Clonal hematopoiesis of indeterminate potential associates with higher risk of thromboembolism in severe COVID-19

Clonal hematopoiesis of indeterminate potential (CHIP) is an age-related somatic mosaicism associated with a pro-inflammatory state and increased cardiovascular morbidity. The aim of the present study was to evaluate the potential association of CHIP with thromboembolic events occurring in severe coronavirus disease 2019 (COVID-19). To this end, peripheral blood samples from 158 consecutive patients positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were collected for next-generation sequencing (NGS) analysis. There were 151 patients eligible for NGS analysis. Mutations were found in 28.5% of patients (N=43/151). A significant association between the presence of clonal hematopoiesis and the occurrence of acute thromboembolic events during COVID-19 infection was observed (odds ratio [OR=2.41], 95% confidence interval [95% CI]: 1.15-5.21; *P*=0.02). However, the increased incidence of thromboembolic events was seen in relation to mutations at high risk of clonal myeloid evolution: low risk: 6/13 (46%), intermediate risk: 15/24 (63%) and high risk: 4/6 (67%). These findings open new perspectives on the management of acute events caused by SARS-CoV-2 and on the understanding of clonal hematopoiesis.

CHIP is an age-related somatic mosaicism characterized by the presence of acquired mutations in myeloid malignancy-associated genes without an underlying diagnosis of hematologic disorder.[1,2] CHIP has recently been linked to a higher risk of cardiovascular diseases,[3] probably because mutations involved in clonal hematopoiesis contribute to systemic inflammation. There is increasing interest in understanding how CHIP may affect patients' outcome in the context of infectious disease. Among other diseases, COVID-19[4] has been associated with a dysregulated immune response characterized by a hyperinflammatory state termed "cytokine storm," leading to severe complications such as acute respiratory distress syndrome, multiorgan failure, and thromboembolic events.[5-7] Indeed, COVID-19 has been characterized by a high incidence of thrombotic complications, including venous thromboembolism, arterial thrombosis, and microvascular thrombosis.[8] Multiple factors contribute to this prothrombotic condition, including endothelial dysfunction, platelet activation, hypercoagulability, and the uncontrolled release of pro-inflammatory cytokines. Notably, clonal hematopoiesis has not been unequivocally associated with venous thromboembolism such as deep vein thrombosis or pulmonary embolism, although some preliminary results support this hypothesis.[9] However, it is plausible that the CHIP-associated pro-inflammatory milieu could serve as an additive or synergistic factor in the development of COVID-19-associated coagulopathy, potentially explaining the increased rates of thrombosis observed in certain high-risk populations.[8]

To further explore the association between CHIP and thrombotic or thromboembolic events during the COVID-19 pandemic, we designed a study to assess the prevalence of CHIP in a population of patients with a diagnosis of severe COVID-19 admitted to the San Raffaele Hospital (Milan, Italy). The study

Table 1. Demographic, clinical and genetic characteristics of the 158 patients studied.

Characteristics	Values
Demographic characteristics	
Male sex, N (%)	118 (74.7)
Caucasian ethnicity, N (%)	145 (91.8)
Age in years, median (IQR)	66 (58-75)
Weight, kg, median (IQR)	78 (70-87.25)
Height, cm, median (IQR)	170 (163-176)
Clinical and laboratory features, median (IQR)	
Hemoglobin value, g/dL	13.7 (12.45-15)
Lymphocyte count, per μL	900 (700-1,200)
LDH, IU/mL	407 (310-580)
C-reactive protein, mg/L	80.3 (35.1-146.5)
NGS results, N of patients (%)	
Presence of mutations detected	43 (28.5)
Oncogenic mutations	8 (18.6)
Possibly oncogenic mutations	35 (81.4)
Type of mutation, N (%)	
Frameshift deletion	6 (14)
Frameshift insertion	2 (4.7)
Non-synonymous single-nucleotide variants	22 (51.2)
Stop-gain mutations	9 (20.9)
Splicing mutations	4 (9.3)
Mutations detected by single gene, N (%)	
ASXL1	5 (11.6)
DDX41	2 (4.7)
DNMT3A	13 (30.2)
IDH1	2 (4.7)
JAK2	1 (2.3)
NF1	1 (2.3)
NRAS	1 (2.3)
PPM1D	2 (4.7)
PTPN11	1 (2.3)
TET2	5 (11.6)
TP53	6 (14)
U2AF1	1 (2.3)
WT1	2 (4.7)
ZRSR2	1 (2.3)

IQR: Interquartile range; LDH: lactate dehydrogenase; NGS: next-generation sequencing.

was conducted according to the Declaration of Helsinki and was approved by the San Raffaele Ethic Committee on April 21, 2021. Subsequently, we analyzed the relative association of single gene abnormalities with the incidence of thrombo-embolic complications and the resulting clinical outcomes.

Peripheral blood samples from 158 consecutive patients positive for SARS-CoV-2 at nasal swab and enrolled to the COVID-BioB Study (ClinicalTrials.gov identifier: NCT04318366) - approved by the San Raffaele Ethic Committee on March 19, 2020 - were tested for molecular abnormalities by NGS analysis using a TruSeq Custom Amplicon Low Input Kit (Illumina)[10] including 72 genes commonly mutated in CHIP and myeloid malignancies. Agilent's SureSelectXT Low Input Target Enrichment capture protocol was followed, and presence in the Catalogue of Somatic Mutations in Cancer (COSMIC) v91 in at least ten myeloid neoplasms (*https://cancer.sanger.ac.uk/cosmic*) was used to identify the oncogenic potential of the variants.

Patients with presence of mutations detected at NGS were grouped according to the type of mutations (high risk: *TP53*; intermediate risk: *DNMT3A, JAK2, TET2, ASXL1*; low risk: other mutations).[11]

The primary objective was to evaluate the prevalence of the different genetic abnormalities that identify CHIP in patients diagnosed with severe COVID-19 and the relative association with clinical outcomes.

Quantitative variables were described as median and interquartile range (IQR) and compared between groups using a Wilcoxon test. Qualitative variables were described as number and percentage and compared between groups using the χ^2 test. In the absence of χ^2 validity, an exact Fisher test was used. To evaluate the impact of the presence of mutations on the incidence of thrombotic events, a logistic model adjusted on age and gender was used. Results are presented as OR and their 95% CI. All tests were two sided with a type I error rate fixed at 5%. The analysis was performed using R statistical software v4.4.0.[12]

All patients were admitted to our Institution due to severe SARS-CoV-2 infection: 118 were male and 40 female and their median age was 66 years (IQR, *TP53* 58-75). Clinical features of the patients are provided in Table 1. Most of the patients (143, 90%) required hospitalization; the remaining were followed as outpatients. All patients were treated with prophylactic doses of low molecular weight heparin, antiviral therapy, immunomodulating agents or convalescent plasma according to local guidelines.

On admission 72.8% had fever (defined as body temperature >37.7°C), with a median body temperature of 37.7°C (IQR 36.32-38.2); the median oxygen saturation was 92% (IQR, 86-96), the median PaO2 was 60 mmHg (IQR, 51-73), the median hemoglobin concentration was 13.7 g/dL (IQR, 12.4-15), the median lymphocyte count was 900/μL (IQR, 700-1,200), the median lactate dehydrogenase concentration was 407 IU/mL (IQR, 310-580), and the median C-reactive protein level was 80.3 mg/L (IQR, 35-146).

Overall, 68 patients (43.6%) developed one or more thrombo-embolic events in concomitance with COVID-19 pneumonia: namely, 60 patients experienced pulmonary embolism, six myocardial infarction, five deep venous thrombosis, three cerebral ischemia and two severe arterial ischemia requiring

Table 2. Univariate and multivariate analyses.

Variables	No mutations N=108	Mutations N=43	OR (95% CI)	*P*
Age in years, median [IQR] (range)	63.5 [57.8-71.2] (37-93)	71 [60.5-77] (43-85)	-	0.054
Gender, N (%) Male Female	 87 (80.6) 21 (19.4)	 26 (60.5) 17 (39.5)	 - -	0.01
Thrombotic events, N (%) No Yes	 65 (60.2) 43 (39.8)	 18 (41.9) 25 (58.1)	 - -	0.04
Death, N (%) No Yes	 85 (78.7) 23 (21.3)	 37 (86) 6 (14)	 - -	0.3
Presence of NGS-detected mutations No Yes	 - -	 - -	 1 2.41 (1.15-5.21)	 0.02
Gender Male Female	 - -	 - -	 1 0.63 (0.28-1.38)	 0.25
Age (continuous by increase of 10 years)	-	-	0.91 (0.67-1.22)	0.52

Statistical analyses: Wilcoxon test for quantitative variables; χ^2 test and exact Fisher test for qualitative variables; logistic model adjusted on age and gender to evaluate the statistical impact of the presence of mutations on the incidence of thrombotic events. OR: odds ratio; 95% CI: 95% confidence interval; IQR: interquartile range; NGS: next-generation sequencing.

limb amputation. 30 patients (21 male and 9 female [19%]) died during hospitalization, 14 of whom due to thrombosis-related complications.

Of the 158 tested patients, seven were excluded from the final analysis: two patients because they were under anticoagulant therapy due to a recent thromboembolic event not related to COVID-19; two patients because of failure of the NGS assay, and three patients because they had been lost from follow up. Variants of uncertain significance or mutations presenting with a variant allele frequency under 2% were not considered significant. Of the 151 evaluable patients, 43 (28.5%) had at least one oncogenic or possibly oncogenic mutated gene: 27, nine, four and three patients had, respectively, one, two, three and four such mutations; in this cohort, 24 (55.7%) patients showed at least one CHIP-related mutation (namely *DNMT3A, ASXL1, TET2*) or *JAK2* mutation, six patients (14%) had a *TP53* gene mutation and the remaining 13 patients carried a mutation on *DDX41, IDH1, PPM1D, WT1, GATA2, NF1, NRAS, PTPN11, U2AF1* or *ZRSR2*. Based on the impact of mutations, 39 (90.7%) of them were exonic: six frameshift deletions (14%), two frameshift insertions (4.7%), 22 non-synonymous single-nucleotide variants (51.2%), and nine stop-gain mutations (20.9%). Only four (9.3%) resulted to be splicing mutations. Further details about the CHIP mutations can be found in *Online Supplementary Table S1*.

Table 2 reports the univariate and multivariate analyses for the main clinical outcomes. The incidence of thromboembolic events was significantly higher in patients harboring NGS-detected mutations (58.1% *vs*. 39.8%, OR=2.41 [95% CI: 1.15-5.21]; *P*=0.02). Importantly, the incidence of thrombosis was higher in those patients carrying mutations, including *DNMT3A, ASXL1, TET2* and in the JAK/STAT pathway genes (15 cases, 60%). Among the nine females with at least one mutation and a concurrent thromboembolic event, *DNMT3A* was the most prevalent mutation (4 patients), followed by *TET2* and *TP53* (2 patients) and *NF1* (1 patient). The presence of at least one mutation

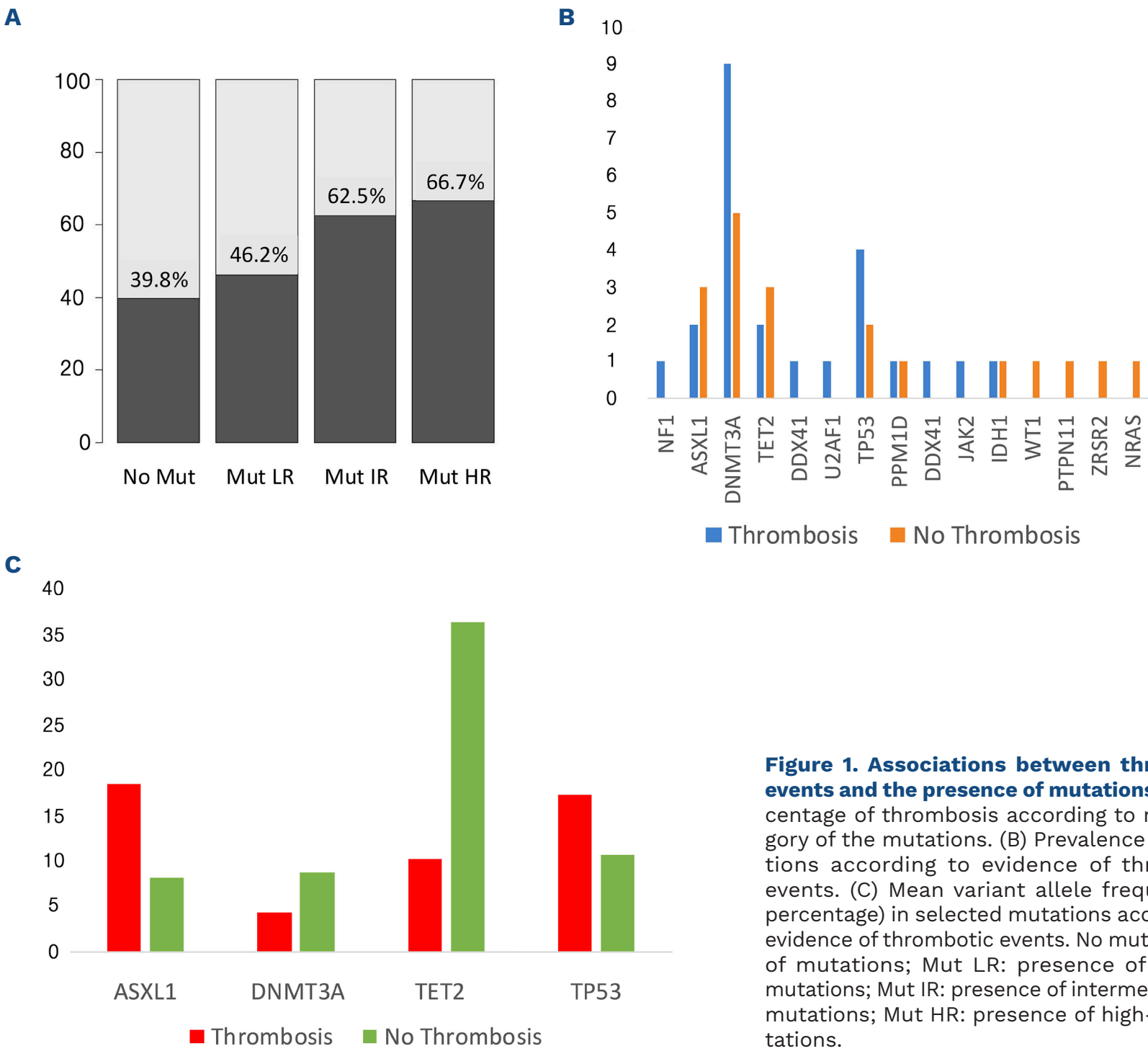

Figure 1. Associations between thrombotic events and the presence of mutations. (A) Percentage of thrombosis according to risk category of the mutations. (B) Prevalence of mutations according to evidence of thrombotic events. (C) Mean variant allele frequency (in percentage) in selected mutations according to evidence of thrombotic events. No mut: absence of mutations; Mut LR: presence of low-risk mutations; Mut IR: presence of intermediate-risk mutations; Mut HR: presence of high-risk mutations.

in the NGS panel was significantly higher in female patients in univariate analysis (39.5% *vs*. 19.4%; *P*=0.01) and non-significant in multivariate analysis (OR=0.63 [95% CI: 0.28-1.38]; *P*=0.25).

The type of mutations based on the hematologic risk of progression (low risk: 6/7 [46%]; intermediate risk: 15/24 [63%], high risk: 4/6 [67%]) was associated with an increasing prevalence of thromboembolic events (Figure 1A). Notably, the same trend was observed when assessing COVID-19 event mortality compared with mutational risk individually and cumulatively with thrombotic events.

Thus, the reported data unveil new aspects of the interaction between CHIP, COVID-19, and thromboembolic complications. Some preliminary studies failed to prove that CHIP carriers experience a higher morbidity and mortality of severe COVID-19,[13,14] while other research suggested that patients with CHIP, especially those harboring mutations in genes involved in epigenetic regulation, may experience more severe COVID-19 outcomes.[15,16] One possible reason for the divergence between these two findings may lie in the different clinical characteristics of the patients in these studies. Despite the relatively low number of patients, our results suggest that CHIP-related mutations may represent an important contributor of disease severity in inflammatory and hypercoagulable conditions, such as severe COVID-19. The mutations driving clonal hematopoiesis are known to promote a pro-inflammatory environment, potentially intensifying the immune dysregulation triggered by SARS-CoV-2 infection and, in turn, more empowered endothelial damage, increased thrombin generation and heightened platelet activation.

Some important questions remain unanswered. It is not yet clear whether CHIP-associated mutations cause thromboembolic complications directly or merely act as a risk modifier in the presence of a subsequent hit of hyperinflammation, such as COVID-19. Furthermore, the relative contribution of different myeloid mutations to thrombosis risk remains debated[3,9] and requires further exploration, as does the role that the use of alternative antithrombotic therapies, such as defibrotide, may play in these cases.[17] Another consideration could be the role of primary prevention in patients with CHIP who show signs of SARS-CoV-2 infection. In any case, our study highlights the need for prospective clinical trials for this subset of patients.

Future studies should focus on elucidating the specific pathways through how mutations, and which ones, influence thromboinflammation during severe infections. It would also be important to determine whether targeted interventions aimed at mitigating CHIP-related inflammation could reduce the incidence of thromboembolic events in this subset of patients. Understanding these mechanisms may open new perspectives for risk stratification and therapeutic interventions in the management of severe infection during inflammatory conditions associated with clonal hematopoiesis.

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Disclosures

No conflicts of interest to disclose.

Contributions

FC, AR and MB designed the study, GMB, AR and MB interpreted data and wrote the manuscript, J-EG performed the statistical analysis, FMA, PA, SM, FE, DP, GC, PRQ, GF, FDC and AAA took care of patients, CT and MGDP were responsible for the analysis of samples. All authors accepted the final version of the manuscript.

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Data-sharing statement

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

References

1. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9-16.
2. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36(7):1703-1719.
3. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med. 2017;377(2):111-121.
4. Zhu N, Zhang D, Wang W, et al. China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733.
5. Grasselli G, Tonetti T, Protti A, et al. Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. Lancet Respir Med. 2020;8(12):1201-1208.
6. Ciceri F, Castagna A, Rovere-Querini P, et al. Early predictors of clinical outcomes of COVID-19 outbreak in Milan, Italy. Clin Immunol. 2020;217:108509.
7. Ciceri F, Beretta L, Scandroglio AM, et al. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. Crit Care Resusc. 2020;22(2):95-97.
8. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol. 2020;7(6):e438-e440.
9. Zon RL, Sekar A, Clapham K, et al. JAK2-mutant clonal hematopoiesis is associated with venous thromboembolism. Blood. 2024;144(20):2149-2154.
10. Rossi M, Meggendorfer M, Zampini M, et al. Clinical relevance of clonal hematopoiesis in persons aged ≥80 years. Blood. 2021;138(21):2093-2105.
11. Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med. 2018;24(7):1015-1023.
12. R Core Team (2024). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
13. Petzer V, Schwendinger S, Haschka D, et al. Clonal hematopoiesis in patients with Covid-19 is stable and not linked to an aggravated clinical course. Am J Hematol. 2021;96(9):E331-E333.
14. Zhou Y, Shalhoub R, Rogers SN, et al. Clonal hematopoiesis is not significantly associated with COVID-19 disease severity. Blood. 2022;140(14):1650-1655.
15. Bolton KL, Koh Y, Foote MB, et al. Clonal hematopoiesis is associated with risk of severe Covid-19. Nat Commun. 2021;12(1):5975.
16. Kessler MD, Damask A, O'Keeffe S, et al. Common and rare variant associations with clonal haematopoiesis phenotypes. Nature. 2022;612(7939):301-309.
17. Ruggeri A, Corrado F, Voza A, et al. Use of defibrotide in COVID-19 pneumonia: comparison of a phase II study and a matched real-world cohort control. Haematologica. 2024;109(10):3261-3268.