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Effectiveness and safety of fibrinolytic therapy in critically ill patients with COVID-19 with ARDS: protocol for a prospective meta-analysis

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ABSTRACT

Introduction The use of fibrinolytic therapy has been proposed in severe acute respiratory distress syndrome (ARDS). During the COVID-19 pandemic, anticoagulation has received special attention due to the frequent findings of microthrombi and fibrin deposits in the lungs and other organs. Therefore, the use of fibrinolysis has been regarded as a potential rescue therapy in these patients. In this prospective meta-analysis, we plan to synthesise evidence from ongoing clinical trials and thus assess whether fibrinolytic therapy can improve the ventilation/perfusion ratio in patients with severe COVID-19-caused ARDS as compared with standard of care.

Methods and analysis This protocol was registered in PROSPERO. All randomised controlled trials and prospective observational trials that compare fibrinolytic therapy with standard of care in adult patients with COVID-19 and define their primary or secondary outcome as improvement in oxygenation and/or gas exchange, or mortality will be considered eligible. Safety outcomes will include bleeding event rate and requirement for transfusion. Our search on 25 January 2022 identified five eligible ongoing clinical trials. A formal search of MEDLINE (via PubMed), Embase, CENTRAL will be performed every month to identify published results and to search for further trials that meet our eligibility criteria.

Dissemination This could be the first qualitative and quantitative synthesis summarising evidence of the efficacy and safety of fibrinolytic therapy in critically ill patients with COVID-19. We plan to publish our results in peer-reviewed journals.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ During a pandemic, evidence synthesis is paramount and this could be the first prospective meta-analysis to assess the safety and efficacy of fibrinolytic therapy as a rescue therapy in critically ill patients with COVID-19.

⇒ As this is a prospective meta-analysis, we defined our outcomes before the results of the specific studies are published, thus reducing selective outcome reporting and publication bias.

⇒ As a limitation, due to the low number of eligible clinical studies some of our preplanned analyses might be omitted from the final analysis due to unavailability of data.

⇒ We do not intend to use individual level data in our analysis but to conduct our analysis based on the results of individual trials.

INTRODUCTION

The COVID-19 pandemic has caused a health crisis all over the world. The number of patients admitted to the hospital and especially in the intensive care unit (ICU) has multiplied.[1] The SARS-CoV-2 caused acute respiratory illness may progress in severe cases to acute respiratory distress syndrome (ARDS). The importance of the interference between inflammatory and haemostatic processes in ARDS has been shown

previously.[2] On the one hand, inflammation may increase the permeability of epithelial barrier of the alveoli, leading to interstitial pulmonary oedema, while the imbalance in coagulation promotes the development of microthrombi in capillaries, which may increase dead-space ventilation further aggravating respiratory failure.[2–4]

With regards to the SARS-CoV-2, the virus enters into alveolar epithelial cells through angiotensin-converting enzyme-2 receptors present on the endothelial cells. The host response to entry of the virus and the induced cell apoptosis will result in a dysregulated hyperinflammatory reaction, a so-called 'cytokine storm', that shifts the balance in coagulation system towards the procoagulant one. Furthermore, the concurrent endothelial damage will release molecules that make the shift more profound, for example, via expression of the tissue factor, von Willebrand factor and factor VIII.[5–7]

This procoagulant state promotes the formation of microthrombi in vessels and

together with the direct lung injury leading to fibrin deposits, these cause a mismatch in the ventilation/perfusion ratio, resulting in perfusion defects and worsening of hypoxemia that might not be improved by mechanical ventilation alone.[8 9]

Autopsies have pointed out the role of immunothrombosis in severe COVID-19 infection. Pulmonary microthrombi were found in 58% of patients with COVID-19; a similar finding to SARS patients (57%) but significantly more when compared with patients with H1N1 influenza (25%).[10]

The use of fibrinolytic agents has already been suggested to enhance perfusion, hence improve oxygenation even before the pandemic in ARDS.[11–13] In a meta-analysis of preclinical studies, Liu *et al* concluded that fibrinolytic therapy improved arterial oxygenation, lung function and reduced inflammatory response.[14] Barret *et al* reviewed a case series on the subject of fibrinolytic therapy in COVID-19 and concluded that tissue plasminogen activator (tPA) therapy showed greater benefit than harm as rescue therapy, but they could not advocate for its use in refractory hypoxemic respiratory failure due to the lack of high-grade evidence.[15]

Objectives

This prospective meta-analysis could be the first to synthesise evidence from ongoing clinical trials to assess whether fibrinolytic therapy as a rescue therapy can have beneficial effects on clinical outcomes of critically ill patients with COVID-19. Our research question is whether fibrinolytic therapy improves the ventilation/perfusion ratio, hence PaO_2/FiO_2 in patients with severe ARDS caused by COVID-19 as compared with standard of care alone without jeopardising safety.

Methods and analysis

Protocol registration

This protocol was registered in PROSPERO international database of prospectively registered systematic reviews (https://www.crd.york.ac.uk/prospero/).

Eligibility of trials

In this study, we will include data from randomised controlled clinical trials (RCTs) and prospective observational studies, which compared fibrinolytic therapy on top of standard of care with standard of care alone. The patient population should be adult patients diagnosed with COVID-19 and ARDS according to the 2012 Berlin definitions[16] who do not have a clear contraindication to fibrinolytic therapy. Table 1 shows a summary of trial eligibility criteria.

Search strategy

A systematic search of trial protocols of ongoing or planned clinical trials was performed in the database of ClinicalTrials.gov, EU Clinical Trial Register, International Clinical Trials Registry Platform, International Standard Randomised Controlled Trial Number registry, Australia and New Zealand Clinical Trial Registry, National Institute of Public Health (NIPH) Clinical Trials Search of Japan IRCT Iranian Registry of Clinical Trials and COVID-NMA database with the following search key: "fibrinoly* OR "fibrinolytic therapy" OR alteplase OR tenecteplase OR reteplase OR tPA". Restriction to COVID-19 trials was used.

Searches to find eligible trial protocols in the above-mentioned databases were initially carried out on 25 October 2021 and updated on 25 January 2022 (figure 1). We found no further eligible protocols compared with

Table 1	Trial eligibility according to PICO	
	Inclusion	**Exclusion**
Population	Adult hospitalised patients with laboratory confirmed (PCR) COVID-19 infection and ARDS according to the Berlin criteria	Children, ARDS caused by non-SARS-COV-2 infection
Intervention	Fibrinolytic therapy (eg, alteplase, tenecteplase) on top of standard of care	Fibrinolytic therapy with other indication (eg, stroke)
Comparator	Standard of care alone	
Primary outcomes	Change in PaO_2/FiO_2 ratio (Horrowitz index) pre-to-post intervention, ventilation-free days; time to ventilator-free state, successful extubation and mortality	
Safety outcomes	Bleeding event rate (major bleeding, clinically relevant non-major bleeding and minor bleeding as per ISTH); requirement for transfusion (packed red blood cell, platelet, fresh frozen plasma, cryoprecipitate, prothrombin complex concentrate)	
Study design	Randomised clinical trials, prospective observational trials	Retrospective trials, case series, case reports, animal studies, conference abstracts

ARDS, acute respiratory distress syndrome; ISTH, International Society on Thrombosis and Haemostasis; PaO_2/FiO_2 ratio, ratio of arterial oxygen partial pressure to fractional inspired oxygen.

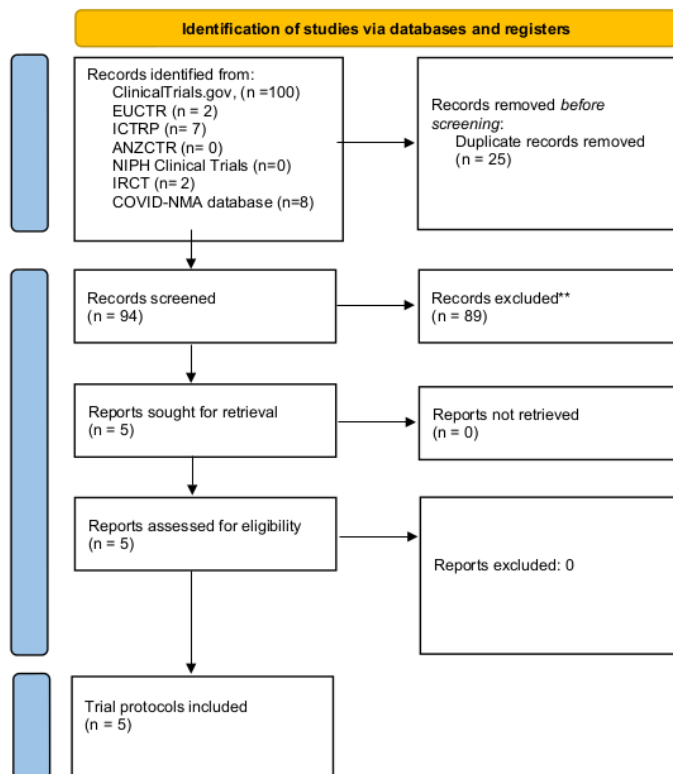PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

Figure 1 PRISMA 2020 flow diagram of the preliminary search. ANZCTR, Australia and New Zealand Clinical Trial Registry; EUCTR, EU Clinical Trial Register; ICTRP, International Clinical Trials Registry Platform; ISRCTN, International Standard Randomised Controlled Trial Number registry; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses.

our first search. In total, five eligible RCT protocols and no prospective observational studies were found. The eligible study protocols are summarised in table 2. We decided to continue our systematic search using the same search key every month until we find at least four eligible RCTs to have their results published, but not later than December 2023.

Systematic search for the published results will be carried out in the following databases: MEDLINE (via PubMed), Embase and the Cochrane Central Register of Controlled Trials (CENTRAL) with the following search key: (covid* OR SARS2 OR SARS-CoV2 OR ncov

Table 2 Eligible study protocols

Identifier	Country	Study design	Arms	Intervention	Comparison	Sample size	Follow-up period
NCT04357730	USA	RCT	2 arms	Alteplase	Standard of care	50	28 days
NCT04640194	Austria, Belgium, Brazil, Denmark France, Germany, Italy, Netherlands, Portugal, Russian Federation, Spain	RCT	3 arms	Alteplase (low dose) on top of standard of care Alteplase (high dose) on top of standard of care	Standard of care	320	28 days
NCT04505592	USA	RCT	2 arms	Tenecteplase	Placebo	60	28 days
IRCT20200415047080N1	Iran	RCT	2 arms	Alteplase	Standard of care	30	28 days
IRCT20200515047456N1	Iran	RCT	3 arms	rtPA	Standard of care	15	30 days

RCT, randomised controlled trial; rtPA, recombinant tissue plasminogen activator.

OR "novel coronavirus" OR covid) AND (fibrinoly* OR "fibrinolytic therapy" OR alteplase OR actylise OR tenecteplase OR TNKase OR reteplase OR retavase OR "tissue plasminogen activator" OR tPA OR rtpa OR PLAT).

If we detect additional relevant keywords during the search process, we will include these in the electronic search strategy and document the changes. We will perform an updated search before submission of the final manuscript and include relevant records in the review.

We will not use any filter or restrictions other than publication year. Only records published in 2020 or later will be included. The reference lists of eligible articles and citing articles will be also screened to capture all relevant studies. Records will be screened based on title, abstract and full text by two independent review authors, using a reference manager software and Cohen's kappa will be calculated after each phase of the selection. Disagreements will be resolved by an independent third investigator.

Outcomes

As main endpoints we defined clinical outcomes that give relevant information about the respiratory support, namely change in PaO2/FiO2 ratio (ratio of arterial oxygen partial pressure to fractional inspired oxygen, Horrowitz index) pre-to-post intervention (24 hours, 48 hours, 72 hours, 7 days and 14 days after); ventilation-free days; time to ventilator-free state (days), successful extubation and mortality (in-hospital, 48 hours, 14-day, 28-day mortality).

Additionally, we enlisted the following safety outcomes: bleeding event rate (Major Bleeding, Clinically Relevant Non-Major Bleeding and Minor Bleeding as per International Society on Thrombosis and Haemostasis); requirement for transfusion (packed red blood cell, platelet, fresh frozen plasma, cryoprecipitate, prothrombin complex concentrate—if any data available).

The following secondary outcomes will be analysed if enough data will be reported: survival to discharge (28 days of hospital stay or until hospital discharge); length of hospital stay (days); length of ICU stay (days); ICU-free days; improvement of the Sequential Organ Failure Assessment (SOFA) score; change in disease severity scores other than SOFA score; the number of vasopressor-free days; the number of patients with newly onset renal failure.

Data extraction

We will perform study selection in accordance with the Cochrane Handbook for Systematic Reviews of Interventions.[17] Records will be screened based on title, abstract and full text by two independent review authors, using a reference manager software. Cohen's kappa will be calculated to measure inter-rater reliability. Disagreements will be resolved by a third independent investigator.

We will create a priori a standardised data collection sheet based on the consensus of methodological and clinical experts. We will extract the following data from the eligible articles: title, first author, year of publication, countries, study design, diagnostic criteria, patient demographics, comorbidities, interventions and the following outcomes change in PaO2/FiO2 ratio, bleeding event rate, requirement for transfusion, mortality (in-hospital, 48 hours, 14-day, 28-day mortality), survival to discharge, length of hospital stay, length of ICU stay, ICU-free days, improvement of SOFA score, change in disease severity scores other than SOFA score, successful extubation, time to ventilator-free state (days), ventilation-free days, number of vasopressor-free days, number of patients with newly onset renal failure). Two independent review authors will extract data using the standardised data collection form, and a third independent reviewer will resolve the disagreements. We will contact the corresponding authors of papers for any missing information.

Statistical analysis

We will use the methods recommended by the working group of the Cochrane Collaboration for data synthesis.[17] The quantitative results will be summarised by calculating mean differences or standardised mean differences for continuous outcomes and OR or risk ratio with 95% CI for dichotomous outcomes using R statistical software (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Random effect model will be applied. Statistical significance is defined as a p less than 0.05.

Key results will be presented using Forest plots. We will test the heterogeneity also with χ^2-test and I^2 statistic; $p<0.1$ is defined to indicate significant heterogeneity.

If there is available data subgroup analysis will be performed according to the different dosing regimens, concomitant therapies, different inflammatory and coagulation profiles and risk factors (eg, age, gender). If enough studies are available, we plan to perform a subgroup analysis, including the data from RCTs and non-randomised trials separately.

Study evaluation

Risk of bias assessment will be done by two independent review authors following the recommendations of the Cochrane Handbook.[17] The Risk of Bias Assessment Tool[18] will be used in case of RCTs and ROBINS-I tool ("Risk Of Bias In Non-randomised Studies - of Interventions")[19] will be used for assessing the quality of nonrandomised studies. The presence of publication bias will be assessed visually by examining a funnel plot as well as statistically by using Egger's regression method if at least eight studies are available.

The quality assessment of the included studies will be performed with GRADE-Pro.[20]

Patient and public involvement

There was no patient or public involvement in the planning of this prospective meta-analysis.

Dissemination

We will publish our findings in peer-reviewed journals according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses statement[21] and present the results at international scientific meetings.

DISCUSSION

During the COVID-19 pandemic, anticoagulation has received special attention due to the frequent findings of microthrombi and fibrin deposits in the lungs and other organs.[8 10] Therefore, the use of fibrinolysis has been regarded as a potential rescue therapy in these patients.[15] Despite the fact that recent numbers of new COVID-19 infections and deaths show a declining tendency, there will still be severe cases admitted to ICUs, where the need for rescue therapy arises due to the vaccination disparity among different regions around the world.[22]

Arachchillage *et al*[23] conducted a retrospective observational study of 12 patients who also showed improvement in PaO_2/FiO_2 after alteplase administration without increased risk of major bleeding events. Orfanos *et al*[24] retrospectively reviewed charts of 15 patients and found decreased physiological dead space, thus improved oxygenation but without significant improvement of PaO2/FiO2 ratio.

Currently, several RCTs are underway that could elucidate whether fibrinolytic therapy has its role in the treatment of critically ill patients with COVID-19-induced ARDS. There is only one phase 2 study published, which enrolled 50 patients who showed large improvements in oxygenation although not statistically significant ones. As there were no severe adverse effects, a phase 3 trial is planned.[25] In another pilot study by Rashidi *et al*[26] conducted a 3-arm open-label RCT, wherein one arm they administered recombinant tPA followed by unfractionated heparin in five patients. As the abovementioned studies remained clinically inconclusive or were underpowered, the question whether fibrinolytic therapy has a role in the treatment of critically ill patients with COVID-19 with refractory hypoxemia is still unclear. This prospective meta-analysis could be the first qualitative and quantitative review about the use of fibrinolytic therapy as a rescue therapy in critically ill patients with COVID-19. As such it could point out whether this therapy has shown efficacy or utility and whether it is safe enough to introduce it as a rescue therapy in the care of critically ill patients with COVID-19.

Strengths and limitations

Our study has potential strengths and limitations that need to be considered. Since this is a prospective meta-analysis, we defined our hypothesis and outcomes before the results of specific studies are known, thus reducing selective outcome reporting and publication bias. Additionally, we defined our statistical plan and subgroup analysis in advance avoiding emphasis on particular results.

On the other hand, by not knowing the specific outcomes and their measurements, we might need to adapt our protocol in the light of published articles when they are available. As at the moment we found only five eligible clinical trials, there is a risk for some outcomes to be omitted from the final analysis due to unavailability of data. Although it is possible to use individual-level data in a prospective meta-analysis, we do not intend to. We will contact corresponding authors if there is missing data as we plan to conduct our analysis based on the results of individual trials.

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REFERENCES

1 Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *The Lancet* 2020;395:1225–8.
2 Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. *Respiration* 2017;93:212–25.
3 Glas GJ, Van Der Sluijs KF, Schultz MJ, *et al*. Bronchoalveolar hemostasis in lung injury and acute respiratory distress syndrome. *J Thromb Haemost* 2013;11:17–25.
4 Günther A, Mosavi P, Heinemann S, *et al*. Alveolar fibrin formation caused by enhanced procoagulant and depressed fibrinolytic capacities in severe pneumonia. *Am J Respir Crit Care Med* 2000;161:454–62.
5 Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. *Inflamm Res* 2020;69:1181–9.

6 Bohn MK, Hall A, Sepiashvili L, *et al*. Pathophysiology of COVID-19: mechanisms underlying disease severity and progression. *Physiology* 2020;35:288–301.

7 Levy JH, Iba T, Olson LB, *et al*. COVID-19: thrombosis, thromboinflammation, and anticoagulation considerations. *Int J Lab Hematol* 2021;43 Suppl 1:29–35.

8 Fahmy OH, Daas FM, Salunkhe V, *et al*. Is Microthrombosis the Main Pathology in Coronavirus Disease 2019 Severity?-A Systematic Review of the Postmortem Pathologic Findings. *Crit Care Explor* 2021;3:e0427.

9 Cronin JN, Camporota L, Formenti F. Mechanical ventilation in COVID-19: a physiological perspective. *Exp Physiol* 2022;107:683–93.

10 Hariri LP, North CM, Shih AR, *et al*. Lung histopathology in coronavirus disease 2019 as compared with severe acute respiratory Sydrome and H1N1 influenza: a systematic review. *Chest* 2021;159:73–84.

11 MacLaren R, Stringer KA. Emerging role of anticoagulants and fibrinolytics in the treatment of acute respiratory distress syndrome. *Pharmacotherapy* 2007;27:860–73.

12 Hardaway RM, Harke H, Tyroch AH, *et al*. Treatment of severe acute respiratory distress syndrome: a final report on a phase I study. *Am Surg* 2001;67:377.

13 Sebag SC, Bastarache JA, Ware LB. Therapeutic modulation of coagulation and fibrinolysis in acute lung injury and the acute respiratory distress syndrome. *Curr Pharm Biotechnol* 2011;12:1481–96.

14 Liu C, Ma Y, Su Z, *et al*. Meta-Analysis of preclinical studies of fibrinolytic therapy for acute lung injury. *Front Immunol* 2018;9:1898.

15 Barrett CD, Moore HB, Moore EE, *et al*. Fibrinolytic therapy for refractory COVID-19 acute respiratory distress syndrome: scientific rationale and review. *Res Pract Thromb Haemost* 2020;4:524–31.

16 Ranieri VM, Rubenfeld GD, ARDS Definition Task Force, *et al*. Acute respiratory distress syndrome: the Berlin definition. *JAMA* 2012;307:2526–33.

17 Higgins JPT, Thomas J, Chandler J. Cochrane Handbook for systematic reviews of interventions version 6.3, 2022. Available: www.training.cochrane.org/handbook

18 Sterne JAC, Savović J, Page MJ, *et al*. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019;366:l4898.

19 Sterne JA, Hernán MA, Reeves BC, *et al*. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016;355:i4919.

20 McMaster University and Evidence Prime. GRADEpro Guideline Development Tool [Software], 2021. Available: gradepro.org

21 Moher D, Altman DG, Liberati A, *et al*. PRISMA statement. *Epidemiology* 2011;22:128.

22 Haider N, Hasan MN, Khan RA. The global case-fatality rate of COVID-19 has been declining disproportionately between top vaccinated countries and the rest of the world. *medRxviv* 2022.

23 Arachchillage DJ, Stacey A, Akor F, *et al*. Thrombolysis restores perfusion in COVID-19 hypoxia. *Br J Haematol* 2020;190.

24 Orfanos S, El Husseini I, Nahass T, *et al*. Observational study of the use of recombinant tissue-type plasminogen activator in COVID-19 shows a decrease in physiological dead space. *ERJ Open Res* 2020;6:00455-2020.

25 Barrett CD, Moore HB, Moore EE, *et al*. Study of alteplase for respiratory failure in SARS-CoV-2 COVID-19: a vanguard multicenter, rapidly adaptive, pragmatic, randomized controlled trial. *Chest* 2022;161:710–27.

26 Rashidi F, Barco S, Rezaeifar P, *et al*. Tissue plasminogen activator for the treatment of adults with critical COVID-19: a pilot randomized clinical trial. *Thromb Res* 2022;216:125–8.