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

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Effectiveness and safety of fibrinolytic therapy in critically ill COVID-19 patients 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 coronavirus disease (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 synthesize 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 to standard of care.

Methods and analysis

This protocol was registered in PROSPERO (CRD42021285281). All randomised controlled trials and prospective observational trials that compare fibrinolytic therapy with standard of care in adult COVID-19 patients 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 5 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 summarizing evidence the efficacy and safety of fibrinolytic therapy in critically ill COVID-19 patients. We plan to publish our results in peer-reviewed journals.

Keywords: COVID-19; ARDS; Respiratory failure; Fibrinolysis; Tissue plasminogen activator

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 COVID-19 patients
- As a limitation, due to the low number of eligible clinical studies some of our pre-planned analyses might be omitted from the final analysis due to unavailability of data.

Introduction

The coronavirus disease (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 severe acute respiratory syndrome coronavirus 2 (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 hemostatic 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, that may increase dead-space ventilation further aggravating respiratory failure.[2,3,4]

With regard to the SARS-CoV-2, the virus enters into alveolar epithelial cells through angiotensin converting enzyme-2 (ACE2) 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 e.g. via expression of the tissue factor, von Willebrand factor (VWF) and factor VIII.[5,6,7]

This procoagulant state promotes the formation of microthrombi and fibrin deposits in various organs.[8] In the respiratory system this causes a mismatch in the ventilation/perfusion ratio resulting in perfusion defects and worsening of hypoxemia that might not be improved by mechanical ventilation alone.[9]

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

The use of fibrinolytic agents has already been suggested to enhance perfusion hence improve oxygenation even before the pandemic in ARDS.[11,12,13] 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 COVID-19 patients. 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 to standard of care alone without jeopardizing safety.

Methods and analysis

Protocol registration

This protocol was registered in PROSPERO international database of prospectively registered systematic reviews with the identifier number CRD42021285281 (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.

Table 1. Trial eligibility according to PICO

	Inclusion	Exclusion
Population	Adult hospitalised patients with suspected or confirmed COVID-19 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

Abbreviations used in the table: ARDS: acute respiratory distress syndrome, PaO2/FiO2 ratio is the ratio of arterial oxygen partial pressure to fractional inspired oxygen, ISTH: International Society on Thrombosis and Haemostasis

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 (ICTRP), International Standard Randomised Controlled Trial Number (ISRCTN) registry, Australia and New Zealand Clinical Trial Registry (ANZCTR), NIPH Clinical Trials Search 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 abovementioned databases were initially carried out on 25 October 2021, and updated on 25 January 2022 (Figure 1. Prisma Flow Diagram). We found no further eligible protocols compared to our first search. In total, 5 eligible RCT protocols and no prospective observational studies were found. The eligible study protocols are summarised in Table 2.

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	3arms	rtPA	Standard of care	15	30 days

Abbreviations used in the table: RCT (randomised controlled trial), rtPA: recombinant tissue plasminogen activator

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 OR “novel coronavirus” OR covid-19) 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. We intend to extract all records from scientific databases and trial registries using the same search key every month until

we find at least 4 of the eligible RCTs to have their results published.

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; 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 SOFA (Sequential Organ Failure Assessment) 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 standardized 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 standardized mean differences for continuous outcomes and odds ratio or risk ratio with 95% confidence interval (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 than0.05.

Key results will be presented using Forest plots. We will test the heterogeneity also with X^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-randomized 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 (RoB 2)[18] will be used in case of randomised controlled trials and ROBINS-I[19] will be used for assessing the quality of nonrandomized 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 8 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 PRISMA 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 amongst different regions around the world.[22]

Arachchillage et al. 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.[23] Orfanos et al. retrospectively reviewed charts of 15 patients and found decreased physiological dead space thus improved oxygenation but without significant improvement of PaO2/FiO2 ratio.[24]

Currently, several randomized controlled trials 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 that 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. conducted a 3-arm open-label randomized controlled trial wherein one arm they administered recombinant tissue plasminogen activator (rtPA) followed by unfractionated heparin in 5 patients.[26] As the abovementioned studies remained clinically inconclusive or were underpowered, the question whether fibrinolytic therapy has a role in the treatment of critically ill COVID-19 patients 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 COVID-19 patients. As such it could point out whether this therapy has shown efficacy or futility and whether it is safe enough to introduce it as a rescue therapy in the care of critically ill COVID-19 patients.

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.

Authors' contributions: KEH drafting of the manuscript, LS: writing of the statistical analysis plan; KO, FD, PH: methodological supervision, ZM: methodological supervision and revision of the manuscript, TK: original idea and critical revision of the manuscript. All of the authors read and approved the final manuscript.

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Competing interest: None declared.

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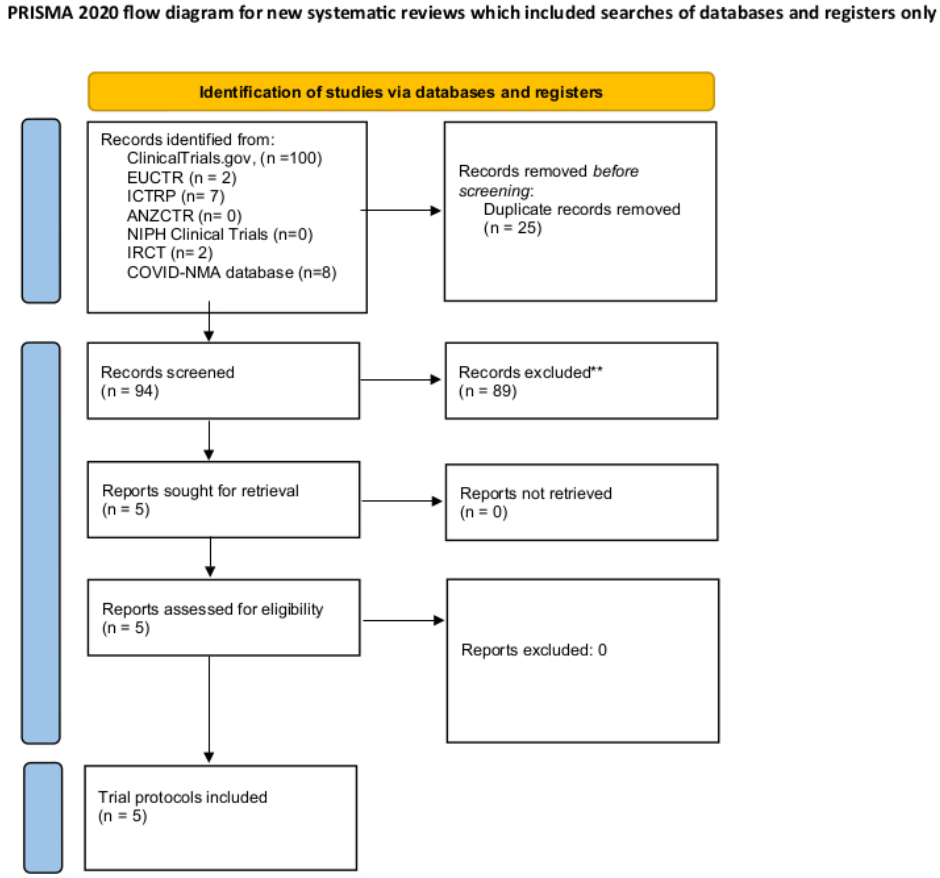

PRISMA 2020 flow diagram (search on 25th January)

Abbreviations used: EUCTR: EU Clinical Trial Register, ICTRP: International Clinical Trials Registry Platform, ISRCTN: International Standard Randomised Controlled Trial Number registry, ANZCTR: Australia and New Zealand Clinical Trial Registry

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	-
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2,4
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	13
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	-
Support:			
Sources	5a	Indicate sources of financial or other support for the review	13
Sponsor	5b	Provide name for the review funder and/or sponsor	13
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	-
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4-5
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	4-5
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5-6
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5-6

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6-7
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	6-7
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7-8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7-8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8-9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	8
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	8
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	8-9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8-9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

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Effectiveness and safety of fibrinolytic therapy in critically ill COVID-19 patients 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 coronavirus disease (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 synthesize 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 to standard of care.

Methods and analysis

This protocol was registered in PROSPERO (CRD42021285281). All randomised controlled trials and prospective observational trials that compare fibrinolytic therapy with standard of care in adult COVID-19 patients 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 5 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 summarizing evidence the efficacy and safety of fibrinolytic therapy in critically ill COVID-19 patients. We plan to publish our results in peer-reviewed journals.

Keywords: COVID-19; ARDS; Respiratory failure; Fibrinolysis; Tissue plasminogen activator

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 COVID-19 patients
- 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 pre-planned 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 coronavirus disease (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 severe acute respiratory syndrome coronavirus 2 (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 hemostatic 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, that may increase dead-space ventilation further aggravating respiratory failure.[2-4]

With regard to the SARS-CoV-2, the virus enters into alveolar epithelial cells through angiotensin converting enzyme-2 (ACE2) 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 e.g. via expression of the tissue factor, von Willebrand factor (VWF) 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 COVID-19 patients; a similar finding to severe acute respiratory syndrome (SARS patients (57%) but significantly more when compared to H1N1 influenza patients (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 COVID-19 patients. 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 to standard of care alone without jeopardizing safety.

Methods and analysis

Protocol registration

This protocol was registered in PROSPERO international database of prospectively registered systematic reviews with the identifier number CRD42021285281 (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.

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

Abbreviations used in the table: ARDS: acute respiratory distress syndrome, PaO2/FiO2 ratio is the ratio of arterial oxygen partial pressure to fractional inspired oxygen, ISTH: International Society on Thrombosis and Haemostasis

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 (ICTRP), International Standard Randomised Controlled Trial Number (ISRCTN) registry, Australia and New Zealand Clinical Trial Registry (ANZCTR), NIPH Clinical Trials Search 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 abovementioned databases were initially carried out on 25 October 2021, and updated on 25 January 2022 (Figure 1. Prisma Flow Diagram). We found no further eligible protocols compared to our first search. In total, 5 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 4 eligible RCTs to have their

results published, but not later than December 2023.

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	3arms	rtPA	Standard of care	15	30 days

Abbreviations used in the table: RCT (randomised controlled trial), rtPA: recombinant tissue plasminogen activator

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 OR “novel coronavirus” OR covid-19) 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 (24h, 48h, 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 SOFA (Sequential Organ Failure Assessment) 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 standardized 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 standardized mean differences for continuous outcomes and odds ratio or risk ratio with 95% confidence interval (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 X^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-randomized 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 (RoB 2)[18] will be used in case of randomised controlled trials and ROBINS-I[19] will be used for assessing the quality of nonrandomized 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 8 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 PRISMA 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 amongst different regions around the world.[22]

Arachchillage et al. 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.[23] Orfanos et al. retrospectively reviewed charts of 15 patients and found decreased physiological dead space thus improved oxygenation but without significant improvement of PaO2/FiO2 ratio.[24]

Currently, several randomized controlled trials 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 that 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. conducted a 3-arm open-label randomized controlled trial wherein one arm they administered recombinant tissue plasminogen activator (rtPA) followed by unfractionated heparin in 5 patients.[26] As the abovementioned studies remained clinically inconclusive or were underpowered, the question whether fibrinolytic therapy has a role in the treatment of critically ill COVID-19 patients 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 COVID-19 patients. As such it could point out whether this therapy has shown efficacy or futility and whether it is safe enough to introduce it as a rescue therapy in the care of critically ill COVID-19 patients.

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.

***Figure 1**: PRISMA 2020 flow diagram of the preliminary search*

Abbreviations used: EUCTR: EU Clinical Trial Register, ICTRP: International Clinical Trials Registry Platform, ISRCTN: International Standard Randomised Controlled Trial Number registry, ANZCTR: Australia and New Zealand Clinical Trial Registry

Authors' contributions: KEH drafting of the manuscript, LS: writing of the statistical analysis plan; KO, FD, PH: methodological supervision, ZM: methodological supervision and revision of the manuscript, TK: original idea and critical revision of the manuscript. All of the authors read and approved the final manuscript.

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Competing interest: None declared.

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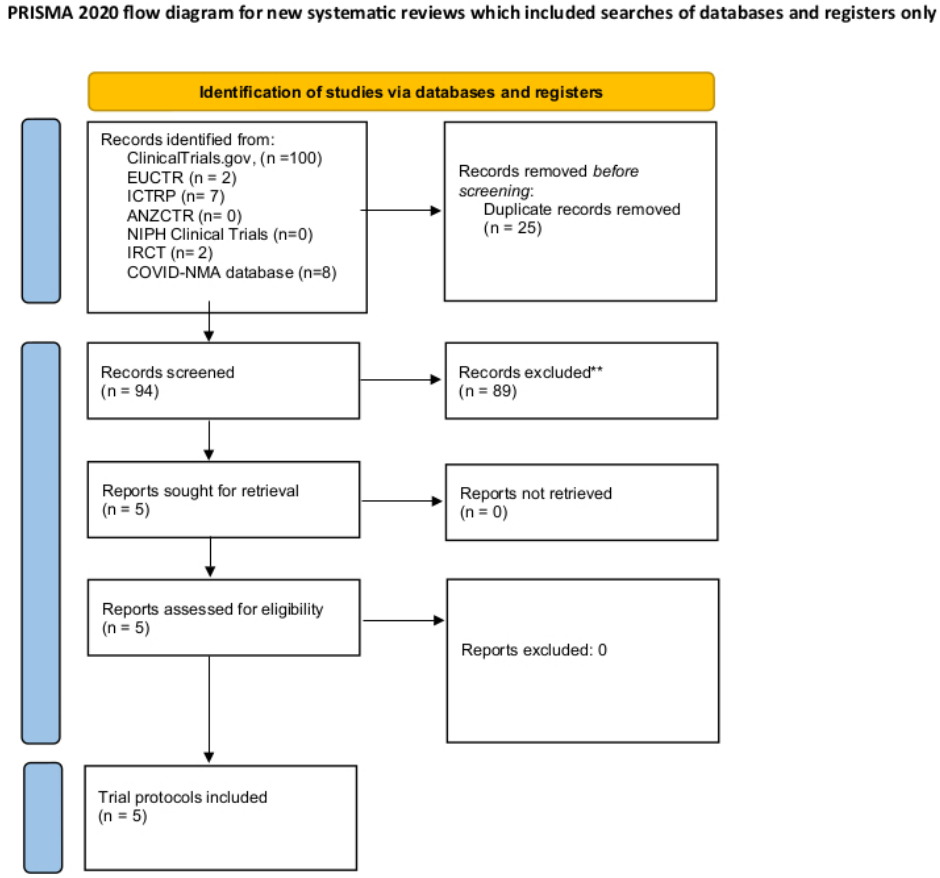

PRISMA 2020 flow diagram (search on 25th January)

Abbreviations used: EUCTR: EU Clinical Trial Register, ICTRP: International Clinical Trials Registry Platform, ISRCTN: International Standard Randomised Controlled Trial Number registry, ANZCTR: Australia and New Zealand Clinical Trial Registry

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	-
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2,4
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	13
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	-
Support:			
Sources	5a	Indicate sources of financial or other support for the review	13
Sponsor	5b	Provide name for the review funder and/or sponsor	13
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	-
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4-5
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	4-5
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5-6
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5-6

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6-7
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	6-7
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7-8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7-8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8-9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	8
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	8
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	8-9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8-9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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