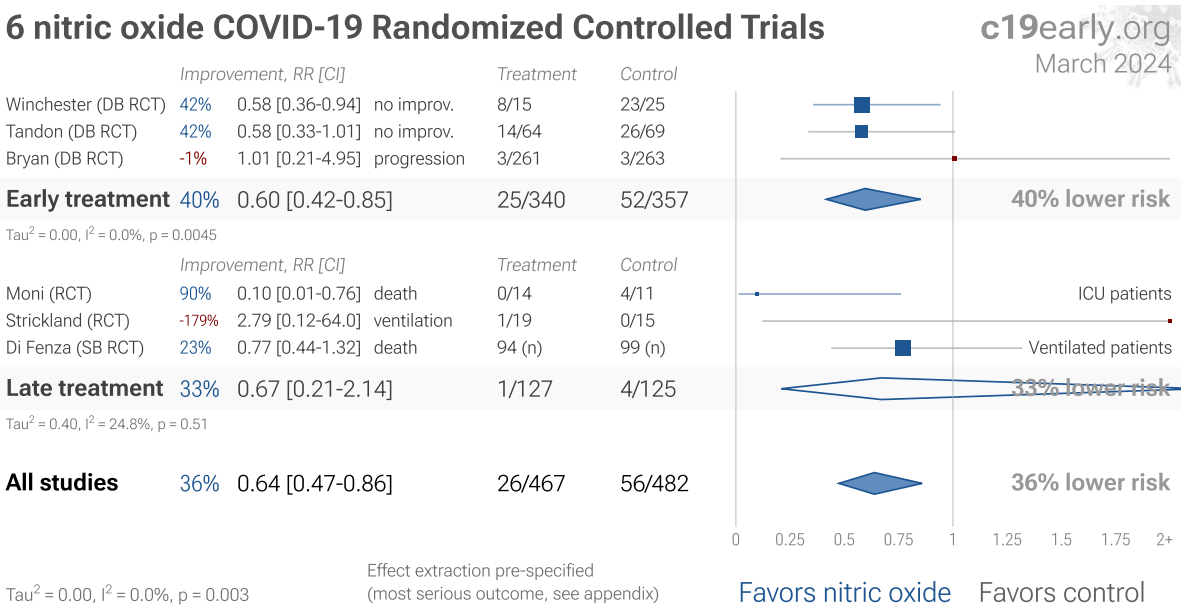


Figure 15. **Random effects meta-analysis for all Randomized Controlled Trials.** This plot shows pooled effects, see the specific outcome analyses for individual outcomes, and the heterogeneity section for discussion. Effect extraction is pre-specified, using the most serious outcome reported. For details of effect extraction see the appendix.

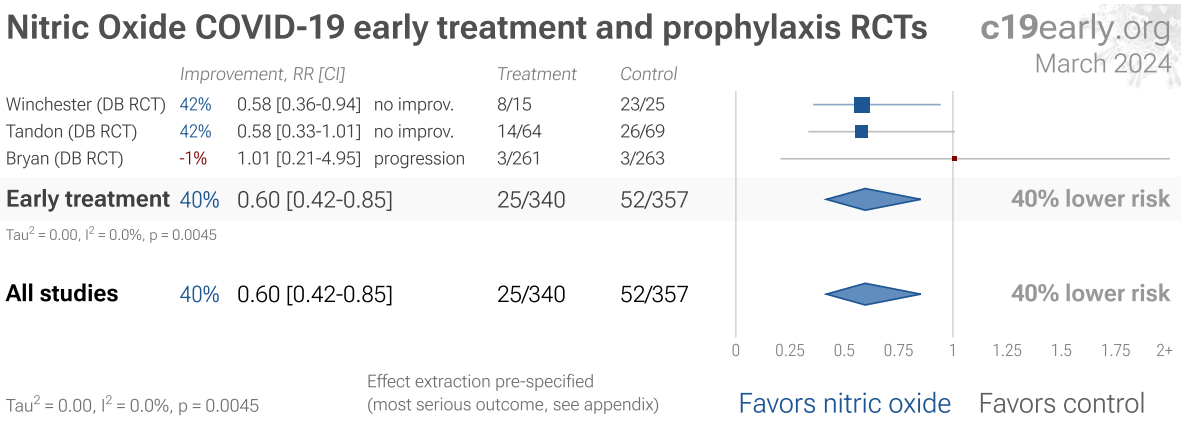


Figure 16. **Random effects meta-analysis for all early treatment and prophylaxis RCTs.** Effect extraction is pre-specified, using the most serious outcome reported, see the appendix for details.

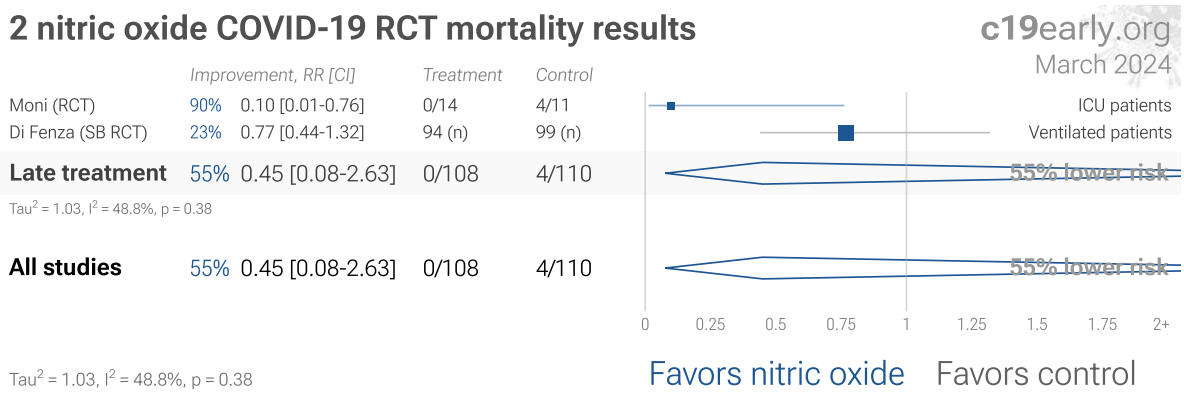


Figure 17. **Random effects meta-analysis for RCT mortality results.**

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* report an 86% reduction in cases for post-exposure prophylaxis, *Hayden* 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* 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 18 shows a mixed-effects meta-regression for efficacy as a function of treatment delay in COVID-19 studies from 66 treatments, showing that efficacy declines rapidly with treatment delay. Early treatment is critical for COVID-19.

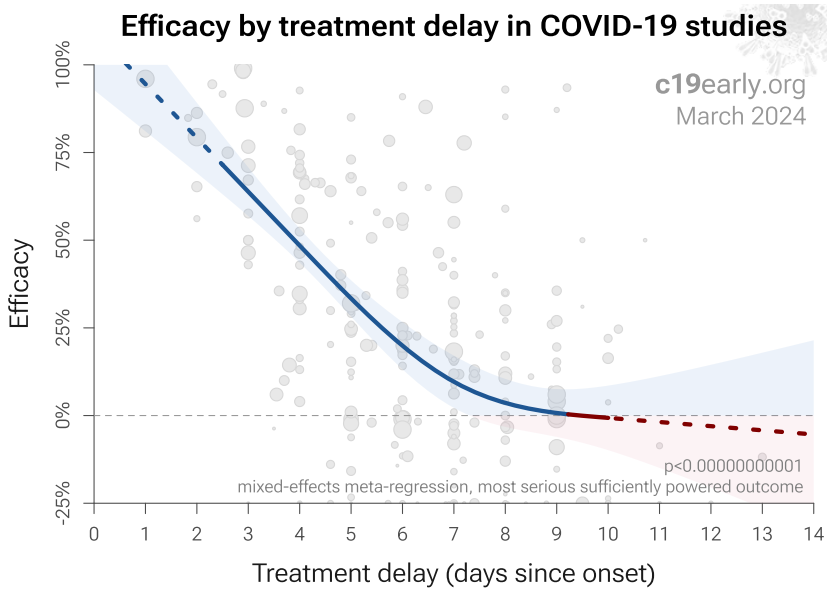


Figure 18. **Early treatment is more effective.** Meta-regression showing efficacy as a function of treatment delay in COVID-19 studies from 66 treatments.

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 (as in *López-Medina*).

Effect measured. Efficacy may differ significantly depending on the effect measured, for example a treatment may be very effective at reducing mortality, but less effective at minimizing cases or hospitalization. Or a treatment may have no effect on viral clearance while still being effective at reducing mortality.

Variants. There are many different variants of SARS-CoV-2 and efficacy may depend critically on the distribution of variants encountered by the patients in a study. 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 viral entry process for the omicron variant has moved towards TMPRSS2-independent fusion, suggesting that TMPRSS2 inhibitors may be less effective *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 anything from supplements, other medications, or other kinds of treatment such as prone positioning.

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

Pooled outcome analysis. We present both pooled analyses and specific outcome analyses. Notably, pooled analysis often results in earlier detection of efficacy as shown in Figure 19. For many COVID-19 treatments, a reduction in mortality logically follows from a reduction in hospitalization, which follows from a reduction in symptomatic cases, etc. An antiviral tested with a low-risk population may report zero mortality in both arms, however a reduction in severity and improved viral clearance may translate into lower mortality among a high-risk population, and including these results in pooled analysis allows faster detection of efficacy. Trials with high-risk patients may also be restricted due to ethical concerns for treatments that are known or expected to be effective.

Pooled analysis enables using more of the available information. While there is much more information available, for example dose-response relationships, the advantage of the method used here is simplicity and transparency. Note that pooled analysis could hide efficacy, for example a treatment that is beneficial for late stage patients but has no effect on viral replication or early stage disease could show no efficacy in pooled analysis if most studies only examine viral clearance. While we present pooled results, we also present individual outcome analyses, which may be more informative for specific use cases.

Pooled outcomes identify efficacy faster. 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. 88% of treatments showing statistically significant efficacy/harm with pooled effects have been confirmed with one or more specific outcomes, with a mean delay of 3.6 months. When restricting to RCTs only, 50% of treatments showing statistically significant efficacy/harm with pooled effects have been confirmed with one or more specific outcomes, with a mean delay of 6.1 months.

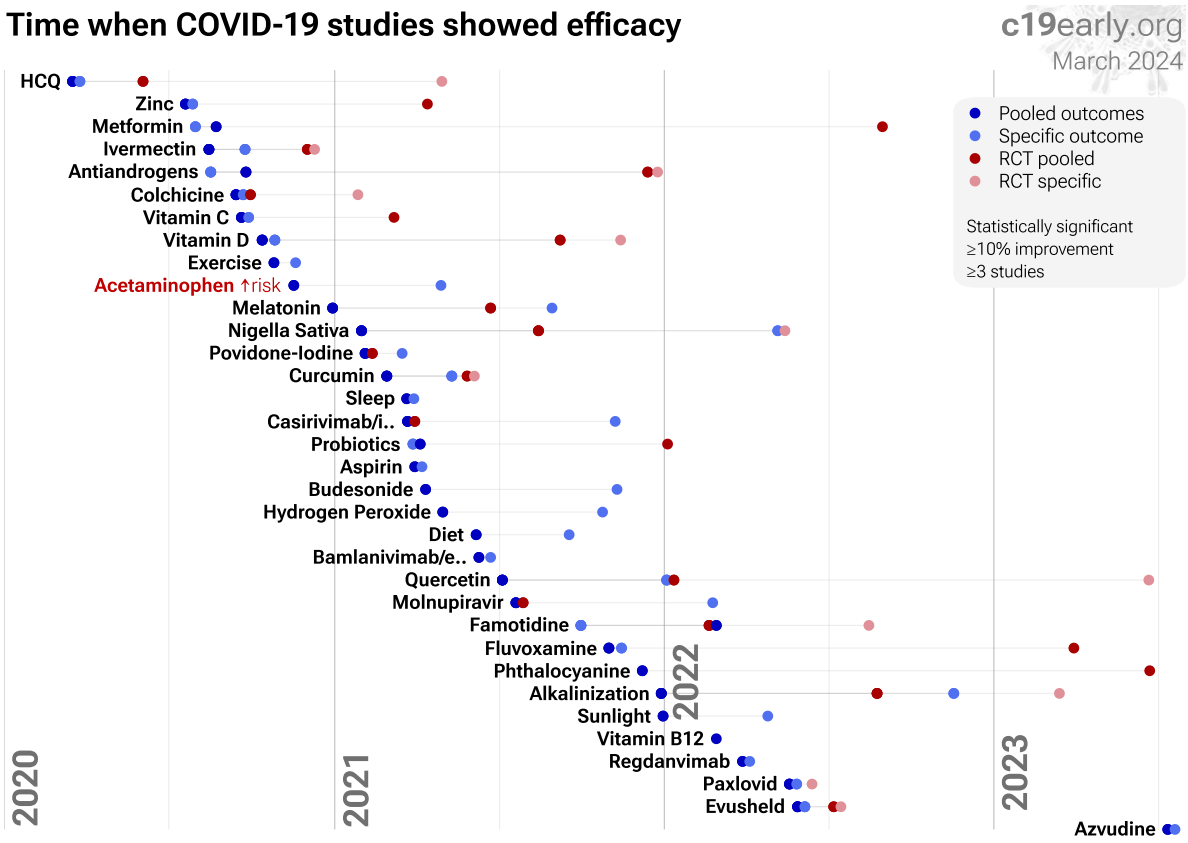


Figure 19. **The time when studies showed that treatments were effective, defined as statistically significant improvement of ≥10% from ≥3 studies.** Pooled results typically show efficacy earlier than specific outcome results. Results from all studies often shows efficacy much earlier than when restricting to RCTs. Results reflect conditions as used in trials to date, these depend on the population treated, treatment delay, and treatment regimen.

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. This may have a greater effect than pooling different outcomes such as mortality and hospitalization. For example a treatment may have 50% efficacy for mortality but only 40% for hospitalization when used within 48 hours. However efficacy could be 0% when used late.

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.

Discussion

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]. For nitric oxide, there is currently not enough data to evaluate publication bias with high confidence.

One method to evaluate bias is to compare prospective vs. retrospective studies. Prospective studies are more likely to be published regardless of the result, while retrospective studies are more likely to exhibit bias. For example, researchers may perform preliminary analysis with minimal effort and the results may influence their decision to continue. Retrospective studies also provide more opportunities for the specifics of data extraction and adjustments to influence results.

Figure 20 shows a scatter plot of results for prospective and retrospective studies. 60% of retrospective studies report positive effects, compared to 67% of prospective studies, consistent with a bias toward publishing negative results. The median effect size for retrospective studies is 14% improvement, compared to 32% for prospective studies, suggesting a potential bias towards publishing results showing lower efficacy.

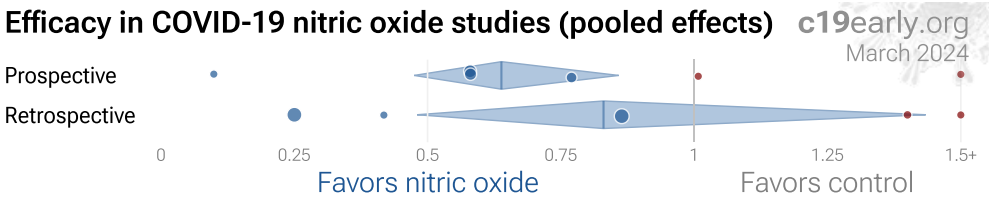


Figure 20. **Prospective vs. retrospective studies. The diamonds show the results of random effects meta-analysis.**

Late treatment bias. Studies for nitric oxide were primarily late treatment studies, in contrast with typical patented treatments that were tested with early treatment as recommended.

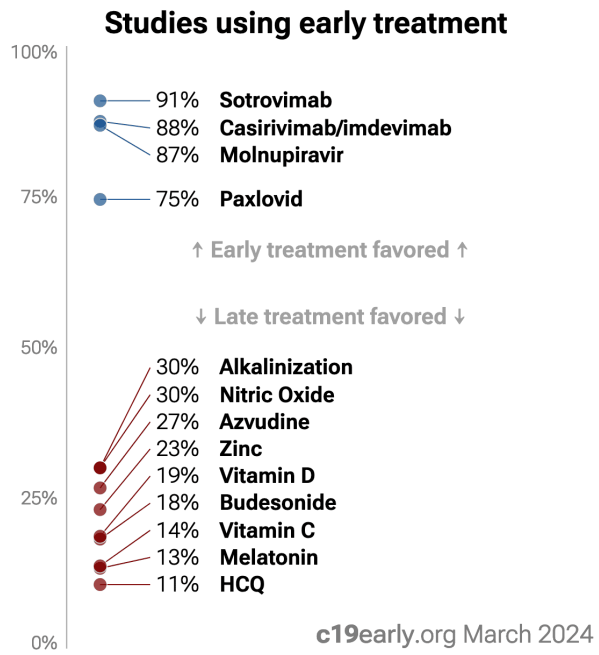


Figure 21. **Patented treatments received mostly early treatment studies, while low cost treatments were typically tested for late treatment.**

Funnel plot analysis. Funnel plots have traditionally been used for analyzing publication bias. This is invalid for COVID-19 acute treatment trials — the underlying assumptions are invalid, which we can demonstrate with a simple example. Consider a set of hypothetical perfect trials with no bias. Figure 22 plot A shows a funnel plot for a simulation of 80 perfect trials, with random group sizes, and each patient's outcome randomly sampled (10% control event probability, and a 30% effect size for treatment). Analysis shows no asymmetry ($p > 0.05$). In plot B, we add a single typical variation in COVID-19 treatment trials — treatment delay. Consider that efficacy varies from 90% for treatment within 24 hours, reducing to 10% when treatment is delayed 3 days. In plot B, each trial's treatment delay is randomly selected. Analysis now shows highly significant asymmetry, $p < 0.0001$, with six variants of Egger's test all showing $p < 0.05$ *Egger, Harbord, Macaskill, Moreno, Peters, Rothstein, Rücker, Stanley*. Note that these tests fail even though treatment delay is uniformly distributed. In reality treatment delay is more complex — each trial has a different distribution of delays across patients, and the distribution across trials may be biased (e.g., late treatment trials may be more common). Similarly, many other variations in trials may produce asymmetry, including dose, administration, duration of treatment, differences in SOC, comorbidities, age, variants, and bias in design, implementation, analysis, and reporting.

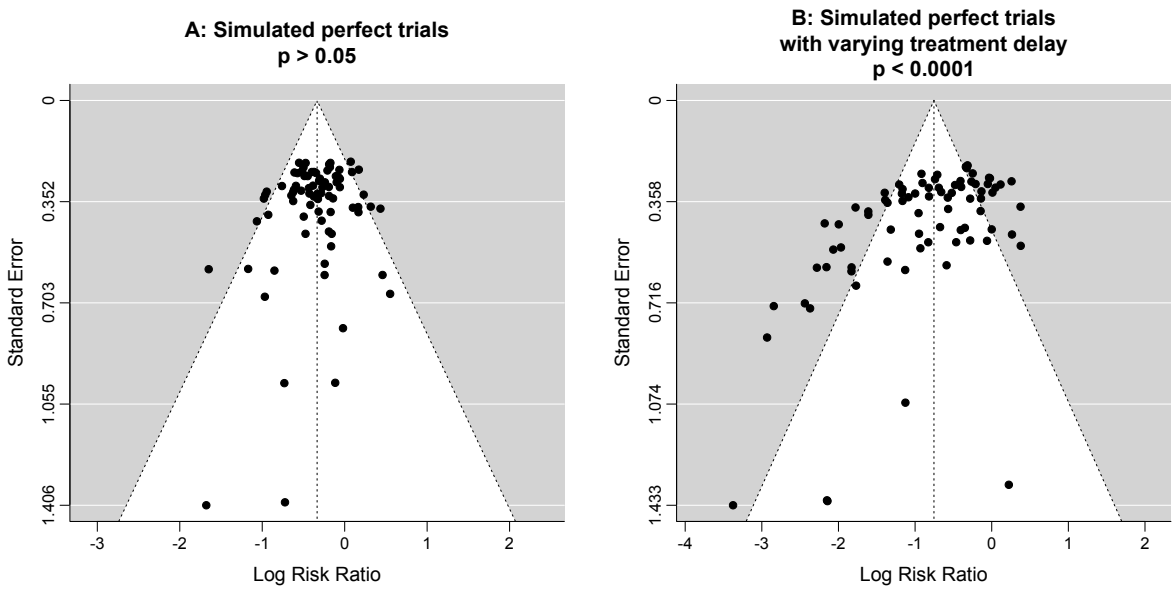


Figure 22. Example funnel plot analysis for simulated perfect trials.

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. Nitric Oxide for COVID-19 lacks this because it is off-patent, has multiple manufacturers, and is very low cost. In contrast, most COVID-19 nitric oxide 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 nitric oxide 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 by 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 affiliated with special interests 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, vaccine, 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 the 11 studies compare against other treatments, which may reduce the effect seen.

Reviews. Multiple reviews cover nitric oxide for COVID-19, presenting additional background on mechanisms and related results, including *Hedenstierna, Oza, Yamasaki, Zhang, Zhao*.

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.

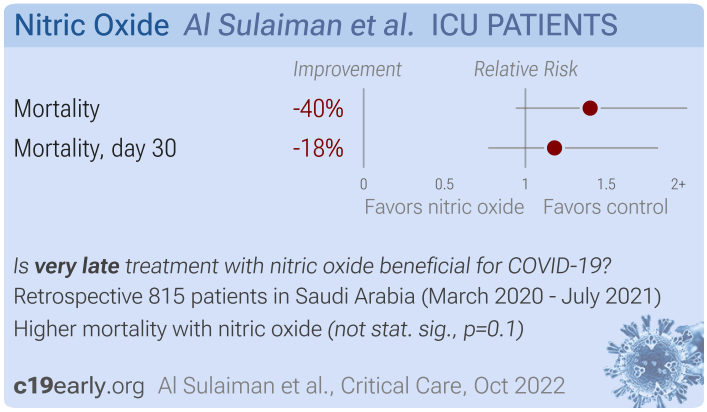
Statistically significant lower risk is seen for cases and viral clearance. 5 studies from 4 independent teams in 4 countries show statistically significant improvements. Meta analysis using the most serious outcome reported shows 25% [-8-48%] lower risk, without reaching statistical significance. Results are similar for Randomized Controlled Trials and worse for peer-reviewed studies. Early treatment shows efficacy while late treatment does not, consistent with expectations for an effective topical nasopharyngeal/oropharyngeal treatment.

Mortality results are negative, however all results to date are from late treatment trials.

Nitric Oxide may affect the natural microbiome, especially with prolonged use.

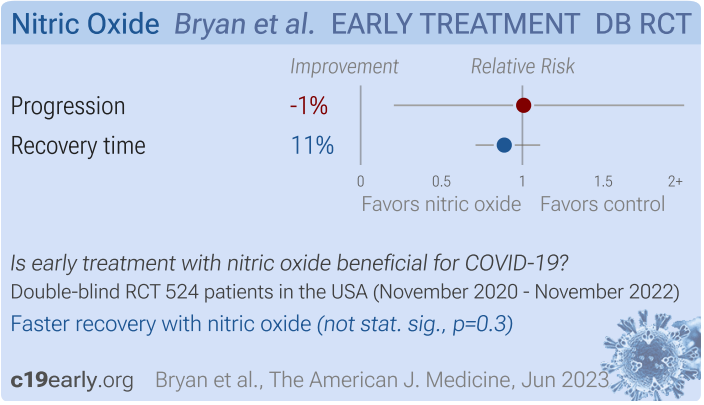
Study Notes

Al Sulaiman



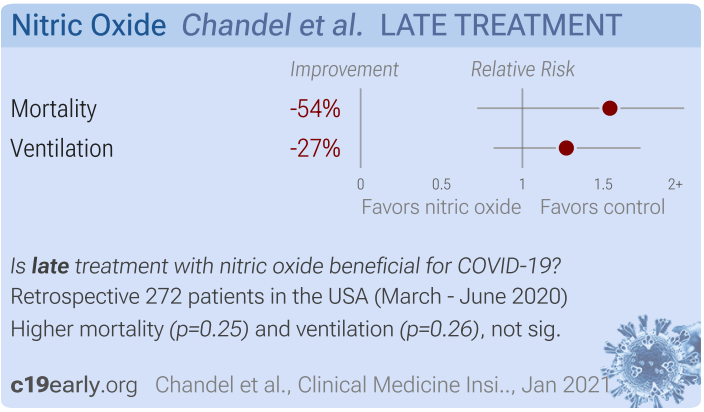
Al Sulaiman: Retrospective 815 COVID-19 ICU patients in Saudi Arabia, showing significant improvement in oxygenation. There was no significant difference in mortality, and ICU and hospitalization time was longer.

Bryan



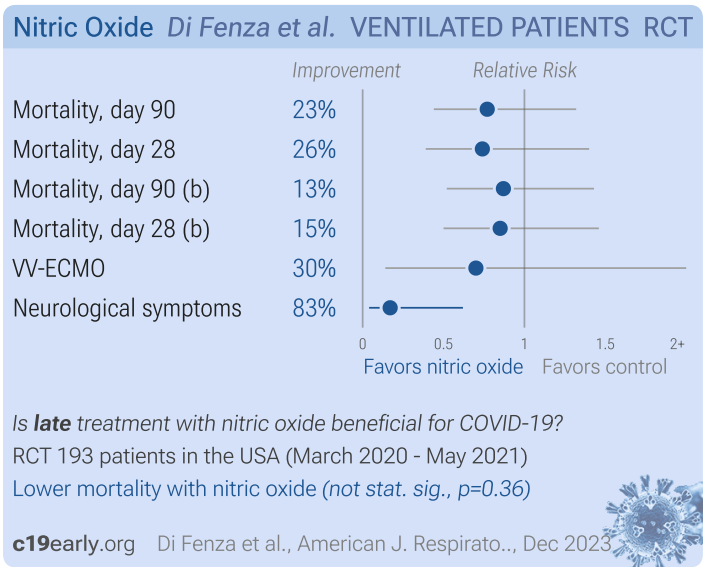
Bryan: RCT 524 outpatients in the USA for a nitric oxide generating lozenge, showing no significant difference in combined hospitalization, ICU admission, intubation, dialysis, and death. There were only 3 events in each arm, all occuring in 2020, with zero events in 2021 or 2022. Recovery was 11% faster with treatment, without statistical significance. Authors note that a higher dose may have been more effective. Trials showing greater efficacy have used a nasal spray.

Chandel



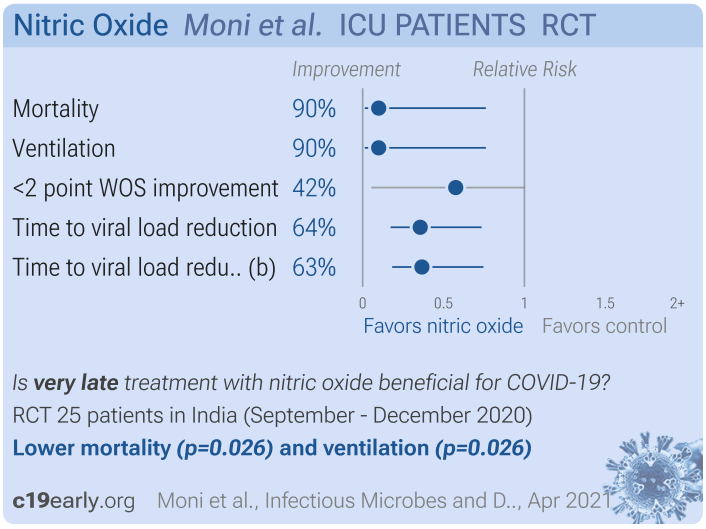
Chandel: Retrospective 272 acute respitory failure patients in the USA treated with high-flow nasal cannula, 66 treated with inhaled nitric oxide, showing increased mortality with inhaled nitric oxide. There were significant differences in the usage of several other treatments between the groups.

Di Fenza



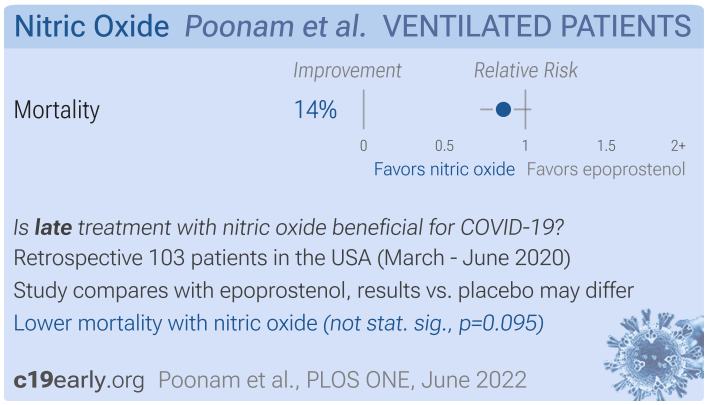
Di Fenza: RCT 193 mechanically ventilated COVID-19 patients showing improved oxygenation at 48 hours but no difference in mortality with high-dose (80ppm) inhaled nitric oxide (NO) for 48 hours. The NO group had a higher proportion attaining PaO2/FiO2 > 300 mmHg and reduced rates of neurologic symptoms at 90 days. NO was associated with faster viral clearance. No serious adverse events were reported with NO.

Moni



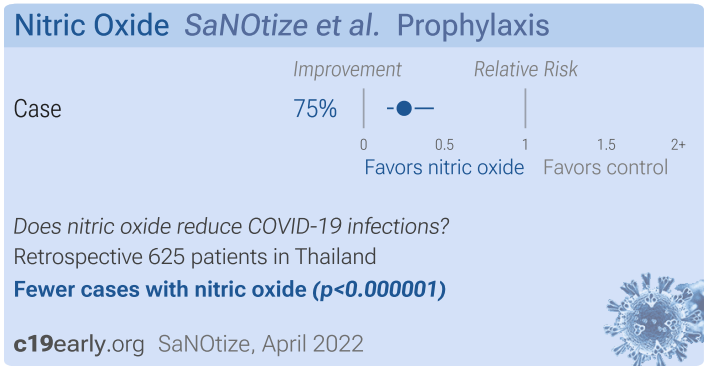
Moni: RCT 29 ICU patients in India, showing improved clinical outcomes and faster viral clearance with inhaled nitric oxide treatment. The treatment group was younger (mean 54 vs. 66) and had more patients on NIV at baseline (29% vs. 18%).

Poonam



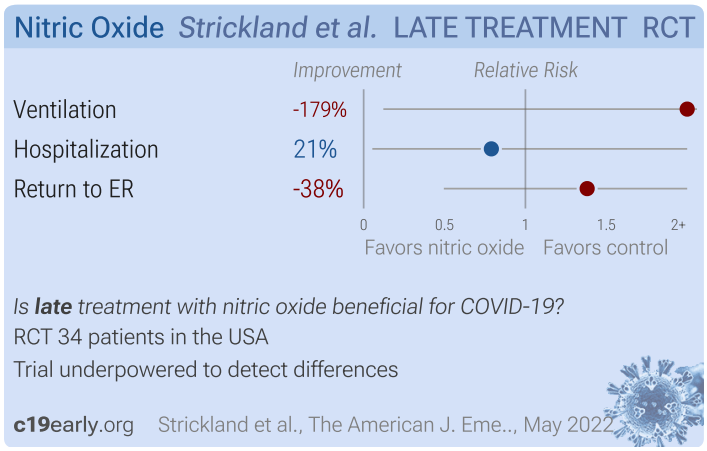
Poonam: Retrospective 103 mechanically ventilated patients, 41 treated with inhaled nitric oxide, and 62 with inhaled epoprostenol, showing no significant difference in outcomes.

SaNOtize



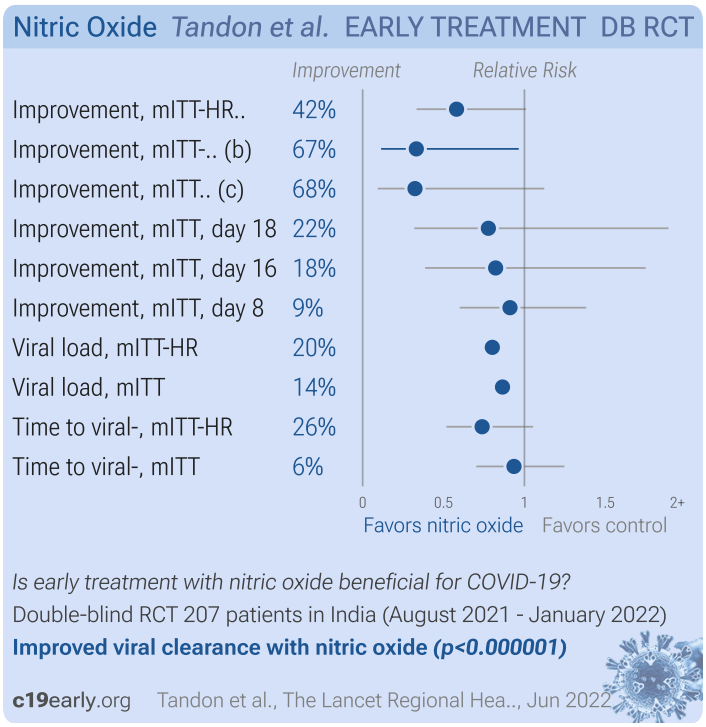
SaNOtize: PEP retrospective 625 university students in Thailand offered nitric oxide nasal spray, showing significantly lower cases for students that chose to use the treatment.

Strickland



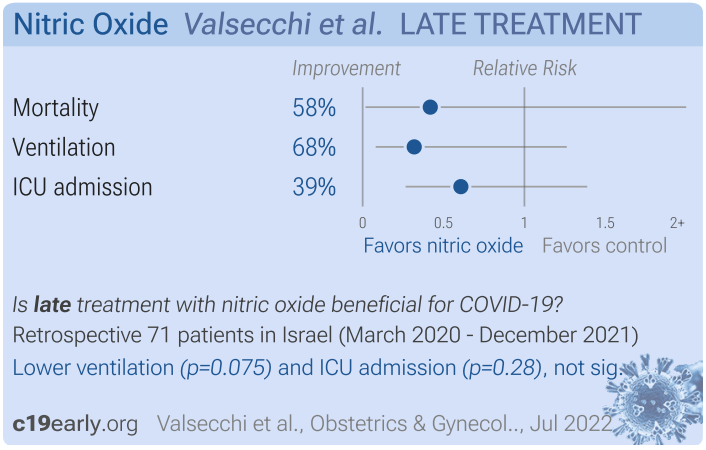
Strickland: Early terminated RCT with 47 ER patients in the USA, less than 12 days of symptoms, showing no significant difference in outcomes with a single high-dose administration of inhaled nitric oxide by mask, 250ppm for 30 min.

Tandon



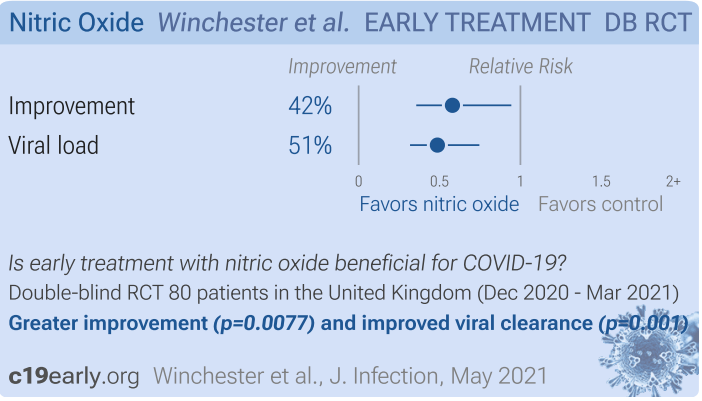
Tandon: RCT with 153 patients treated with a nitric oxide nasal spray, and 153 placebo patients, showing faster viral clearance with treatment. NO generated by a nasal spray (NONS) self-administered six times daily as two sprays per nostril (0.45mL of solution/dose) for seven days.

Valsecchi



Valsecchi: Retrospective 71 hospitalized patients in Israel, 20 treated with inhaled nitric oxide, showing

Winchester



Winchester: RCT with 40 nitric oxide and 40 placebo patients in the UK, showing faster viral clearance and greater improvement with treatment.

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 nitric oxide 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 nitric oxide 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 (B)*. 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 (5.19.0).

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].

We received no funding, this research is done in our spare time. We have no affiliations with any pharmaceutical companies or political parties.

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

Bryan, 6/24/2023, Double Blind Randomized Controlled Trial, placebo-controlled, USA, peer-reviewed, 3 authors, study period 1 November, 2020 - 30 November, 2022, trial NCT04601077 (history).	risk of progression, 0.8% higher, RR 1.01, $p = 1.00$, treatment 3 of 261 (1.1%), control 3 of 263 (1.1%), combined hospitalization, ICU admission, intubation, dialysis, and death.
	recovery time, 11.2% lower, relative time 0.89, $p = 0.30$, treatment 261, control 263.
Tandon, 6/29/2022, Double Blind Randomized Controlled Trial, placebo-controlled, India, peer-reviewed, 10 authors, study period 10 August, 2021 - 25 January, 2022, trial CTRI/2021/08.	risk of no improvement, 41.9% lower, RR 0.58, $p = 0.06$, treatment 14 of 64 (21.9%), control 26 of 69 (37.7%), NNT 6.3, mITT high risk, day 18.
	risk of no improvement, 66.8% lower, RR 0.33, $p = 0.04$, treatment 4 of 64 (6.2%), control 13 of 69 (18.8%), NNT 7.9, mITT high risk, day 16.
	risk of no improvement, 67.7% lower, RR 0.32, $p = 0.08$, treatment 3 of 64 (4.7%), control 10 of 69 (14.5%), NNT 10, mITT high risk, day 8.
	risk of no improvement, 22.3% lower, RR 0.78, $p = 0.63$, treatment 8 of 105 (7.6%), control 10 of 102 (9.8%), NNT 46, day 18, modified intention-to-treat.
	risk of no improvement, 17.8% lower, RR 0.82, $p = 0.67$, treatment 11 of 105 (10.5%), control 13 of 102 (12.7%), NNT 44, day 16, modified intention-to-treat.
	risk of no improvement, 8.9% lower, RR 0.91, $p = 0.76$, treatment 30 of 105 (28.6%), control 32 of 102 (31.4%), NNT 36, day 8, modified intention-to-treat.

	viral load, 19.8% lower, relative load 0.80, $p < 0.001$, treatment mean 2.62 (±0.145) n=64, control mean 2.1 (±0.141) n=69, mITT high risk, day 8.
	viral load, 13.5% lower, relative load 0.86, $p < 0.001$, treatment mean 2.51 (±0.114) n=105, control mean 2.17 (±0.118) n=102, day 8, modified intention-to-treat.
	time to viral-, 26.1% lower, relative time 0.74, $p = 0.09$, treatment 64, control 69, inverted to make RR<1 favor treatment, mITT high risk, Kaplan–Meier.
	time to viral-, 6.5% lower, relative time 0.94, $p = 0.66$, treatment 105, control 102, inverted to make RR<1 favor treatment, Kaplan–Meier, modified intention-to-treat.
Winchester, 5/13/2021, Double Blind Randomized Controlled Trial, placebo-controlled, United Kingdom, peer-reviewed, 4 authors, study period 15 December, 2020 - 31 March, 2021.	risk of no improvement, 42.0% lower, RR 0.58, $p = 0.008$, treatment 8 of 15 (53.3%), control 23 of 25 (92.0%), NNT 2.6.
	viral load, 51.3% lower, relative load 0.49, $p = 0.001$, treatment 40, control 40, AUC relative mean change.

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.

Al Sulaiman, 10/3/2022, retrospective, Saudi Arabia, peer-reviewed, mean age 62.5, 29 authors, study period 1 March, 2020 - 31 July, 2021.	risk of death, 40.0% higher, HR 1.40, $p = 0.10$, treatment 44 of 56 (78.6%), control 52 of 125 (41.6%), adjusted per study, in-hospital mortality, multivariable, Cox proportional hazards.
	risk of death, 18.0% higher, HR 1.18, $p = 0.45$, treatment 41 of 56 (73.2%), control 44 of 122 (36.1%), adjusted per study, multivariable, Cox proportional hazards, day 30.
Chandel, 1/31/2021, retrospective, USA, peer-reviewed, 14 authors, study period 1 March, 2020 - 9 June, 2020.	risk of death, 54.1% higher, RR 1.54, $p = 0.25$, treatment 12 of 66 (18.2%), control 36 of 206 (17.5%), adjusted per study, odds ratio converted to relative risk, multivariable.
	risk of mechanical ventilation, 27.2% higher, RR 1.27, $p = 0.26$, treatment 29 of 66 (43.9%), control 79 of 206 (38.3%), adjusted per study, odds ratio converted to relative risk, multivariable.
Di Fenza, 12/15/2023, Single Blind Randomized Controlled Trial, placebo-controlled, USA, peer-reviewed, 54 authors, study period 22 March, 2020 - 21 May, 2021, trial NCT04306393 (history).	risk of death, 23.0% lower, RR 0.77, $p = 0.36$, treatment 94, control 99, including additional covariates with SMD > 0.20, day 90, Table E1.
	risk of death, 26.0% lower, RR 0.74, $p = 0.36$, treatment 94, control 99, including additional covariates with SMD > 0.20, day 28, Table E1.
	risk of death, 13.0% lower, RR 0.87, $p = 0.60$, treatment 94, control 99, day 90.

	risk of death, 15.0% lower, RR 0.85, *p* = 0.56, treatment 94, control 99, day 28.
	VV-ECMO, 30.0% lower, RR 0.70, *p* = 0.67, treatment 94, control 99.
	neurological symptoms, 83.0% lower, RR 0.17, *p* = 0.01, treatment 94, control 99, day 90.
Moni, 4/20/2021, Randomized Controlled Trial, India, peer-reviewed, 16 authors, study period September 2020 - December 2020, average treatment delay 6.78 days, trial ISRCTN16806663.	**risk of death, 90.1% lower, RR 0.10, *p* = 0.03**, treatment 0 of 14 (0.0%), control 4 of 11 (36.4%), NNT 2.8, relative risk is not 0 because of continuity correction due to zero events (with reciprocal of the contrasting arm), day 28.
	risk of mechanical ventilation, 90.1% lower, RR 0.10, *p* = 0.03, treatment 0 of 14 (0.0%), control 4 of 11 (36.4%), NNT 2.8, relative risk is not 0 because of continuity correction due to zero events (with reciprocal of the contrasting arm), day 28.
	risk of <2 point WOS improvement, 42.5% better, RR 0.58, *p* = 0.47, treatment 3 of 14 (21.4%), control 7 of 11 (63.6%), NNT 2.4, adjusted per study, inverted to make RR<1 favor treatment, odds ratio converted to relative risk, day 14.
	time to viral load reduction, 64.4% lower, RR 0.36, *p* = 0.005, treatment 14, control 11, adjusted per study, inverted to make RR<1 favor treatment, N gene.
	time to viral load reduction, 63.4% lower, RR 0.37, *p* = 0.005, treatment 14, control 11, adjusted per study, inverted to make RR<1 favor treatment, Orf1ab gene.
Poonam, 6/27/2022, retrospective, USA, peer-reviewed, 5 authors, study period 1 March, 2020 - 30 June, 2020, this trial compares with another treatment - results may be better when compared to placebo.	**risk of death, 13.6% lower, RR 0.86, *p* = 0.10**, treatment 32 of 41 (78.0%), control 56 of 62 (90.3%), NNT 8.1.
Strickland, 5/4/2022, Randomized Controlled Trial, placebo-controlled, USA, peer-reviewed, 8 authors.	**risk of mechanical ventilation, 178.9% higher, RR 2.79, *p* = 1.00**, treatment 1 of 19 (5.3%), control 0 of 15 (0.0%), continuity correction due to zero event (with reciprocal of the contrasting arm).
	risk of hospitalization, 21.1% lower, RR 0.79, *p* = 1.00, treatment 1 of 19 (5.3%), control 1 of 15 (6.7%), NNT 71.
	return to ER, 38.2% higher, RR 1.38, *p* = 0.72, treatment 7 of 19 (36.8%), control 4 of 15 (26.7%).
Valsecchi, 7/7/2022, retrospective, Israel, peer-reviewed, 20 authors, study period March 2020 - December 2021.	**risk of death, 58.2% lower, RR 0.42, *p* = 1.00**, treatment 0 of 20 (0.0%), control 1 of 51 (2.0%), NNT 51, relative risk is not 0 because of continuity correction due to zero events (with reciprocal of the contrasting arm).
	risk of mechanical ventilation, 68.1% lower, RR 0.32, *p* = 0.08, treatment 2 of 20 (10.0%), control 16 of 51 (31.4%), NNT 4.7.

	risk of ICU admission, 39.3% lower, RR 0.61, p = 0.28, treatment 5 of 20 (25.0%), control 21 of 51 (41.2%), NNT 6.2.

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.

SaNOtize, 4/30/2022, retrospective, Thailand, preprint, 1 author.	risk of case, 75.0% lower, RR 0.25, p < 0.001, treatment 13 of 203 (6.4%), control 108 of 422 (25.6%), NNT 5.2.

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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