Use of defibrotide in COVID-19 pneumonia: comparison of a phase II study and a matched real-world cohort control

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Running Heads: “Use of defibrotide in COVID-19 pneumonia”

Annalis a R u g g e r i 1* , Francesco C or rad o 2,3* , Antonio V o z a 2,4 , L ee-J en W ei 5 , G loria Ca t a la no 1, C arm ine L ib eratore 1, R os a mar ia N itti 1, C arlo F ed eli 7, A le s s an dro B r u no 1, E leon or a Ca lab retta 2,3, F abio G ig l io 1, F abio S ciu tti 6, F r an ces ca L unghi 1, G iov a nn i L and o ni 7,8, A l e s s io A gh emo 2,9, M ass im o I acl obelli 10, P a tri z ia R o v er e Q uerini 9,11, P a ul G. R ichar d son 12, A n dre a A ss an e l li 1, J ac o p o Pe c c at or i 1, F ab io Ci cer i 1,9#, C ar m elo C ar lo-St e l la 2,3#

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D ata S haring Statement

O riginal data may be available upon specific request to the corresponding author.

C ontributions: A R, J P, F C and C CS designed the study and wrote the manuscript, A R and F C o collected data, F C o and L J W performed statistical analyses and wrote the manuscript, A R, F C o, L J W, F C and C CS analyzed and interpreted data, A V, G C, C L, R N, C F, A B, E C, F G, F A, F L, G L, A A, P R Q, A A s, were involved in patients recruitment, M I and P R provided significant advice throughout the study. A ll authors had access to the primary data and participated in editing the manuscript.

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**Trial registration:** ClinicalTrials.gov NCT04335201

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ABSTRACT
The coronavirus disease 2019 (COVID-19) pandemic led to an unprecedented burden on healthcare systems around the world and a severe global socioeconomic crisis, with more than 750 million confirmed cases and at least 7 million deaths reported by 31st December 2023. The DEFI-VID19 study (ClinicalTrials.gov NCT04335201), a phase II, single-arm, multicenter, open-label trial was designed in mid-2020 to assess the safety and efficacy of defibrotide in treating patients with COVID-19 pneumonia. Defibrotide was administered at a dose of 25 mg/kg/d intravenously, divided into four daily doses over a planned 14-day period for patients with COVID-19 pneumonia receiving non-invasive ventilation. The primary endpoint was Respiratory Failure Free Survival (RFFS); Overall Survival (OS), the number of post-recovery days, and adverse events were the secondary endpoints. For comparison, a contemporaneous control cohort receiving standard of care only was retrospectively selected by applying the eligibility criteria of the DEFI-VID19 trial. To adjust for the imbalance between the two cohorts in terms of baseline variable distributions, an outcome regression analysis was conducted. In adjusted analysis, patients receiving defibrotide reported a trend towards higher RFFS (HR=0.71[0.95CI: 0.34 to 1.29, \(P= .138\)]) and OS (HR=0.78[0.95CI: 0.33 to 1.53, \(P= .248\)]) and showed a significantly increased number of post-recovery days (difference in means: 3.61[0.95CI: 0.97 to 6.26, \(P= .0037\)]). Despite concomitant thromboprophylaxis with low molecular weight heparin, the safety profile of defibrotide proved to be favorable. Taken together, our findings suggest that defibrotide may represent a valuable addition to the COVID-19 therapeutic options.
INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, the global outbreak of a viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), led to an unprecedented burden on healthcare systems around the world and a severe global socioeconomic crisis. On 30 January 2020 COVID-19 was declared a Public Health Emergency of International Concern (PHEIC) with an official death toll of less than 200. By 1st August 2023, there have been more than 750 million confirmed cases of COVID-19, including at least 7 million deaths reported by the WHO. The current epidemiological landscape reflects the implementation of extensive policies of vaccination and the approval of a limited number of therapeutic agents. The adoption of vaccination policies is estimated to have prevented nearly 14 million deaths from COVID-19 between Dec 8, 2020, and Dec 8, 2021. Baricitinib, IL-6 receptor blockers, and corticosteroids emerged as the main drugs demonstrating a reduction in mortality among patients with severe COVID-19 pneumonia. Conversely, remdesivir has shown the potential to expedite the time to recovery in patients with mild to severe cases of COVID-19 pneumonia, but without a clear survival advantage.

The DEFI-VID19 clinical trial was designed in mid-2020, at a time when an effective vaccine was not yet available, and COVID-19 presented as an uncontrolled, devastating, and widespread disease. In the absence of established therapeutic strategies, the mainstay of COVID-19 management was supportive care, with acute respiratory distress syndrome (ARDS) as the primary cause of mortality. COVID-19 related ARDS is uniquely associated with distinct pathobiological pathways, with angiocentric inflammation, microangiopathy, cytokine release syndrome and hypercoagulation emerging as the primary factors influencing the severity of the disease. Moreover, specific inflammatory biomarkers, such as ferritin and IL-6 had been found to be independent predictors of fatality in COVID-19 further suggesting the central role of endotheliitis in the pathophysiology of the disease. The endothelial damage and cytokine profile associated with severe COVID-19 pneumonia suggested significant parallels with the pathophysiology of Veno-Occlusive Disease (VOD), also referred to as Sinusoidal Obstruction Syndrome (SOS). VOD/SOS is a syndrome characterized by endothelial damage to hepatic sinusoids and subsequent hepatocellular injury with subsequent necrosis, which is most commonly seen after bone marrow transplantation. Both conditions involve reactive endothelial activation, pro-inflammatory responses, cytokine release, progressive endotheliopathy and multi-organ dysfunction. The pathological similarities extend further, with lung lesions in VOD/SOS exhibiting features akin to those observed in COVID-19 patients. 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 VOD/SOS with either pulmonary or renal dysfunction after hematopoietic cell transplantation (HCT)\textsuperscript{15}. Defibrotide has demonstrated profibrinolytic, antithrombotic, anti-inflammatory, and angio-protective properties\textsuperscript{16}. Given its potential therapeutic effects and the similarities between VOD/SOS and COVID-19, the DEFI-VID19 study was designed to evaluate the safety and efficacy of defibrotide in treating SARS-CoV-2-related ARDS, as part of several international studies evaluating the role of defibrotide in this setting and the underlying hypotheses of ameliorating endotheliitis, modulating cytokine release and reversing microangiopathy\textsuperscript{17–19}

The DEFI-VID19 study was conceived as a phase II, single-arm trial. Subsequently, a comparison group was retrospectively built by selecting an observational cohort of patients who received standard of care alone.

**METHODS**

DEFI-VID19 was a phase II, prospective, interventional, single-arm, multicenter, open label trial conducted at IRCCS-San Raffaele Scientific Institute (Milan), IRCCS-Humanitas Research Hospital (Milan), IRCCS- Policlinico San Matteo (Pavia). Approval was provided by the National IRB for COVID-19 trials at Institute Spallanzani (Rome) and by the Italian Agency for Drug (AIFA). All patients provided written informed consent. The trial was registered at ClinicalTrials.gov (ID: NCT04335201).

**DEFI-VID19 eligibility criteria**

Inclusion criteria included documented COVID-19 pneumonia: defined as a positive real-time reverse transcription polymerase chain reaction from upper respiratory tract specimens (nasopharyngeal or throat swabs) and/or imaging at computed tomography scan suggestive of COVID-19 pneumonia; oxygen saturation (SaO\textsubscript{2}) of 92\% or less without oxygen support, or reduction of 3\% from the basal value of SaO\textsubscript{2}, or a ratio of the partial pressure of oxygen (PaO\textsubscript{2}) to the fraction of inspired oxygen (FiO\textsubscript{2}) (PaO\textsubscript{2}/FiO\textsubscript{2}) below 300; any gender; age $\geq$ 18 years; written informed consent.

Exclusion criteria included onset of COVID-19 pneumonia $>$ 14 days; orotracheal intubation; uncontrolled systemic infections (other than COVID-19); concomitant use of thrombolytic therapy; concomitant systemic anticoagulant therapy (e.g., heparin, warfarin, direct thrombin inhibitors, and direct factor Xa inhibitors); haemodynamic instability; hypersensitivity to the active substance or to any of the excipients of the experimental drug. patients who, based on
the investigator's clinical judgment, were not able to receive the treatment; pregnancy or breast feeding.

**DEFI-VID19 study design**
Defibrotide was administered at a dose of 25 mg/kg/d intravenously (IV) fractionated in four doses daily for a planned treatment period of 14 days. Details are provided in the supplementary methods.

**Observational data selection criteria**
A contemporary cohort of patients admitted to the IRCCS – Humanitas Research Hospital with Covid-19 pneumonia was retrospectively screened for meeting the eligibility criteria of the DEFI-VID19 trial. After applying the eligibility criteria, 153 patients qualified as controls.

**Endpoints**
The primary endpoint was the Respiratory Failure Free Survival (RFFS) defined as the time from day one until respiratory failure. Respiratory failure was defined as follows: intensive Care Unit (ICU) admission or death within day 14; the persistence of respiratory distress despite oxygen therapy at day 14 and for patients with a day1 P/F ratio >200: a P/F ratio <200 at day 14. Respiratory distress was operationally defined as a respiratory rate > 24/min. The number of post-recovery days was defined as the number of days after hospital discharge out of a predefined time window of 28 days from day 1. A value of zero post-recovery days was assigned to patients who were still hospitalized or died before day 28. Secondary endpoints and biological markers evaluated are indicated in the Supplementary methods.

**Statistical methods**
Statistical analyses were conducted using GraphPad Prism (version7.5) and R version 4.2.2. To account for the potential imbalance between the two cohorts in terms of baseline variable distributions, an outcome regression analysis was conducted. Specifically, as previously described by Richardson et al, a survival Cox prediction model was developed using the data from the observational cohort. A set of clinically relevant baseline covariates was selected.
RESULTS

Patients’ characteristics

Overall, 52 patients were enrolled in the DEFI-VID19 trial from September 2020 to April 2021; 48 were evaluated for efficacy and safety; 4 patients were excluded due to screen failure (n=2) or withdrawal of informed consent at day 2 after Defibrotide was initiated (n=2). The distribution of patients’ baseline characteristics according to Defibrotide exposure is shown in Table 2. DEFI-VID19 patients were younger than patients who did not receive defibrotide (median, 60.5 vs. 72.7 years old), had fewer cardiovascular diseases (6.2% vs. 21.6%), hypertension (33.3% vs 54.9%), and higher PaO2:FiO2 ratio at baseline (210.5 vs. 164). Median values of C-reactive protein (mg/dL), D-dimer(ng/mL), Ferritin (ng/mL), Interleukin-6 (pg./mL), Lactate Dehydrogenase (UI/L) and platelet counts (10^9/L) for the DEFI-VID19 cohort are reported in Table 2. At study entry, all treated and untreated patients received oxygen support either through a high-flow nasal cannula (HFNC) or continuous positive air pressure (CPAP) and were assigned a score of 5 on the WHO ordinal scale for clinical activity. The median time from the diagnosis to the start of Defibrotide was 4 days (range 1-11). Therapy duration ranged from 1 to 14 days (median, 8 days). 41 patients received less than 14 days of treatment because of the independence from oxygen therapy or discharge before day 14(n=29), disease progression(death(n=3) or the need for invasive ventilation (n=5)) or the requirement of full anticoagulant therapy(n=4). No other COVID-19 experimental therapy, including monoclonal antibodies, was given to patients while they received Defibrotide. Every patient in the study group received Dexamethasone (6 mg/dose) and antiviral therapy according to the current local guidelines. All DEFI-VID19 patients also received thromboprophylaxis with Low Molecular Weight Heparin (LMWH) according to each institution’s standards of care.

DEFI-VID19 Safety

Forty-eight patients were evaluated for safety. The infusions were generally well tolerated, with no infusion-related reactions reported. No significant hemorrhagic or bleeding episodes occurred during study therapy. Thirteen patients (27.08%, 0.95CI: 16.6 to 41) developed a serious adverse event (SAE). Twelve (18.7%, 0.95CI: 10.2 to 32) developed respiratory failure, which was associated with death for seven patients. Four patients (8.3%, 0.95 CI: 3.3
to 19.55) developed thrombotic events that lead to the initiation of a full anticoagulant therapy and withdrawal of Defibrotide treatment per protocol. One thrombotic event, a pulmonary embolism, proved to be fatal, despite prophylactic LMWH. However, no serious adverse event was deemed to be associated with or related to the administration of Defibrotide, and importantly no bleeding with concomitant LMWH was reported. The median time of onset to SAEs was 6 days (range 2-12).

**Respiratory Failure-Free Survival**

Respiratory Failure Free Survival Rate at day 14 was 0.75 (95%CI:0.68 to 0.88) in the DEFI-VID19 cohort and 0.50 (95%CI:0.43 to 0.59) in the Observational cohort. In unadjusted analysis, the respiratory failure-free survival was significantly higher in DEFI-VID19 patients (HR: 0.43, 95%CI [0.23, 0.79], P=.005) (Figure1).

In the DEFI-VID19 cohort, all Respiratory Failure events were represented by death or ICU admission before Day 14 regardless of baseline P/F ratio. Within the observational cohort, 68 out of 76 cases of Respiratory Failure were characterized by death or ICU admission before Day 14, or by a deterioration of the P/F ratio from over 200 on Day 1 to below 200 by Day 14. The remaining 8 cases were marked by persistent respiratory distress at Day 14 despite oxygen therapy. Notably, among these 8 cases, 7 had a baseline P/F ratio below 200, which persisted below 200 after 14 days. In all these instances, respiratory rates were confirmed to be above 24 in at least 2 measurements throughout the day.

An outcome regression model was built with data from the observational cohort using a standard statistical variable selection process. For the overall analysis, the variables selected to be adjusted in the prediction model were age (P = .0004) baseline P/F ratio (P = .078) and the presence of malignancies (P = .079), and respiratory disease (P= .074) (Supplementary Table 1A). The C-index for this fitted model was C=0.67 (95%CI: 0.59 to 0.75). Adjusted HR for RFFS between the DEFI-VID19 population and “standard of care” (SOC) cohorts was HR=0.71 [0.95CI: 0.34 to 1.29] (P=.138). Therefore, after adjusting for potentially confounding baseline covariates, we observed a tendency towards a higher RFFS in the DEFI-VID19 group (Figure 2).

In the DEFI-VID 19 cohort the median Day1 D-Dimer value was of 450 ng/mL(Q1-Q3 285.5-1337.7), with no significant difference between patients who developed Respiratory Failure and those who did not (median 515 vs 441 ng/mL; p value 0.26). However, at the end of treatment, patients in respiratory failure showed significantly higher values of D-Dimer compared to the responding patients (median 2632,5 vs 380 ng/mL, p value=0.0005).
Overall Survival
The overall survival (OS) of patients enrolled in the DEFI-VID19 trial was 0.90 (0.95CI:0.81 to 0.98) at day 28 and 0.83 (0.95CI:0.73 to 0.95) at day 60. OS of patients receiving only SOC was 0.60 (0.95CI:0.53 to 0.68) at day 28 and 0.59 (0.95CI:0.52 to 0.68) at day 60. In unadjusted analysis, the Overall Survival was significantly higher in patients treated with Defibrotide (HR 0.33, 95%CI:0.16 to 0.69, P= .003) (Figure 1). The variables selected to be adjusted in the survival prediction model were age (P < .0001), baseline P/F ratio (P = .034), the presence of malignancies (P = .009), and respiratory diseases (P=. .0007) (Supplementary Figure 1B). The C-index for this fitted model was 0.81 (95%CI: 0.76 to 0.87). Adjusted HR for OS between the DEFI-VID19 population and SOC cohorts was HR=0.78 (0.95CI: 0.33 to 1.53) P= .248. Therefore, in the adjusted analysis we noted a trend towards an increased OS in the DEFI-VID19 cohort (Figure 2).

Post-Recovery Days
DEFI-VID19 patients showed a mean number of post-recovery days of 11.60 [95%CI: 9.25 to 13.96] while patients from the observational cohort had a mean of 5.29 post-recovery days [95%CI:4.19 to 6.39]. In unadjusted analysis, the difference in means was statistically significant [6.32, 0.95CI 3.94 to 8.69, P< .0001] (Figure 3). A linear prediction model was built with data from the Observational cohort and the variables selected to be adjusted in the prediction model were age (P < .0001), the presence of malignancies (P = .026), and respiratory diseases (P=. .002) (Supplementary Figure 1C). The Mean Absolute Error (MAE) for this fitted model was 4.32. By entering these covariates values for each DEFI-VID19 patient we obtained individual predicted values of post-recovery days. The mean value of predicted post-recovery days was 7.99 (0.95 CI: 6.94 to 9.05). The adjusted analysis confirmed that DEFI-VID19 patients had a significantly higher number of post-recovery days compared to patients receiving SOC, with a difference in means of 3.61 [0.95CI: 0.97 to 6.26, P= .0037] (Figure 3). Of note, at the time of discharge, patients treated with Defibrotide showed no limitation in activities and did not require home oxygen therapy (WHO score of 1).

DISCUSSION
We report the first study on the safety and efficacy of Defibrotide therapy in patients with severe COVID-19 pneumonia who were receiving non-invasive ventilation. The results from the phase-II DEFI-VID19 trial were promising, demonstrating a favorable safety profile, a day-14 RFFS of 75%, a 60-day OS of 83%, and a mean number of post-recovery days of 12. Acknowledging that comparison between studies is challenging because of potential differences in patient characteristics and across different hospital systems, the comparison of DEFI-VID19 results with a carefully selected observational cohort of patients who received standard of care proved informative. Specifically, using data from patients who were treated for COVID-19 pneumonia during the same period as those enrolled in the DEFI-VID19 study allowed us to minimize various confounders and biases that could have been introduced because of changes in patient management over time. Additionally, we screened healthcare records excluding patients who did not receive CPAP and/or HFNO and then selected potential control patients based on the DEFI-VID19 eligibility criteria. Nevertheless, despite these measures, the 153 control patients were older, had more cardiovascular comorbidities and a lower PaO2:FiO2 ratio at baseline. One may argue that the controls were selected from one center, however more than 95% of patients enrolled into the study came from 2 centers in the same city, with common strategies in patients management and care. To correct for additional confounding factors, a tailored, model-based approach was used, as previously described by Torbicki et al. Other novel statistical methods employed for making adjustments of baseline covariates, such as propensity score matching, inverse probability of treatment weighting (IPTW), and the use of double robustness were considered. However, the outcome regression employed in our study appeared to be the most intuitive procedure based on the fundamental regression analysis prediction principle.

We are aware of some limitation of our study namely that the ICU transfer could be considered as a subjective parameter, however the unprecedent situation during the COVID pandemic the ICU admission was considered as clinically relevant according to the Italian policy.

Moreover, the propensity score and IPTW methods proved ineffective, as the score sets from the two groups of patients varied significantly. In aggregate, after adjusting for potential confounding baseline variables, we found that the DEFI-VID19 cohort exhibited a trend towards increased RFFS (HR=0.71, P= .138) and OS (HR=0.78, P=.248) when compared to patients receiving SOC only. Furthermore, there was a clear advantage in terms of time to
recovery, as indicated by a difference in means of post-recovery days of 3.61 [0.95CI: 0.97 to 6.26, P= .0037]. Notably, at the time of discharge, patients treated with Defibrotide showed no limitation in activities and did not require home oxygen therapy (i.e., a WHO score of 1). In another study involving 13 critically ill patients with COVID-19-associated ARDS who were receiving invasive ventilation, the administration of Defibrotide was found to be safe, and no instances of hemorrhagic events were recorded \(^{27}\), with improved survival over that expected in such a sick population. It is worth noting that only three patients in this US study received prophylactic low molecular weight heparin (LMWH), whereas all patients in the DEFI-VID19 trial received prophylaxis with LMWH. Despite this difference in LMWH administration, no bleeding was observed in our DEFI-VID19 patient group, supporting the observed safety of Defibrotide in this setting. In addition, due to its favorable pharmacokinetic profile, Defibrotide is not contraindicated in patients with end-stage renal disease\(^{28}\) or liver dysfunction\(^{29}\), in contrast to other approved drugs for severe COVID-19 pneumonia, such as Baricitinib, Remdesivir, and Tocilizumab.

Accumulating evidence supports the central role of lung microvascular endothelial damage in the pathophysiology of COVID-19 related ARDS \(^{7,8,10}\). As a result of its angio-protective properties\(^{16,17–19}\), the early administration of Defibrotide has the potential to mitigate Sars-Cov-2 induced endothelial injury and improve clinical outcomes, as illustrated in this study. Specifically, Defibrotide may represent a valuable addition to the COVID-19 therapeutic options based upon our results and others.\(^{27}\) Clinical benefit was reflected in our trial by its positive effect on reducing the time to discharge in patients with COVID-19 pneumonia receiving non-invasive ventilation, an improved OS using a novel case-control methodology, and a manageable safety profile. Further studies are therefore warranted to confirm these findings, both in the setting of COVID-19 and potentially in other related viral and post infectious syndromes characterized by endothelial injury\(^{18,19}\).
REFERENCES


<table>
<thead>
<tr>
<th>Clinical Status</th>
<th>Activity and Respiratory Support</th>
<th>Ordinal score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambulatory</strong></td>
<td>No limitation of activity</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Activities limited</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hospitalized</strong></td>
<td>No oxygen therapy</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Oxygen by mask or nasal cannula</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Non-invasive ventilation or high-flow oxygen</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Intubation or mechanical ventilation</td>
<td>6</td>
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<tr>
<td><strong>Deceased</strong></td>
<td>Mechanical ventilation plus one of the following: pressors, ECMO, or dialysis</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>8</td>
</tr>
</tbody>
</table>

ECMO=extracorporeal membrane oxygenation.
<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>DEFI-VID 19 cohort</th>
<th>Observational cohort</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>48</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>Age (median [Q1-Q3])</td>
<td>60.5 [53.75, 70.25]</td>
<td>72.7 [62.6, 79.7]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex Male, n(%)</td>
<td>35 (72.9)</td>
<td>109 (71.2)</td>
<td>0.97</td>
</tr>
<tr>
<td>BMI (median [Q1-Q3])</td>
<td>26.9 [25.00, 29.06]</td>
<td>27 [24.6, 29.7]</td>
<td>0.79</td>
</tr>
<tr>
<td>Comorbidities (median [Q1-Q3])</td>
<td>0.5 [0.0, 1.0]</td>
<td>1.0 [0.0, 2.0]</td>
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<td>Cardiovascular diseases (n%)</td>
<td>3 (6.2)</td>
<td>33 (21.6)</td>
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<tr>
<td>Hypertension (n%)</td>
<td>16 (33.3)</td>
<td>84 (54.9)</td>
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</tr>
<tr>
<td>Diabetes (n%)</td>
<td>7 (14.6)</td>
<td>32 (20.9)</td>
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<tr>
<td>Malignancies (n%)</td>
<td>2 (4.2)</td>
<td>14 (9.2)</td>
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<tr>
<td>Respiratory diseases (n%)</td>
<td>5 (10.4)</td>
<td>18 (11.8)</td>
<td>1</td>
</tr>
<tr>
<td>WHO score day 1 (Score 5, n%)</td>
<td>48 (100)</td>
<td>153 (100)</td>
<td>1</td>
</tr>
<tr>
<td>P/F ratio day 1 (median [Q1-Q3])</td>
<td>210.5 [135.2, 272.5]</td>
<td>164.0 [125.0, 231.0]</td>
<td>0.08</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/dL) (median [Q1-Q3])</td>
<td>4.9 [2.5, 11.0]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D-Dimer (ng/mL) (median [Q1-Q3])</td>
<td>450.0 [285.5, 1337.7]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/mL) (median [Q1-Q3])</td>
<td>854.5 [496.7, 1173.5]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml) (median [Q1-Q3])</td>
<td>21.3 [11.0, 33.2]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Platelets count (10⁹/L) (median [Q1-Q3])</td>
<td>231 [191, 336]</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase (IU/L) (median [Q1-Q3])</td>
<td>415.0 [294.0, 509.0]</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

BMI= Body Mass Index, IL-6=interleukin-6, IQR=interquartile range, n=number, P/F ratio= ratio of the partial pressure of oxygen to the fraction of inspired oxygen.
Figure Legends

Figure 1. Probability of Overall Survival (OS) (Fig 1A) Unadjusted Respiratory Failure Free Survival (RFFS) (Fig 1B) for patients receiving defibrotide and control group. Unadjusted Kaplan-Meier curves for RFFS and OS show significantly better outcomes for patients receiving defibrotide in addition to standard of care.

Figure 2. Probability of Overall Survival (OS) (Fig 2A) Unadjusted Respiratory Failure Free Survival (RFFS) (Fig 2B) for patients receiving defibrotide and control group. After adjusting for potentially confounding baseline covariates, DEFI-VID19 patients exhibited a trend towards an increased RFFS (HR: 0.71, 0.95CI: 0.34 to 1.29, P=.138) and OS (HR: 0.78, 0.95CI: 0.33 to 1.53, P=.248).

Figure 3. Observed and predicted numbers of post-recovery days. Mean values and 95% Confidence Intervals are shown in the plot. DEFI-VID19 patients (red dots) showed a significantly higher number of post-recovery days after adjusting for potentially confounding baseline covariates (green dots; difference in means: 3.61, 0.95CI: 0.97 to 6.26, P=.0037).
Supplementary Methods

DEFI-VID19 study design

Defibrotide was administered at a dose of 25 mg/kg/d intravenously (IV) fractionated in four doses daily for a planned treatment period of 14 days. Each dose was diluted prior to use with a 5% dextrose or a sodium chloride 9 mg/mL (0.9%) solution to reach a concentration in the range of 4 to 20 mg/mL, and infused over a 2-hours period. WHO ordinal scores for clinical activity were assigned to patients on the first day of treatment, then daily throughout the study period (Table 1)\(^{20}\). Patients who reached independence from oxygen therapy for 48 hours consecutively, or were discharged, before day 14 were allowed to discontinue defibrotide at that time, without completing the 14-day course. Defibrotide therapy was withheld in case patients displayed signs of bleeding and discontinued if extracorporeal membrane oxygenation therapy (ECMO), mechanical ventilation, or full anticoagulant therapy were required. Complete blood counts, serum chemistry findings, D-dimer levels, prothrombin time (PT), partial thromboplastin time (PTT), and serum fibrinogen levels were required daily while receiving Defibrotide therapy. The use of fresh frozen plasma-cryoprecipitate, platelet transfusions, or both was allowed if clinically indicated during therapy. The level and type of supplemental oxygen support and arterial blood gas (ABG) analysis were recorded daily until the end of treatment and again on day 14 from study entry.

Observational data selection criteria

A contemporary cohort of patients admitted to the IRCCS – Humanitas Research Hospital with Covid-19 pneumonia was retrospectively screened for meeting the eligibility criteria of the DEFI-VID19 trial. The IRCCS – Humanitas Research Hospital clinical data warehouse was employed to collect all patients admitted with COVID-19 pneumonia in the period from November 1, 2020 to March 31, 2021. In accordance with DEFI-VID19 exclusion criteria patients directly admitted to the ICU or who died at admission, or the next day were automatically excluded from the list of potentially eligible controls. Patients enrolled in other experimental studies were excluded as well. Most importantly, all DEFI-VID19 patients required Continuous Positive Airways Pressure (CPAP) or high-flow oxygen therapy (HFNO) at the study entry and were assigned a WHO score of 5 according to the WHO ordinal scale for clinical activity\(^{20}\). Therefore, patients who had never received CPAP or high-flow oxygen therapy were excluded from the list of potential control patients. The list of 980 remaining patients was systematically manually abstracted from the electronic medical charts by a trained physician (FC) who applied the inclusion and exclusion criteria of the DEFI-VID19 trial. This was deemed necessary to avoid selection biases and immortal-time bias that often arises from mishandling the start of follow-up
in such analyses. The earliest time each potential control patient met the DEFI-VID19 eligibility criteria was considered as day 0 (i.e., the date the patient would have given the informed consent) and the following day was considered as the start of the follow-up (day 1). Since all DEFI-VID19 patients met the eligibility criteria within seven days from the hospital admission, electronic health records were screened from the admission day up to the seventh day. After applying the eligibility criteria, 153 patients qualified as controls.

The following variables were collected for patients in the DEFI-VID19 trial and in the control group: age; sex; the first recorded body-mass index (BMI); oxygen saturation in room air at hospital admission; the partial pressure of arterial oxygen (PaO₂) at day 1; the fraction of inspired oxygen (FiO₂) at day 1; PaO₂:FiO₂ at day 1 (P/F ratio); the presence of hypertension, cardiovascular diseases, diabetes, respiratory diseases, malignancies, neurological diseases, and chronic kidney diseases. For DEFI-VID19 patients, the complete blood count and the coagulation profile (PT, PTT, Fibrinogen) were assessed daily during treatment, as part of the safety evaluation. Among biomarkers of endotheliitis, D-dimer was assessed daily, whereas serum levels of C-Reactive Protein (CRP) and Interleukin-6 (IL-6) were measured weekly.

**Endpoints**

Secondary endpoints included overall survival at 60 days, the number of post-recovery days, and the rate of adverse events. Overall survival (OS) was defined as the time from day one until death from any cause, with data on OS censored on day 60 for patients who were still alive.

An exploratory analysis of the longitudinal change of coagulation indexes (PT, PTT, Fibrinogen) and endotheliitis biomarkers (D-Dimer, CRP, IL-6) in patients receiving Defibrotide is currently ongoing. To comprehensively describe the trends of these biomarkers serum levels and unveil their potential association with clinical outcomes we plan to integrate these data into larger datasets under the DEFACOVID study group, thereby generating more comprehensive and meaningful insights.

**Statistical methods**

Statistical analyses were conducted using GraphPad Prism (version 7.5) and R version 4.2.2. Unadjusted comparison of RFFS, OS and post-recovery days between the two cohorts was first performed. RFFS and 60 days OS for the DEFI-VID19 and Observation cohorts were estimated based on the Kaplan-Meier method. The mean number of post-recovery days was estimated, and 95% Confidence Intervals were assessed for both groups and also the group difference. A Cox proportional hazards regression model with RFFS as the outcome and study population as the
only independent variable was conducted to compare the RFFS between the two cohorts. To account for the potential imbalance between the two cohorts in terms of baseline variable distributions, an outcome regression analysis was conducted\(^{23}\). Specifically, as previously described by Richardson et al\(^{24}\), a survival Cox prediction model was developed using the data from the observational cohort. A set of clinically relevant baseline covariates was selected\(^{25}\). In particular, we focused on: day 1 P/F ratio, age, body mass index, and the presence of cardiovascular comorbidities (i.e., congestive heart failure), respiratory diseases (asthma and COPD), hypertension, diabetes mellitus or malignancies. The standard stepwise regression procedure was employed to derive a parsimonious, final model\(^{23}\). The C-index for this fitted model was examined to assess the model's fit. Assuming this model was transportable to the DEFI-VID19 population, a predicted survival curve for each patient in DEFI-VID19 was obtained by plugging in its corresponding baseline covariates. The predicted survival curves were averaged over the DEFI-VID19 patients to obtain the RFFS profile from “standard of care”. The estimated hazard ratio (HR) between this average curve and the Kaplan-Meier curve from DEFI-VID19 was obtained by fitting a Cox proportional hazards model. The CI for HR and its nominal P value were obtained via the bootstrapping method applied to the DEFI-VID19 and Observational populations. The same process was performed with OS as the outcome variable. Similarly, a linear regression model was conducted with post-recovery days as the outcome and the study population as the only independent variable. The same set of baseline covariates described above was used and a standard stepwise regression was performed. The Mean Absolute Error (MAE) was examined to assess model fit. The number of post-recovery days for each patient in DEFI-VID19 was predicted by plugging in its corresponding covariates. The difference between the predicted mean value and the actual mean value from the DEFI-VID19 study was estimated. The CI for the difference in means and its nominal p value were obtained via the bootstrapping method applied to the DEFI-VID19 and Observational populations.
### A  
**Stepwise Regression Analysis - Respiratory Free Survival**

<table>
<thead>
<tr>
<th>Selected Variables</th>
<th>Hazard Ratio</th>
<th>SE</th>
<th>z</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(n)</td>
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<td>0.011</td>
<td>3.53</td>
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<td>P/F ratio Day1(n)</td>
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<td>0.0739</td>
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<td>Cardiovascular diseases(Yes/No)</td>
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<td>1.35</td>
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### B  
**Stepwise Regression Analysis - Overall Survival**

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<td>BMI(n)</td>
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<td>0.0315</td>
<td>0.61</td>
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### C  
**Stepwise Regression Analysis - Number of Post-Recovery Days**

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<thead>
<tr>
<th>Selected Variables</th>
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<th>SE</th>
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<td>(Intercept)</td>
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<td>2.58</td>
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<td>Age(n)</td>
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<td>&lt;0.0001</td>
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**Legend of Supplementary Table 1 Stepwise Regression Analysis**

n=Number; SE= Standard Error; z= Z-scores, t= T-scores, BMI body mass index