CHEST

Defibrotide Therapy for SARS-CoV-2 ARDS

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BACKGROUND: SARS-CoV-2-related ARDS is associated with endothelial dysfunction and profound dysregulation of the thrombotic-fibrinolytic pathway. Defibrotide is a polyanionic compound with fibrinolytic, antithrombotic, and antiinflammatory properties.

RESEARCH QUESTION: What is the safety and tolerability of defibrotide in patients with severe SARS-CoV-2 infections?

STUDY DESIGN AND METHODS: We report a prospective, open-label, single-center safety trial of defibrotide for the management of SARS-CoV-2-related ARDS. Eligible participants were 18 years of age or older with clinical and radiographic signs of ARDS, no signs of active bleeding, a serum D-dimer of more than twice upper limit of normal, and positive polymerase chain reaction-based results for SARS-CoV-2. Defibrotide (6.25 mg/kg/dose IV q6h) was administered for a planned 7-day course, with serum D-dimer levels and respiratory function monitored daily during therapy.

RESULTS: Twelve patients (median age, 63 years) were treated, with 10 patients receiving mechanical ventilation and 6 receiving vasopressor support at study entry. The median D-dimer was 3.25 μg/ml (range, 1.33-12.3) at study entry. The median duration of therapy was 7 days. No hemorrhagic or thrombotic complications occurred during therapy. No other adverse events attributable to defibrotide were noted. Four patients met the day 7 pulmonary response parameter, all four showing a decrease in serum D-dimer levels within the initial 72 h of defibrotide therapy. Three patients died of progressive pulmonary disease 11, 17, and 34 days after study entry. Nine patients (75%) remain alive 64 to 174 days after initiation of defibrotide. Day 30 all-cause mortality was 17% (95% CI, 0%-35%). All patients with a baseline Pao_2 to Fio_2 ratio of $\geq$ 125 mm Hg survived, whereas the three patients with a baseline Pao_2 to Fio_2 ratio of $<$ 125 mm Hg died.

INTERPRETATION: The use of defibrotide for management of SARS-CoV-2-related ARDS proved safe and tolerable. No hemorrhagic or thrombotic complications were reported during therapy, with promising outcomes in a patient population with a historically high mortality rate.

TRIAL REGISTRY: ClinicalTrials.gov; No.: NCT04530604; URL: www.clinicaltrials.gov

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KEY WORDS: ARDS; COVID-19; defibrotide; SARS-CoV-2

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ABBREVIATIONS: EC = endothelial cell; HCT = hematopoietic cell transplantation; NET = neutrophil extracellular trap; PAI-1 = plasminogen activator inhibitor 1; tPA = tissue plasminogen activator; WHO = World Health Organization

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Take-home Points

Study Question: What is the safety and tolerability of defibrotide in patients with SARS-CoV-2-related ARDS?

Results: A prospective, open-label trial showed that defibrotide therapy in patients with SARS-CoV-2-related ARDS was associated with no hemorrhagic or thrombotic complications and high survival rates (75%).

Interpretation: Defibrotide was safe and tolerable in this clinical setting, with promising outcomes in a patient population with a historically high mortality rate.

The COVID-19 pandemic, caused by SARS-CoV-2, has affected > 300 million individuals, with 5.5 million deaths reported.[1] Comorbidities that increase disease severity include older age, diabetes, hypertension, and prior pulmonary or cardiovascular disease.[2,3] One commonality in these disorders is chronic endothelial cell (EC) dysfunction, with EC dysfunction being a major factor in the pathogenesis and outcomes related to SARS-CoV-2 infections.[4-6] Ultimately, EC dysfunction leads to end-organ damage in the setting of microvascular and macrovascular emboli, potentially resulting in acute pulmonary, hepatorenal, myocardial, and neurologic sequelae.[7,8]

Autopsy data from patients with SARS-CoV-2 pneumonitis have identified diffuse alveolar damage, widespread microscopic thrombi, and associated organ infarcts, including hepatic sinusoidal damage, renal thrombotic microangiopathy, and myocardial infarction, even in patients who had received anticoagulation.[9-11] Severe SARS-CoV-2 infections have been associated with elevated D-dimer levels, with increasing mortality as the D-dimer value rises, and mortality rates of > 50% with values of more than sixfold of the upper limit of normal.[12] Approximately 25% of patients requiring hospitalization for SARS-CoV-2-related pneumonia may exhibit a D-dimer level of > 0.5 μg/L, with elevated levels often persisting for several months in patients.[13] Of note, hemorrhagic symptoms are not common with SARS-CoV-2 infections, with infrequent reports of pulmonary or gastrointestinal bleeding.[14,15]

Defibrotide is a polydisperse mixture of porcine-derived single-stranded oligonucleotides that was approved by the Food and Drug Administration in March 2016 for the treatment of hepatic venoocclusive disease/sinusoidal obstruction syndrome with either pulmonary or renal dysfunction after hematopoietic cell transplantation (HCT).[16] Defibrotide displays endothelial-stabilizing properties, with profibrinolytic, antiinflammatory, and endothelial adhesion effects, leading to increases in tissue plasminogen activator (tPA) and reductions in plasminogen activator inhibitor 1 (PAI-1) levels.[17-24] The agent has antiinflammatory effects, potentially modulated through decreased activation of p38 mitogen-activated protein kinase signaling pathways, the downregulation of intercellular adhesion molecule 1 expression in ECs, as well as suppression of heparanase expression in a variety of experimental models.[21-24] The agent may play a role in reducing oxidative stress, increasing nitric oxide generation, and inhibiting the generation of reactive oxygen species, potential key mediators of the endothelial injury associated with SARS-CoV-2 infections.[25] Based on this rationale, we completed a safety trial of defibrotide for the management of patients with SARS-CoV-2-related ARDS.

Study Design and Methods

A prospective, single-center, open-label trial to evaluate the safety of defibrotide for the treatment of patients with SARS-CoV-2 pneumonitis was performed. The trial was approved by the institutional review board at Michigan Medicine, with written informed consent required from patients or their legally authorized representative. In situations in which a patient was incapable of providing consent (ie, the patient was sedated and receiving mechanical ventilation), the patient's legally authorized representative provided consent. The trial was registered at ClinicalTrials.gov (Identifier: NCT04530604).

Eligibility

Eligible patients showed evidence of an active COVID-19 infection confirmed via real-time reverse transcription polymerase chain

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TABLE 1] WHO Ordinal Scale for Clinical Activity[26]

Clinical Status	Activity and Respiratory Support	Ordinal Score
Ambulatory	No limitation of activity	1
	Activities limited	2
Hospitalized	No oxygen therapy	3
	Oxygen by mask or nasal cannula	4
	Noninvasive ventilation or high-flow oxygen	5
	Intubation or mechanical ventilation	6
	Mechanical ventilation plus one of the following: pressors, ECMO, or dialysis	7
Deceased	Death	8

ECMO = extracorporeal membrane oxygenation; WHO = World Health Organization.

reaction from nasopharyngeal or lung lavage samples. Patients were 18 to 70 years of age with a serum D-dimer level of ≥ 2.0 μg/mL and ARDS, with radiographic evidence of bilateral lung disease, impaired oxygenation (Pao_2 to Fio_2 ratio ≤ 300 mm Hg), or both. Patients were ineligible if they had received thrombolytic therapy, anticoagulants, or both within 12 h of study entry, excluding heparin flushes for centrally placed catheters. Patients with clinical evidence of active bleeding or hemorrhage within the prior 72 h, mechanical ventilation for > 96 h consecutively, thrombocytopenia (platelets < 50,000/mm^3), hypofibrinogenemia (< 150 mg/dL), uncontrolled infection other than SARS-CoV-2, hemodynamic instability defined as the use of two or more vasopressors, the use of extracorporeal membrane oxygenation, or pregnancy additionally were excluded. No exclusion criteria were based on hepatic or renal function, including the use of hemodialysis. Transfusions of platelets to attain a level of > 50,000/μL or infusions of fresh frozen plasma (or cryoprecipitate) to attain a serum fibrinogen > 150 mg/dL were not allowed to meet eligibility criteria.

After the first three patients had enrolled, the study was amended (amendment version 1.0) to include patients older than 70 years, to lower the D-dimer threshold to more than twice the upper limit of normal, and to allow patients receiving mechanical ventilation for ≤ 7 d consecutively to enroll. After the ninth patient had enrolled, the study was amended (amendment version 2.0) to allow the use of nontherapeutic doses of heparin (≤ 7,500 units every 8 h) or low-molecular-weight heparin (≤ 1 mg/kg/d) concurrent with defibrotide therapy. Thrombolytic treatment, anticoagulant treatment, or both at therapeutic doses within 12 h of study entry remained an exclusion criterion.

Study Design

Defibrotide was administered at a dose of 25 mg/kg/d IV divided in four doses daily for a planned duration of 7 days (28 doses). Individual doses were diluted in 0.9% sodium chloride or 5% dextrose in water, mixed to a concentration of 4 to 20 mg/mL, and infused with a 0.2-μm in-line filter over a 2-h period. No other medications were coadministered through the same IV line during the defibrotide infusion.

Clinical ordinal scores for activity and respiratory support were assigned to patients at baseline, then daily throughout the study period (Table 1).[26] Response to therapy was defined as a reduction in the World Health Organization (WHO) ordinal score of ≥ 2 points for 48 h consecutively or a complete cessation of supplemental oxygen support by day 7 of therapy. Patients who met the response parameter (or were discharged) before day 7 were allowed to discontinue defibrotide at that time, without completing the 7-day course. Patients with a ≥ 20% reduction in supplemental oxygen requirement (% Fio_2) by day 7 were allowed to receive an additional 7 days of therapy, for a maximum 14-day course. No outpatient dosing was allowed.

Defibrotide therapy was withheld if a patient demonstrated signs of bleeding as defined by International Thrombosis and Haemostasis criteria.[27] Blood-tinged endotracheal secretions, microscopic hematuria, or mild menorrhagia did not require withholding defibrotide, unless the treating medical team deemed that the event required medical intervention. Patients with CNS or alveolar hemorrhage were required to discontinue therapy immediately, without the option to resume. Defibrotide was withheld for surgical procedures or to accommodate central line placements, the agent being withheld > 2 h before the procedure and for 12 to 24 h after the procedure, based on the discretion of the medical team. No additional doses were given to account for doses withheld during this period. Defibrotide therapy was discontinued if a patient initiated extracorporeal membrane oxygenation therapy.

Hematologic parameters (CBC counts), serum chemistry findings, D-dimer levels, prothrombin time, and serum fibrinogen levels were required daily while receiving defibrotide therapy. Platelet transfusions, use of fresh frozen plasma-cryoprecipitate, or both were allowed if clinically indicated during therapy. Pulmonary indexes (level and type of supplemental oxygen support) were recorded daily until cessation of therapy and again at day 14 from study entry. Biomarkers of hemostasis, including PAI-1 and tPA, were obtained at day 1 (baseline) and day 4 of the study, provided the patients were still receiving defibrotide therapy at that time.

Statistical Analysis

The study was designed as a feasibility study to examine the use of defibrotide in patients with SARS-CoV-2 ARDS. The primary study end point was the occurrence of major toxicity, including hemorrhagic complications during study therapy. Secondary end points included overall survival, day 7 response, day 14 survival, and day 14 ventilator-free survival, with ventilator-free survival summarized by the proportion of patients alive and ventilator free on day 14 of study. Stopping rules for hemorrhagic complications and day 14 all-cause mortality were present, with the study suspended if three of the first six patients demonstrated a hemorrhagic event or if five of the first six patients died by day 14. Patients who received at least one dose of defibrotide were considered evaluable for the primary and secondary end points. Toxicity assessments using Common Terminology Criteria for Adverse Events version 5.0 were used. Overall survival was

determined using the Kaplan-Meier method, with survival defined from the time of study entry to the date of death or last contact. The study was designed to have a planned sample size of 12 patients, and the sample size was deemed reasonable to assess feasibility. A data safety monitoring committee reviewed all toxicity data and response assessments.

Results

Thirteen patients were enrolled between October 2020 and March 2021, with 12 patients (median age, 63 years; age range, 35-73 years) treated (Table 2). One patient demonstrated positive blood culture results after consent was signed but before receipt of the first dose of defibrotide. The patient was deemed ineligible and was replaced in the study.

The median time from diagnosis of SARS-CoV-2 to study entry was 9 days (range, 1-26 days), with supplemental oxygen given a median 7 days (range, 3-26 days) before enrollment. Ten of the 12 patients required mechanical ventilation and six patients required vasopressor support (WHO ordinal score of 7) at study entry (Table 2). The median F_{IO_2} was 55% (range, 40%-75%), median positive end-expiratory pressure was 18 cm (range, 10-18 cm), and median Pa_{O_2} to F_{IO_2} ratio was 137 mm Hg (range, 83-200 mm Hg) in the 10 patients receiving mechanical ventilation. The median D-dimer level was 3.25 μg/mL (range, 1.33-12.3 μg/mL), median serum fibrinogen level was 637 mg/dL (range, 250-1,208 mg/dL), and median platelet count was 226 K/μL (range, 70-341 K/μL) at study entry. Dexamethasone (6 mg/dose) and a 5-day course of remdesivir were begun before defibrotide therapy in all patients. The dexamethasone was started a median of 4 days (range, 1-21 days) before defibrotide and continued for a median of 8 days after defibrotide therapy had started. Three patients (patients 10, 11, and 12) received a single dose of tocilizumab within 1 week of study entry. No patients who were responding clinically to dexamethasone therapy, remdesivir therapy, tocilizumab therapy, or a combination thereof were included in the study. No other COVID-19-targeted therapy, including monoclonal antibodies, was given to patients while they received defibrotide. Common comorbidities included diabetes mellitus (n = 7), hypertension (n = 7), obesity (n = 4), sleep apnea (n = 4), COPD (n = 4), hyperlipidemia (n = 3), and immune deficiency disorders (n = 2).

Therapy duration ranged from 1 to 14 days (median, 7 days), with six patients receiving fewer than 7 days of therapy because of meeting the response criteria (n = 3), ventilator-associated infections (n = 2), or progressive

TABLE 2] Patient Demographics and Baseline Laboratory Parameters

Patient No.	Age (y)	Gender	Prior Therapy	Oxygen Support	F_{IO_2} (%)	Pressor[a]	WHO[b]	Onset (d)[c]	D-Dimer (μg/mL)	Platelets (K/μL)	Pa_{O_2} to F_{IO_2} Ratio	Anticoagulant[d]
1	64	M	D, R	MV	40	Yes	7	23	2.34	70	200	None
2	36	M	D, R	MV	60	No	6	16	3.23	284	173	None
3	35	M	D, R	MV	75	Yes	7	5	12.3	224	124	None
4	58	M	D, R	MV	70	Yes	7	21	3.55	250	91	None
5	64	M	D, R	NC	35	No	4	14	14.9	320	NA[e]	None
6	48	F	D, R	MV	50	No	6	3	2.02	341	194	None
7	73	M	D, R	NC	40	No	4	2	2.84	275	155	None
8	62	M	D, R	MV	50	No	6	18	7.09	169	130	None
9	73	M	D, R	MV	50	Yes	7	1	3.27	188	112	None
10	70	F	D, R, T	MV	60	Yes	7	14	3.85	151	158	LMWH
11	51	M	D, R, T	MV	75	No	6	5	1.33	94	145	LMWH
12	64	M	D, R, T	MV	70	Yes	7	26	1.41	228	83	Heparin

D = dexamethasone; Dx = diagnosis; F = female; M = male; MV = mechanical ventilation; NA = not available; NC = nasal cannula; R = remdesivir; T = tocilizumab.

[a]Requirement for vasopressor support at study entry.

[b]World Health Organization ordinal score at study entry.

[c]Time (d) from diagnosis of SARS-CoV-2 to onset of study therapy.

[d]Patients 10 and 11 received low-molecular-weight heparin at < 1.0 mg/kg/d. Patient 12 received subcutaneous heparin at prophylactic dosing.

[e]Not available for patient 5. Venous blood gas measurements had been obtained from this patient.

TABLE 3] Outcomes and Adverse Events

Patient No.	Therapy Duration (d)	Adverse Events[a]			WHO Score Day 30[b]	Status	Overall Survival (d)
		Bleeding	Thrombotic	Other			
1	4	None	None	None	4	Alive	231+
2	4	None	None	None	1	Alive	220+
3	2[c]	None	None	Pneumonitis	4	Alive	175+
4	14	None	None	Pneumonitis	8	Died	17
5	7	None	None	None	2	Alive	142+
6	6	None	None	None	2	Alive	157+
7	6	None	None	None	1	Alive	140+
8	7	None	None	None	3	Alive	64+
9	7	None	None	Pneumonitis	8	Died	34
10	7	None	None	Pneumonitis	6	Alive	96+
11	7	None	None	Pneumonitis	7	Alive	75+
12	4	None	None	Pneumonitis	8	Died	11

WHO = World Health Organization.
[a]Serious adverse events occurring during study therapy or within 7 d after therapy completion. Pneumonitis: bacterial (n = 5), fungal (n = 1).
[b]WHO ordinal score 30 d after completion of study therapy.
[c]A blood culture obtained before therapy became abnormal on day 2 of defibrotide therapy. The defibrotide was discontinued at that time and was not reinstituted. The patient's F_{IO_2} requirement had declined from 75% to 50% by day 2 of therapy. The patient was ineligible to continue study drug further.

pulmonary disease (n = 1) (Table 3). The infusions were well tolerated, with no infusion-related reactions reported. All defibrotide doses were given as scheduled, with minor adjustments in the timing (± 1-2 h) required given the complexities of ICU care for patients with positive SAR-CoV-2 findings. The first nine patients received defibrotide without any other anticoagulation being given. After approval of amendment 2.0, patients 10, 11, and 12 received defibrotide in conjunction with prophylactic doses of IV (n = 1) or low-molecular-weight heparin (n = 2).

Toxicity

No hemorrhagic or bleeding episodes occurred during study therapy, including the three patients (patients 10, 11, and 12) who received defibrotide with concurrent heparin prophylaxis (Table 3). Conversely, no thrombotic events developed while patients were receiving therapy, including the nine patients to whom defibrotide was given without other anticoagulants. Six patients demonstrated a secondary pneumonia during or within 7 days of completion of study therapy, including positive endotracheal culture results for *Klebsiella aerogenes* (n = 2), *Escherichia coli* (n = 2), *Enterobacter cloacae* (n = 1), and *Pseudomonas aeruginosa* (n = 1).

Clinical Response

Four patients (patients 1, 5, 6, and 7) met the day 7 response parameter, with two patients (patients 5 and 6)

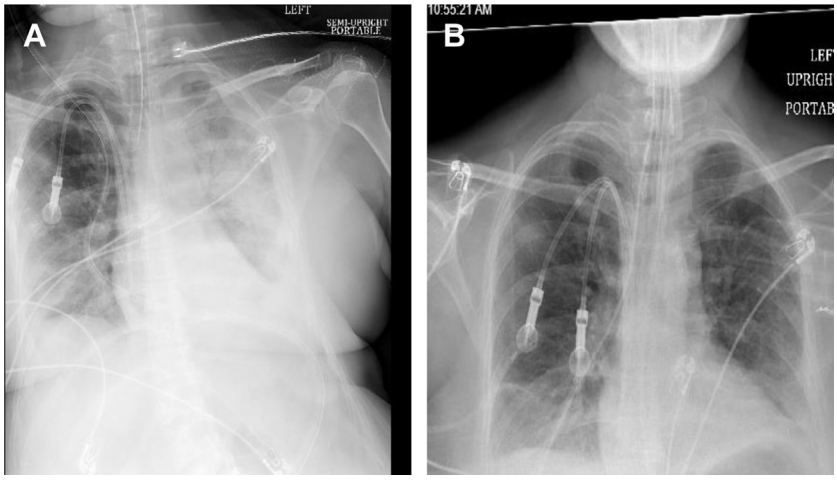

Figure 1 – *A, B, Chest radiographs from patient 6 obtained at study entry (A) and day 3 (B). At the time of study entry, the patient was mechanically ventilated, on 50% F_{IO_2}, with positive end-expiratory pressure of 10 cm H_2O. The patient subsequently was extubated by day 4, and all supplemental oxygen support was removed by day 7.*

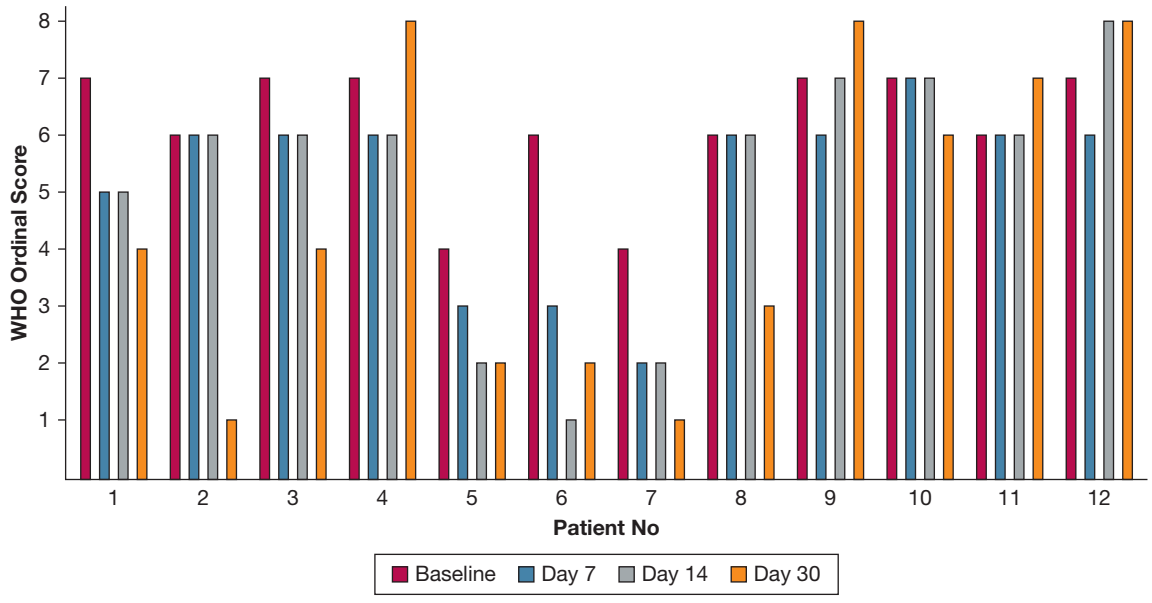

Figure 2 – *Bar graph showing WHO ordinal scores obtained at baseline, day 7, and day 14 from study entry. The day 30 score was obtained 30 days after completion of study therapy. WHO = World Health Organization.*

achieving complete cessation of oxygen support within this 7-day period. Patient 6, a 48-year-old woman with severe myasthenia gravis, was extubated after 4 days of defibrotide therapy and discontinued all supplemental oxygen by day 7 (Fig 1). Overall, six patients remain without supplemental oxygen support 64 to 174 days from study entry. Seven of the 12 patients showed a two-point or more decline in WHO ordinal scores within 30 days of completion of study therapy (Fig 2). All four patients who met the day 7 response parameter showed a decrease in serum D-dimer values within 72 h of initiation of defibrotide therapy, including patient 5, whose D-dimer level decreased from 14.9 to 5.81 μg/mL within the initial 24 h of therapy (Table 4). Two patients (patients 4 and 9) showed a threefold increase in D-dimer levels within 72 h of starting therapy. Both patients died of progression of SARS-CoV-2 ARDS. One of two patients (patients 3 and 5) with a baseline D-dimer level of $>$ 10 μg/mL responded, whereas both patients (patients 11 and 12) with a baseline D-dimer level of $<$ 2.0 μg/mL did not meet the day 7 response parameter.

All-Cause Mortality and Survival

Nine patients (75%) remain alive in an outpatient setting after the primary hospitalization for SARS-CoV-2 ARDS, including seven of 10 male and both female patients (Table 3, Fig 3). Eleven patients (92%) were alive at the day 14 end point, with a day 14 ventilator-free survival of 33%. Estimated all-cause mortality was 17% (95% CI, 0%-35%) and 25% (95% CI, 0%-46%) by day 30 and day 60, respectively, after initiation of study therapy. Deaths resulting from progression of SARS-CoV-2 ARDS occurred in three patients at 11, 17, and 34 days from study entry. The three patients with the lowest Pao_2 to Fio_2 ratios at study entry (83 mm Hg, 91 mm Hg, and 112 mm Hg) each died, whereas all patients with baseline Pao_2 to Fio_2 ratios of $>$ 125 mm Hg survived. Both patients who received mechanical ventilation for $>$ 5 days before study entry died.

Markers of Hemostasis

Total PAI-1 and tPA levels were available for seven of the 12 patients. At baseline, the median PAI-1 level was 167 ng/mL (range, 105-264 ng/mL), decreasing to 104 ng/mL (range, 55-166 ng/mL) by day 4 of therapy. All seven patients exhibited a decline in PAI-1 levels within the initial 1 week of therapy. Total tPA levels increased from a median of 3.02 ng/mL (range, 0.72-36.1 ng/mL) at baseline to 4.5 ng/mL (range, 1.1-8.2 ng/mL) by day 4, increasing in 5 of 7 patients. Given the small sample sizes, no definitive correlation of PAI-1 or tPA levels with outcome or response could be determined.

Discussion

We report the first safety study of defibrotide therapy in a trial of critically ill patients with SARS-CoV-2-associated ARDS. The lack of hemorrhagic or

TABLE 4] D-Dimer Values at Baseline, Day 3, and Day 7 From Study Entry

Patient No.	Baseline	Day 3	Day 7	Status
1	2.34	1.65	3.61	Alive
2	3.23	1.75	5.85	Alive
3	12.3	1.83	2.14[a]	Alive
4	3.55	11.1	12.2[a]	Died
5	14.9	5.81	2.69	Alive
6	2.02	1.47	1.68	Alive
7	2.85	1.69	1.39[a]	Alive
8	7.09	3.92	6.07	Alive
9	3.27	11.68	31.9	Died
10	3.85	2.56	4.67	Alive
11	1.33	2.31	NA	Alive
12	1.41	1.74	NA	Died

NA = not available.
[a]Day 6 instead of day 7 value obtained.

thrombotic complications, or both, the response to therapy, and overall survival were promising in this prospective open-label trial.

Historically, the mortality for patients with SARS-CoV-2 ARDS is high, with day 28 mortality rates of 26% to 61.5% in patients requiring mechanical ventilation.[27-32] The median duration from ICU care to death often is short: within 7 days in several reports.[28-30] In a prospective cohort study of 4,643 critically ill adults with COVID-19 in northern Europe, 63% were intubated within 24 h of ICU admission, with 80% requiring mechanical ventilation during the ICU course.[29] Mortality rates paralleled the severity of the ARDS at the time of ICU admission, with mortality rates of 30%, 34%, and 50%, respectively, for mild, moderate, and severe ARDS. In comparison, day 30 mortality was 17% (95% CI, 0%-35%) in the present study with defibrotide. Although this study was not designed to assess efficacy, the day 30 and 60 survival are promising considering the high acuity of disease in patients at study entry.

Defibrotide was safe in this patient population, with no major adverse events attributable to the agent noted. Defibrotide is currently approved by the Food and Drug Administration for the treatment of adult and pediatric patients with venoocclusive disease/sinusoidal obstruction syndrome with renal or pulmonary dysfunction after HCT. Common toxicities (any grade) reported in HCT recipients included hypotension (37%), diarrhea (24%), vomiting (18%), epistaxis (14%), pulmonary alveolar hemorrhage (9%), gastrointestinal hemorrhage (9%), septicemia (7%), and cerebral hemorrhage (2%), with grade 4 to 5 pulmonary hemorrhage noted in 8% of patients.[16] The agent was well tolerated in the current study, with no hypotension, gastrointestinal toxicity, or pulmonary hemorrhage noted. No bleeding (pulmonary or nonpulmonary) occurred during study therapy in any patient, including the three patients who received concurrent heparin prophylaxis.

The incidence of ventilator-associated pneumonia in this patient population (33%) was similar to that in published reports in patients requiring ICU-level care for SARS-CoV-2 infection, in which secondary infection rates of 21% to 58% have been reported.[29-33] Considering that patients in this study all were receiving concurrent systemic corticosteroids, the infection rate for these patients was promising, with secondary infections primarily resulting from gram-negative pathogens. Furthermore, the use of defibrotide has not been associated with an increased risk of infections in other (non-SARS-CoV-2) patient populations, particularly HCT recipients.[16]

Although the current study followed dosing guidelines established in HCT recipients (6.25 mg/kg IV q6h), we used a much shorter course of therapy (7-14 days) than typically given to HCT recipients, in which 21 days of therapy are administered routinely.[16] The use of a longer therapy course (> 7 days) may not be required, because all responders did so within 7 days of study initiation. Ongoing trials in the critical care setting will evaluate the optimal duration of therapy in different patient subgroups.[34,35] We also are unable to address whether defibrotide can be given safely with other fibrinolytics or

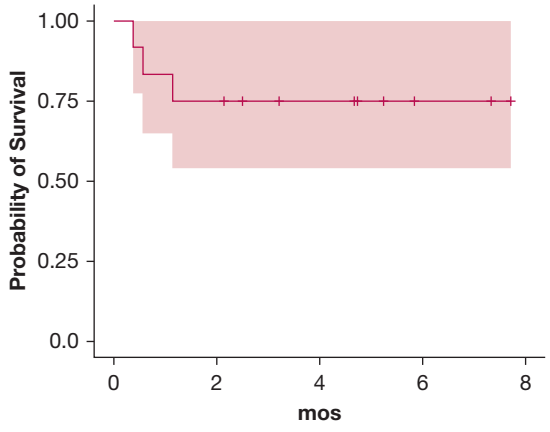

Figure 3 – *Graph showing overall survival from time of study entry. Shaded area denotes pointwise 95% CI.*

anticoagulants because only three of 12 patients received prophylactic heparin concurrent with defibrotide therapy. Importantly, none of the three experienced any bleeding complications. Preliminary results from other ongoing defibrotide trials have shown the combination of defibrotide with anticoagulants to be feasible.[34,35]

The potential endothelial protective effects of defibrotide may be a key feature in treating the vasculopathy associated with SARS-CoV-2 infections. Furthermore, the usefulness of defibrotide as an alternative to heparin for the prevention of thromboembolic complications in patients with severe SARS-CoV-2 infection is an intriguing possibility. Several studies have shown that defibrotide is at least as effective as heparin for thromboprophylaxis in other clinical settings.[36-38] An open-label, multicenter study comparing outcomes in postoperative patients (n = 4,810) receiving either defibrotide (n = 2,810) or heparin prophylaxis (n = 2,000) noted a lower incidence of postsurgical DVT and pulmonary embolism in those receiving defibrotide as thromboprophylaxis (DVT, 1.17% vs 2.35% [$P = .002$]; pulmonary embolism, 0.53% vs 1.15% [$P = .025$]).[37] The ability to use defibrotide therapy safely as the sole antithrombotic agent in patients with SARS-CoV-2 ARDS was a promising finding in our trial.

From a mechanistic standpoint, defibrotide is an attractive agent for investigation into the management of the thromboinflammation associated with SARS-CoV-2 infections through its modulation of cytokine release and other markers of endothelial stress. Specifically, both preclinical and clinical data indicate that the agent may augment fibrinolysis, decrease thrombin generation, reduce PAI-1 levels, reduce oxidative stress, and decrease the platelet activation seen in prothrombotic states. Another potential mechanism of action may involve inhibition of neutrophil extracellular trap (NET) formation in SARS-CoV-2-affected patients, with the severity of SARS-CoV-2 infections directly correlating with NET overexpression.[39-43] Histone generation during NET formation already has been shown to lead to EC injury in other clinical settings.[44] Preclinical models by investigators at our center have shown that defibrotide directly neutralizes NET-derived cationic proteins (histones), inhibits the activation and permeability of cultured ECs, and protects ECs from histone-induced cell death.[45]

Given its small sample size, this pilot study has several potential limitations. The study was not designed to address the efficacy of defibrotide in this clinical setting. In addition, the study could not address the role of concurrent anticoagulation, with only three of 12 patients receiving heparinization during study therapy. Furthermore, defibrotide was given in conjunction with only prophylactic, but not therapeutic, doses of heparin in our study. We do not know the impact of defibrotide when combined with therapeutic heparin dosing.

For patients with SARS-CoV-2 infections, defining both the optimal patient population and the optimal timing for initiation of defibrotide remain under investigation, with larger phase 2 and phase 3 clinical trials required. Several trials currently are in progress, including a randomized, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT04348383) and two trials targeting patients with high-acuity disease (ClinicalTrials.gov Identifiers: NCT04652115 and NCT04335201). Despite the small sample size of this pilot study, it is reasonable to infer that defibrotide therapy could be considered in patients with less acute pulmonary disease (ie, WHO ordinal scores < 6). Our results support this assumption, in which all patients with severe pulmonary disease at baseline (Pao_2 to Fio_2 ratio < 125 mm Hg) died within 60 days of therapy and all patients with a baseline Pao_2 to Fio_2 ratio of > 125 mm Hg survived. Preliminary results from an Italian trial likewise show significant promise for both safety and efficacy in less severely ill patients with SARS-CoV-2 pneumonitis.[35] Given that SARS-CoV-2-related thromboinflammation, complement activation, and resultant endothelial damage may begin early in a patient's clinical course, one might consider initiating defibrotide therapy in any patient hospitalized for SARS-CoV-2 in whom prophylactic heparinization is being considered. Combining an immunomodulatory and antithrombotic agent such as defibrotide with prophylactic doses of low-molecular-weight heparin may be an attractive option to consider in this clinical situation.[22,25,46] Our findings should motivate additional studies examining earlier initiation of defibrotide therapy, specifically targeting those with lower WHO ordinal scores.

Interpretation

Defibrotide therapy in patients with SARS-CoV-2 ARDS was well tolerated, with no hemorrhagic complications noted and promising response rates seen.

Acknowledgments

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