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

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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[21]. 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(day1). 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 (PaO_2) at day 1; the fraction of inspired oxygen (FiO_2) at day 1; PaO_2:FiO_2 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.

Supplementary Table 1.Stepwise Regression Analysis

A				
	Stepwise Regression Analysis - Respiratory Free Survival			
Selected Variables	**Hazard Ratio**	**SE**	**z**	**P-value**
Age(n)	1.04	0.011	3.53	0.0004
P/F ratio Day1(n)	0.99	0.001	-1,76	0.0778
Malignancies(Yes/No)	1.79	0.33	1.75	0.0787
Respiratory Diseases(Yes/No)	1.77	0.32	1.78	0.0739
Cardiovascular diseases(Yes/No)	1.41	0.26	1.35	0.1756
Diabetes(Yes/No)	1.38	0.26	1.25	0.2122

B				
	Stepwise Regression Analysis - Overall Survival			
Selected Variables	**Hazard Ratio**	**SE**	**z**	**P-value**
Age(n)	1.1	0.016	5.83	<0.00001
P/F ratio Day1(n)	1	0.002	-2,11	0.034
Malignancies(Yes/No)	2.57	0.359	2.62	0.008
Respiratory Diseases(Yes/No)	3.1	0.336	3.36	0.0007
Cardiovascular diseases(Yes/No)	1.6	0.274	1.70	0.08
Diabetes(Yes/No)	1.4	0.289	1.20	0.22
BMI(n)	1.02	0.0315	0.61	0.54

C				
	Stepwise Regression Analysis - Number of Post-Recovery Days			
Selected Variables	**Coefficient**	**SE**	**t**	**P-value**
(Intercept)	2.5	2.58	9.64	<0.00001
Age(n)	-0,26	0.03	-7,1	<0.00001
Diabetes(Yes/No)	-1,72	1.14	-1,5	0.13
Malignancies(Yes/No)	-3,60	1.60	-2,24	0.02
Respiratory Diseases(Yes/No)	-4,70	1.44	-3,26	0.001

Legend of Supplementary Table 1 Stepwise Regression Analysis

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