Supplementary file

Systematic Review Protocol

Title of the review	Efficacy and safety of nitazoxanide in treating SARS-CoV-2 infection: A systematic review and meta-analysis of blinded, placebo-controlled, randomized clinical trials
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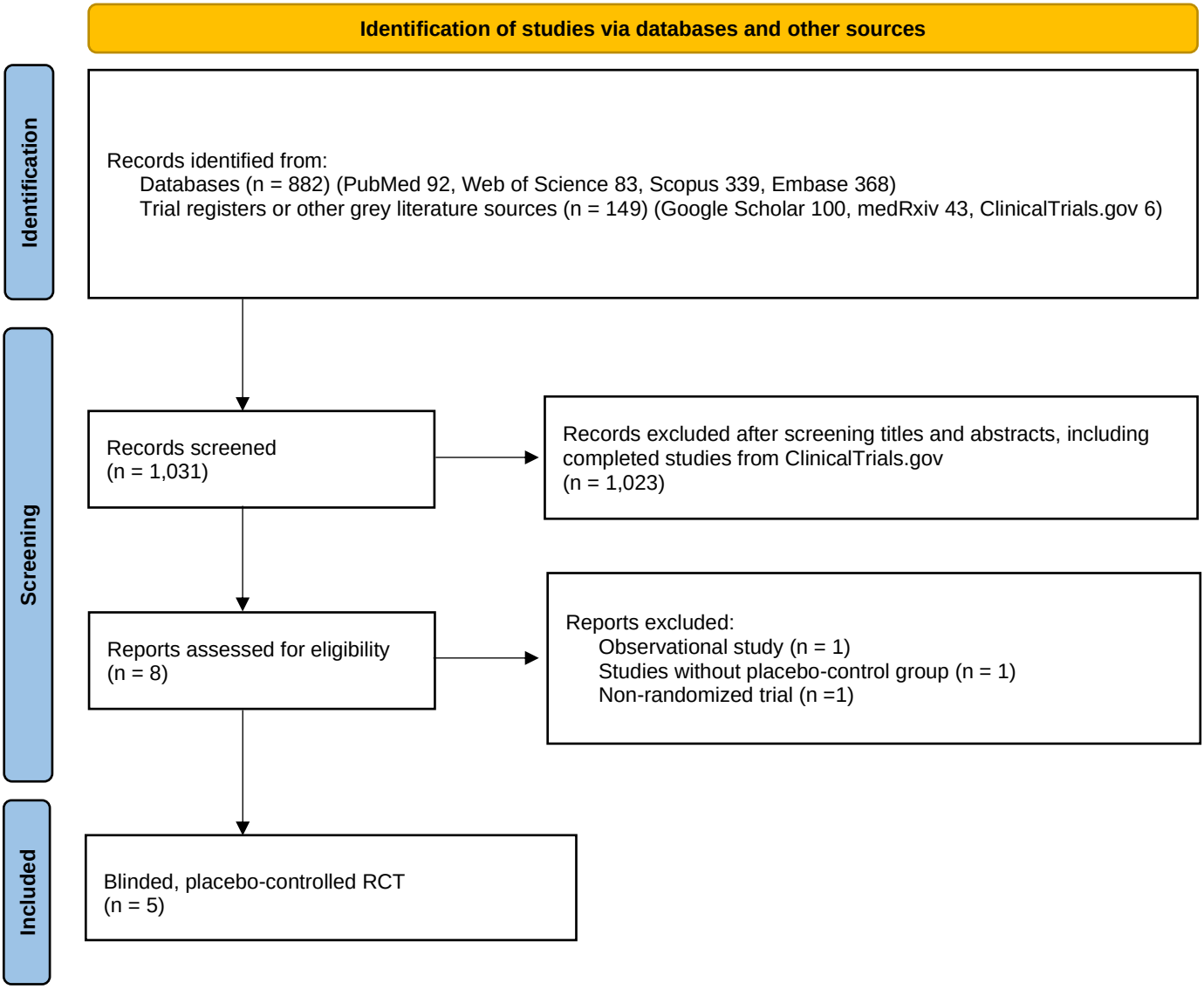
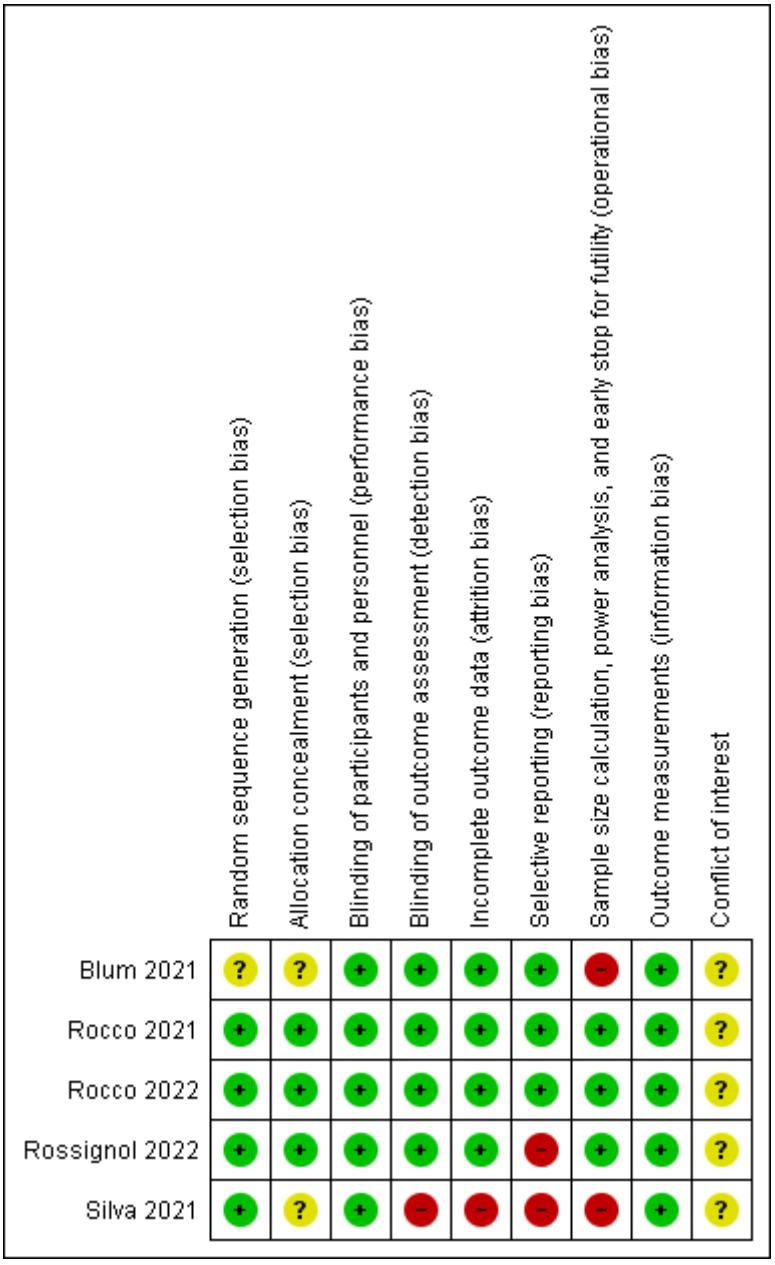
1. Background	
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel RNA virus associated with an acute pulmonary disease known as COVID-19. Given the lack of effective and safe antiviral agents against SARS-CoV-2, drug repurposing has played a critical role in the identification of rapidly available therapeutic solutions in treating patients with the disease (Heimfarth et al., 2020). After a comprehensive review published by Sanders and colleagues in April 2020 in the JAMA and a letter to the editor published by our research group in July 2020 in the American Journal of Physiology - Lung Cellular and Molecular Physiology calling attention to the potential antiviral effects of nitazoxanide and the need for high-quality trial evidence of this thiazolide antiparasitic drug in the treatment of SARS-CoV-2 infection, 28 interventional studies were registered on ClinicalTrials.gov, of which eight were completed or published at the time of writing this protocol. Aim This systematic review and meta-analysis aimed to synthesize the available evidence on the efficacy and safety of nitazoxanide as a treatment option in patients with COVID-19.	
2a. Criteria for including studies in the review based on PICOT elements	
P (population)	Individuals with COVID-19
I (intervention)	Nitazoxanide
C (comparison)	Placebo
O (outcomes)	Primary: death Secondary: viral load, positive RT-PCR status, composite measure of disease progression (ICU admission or invasive mechanical ventilation), serum biomarkers of inflammation (white blood cells, neutrophils, lymphocytes, C-reactive protein, D-dimer, lactate dehydrogenase, IL-6, IL-8, TNF-α), and any adverse events
T (study type)	Blinded, placebo-controlled, RCTs
2b. Criteria for excluding studies in the review	
Overlapping populations, open-label trials, observational studies, and trials testing drug associations	
3. Search methods	
Electronic databases	PubMed, Web of Science, Scopus, Embase
Preprint servers	medRxiv
Registries	ClinicalTrials.gov
Other methods used for identifying relevant research	Google Scholar (100 first results) Reference lists of all eligible studies and reviews
Search strategy	(nitazoxanide) AND (COVID-19 OR "2019-nCoV Infection" OR "Coronavirus Disease-19" OR "2019-nCoV Disease" OR SARS-CoV-2)
Language restriction	No
Filters	No
Search date	From January 1, 2020 to May 23, 2022

4. Methods of review	
Study selection	Two reviewers will be screening titles and abstracts independently of each other. Disagreements should be resolved by consensus 1st step: Initial screening of titles and abstracts 2nd step: Full reading of potential papers for inclusion
Risk of bias assessment	Cochrane guideline for RCTs: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. Include as "other biases": sample size calculation, power analysis, and early stopping for futility (operational bias), outcome measurements (information bias), and the authors' financial or non-financial conflicts of interest that could appear to affect the judgment of research team when designing, conducting, or reporting study
Data extraction	Two main reviewers. The reviewers must add information to a standardized data extraction worksheet in Excel. The following information must be extracted: registry of the study protocol, demographic characteristics of study participants, pre-existing medical conditions, treatment arms, nitazoxanide protocol, concomitant medications, follow-up duration, and outcome data. For dichotomous outcomes: the number of events and individuals in each treatment group. For continuous outcomes: means and standard deviations for each study group
5. Meta-analysis	
Data synthesis	Dichotomous variables: relative risk Continuous variables: standardized mean difference * Viral load: change from baseline
Statistical heterogeneity	I^2
Method	Random or fixed-effects model. In the case of heterogeneity, the random-effects model will be used
Additional analyses	Funnel plot: if the number of studies > 10; Subgroup analysis: not planned; Sensitivity analysis: "leave one out" method
Results presentation	Forest plot
Software	Review Manager, version 5.3 (Cochrane IMS)
6. Strength of evidence	
GRADE system (https://www.gradepro.org/)	
Factors that can reduce the quality of the evidence	
Risk of bias across studies	↓ 1 or 2 levels
Inconsistency of results	↓ 1 or 2 levels
Indirectness of evidence	↓ 1 or 2 levels
Imprecision	↓ 1 or 2 levels
Influence of small trials (< 100 patients)	↓ 1 or 2 levels
Factors that can increase the quality of the evidence	
Large magnitude of effect	↑ 1 or 2 levels
All plausible confounding would reduce the demonstrated effect or increase the effect if no effect was observed	↑ 1 level
Dose-response gradient	↑ 1 level



eFig. 1. PRISMA flow chart of studies screened and included.



eFig. 2. Risk of bias assessment.

eTable 1. Strength of evidence for efficacy and safety of nitazoxanide in treating patients with COVID-19.

Outcomes	Risk of bias	Inconsistency	Indirectness	Imprecision	Influence of small trials	Large effect	Quality of evidence
Viral load	Not serious	Serious	Not serious	Not serious	No	No	⊕⊕⊕◯
Positive RT-PCR status	Not serious	Not serious	Not serious	Not serious	No	No	⊕⊕⊕⊕
Composite measure of disease progression	Not serious	Not serious	Not serious	Serious	No	No	⊕⊕⊕◯
Death	Not serious	Not serious	Not serious	Serious	No	No	⊕⊕⊕◯
Serum inflammatory biomarkers							
WBC	Not serious	Not serious	Serious	Not serious	No	No	⊕⊕⊕◯
Neutrophils	Not serious	Not serious	Serious	Serious	No	No	⊕⊕◯◯
Lymphocytes	Not serious	Not serious	Serious	Not serious	No	No	⊕⊕⊕◯
LDH	Not serious	NA	Serious	Not serious	NA	No	-
IL-6	Not serious	Serious	Serious	Serious	Yes	No	⊕◯◯◯
IL-8	Not serious	Serious	Serious	Serious	Yes	No	⊕◯◯◯
TNF-α	Not serious	NA	Serious	Not serious	NA	No	-
CRP	Not serious	Serious	Serious	Serious	No	No	⊕◯◯◯
D-dimer	Not serious	Not serious	Serious	Not serious	No	No	⊕⊕⊕◯
Any adverse events	Not serious	Not serious	Serious	Not serious	No	No	⊕⊕⊕◯

WBC, white blood cells. LDH, lactate dehydrogenase. CRP, C-reactive protein. NA, not applicable.
Certainty: ⊕ very-low; ⊕⊕ low; ⊕⊕⊕ moderate; ⊕⊕⊕⊕ high.