Appendix: Thrombosis, major bleeding, and survival in COVID-19 supported by VV- ECMO in the first vs second wave- multicentre observational study in the UK.

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Appendix S1: Study participating centres.

Royal Brompton & Harefield NHS Foundation Trust, London, UK

Royal Papworth Hospital NHS Foundation Trust, Cambridge, UK

University Hospitals of Leicester NHS Trust, Leicester, UK

NHS Grampian, Aberdeen, UK

Table S1. Criteria for the referral of patients to ECMO centres NHS England[1]

Inclusion criteria for referral (in **bold**) and additional considerations for ECMO in all patients regardless of aetiology:
• Potentially reversible severe respiratory failure – for example, PaO2/FiO2 <6.7 kPa for ≥3 hours or PaO2/FiO2 <10 kPa for ≥6 hours[2]
• Lung injury score (Murray Score) ≥ 3 [3]
• Uncompensated hypercapnia with a pH ≤7.20 despite respiratory rate >35/min or due to life threatening airway disease – for example, asthma or airway trauma, air leak[2]
• Failed trial of ventilation in prone position ≥ 6 hours (unless contraindicated)
• Failed airway pressure release ventilation (APRV) or high PEEP ventilation strategy ≥ 6 hours (unless contraindicated)
• If RESP score ≤ 3 or receiving invasive mechanical ventilation >7 days ECMO should only be considered after agreement across two centrally commissioned ECMO centres[2]

Appendix S2. ECMO cannulation

Patients in this study were cannulated using either bifemoral venous access, femoral – jugular or bicaval access. The patients were retrieved to the ECMO centres using mobile ECMO in the majority of cases.

Appendix S3. Definition of Heparin induced thrombocytopenia (HIT) and diagnosis.

A screening test was performed for patients who developed thrombocytopenia with a clinical picture suggestive of HIT, regardless of thrombosis status. The screening test was positive when pre-test probability was ≥4 (calculated by two clinicians). All centres used the 4Ts score to calculate the pre-test probability score. This score was calculated by two different clinicians. The team proceeded to a formal laboratory test for HIT if the pre-test probability score was 4 or greater. We have previously shown that Low PTPS does not reliably exclude HIT/HITT in patients on ECMO and those with a decline in platelet count suggestive of HIT/HITT should be promptly investigated even if the PTPS is low. Therefore, a combination of 4T score and careful clinical assessment by a haematologist was performed if there is falling platelet count suggestive of HIT with laboratory assessment to confirm or exclude HIT. Samples from patients with a positive screen result were tested using an enzyme-linked immunoabsorbent assay (ELISA; HYPHEN BioMed, Neuville-sur-Oise, France) in all centres and with further confirmatory tests such Hemosil AcuStar HIT-IgG (PF4-H; Werfen, Warrington, Cheshire, UK) which is an automated chemiluminescent immunoassay or platelet aggregation assay. Although a functional assay, such as the serotonin release assay (SRA) is considered as the

gold standard test for confirming HIT, this was not easily available in the UK. Latex immunoturbidimetric assay (LIA) used for HIT screening in our study has shown a sensitivity of 97.4% and specificity of 94.0% in a prospective study using the SRA test [4] . In a systematic review and meta-analysis, it has been shown that Hemosil AcuStarHIT-IgG assay has sensitivity (> 95%) and specificity (> 90%) in the diagnosis HIT. Values ≥1.00 U/mL were considered as positive. Patients with HIT were anticoagulated with argatroban.

Appendix S4. Transfusion and haemostatic support

Transfusion thresholds in the context of VV-ECMO remain controversial. Centres used thresholds of haemoglobin 70–100 g/l, assessing for other factors such as underlying cardiac disease (12). Platelet transfusion was given targeting a platelet count of ≥50x109/l in the absence or ≥100x109/l in the presence of bleeding. Fresh frozen plasma (FFP) or cryoprecipitate was given in the presence of major bleeding or clinically relevant non-major bleeding (CRNMB) associated with prolonged prothrombin time (PT) or APTT after discontinuation of anticoagulation. Fibrinogen concentrate or cryoprecipitate was given to target fibrinogen level of 15-20 g/l in the presence of bleeding. In addition to blood product support, further haemostatic support with intravenous tranexamic acid 1g once only or three times daily was given in the presence of major bleeding or CRNMB (especially in the mucosal surfaces) and discontinued promptly when the bleeding stopped. Intravenous vitamin K (10 mg IV one to three days) was given in patients with PT prolongation with or without evidence of liver disease only if there was evidence of major bleeding or CRNMB.

Appendix S5. Management of pulmonary haemorrhage

Management of pulmonary haemorrhage depends on the severity of the bleeding and the potential contributory factors to the bleeding. Management comprised correction of any bleeding tendency and haemodynamic stabilisation, followed by localisation of the bleeding site through clinical history, imaging studies, and bronchoscopy. In bleeding that could not be managed by medical intervention and supportive haemostatic measures, bronchoscopic intervention and bronchial arterial embolization were used to control the bleeding. Where such interventions were not possible, blood was left in the airway (sometimes occluding the airway) as the patient was receiving VV-ECMO.

Supplementary tables

Table S2. Univariate and multivariate analysis of factors for survival of the overall cohort (P<0.05)

Factor	N	Probability of survival at day 180 % (95%CI)	P-value	Hazard Ratio (95%CI)	P-value
Age					
<42	83	72.3% (63.3-82.6)		1.00	
42-48	86	67.4% (58.2-78.1)	**0.014**	1.01 (0.57-1.78)	0.96
49-55	76	59.2% (49.1-71.4)		1.47 (0.85-2.55)	0.2
>55	64	46.9% (36.1-60.8)		2.29 (1.33-3.93)	**0.003**
Creatinine					
Normal	112	63.4% (55.1-73.0)		1.00	
Below normal	114	69.3% (61.3-78.3)	**.003**	1.13 (0.72-1.79)	0.6
Above normal	77	49.4% (39.4-61.9)		1.91 (1.19-3.08)	**0.008**
Platelets					
Normal	225	64.7% (58.8-71.3)			
Below normal	45	46.7% (34.1-63.8)	**0.038**		
Above normal	34	67.6% (53.6-85.3)			

Table S3. Immunomodulatory treatment given during study period.

Treatment	Cohort 1 (%) (N=159)	Cohort 2 (%) (N=150)	Total (%) (N=309)
Steroids	85 (53.5)	120 (80)	205 (66.3)
Tocilizumab	6 (3.4)	20 (13.3)	26 (8.4)
Intravenous immunoglobulin	4 (2.5)	1 (0.7)	5 (1.6)
Plasmapheresis *	12 (7.5)	7 (4.7)	19 (6.1)

***** *The plasma exchange was done with the intension to remove cytokines from plasma that may have contributed to uncontrolled inflammation in some of the patients. Generally, exchange plasma volumes of 1.5 times the estimated circulating plasma volume and replacement fluid consist of 3-5% albumin and transfusion of couple of units of fresh frozen plasma at the end of the plasma exchange. This has been shown to effective in patients with severe COVID-19[5] although this may not be case anymore.*

Table S4. Univariate and multivariate analysis of factors for arterial and/or venous thrombosis (P<0.05)

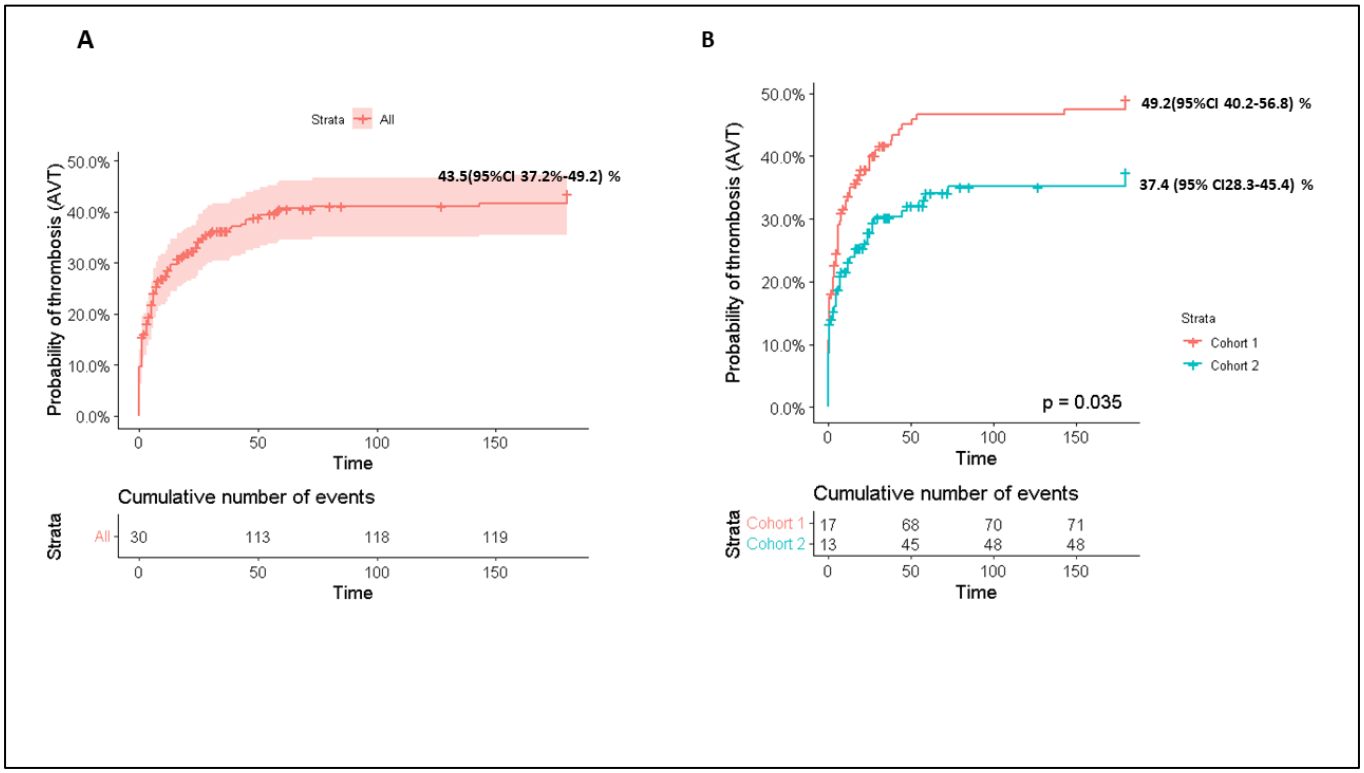
Factor	N	Probability of thrombosis at day 180 % (95%CI)	P-value	Hazard Ratio (95%CI)	P-value
Troponin			0.004		
Normal	63	25.7% (13.3%-36.3%)		1.00	
Above normal	148	48.6% (39.3%-56.5%		2.08 (1.17-3.70)	**0.013**
Haemoglobin			0.037		
Normal	77	41.4% (19.4-41.7%)		1.00	
Below normal	214	38.2% (41.5% -56.0%)		1.34 (0.71-2.50)	0.4
Above normal	12	48.2% (10.1%-62.4%)		6.77 (1.87-24.5)	**0.004**
D-Dimer			0.002		
Normal range	61	26.6% (13.9-37.5%)		1.00	**0.02**
Above normal	158	53.6 (44.6%-61.1%%)		2.04 (1.12-3.71)	

Table S5. Univariate and multivariate analysis of factors for major bleeding (P<0.05)

Factor	N	Probability of major bleeding at day 180 % (95%CI)	P-value	Hazard Ratio (95%CI)	P-value
Platelets					
Normal	224	28.2% (21.7%-34.1%)		1.00	
Below normal	45	56.9% (37.5%-70.3%)	0.001	3.5 (1.9-6.8.)	**<0.001**
Above normal	34	21.7% (18.0%-35.7%)		0.55 (0.27-1.11)	0.094

Normal platelet count is defined 150-400x109/L. All blood results referenced in this study were the baseline blood results taken on admission to the ECMO centre. In the absence of clinically relevant bleeding, generally platelet count of 50x109/L was maintained during VV-ECMO.

Figure S1. Probability of thrombosis (arterial or venous, AVT) in the overall cohort (A) and comparison between the two cohorts (B) supported by VV-ECMO



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