Fibrinolytic Therapy for Refractory COVID#19 Acute Respiratory Distress Syndrome: Scientific Rationale and Review

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Fibrinolytic Therapy for Refractory COVID-19 Acute Respiratory Distress Syndrome: Scientific Rationale and Review

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Short Running Title: *Tissue Plasminogen Activator (tPA) to Treat COVID-19 ARDS*

Conflicts of Interest: CDB, HBM, EEM, and MBY have patents pending related to both coagulation/fibrinolysis diagnostics and therapeutic fibrinolytics, and are passive co-founders and holds stock options in Thrombo Therapeutics, Inc. HBM and EEM have received grant support from Haemonetics and Instrumentation Laboratories. EEM holds a grant from Genentech. MBY has previously received a gift of Alteplase (tPA) from Genentech, and owns stock options as a co-founder of Merrimack Pharmaceuticals. JB and JA are co-founders of Applied BioMath and own stock. FH owns stock in Applied BioMath. LAV has received grant support from Genentech. PCM, PKM, AS, DL, RM and DST have nothing to disclose.

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Essentials

- COVID-19 has caused thrombotic coagulopathy and respiratory failure in unprecedented numbers
- Pulmonary microvascular thrombosis is particularly prominent in COVID-19 respiratory failure
- Animal and limited human data support a role for fibrinolytic therapy in respiratory failure
- Urgent study of fibrinolytic therapy is needed. A Phase 2a trial is pending (NCT 04357730).

Abstract

The COVID-19 pandemic has caused respiratory failure and associated mortality in numbers that have overwhelmed global health systems. Thrombotic coagulopathy is present in nearly three-quarters of COVID-19 patients admitted to the intensive care unit, and both the clinical picture and pathologic findings are consistent with microvascular occlusive phenomena being a major contributor to their unique form of respiratory failure. Numerous studies are ongoing focusing on anti-cytokine therapies, antibiotics and anti-viral agents, but none to-date have focused on treating the underlying thrombotic coagulopathy in an effort to improve respiratory failure in COVID-19. There is animal data and a previous human trial demonstrating a survival advantage with fibrinolytic therapy to treat ARDS. Here we review the extant and emerging literature on the relationship between thrombotic coagulopathy and pulmonary failure in the context of COVID-19 and present the scientific rationale for consideration of targeting the coagulation and fibrinolytic systems to improve pulmonary function in these patients.

Keywords: COVID-19; Acute Respiratory Distress Syndrome (ARDS); Tissue Plasminogen Activator (tPA); Pulmonary Failure; Fibrinolysis

Introduction

As the Coronavirus disease 2019 (COVID-19) pandemic accelerates, cases have grown exponentially around the world. Other countries' experience suggests that 5-16% of COVID-19 in-patients will undergo prolonged intensive care(1-3), with 50-70% needing mechanical ventilation (MV)(3, 4),(5), threatening to overwhelm hospital capacity(6).

While COVID-19 overall mortality likely ranges from 1-5%, this is much higher in COVID-19-induced Acute Respiratory Distress Syndrome (ARDS) patients (22-64%)(3, 4, 7). There are currently few proven ARDS therapies other than low-tidal-volume(8) and prone positioning(9) MV. Most current trials on clinicaltrials.gov for COVID-19-induced ARDS aim at modulating the inflammatory response. Sarilumab and tocilizumab that block IL-6 effects are being tested in randomized controlled trials (RCT) for patients hospitalized with severe COVID-19 ((9);NCT04317092). The World Health Organization international trial SOLIDARITY will test remdesivir, chloroquine plus hydroxychloroquine, lopinavir plus ritonavir, and lopinavir plus ritonavir and interferon-beta. However, studies targeting the coagulation system, which is intrinsically intertwined with the inflammatory response are lacking(10-14).

Fibrinolysis, ARDS and the Possible Role of Fibrinolytic Therapy in COVID-19

Our group has shown that low fibrinolysis is associated with ARDS(15-19), and COVID-19 Intensive Care Unit (ICU) patients have now been shown on thromboelastography to universally have lower levels of fibrinolysis than the reference population(20). Studies starting decades ago have demonstrated the systemic and local effects of dysfunctional coagulation, specifically related to fibrin, in ARDS(11, 13, 14, 21, 22). ARDS, regardless of cause, is associated with fibrin deposition in airspaces and fibrin-platelet microthrombi in the pulmonary vasculature(23-25), which is also consistently observed in the lung microvasculature of COVID-19 patients(26-28). This pathologic fibrin deposition reflects a dysfunctional clotting system, with enhanced clot formation and propagation as well as fibrinolysis suppression(29-31), largely due to tissue factor produced by alveolar epithelial cells and macrophages(32), and high levels of plasminogen activator inhibitor-1

(PAI-1) produced by endothelial cells or activated platelets(33, 34). Consistent with this, prothrombin time prolongation, elevated D-dimer and fibrin degradation products along with uniquely elevated fibrinogen levels have been reported in severely ill COVID-19 patients, particularly in non-survivors(3, 4, 20, 35-38). Similar findings have been observed in sepsis(29, 39), endotoxemia(40), and extensive tissue disruption(18), in which early activation of coagulation and fibrinolysis is followed by late fibrinolytic shutdown and endothelial dysfunction. It is also consistent with an initial viral infection of airway epithelial cells, with later spread to endothelial cells which has now been shown to occur in COVID-19(41), both of which express the receptor protein for the virus, angiotensin-converting-enzyme-2 (ACE2)(42). Furthermore, it has now also been shown that critically ill COVID-19 patients universally demonstrate hypercoagulable findings on viscoelastic assays relative to the reference population, with shortened reaction time (R-time), increased α-angle, increased maximal amplitude, and in virtually all cases a reduced level of fibrinolysis on thromboelastography(20).

Targeting the coagulation and fibrinolytic systems to improve ARDS and associated pulmonary clot formation syndromes has been described(34, 43-47), and tested in animal models (48-51), and in light of the mounting findings in COVID-19 as described above may also have a role in the management of COVID-19 respiratory failure. In 2001, Hardaway and colleagues described a small, non-controlled human trial in severe ARDS, showing that uro/streptokinase led to remarkable improvement in oxygen requirements without bleeding events(52). Tissue-type plasminogen activator (tPA) is a more modern fibrinolytic approach with higher clot lysis efficacy without increased bleeding risk. A meta-analysis of acute lung injury in animals showed that, compared to controls, tPA improved survival, arterial pO_2 and pCO_2 better than either uPA or plasmin, although none of the studies included viral-induced ARDS(50). In other studies, intra-airway delivered tPA improved survival and morbidity associated with acute plastic bronchitis crisis, in which intra airway clotting occurs(46, 53-60). Both nebulized and direct instillation of tPA into the airways via bronchoscopy have been used off-label to treat fibrin airway casts(61). In a lethal animal model of both severe pulmonary microvascular thrombi and severe bronchial fibrin casts, treatment with airway tPA resulted in improved survival, dissolved airway casts, and normalized pO_2 and pCO_2(62, 63). However, the mounting evidence specific to COVID-19 that shows pulmonary microvascular

thrombosis as a predominant finding(26-28) combined with normal lung compliance and high Alveolar-arterial oxygen gradients(64) all suggest intravascular delivery may be the more appropriate delivery route, with concern that intra-airway delivery via intratracheal instillation or nebulized solutions may increase the risk to healthcare workers by exposing them to infectious airway secretions.

Taken together, the extant data on fibrinolytic therapy in ARDS combined with the thrombotic coagulopathy and clinical findings consistent with pulmonary vascular thrombo-occlusive disease in COVID-19 suggest that manipulation of the fibrinolytic system through administration of tissue plasminogen activator (tPA) may have a role in the therapy of severe, medically refractory COVID-19-induced ARDS. Importantly, such an approach is non-exclusive and could be used in patients who have been treated with other experimental agents including anti-IL6 receptor blockers and other immune modulators, antibiotics, and anti-viral agents.

Risk Considerations for Fibrinolytic Therapy in COVID-19 ARDS

The main risk if fibrinolytic therapy were considered for treatment of severe, medically refractory hypoxemia in COVID-19 respiratory failure is bleeding. The bleeding risk can be estimated from its use in myocardial infarction (MI) and sub-massive pulmonary embolism. In the largest available prospectively collected data set of intravenous (IV) alteplase for non-stroke indication (GUSTO trial; myocardial infarction), the risk of hemorrhagic stroke was 0.7% and severe/life-threatening bleeding was 0.4% in the group that received 50mg of alteplase over 90 minutes followed immediately by a 5,000u bolus of IV unfractionated heparin and a therapeutic heparin drip (n = 10, 396 patients)(65). In a trial of high-dose Alteplase (100 mg over 2 hours) given concomitant with therapeutic systemic heparinization for sub-massive pulmonary embolism the rate of major bleeding was 0.8% and none of the 118 patients in the Alteplase arm of the trial developed a hemorrhagic stroke(66). These two patient groups are expected to be relatively similar to those with severe COVID-19 illness regarding comorbidities and the absence of active stroke, which increases the risk of hemorrhagic conversion. There are multiple studies that quote higher risks of "major bleeding," with a commonly referenced meta-analysis by Chatterjee et al(67) quoting a major bleeding risk with tPA in pulmonary embolism as being 9.24% relative to 3.42% for anticoagulation alone. The majority

of the patients in this meta-analysis came from a single study (the PEITHO study) that used tenecteplase(68), which is resistant to plasminogen activator inhibitor-1 (PAI-1) inhibition(69). Additionally, many of the included studies had no pre-specified definition of a major bleeding event, several included studies considered any blood transfusion as a major bleed, and none used hemodynamic parameters or massive transfusion protocol activation as criteria. With respect to COVID-19 ICU patients on mechanical ventilation where death is as likely as survival, life-threatening hemorrhage is the most relevant for consideration, which again suggests the GUSTO study of over 40,000 patients including over 10,000 patients in the Alteplase bolus plus heparin group is likely the most relevant for risk considerations for fibrinolytic therapy in severe, medically refractory hypoxemic respiratory failure in COVID-19(65). Furthermore, given the profound hypercoagulable/thrombotic coagulopathy in the majority of critically ill COVID-19 patients(20, 35, 38, 70, 71) where bleeding is quite rare and thrombosis predominates, the risk of systemic fibrinolysis therapy may be even lower. Similarly, while we posit that intravascular delivery of tPA would likely be more effective, if intra-airway tPA were to be pursued and effective the available case reports of tPA airway delivery have thus far showed no bleeding events (46, 61).

In addition to bleeding events, there is also risk specific to the COVID-19 betacoronavirus itself. Cell entry by this virus requires cleavage of a trimeric spike(S) glycoprotein that protrudes from the viral envelope, generating two subunits (S1 and S2), followed by additional cleavage within S2(72). The S1 subunit provides the receptor binding domain, which binds to ACE2 on the cell surface(73, 74). The S2 subunit then drives fusion between the virus and the cell membranes, resulting in viral entry. S protein cleavage at the S1/S2 junction and within S2 occurs through host cell proteases, primarily TMPRSS2, at specific Arginine-Serine sequences(72). Plasmin, like TMPRSS2, also favors cleavage between Arginine-Serine sequences (75), thus tPA administration with plasmin generation could conceivably enhance viral infectivity. However, patients in COVID-19 terminal stages are likely already massively infected with high viral loads, where the profound hypoxemic respiratory failure resulting from pulmonary microvascular thrombosis and the host inflammatory response to the virus is the imminent threat to life rather than the virus itself. As such, we posit that plasmin activation effects on viral entry or decreased clearance would be minor

compared with salvage of pulmonary gas exchange and correction of profound hypoxemic respiratory failure to prevent imminent death by restoring pulmonary microvascular patency.

Practical Considerations if Fibrinolytic Therapy Were Considered in COVID-19 ARDS

In the absence of effective therapies for medically refractory COVID-19 hypoxemic respiratory failure where critical care physicians have exhausted all available treatment options in a dying patient, patients with pro-thrombotic presentations, normal lung compliance on the ventilator, and high Alveolar-arterial oxygen gradients could potentially be considered for fibrinolytic therapy, as such a clinical presentation is suggestive of vascular occlusive disease as a primary cause of hypoxemia. It should be noted that in a disease entity only several months old such an approach has no controlled trial data so must be considered with extreme caution, although there are now a handful of case reports which so far have demonstrated temporal associations with alteplase administration and improvement of critically ill COVID-19 patients' respiratory status (emphasis: a causal relationship in uncontrolled case reports cannot be inferred)(76) (Poor et al, in review; Barrett et al, in review). The large experience using IV tPA for strokes, MIs and pulmonary emboli(66, 77, 78) may serve as useful guides in such desperate scenarios of COVID-19 respiratory failure that this approach were considered in the absence of controlled trial data. Our suggestion in such a situation is to consider an initial IV bolus dose of 50 or 100 mg of alteplase over 2 hours, concomitant with, or immediately followed by systemic anticoagulation with heparin. Multiple sites across the United States have already taken this approach (fibrinolytic therapy) in severe, medically refractory COVID-19 hypoxemic respiratory failure, and the specifics around the use of heparin vary from fully therapeutic heparin drips (PTT goal 60-80 seconds) while tPA is infusing, to 500u/hr heparin while tPA is infusing following by full anticoagulation after tPA is finished, to starting therapeutic heparin right after tPA finishes infusing with no heparin during the actual tPA administration period. As a rationale for dosing with respect to tPA (alteplase), we performed pharmacokinetic simulations on two test 'subjects' (75kg and 60kg), and found that the 50 and 100mg bolus dose regimens would quickly achieve tPA plasma concentrations above median PAI-1 levels in injured patients (200ng/mL, 4.7nM) (Moore et al., unpublished and (79)) (Figure 1). Importantly, this pharmacokinetic model also supports a re-dosing strategy at 12 hours or later in transient responders (e.g. those that may have re-

thrombosed due to inadequate anticoagulation, as suspected in the case series observations by Wang et al(76)) since by this time the plasma levels of tPA from the first bolus have fallen below the level of circulating tPA in normal patients(80). A Phase II clinical trial will be required to confirm these estimates and is now planned (discussed below). While we believe that intravascular delivery of tPA is likely a more appropriate route of administration in COVID-19 respiratory failure if fibrinolytic therapy were considered, if intra-airway tPA delivery were to be pursued we suggest 50mg (or 0.7 mg/kg) of tPA instilled into the airways, preferentially by bronchoscopy, followed by repeat dosing every 4-8 hours as needed for sustained improvement of oxygen requirements. This regimen is based on empiric guidelines for treatment of plastic bronchitis patients at the Children's Hospital of Colorado, as well as multiple case reports and animal studies(51, 54, 56, 61). The same exclusion criteria for MI treatment should apply, with patients maintained on a heparin infusion after tPA treatment completion to prevent re-accumulation of fibrin clot in the lung microvasculature. A possible algorithm for consideration of fibrinolytic therapy in severe, medically refractory COVID-19 respiratory failure is shown in Figure 2, with the key points being that in the absence of controlled trial data such an approach only be considered in patients with persistent, refractory hypoxemic respiratory failure despite maximal management strategies, have evidence of a hypercoagulable state, and have normal lung compliance with high Alveolar-arterial oxygen gradients that suggest the patients hypoxemia likely has a vascular occlusive component. While this scenario of a hypercoagulable state, normal lung compliance, and high Alveolar-arterial oxygen gradients is seen in the majority of patients with COVID-19 respiratory failure and microvascular thrombosis is present in the majority of autopsies, the possibility of macrovascular pulmonary embolism is not insignificant(71, 81) and similarly may improve after fibrinolytic therapy. As discussed above, such an approach involves risk, but such risk in carefully selected patients is likely outweighed by certainty of death in the proposed population and justifies consideration of salvage tPA therapy when all other therapeutic options are exhausted. We would encourage all those who are inclined to treat critically ill COVID-19 patients with tPA for refractory respiratory failure to track the success or failure of this approach and report their clinical outcomes.

Summary and Conclusions

In the present COVID-19 crisis facing a disease entity that has only existed for several months, physicians (particularly critical care physicians) are faced with large numbers of patients in profound, medically refractory hypoxemic respiratory failure with multiple clinical clues and autopsy reports that suggest a significant pulmonary microvascular thrombotic component. Level 1 evidence from randomized controlled trials for managing COVID-19 and its associated severe, refractory hypoxemic respiratory failure is months, if not years, away. As such, clinicians facing life-and-death situations in critically ill COVID-19 patients must treat them using clinical reasoning based on observation of the patient's physiology, as the standard protocols and best practice "pathways" that modern medicine has become dependent on simply do not exist yet in this emerging and lethal disease. If tPA fibrinolytic therapy were used in decompensating patients with no options for escalation of care, and shown to be effective with a greater risk of benefit than harm, such an approach could be rapidly broadened globally due to tPA's availability at most medical centers. While we cannot specifically advocate for its use in a systematic way at this time, and caution against broad implementation of this approach in the absence of controlled trial data, such an approach should at least be known to clinicians treating critically ill COVID-19 patients in the event that they have an immenently dying patient meeting the criteria outlined above and in Figure 2 and have exhausted all other options. Formal study of this potential therapy is urgently needed. A Phase IIa multi-center randomized controlled trial of alteplase therapy in severe, medically refractory hypoxemic respiratory failure in COVID-19 is now planned (ClinicalTrials.gov NCT 04357730).

Figure Legends

Figure 1. Pharmacokinetic simulations of tPA levels over time relative to its native inhibitor (PAI-1) in COVID-19 patients based on various body mass and bolus/maintenance doses of tPA. The model assumes a plasma clearance of 8.3 min, and a terminal half-life of 88 min (82).

Figure 2. Possible algorithm for consideration of fibrinolytic therapy in severe, medically refractory COVID-19 respiratory failure.

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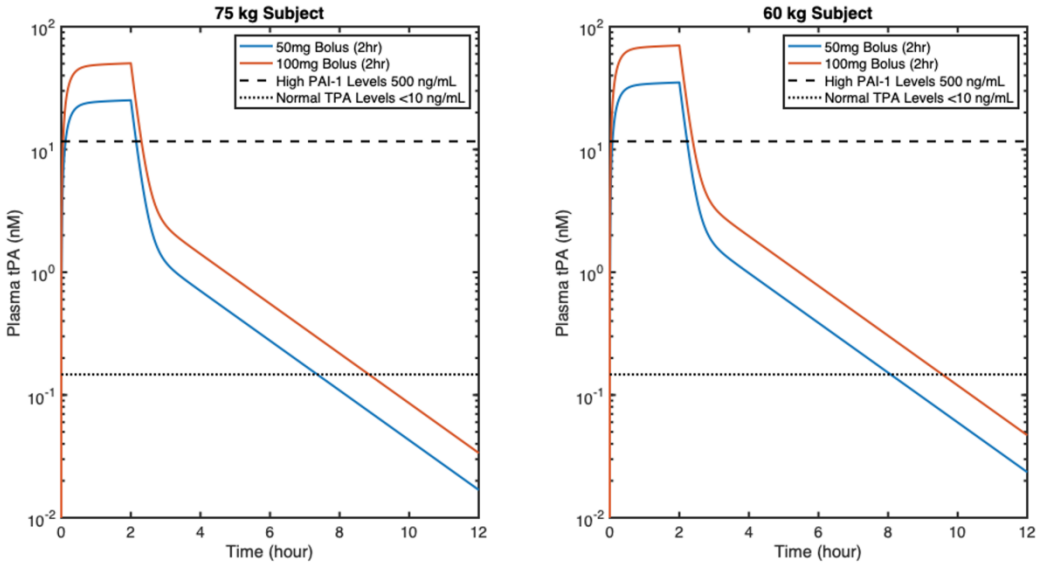

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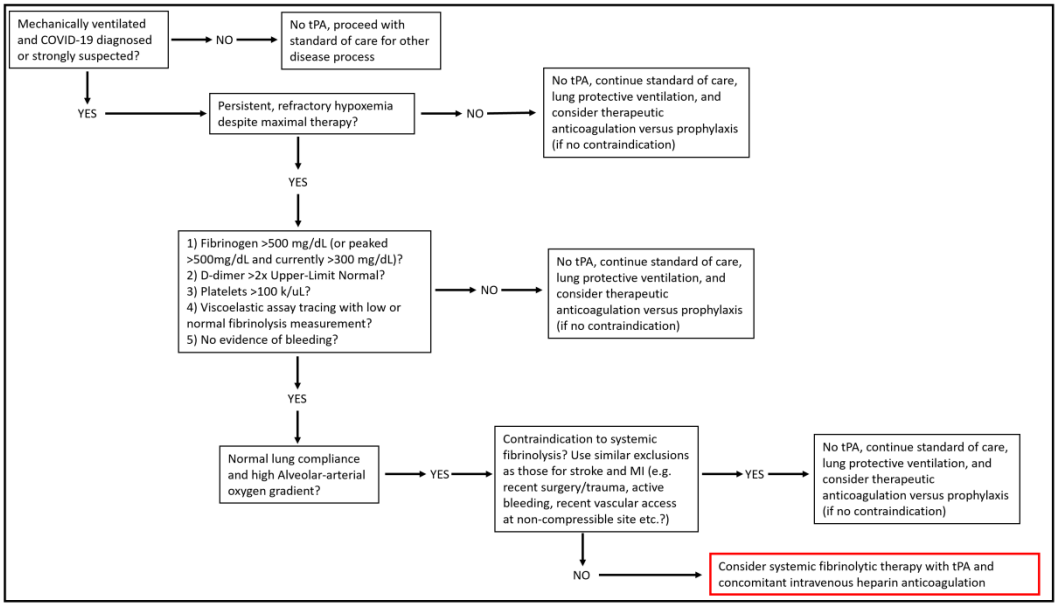

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