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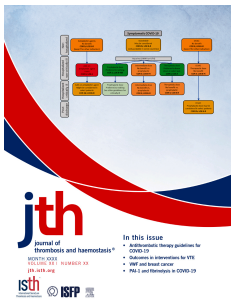

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PII: S1538-7836(23)00520-2

DOI: https://doi.org/10.1016/j.jtha.2023.06.034

Reference: JTHA 335

To appear in: *Journal of Thrombosis and Haemostasis*

Received Date: 18 March 2023

Revised Date: 20 June 2023

Accepted Date: 28 June 2023

Please cite this article as: Arachchillage DJ, Weatherill A, Rajakaruna I, Gaspar M, Odho Z, Isgro G, Cagova L, Fleming L, Ledot S, Laffan M, Szydlo R, Jooste R, Scott I, Vuylsteke A, Yusuff H, Thrombosis, major bleeding, and survival in COVID-19 supported by VV- ECMO in the first vs second wave- multicentre observational study in the UK., *Journal of Thrombosis and Haemostasis* (2023), doi: https://doi.org/10.1016/j.jtha.2023.06.034.

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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Highlights

- Veno-venous extracorporeal membrane (VV-ECMO) can be lifesaving in severe COVID-19 patients.
- Outcomes of VV-ECMO supported patients were compared in 1st vs 2nd waves of COVID-19 pandemic.
- 2nd wave had higher mortality, circuit thrombosis & major bleeding but lower venous thrombosis.
- Major bleeding, arterial and circuit thrombosis were associated with increased mortality.

Abstract

Background: Bleeding and thrombosis are major complications of veno-venous extracorporeal membrane (VV-ECMO).

Objectives: To assess thrombosis, major bleeding (MB) and 180-day in patients supported by VV-ECMO between first (1st March-31st May 2020) and second (1st June 2020-30th June 2021) waves of the COVID-19 pandemic.

Patients/Methods

Observational study of 309 consecutive patients (≥18years) with severe COVID-19 supported by VV-ECMO in four nationally commissioned ECMO centres, UK.

Results

Median age was 48 (19–75)years and 70.6% were male. Probabilities of survival, thrombosis, and MB at 180 days in the overall cohort were 62.5% (193/309), 39.8%(123/309) and 30%(93/309). In multivariate analysis, age >55 years (HR 2.29 [1.33-3.93],*p*=0.003) and elevated creatinine (HR 1.91 [1.19-3.08],*p*=0.008) were associated with increased mortality.

Corrected for duration of VV-ECMO support, arterial thrombosis alone (HR 3.0 [95% CI1.5-5.9], P= 0.002) or circuit thrombosis alone (HR 3.9 [95% 2.4-6.3], P<0.001), but not venous thrombosis, increased mortality. MB during ECMO had 3-fold risk (95% CI 2.6-5.8, P<0.001) of mortality.

The first wave cohort had more males (76.7% vs 64%, p=0.014), higher 180-day survival (71.1% vs 53.3% p=0.003), more venous thrombosis alone (46.4% vs 29.2%, p=0.02) and lower circuit thrombosis (9.2% vs 28.1%, p<0.001). The second wave cohort received more steroids (121/150 [80.6%] vs 86/159 [54.1%], p<0.0001) and Tocilizumab (20/150 [13.3%] vs 4/159 [2.5%] p=0.005).

Conclusions

MB and thrombosis are frequent complications in patients on VV-ECMO and significantly increase mortality. Arterial thrombosis alone or circuit thrombosis alone increased mortality whilst venous thrombosis alone had no effect. MB during ECMO support increased mortality 3.9-fold.

Key words : COVID-19, Extracorporeal membrane oxygenation, Hemorrhage, thrombosis, mortality

Introduction

Veno-venous extracorporeal membrane oxygenation (VV-ECMO) should be considered for patients with acute respiratory failure due to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection refractory to optimal conventional management including mechanical ventilation(1-4)

Severe Coronavirus Disease 2019 (COVID-19) carries a higher risk of thrombosis compared to other viral pneumonias such as influenza(5, 6). The use of VV-ECMO is associated with high rates of thrombosis and haemorrhage which vary between centres. Based on ELSO registry data, rates of thrombosis and bleeding in patients supported with VV-ECMO for non-COVID-19 were around 25.3% and 23.4%, respectively(7). This is due to a myriad of factors which may include contact activation, disease related endothelial dysfunction, sepsis-induced coagulopathy, acquired von-Willebrand syndrome, platelet dysregulation, consumption of coagulation factors and anticoagulation(7-11).

COVID-19 infection progressed through several waves, each with distinct transmission and virulence characteristics. With accumulating evidence from multiplatform clinical studies such as the RECOVERY trial(12, 13) and REMAP-CAP(14, 15), an increasing number of hospitalised patients with COVID-19 received immunomodulatory therapy such as corticosteroid and tocilizumab and early anticoagulation, particularly after the first wave of the pandemic(12, 13). More importantly, with the introduction of mass vaccination against COVID-19 in early 2021, the severity of disease reduced in those who received vaccination(16).

No multicentre study has yet examined the impact of thrombosis and haemorrhage on 180-day mortality in patients with COVID-19 supported by VV-ECMO during the first and

second waves of the pandemic, defined as 1st March 2020 to 31st May 2020, and 1st June 2020 to 30th June 2021 respectively. The 180-day follow-up of the last patient included into the study was 31st December 2021... Previous studies assessing outcomes (including thrombosis and bleeding) of patients with COVID-19 supported by ECMO had a significantly shorter (90days)(17, 18) than the follow-up duration of the present study (180 days).

This remains important because of the ongoing clinical need and lack of international consensus on optimal anticoagulation and monitoring strategies for this patient group. The primary aim of this study was to compare the 180-day probability of thrombosis, major bleeding and mortality in patients supported by VV-ECMO between first and second waves of the COVID-19 pandemic in the UK.

Therefore, in the two waves we assessed:

1. Overall mortality, thrombosis, and major bleeding
2. Factors affecting survival, thrombosis, and major bleeding.
3. The effect of thrombosis and bleeding on survival.
4. Differences in these outcomes between the first and second wave cohorts

Methods

Study design

This was a multicentre observational study using data collected from four of the six UK centres (**appendix page 1**) nationally commissioned to provide ECMO for adult patients with acute respiratory failure **(Table S1).** A total of 309 consecutive adult patients were supported by VV-ECMO for at least 48 hours between 1st March 2020 and 31st December 2021 (spanning the first and second waves of the pandemic). Patients were assigned to the first wave cohort (Cohort 1) if ECMO was initiated between 1st March 2020 and 31st May 2020, and second

wave cohort (Cohort 2) if ECMO was initiated between 1st June 2020 and 30th June 2021, inclusive. All patients had SARS-CoV-2 infection confirmed by RT-PCR positive nasal swabs or nasopharyngeal or lower respiratory tract aspirates.

The study was approved by the Human Research Authority (HRA), Health and Care Research Wales and the local Caldicott Guardian in Scotland (20/HRA/1785). All patients lacked capacity and the need for informed consent was waived because of the observational nature of the study.

Data collection

Data containing demographics, medical history, treatment and clinical course were collected retrospectively from prospectively acquired databases by clinicians involved in patient care using a standardised case record form (CRF) which was submitted to a central electronic database (RED-cap v100.10; Vanderbilt University, Nashville, TN, USA) hosted by Imperial College London (Coagulopathy in COVID19 - A Multi-Centre Observational Study in UK - Full Text View - ClinicalTrials.gov NCT04405232). CA-COVID-19 is a UK multicentre study set up to assess the natural history of COVID-19 from hospital admission to 180 days thereafter. Patients with VA-ECMO are managed differently in relation to anticoagulation intensity (higher intensity compared to VV-ECMO and some patients may receive antiplatelet treatment in addition to anticoagulation. To include a uniform population of patients, this study included only those patients supported with VV-ECMO. Patients supported with veno-arterial (VA)-ECMO or combination of VV-ECMO and VA-ECMO were excluded. All study patients had ended VV-ECMO support and completed the follow-up up to 180days after VV-ECMO.

Anticoagulation

In the absence of robust evidence for intensification of anticoagulation during VV-ECMO in the context of COVID-19, ECMO centres in the United Kingdom maintained anticoagulation protocols that were in use prior to the pandemic. First line anticoagulation was intravenous unfractionated heparin (UFH). Argatroban was used in the context of heparin-induced thrombocytopenia (HIT) or when target anti-Xa levels were difficult to achieve despite appropriate dose titration. Precise anticoagulation protocols were not specified as part of the study design, but in general heparin anti-Xa levels of 0.2-0.3 IU/mL, or equivalent activated thromboplastin time (APTT), were targeted in the absence of significant bleeding. In the context of thrombosis identified at initiation or during ECMO, therapeutic heparin anti-Xa levels of up to 0.3-0.7 IU/mL, or equivalent APTT, were targeted according to local clinical discretion. Target APTT was based on the local reference range corresponding to heparin anti–factor Xa chromogenic assay and established for specific APTT reagents. Diagnosis of heparin induced thrombocytopenia (HIT), Transfusion and haemostatic support are described in appendix page 1. Patients who developed HIT were managed with argratroban. Although, it is not ideal to monitor argatraban using APTT, argatroban level was not widely available. Therefore, in general, APTT was used to monitor the anticoagulant effect of argatroban.

Definitions of clinical outcomes

Bleeding was defined according to the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee [SSC]) criteria for major or clinically relevant non-major bleeding in non-surgical patients(20, 21) Thrombotic events were defined as image-confirmed pulmonary embolism (PE), deep vein thrombosis (DVT), arterial thrombosis, or thrombosis of the ECMO circuit. Circuit thrombosis is defined as thrombosis in the oxygenator signified by high transmembrane pressure or thrombosis in other parts requiring a change in the circuit or clinical intervention. Heparin induced thrombocytopenia (HIT) was defined and diagnosed as detailed in appendix page 1

Routine whole body computed tomography (CT) was performed within 24hours of admission for VV-ECMO. Thereafter, imaging for bleeding or thrombosis was performed when clinically indicated. If acute neurological injury was suspected, non-contrast CT of the brain was used in the first instance to identify intracranial haemorrhage (ICH).

Statistical analysis.

Standard descriptive parameters were calculated for categorical and quantitative variables and presented as frequencies with percentages, or medians with a range. Survival probabilities were calculated using the Kaplan–Meier method, and groups compared using the log-rank test. Variables identified from univariate analyses with P values < 0.2 were entered into a backward stepping Cox regression analysis to find independent prognostic factors significant at $P < 0.05$.

To assess the influence of complications following initiation of VV-ECMO on the risk of mortality, each complication was independently entered as a time-dependent variable. Identification of significant independent prognostic factors for the thrombosis and major bleeding required the use of the cumulative incidence procedure with Gray's test to compare groups, and the Fine and Gray model for the multivariate setting. Death in the absence of thrombosis or major bleeding was considered the competing event.

Multiple imputation was used to account for missing laboratory values (<10%) but not for comorbidities or clinical outcomes. The multiple imputation by chained equation (MICE) technique with its regression imputation model was used for this imputation with ten iterative cycles. Once imputation was done, results were reviewed for each imputed feature to make sure that the imputation has generated plausible data. All tests were two sided, and P values <0.05 were deemed statistically significant. Analyses were performed using either SPSS version 27 (SPSS v27; IBM, Armonk, NY, USA), R (v4.0.3, Open-source software) or Stata (v17, StataCorp LLC, College Station, TX, USA) and open-source software programming

languages and libraries (Python [v3.7, Open-source software], panda [v1.3.3, Open-source software], numpy [v1.21.2, Open-source software], scikit learn [v0.22.1, Open-source software]).

Results

Study population, ECMO duration and immunomodulatory therapy

A total of 309 consecutive adult patients were included, 159 and 150 of whom were admitted during the first and second waves, respectively. The median age of the overall study population was 48 (range 19-75) years, and the majority were male (70.6%), with a larger male majority in the first wave cohort (76.7% versus 64%, p=0.014) **(Table 1).** There were no differences in body mass index (BMI), ethnicity or comorbidities between the first and the second wave cohorts. The first wave cohort had a higher percentage of ex- or current smokers (17% versus 5.4%, p=0.001). Multiple differences were observed in blood results at the time of initiation of VV-ECMO between the two cohorts **(Table 2)** but were not associated with the study outcomes in multivariate analysis.

The median duration on VV-ECMO in the overall study population was 17 (2-153) days. Patients in the second cohort had a significantly longer duration of VV-ECMO support compared to the first cohort (19.5 [2-127] days vs 14 [2-153] days, p=0.016). The emergence of data supporting immunomodulatory therapy for COVID-19(12, 13) meant that more patients in the second cohort received steroids (121/150 [80.6%] versus 86/159 [54.1%], p<0.0001), as well as tocilizumab (20/150 [13.3%] versus 4/159 [2.5%], p=0.005) **(Table S3).**

Transfusion and haemostatic support

Across the whole study population, 268(87%) patients received at least one transfusion of blood products: 250(80.9%) received red cells, 26(8.4%) platelets, 14(4.5%) fresh frozen plasma, and 10 (3.2%) cryoprecipitate. Thirty-four (11.0%) patients received tranexamic acid.

180-day Survival and factors contributing to mortality.

Overall, 180-day survival was 62.5% (95% CI 57.3-68.1) (**Figure 1A).** Table 2 shows univariate and multivariate analyses of the baseline characteristics that were associated with survival. Increasing age was associated with reduced survival; age >55 years on admission was associated with a 2.29-fold (1.33-3.93, *p*=0.003) increased probability of mortality at 180 days from the initiation of VV-ECMO. Creatinine above normal was associated with a 1.91-fold (1.19-3.08, *p*=0.008) increased 180-day probability of mortality. Although thrombocytopenia at the time of initiation of VV-ECMO was associated with reduced survival on univariate analysis, this was not significant on multivariate analysis **(Table S2).**

Thrombosis and heparin induced thrombocytopenia.

Arterial and/or venous thrombotic events were identified in 123(39.8%) patients **(Figure S1A).** 180-day probability of arterial or venous thrombosis was 43.5% (95% CI 37.2%-49.2%). Twenty-eight (9.1%) patients developed arterial thrombosis, 14/28 (50.0%) of whom developed strokes, 4(14.3%) peripheral arterial disease, and 10 (35.7%) arterial thrombosis elsewhere such as mesenteric ischaemia or myocardial infarction. 118 (38.1%) patients developed VTE, 91(77.1%) of whom developed PE/pulmonary thrombosis, 8 (6.8%) solitary lower limb DVT, 10 (8.5%) DVT elsewhere, and 9(7.6%) both DVT and PE. 46/309(14.9%) patients had likely oxygenator failure due to thrombosis. Both arterial and venous thrombotic events were diagnosed in 15(4.9%) patients. 10.0% (31/309) of patients developed HIT with 180-day probability of 11.4% (95% CI 7.5-15.1%). All patients who developed HIT were treated with argatroban and none of these patients developed major bleeding. Probabilities of 180-day overall thrombotic events, venous, arterial, circuit thrombosis and heparin induced thrombocytopenia are presented in **Table 4.**

Table S4 shows that baseline Troponin I, haemoglobin and D-dimer above the normal reference values were associated with increased risk of arterial and/or venous thrombosis (HR 2.08 [1.17-3.70], 6.77 [1.87-24.5] and 2.04 [1.12-3.71], respectively).

Major and minor haemorrhage

The overall cumulative incidence of major bleeding at 180 days was 32.8%(95%CI 38.1%-27.0%) **(Table 4 and Figure 1B).** Major bleeding was identified in 93(30.1%) patients, of whom 30(32.3%) developed ICH, 23(24.7%) pulmonary haemorrhage, 14(4.5%) gastrointestinal haemorrhage, and 26(8.4%) major haemorrhage elsewhere. Platelet count below normal was the only baseline blood result associated with major bleeding on multivariate analysis (**Table S5**). Baseline Heparin anti-Xa level was not associated with major bleeding on univariate or multivariate analysis.Clinically relevant non-major bleeding (CRNMB) was identified in 108 (35.0%) patients and 29 patients suffered both major and non-major haemorrhagic events. Overall, 180-day probability of CRNMB was 38.8% (95% CI 32.6%-44.3%).

Impact of thrombosis and major bleeding on 180-day survival

Table 3 shows the association of thrombotic and haemorrhagic events with 180-day survival, both adjusted and unadjusted for age and creatinine at the time of admission.

Arterial and circuit thrombosis were associated with significantly increased mortality, with (adjusted for age and raised creatinine) hazard ratios of 3.0 (1.5-5.9, p=0.002) and 3.9 (2.4-6.3, p<0.001), respectively. Nine of 14 patients (64.3%) who developed ischaemic stroke subsequently died. While 13 of the 31 (41.9%) patients who developed HIT later died, neither HIT nor venous thromboembolism were associated with mortality on multivariate analysis.

Major bleeding was strongly associated with mortality, with an adjusted hazard ratio of 3.9 (2.6-5.8, p<0.001). Out of 116 patients who died, 49.1% (57/116) had major bleeding and of these major bleeding was recorded as the cause of death in 42.1% (24/57). Nineteen (63.3%) cases of ICH, 15(65.2%) cases of pulmonary haemorrhage, and 9 (64.3%) cases of GI haemorrhage subsequently died, with significant adjusted hazard ratios of 2.4(1.3-4.4), 3.7(2.0-

6.7) and 3.3(1.6-6.7), respectively for these complications (**Table 3).** Clinically relevant non-major bleeding was not associated with mortality. ECMO duration was not associated with mortality.

Comparison of outcomes between the two cohorts

The 180-day survival probability was greater in the first wave cohort (Cohort 1) than the second wave cohort (Cohort 2) (71.1% versus 53.3%,*p*=0.003) **(Table 4 and Figure1C).** The 180-day probability of circuit thrombosis was significantly higher in Cohort 2 (28.1% versus 9.2%, *p*<0.001). The 180-day probability of venous thrombosis was significantly greater in Cohort 1 (46.4% versus 29.2%,*p*=0.002) **(FigureS1B**) and the difference in the 180-day probabilities of arterial thrombosis was not significant. There was a trend towards higher 180-day probability of major bleeding in Cohort 2 (39.2% versus 27.1%, p=0.084) (**Figure1D)**. This coincides with the survival curve **(Figure 1C)**.

Transfusion and haemostatic support between two cohorts

A significantly higher proportion of patients in the cohort 2 received platelet transfusion compared to first cohort (p=0.007). However, there were no differences in the number of red cell units, or the proportions of patients received fresh frozen plasma (FFP), cryoprecipitate, fibrinogen concentrate or tranexamic acid between the two cohorts. Comparison of the transfusion and haemostatic support is presented in Table 5.

Discussion

In this multicentre observational study comparing thrombosis, major bleeding, and mortality in patients with severe COVID-19 supported by VV-ECMO between first and second waves in the UK, there was a lower 180-day survival probability in the second wave cohort. There was a trend towards higher major bleeding rate in Cohort 2, especially after day-50 on VV-ECMO, which coincides with the divergence of survival curves. Cohort 2 also had a higher

risk of developing circuit thrombosis, which was strongly associated with mortality. These occurred despite consistent anticoagulation protocols and no difference in the type of VV-ECMO circuits used between the two waves. There was a higher rate of VTE in Cohort 1, and Cohort 2 were more frequently treated with steroids and tocilizumab. There were no differences in body mass index (BMI), ethnicity or comorbidities between the first and second cohorts. Although there were multiple differences in blood results at the time of initiation of VV-ECMO between the cohorts, none of these were associated with the study outcomes in multivariate analysis. Duration of VV-ECMO support was significantly longer for Cohort 2 than Cohort 1, but VV-ECMO duration was not associated with mortality.

Overall, there were high rates of thrombosis and major bleeding. Arterial thrombosis, circuit thrombosis and major haemorrhage were significant predictors of reduced survival at 180 days, independent of age and renal impairment at baseline which were also associated with reduced survival.

The overall mortality (37.5%) in this study was comparable to that found in large studies of patients receiving VV-ECMO support both for COVID-19 (37.4%) and for other indications pre-COVID-19 pandemic (34.9%)(7, 22).

Other studies of COVID-19 patients supported by ECMO found high rates of bleeding but reported a lower thrombosis rate than that identified here(23-25). The higher rate of thrombosis in this study could be due to differences in imaging or anticoagulation protocols or the longer follow-up period of 180 days. A nationwide cohort study (The ECMOSARS registry, France) included 620 COVID-19 patients supported by ECMO (568 VV-ECMO and 52 VA-ECMO) and reported that 29% experienced one or more bleeding events, 16% one or more thrombotic event and 20% both bleeding and thrombosis. ICH was detected in 8%. The presence of major bleeding was associated with a 2.91-fold risk of in-hospital mortality [95% CI 1.94–4.4]), with presence of ICH having the highest risk (OR 13.5[95% CI 4.4–41.5]), but thrombosis had no

effect on mortality. However, the study assessed these outcomes only until 90days after the initiation of ECMO(23). Our study found only arterial and circuit thrombosis to be significantly predictive of mortality with hazard ratios of 3.0 (1.5-5.9) and 3.9 (2.4-6.3), respectively. One recent large study of non-COVID-19 patients supported with VV-ECMO did find ischaemic stroke to be highly predictive of mortality with an adjusted hazard ratio of 4.5(7).

This study presents an adjusted 180-day probability of arterial thrombosis of 10.9% (6.9%-14.7%) with ischaemic stroke the most frequent arterial event (14/28). Arterial thrombosis is a feature of severe COVID-19 outside the context of ECMO; it has been reported to occur in 3% of cases in the intensive care unit(26, 27). This study suggests both venous and arterial thrombosis are a major concern in patients supported with VV-ECMO for severe COVID-19.

A much lower rate of arterial events such as ischaemic stroke was reported in non-COVID-19 patients supported by VV-ECMO in data derived from the Extracorporeal Life Support Organization (ELSO) registry(7). However, this registry does not collect data on certain important bleeding and thrombotic complications including upper respiratory bleeding, deep vein thrombosis, or pulmonary embolism and there is also a possibility of under detected and under reported arterial events if CT scans were not performed systematically in some centres (28-30).

The adjusted 180-day probability of circuit thrombosis was 18.1% (13.2%-22.8%) which is similar to incidence rates reported in recent large studies of COVID-19 and non-COVID-19 patients supported by ECMO(7, 23). However, this study uniquely found circuit thrombosis to be an independent indicator of mortality on multivariate analysis (HR 3.9 [2.4-6.3]). Although the reason for this is not clear, circuit thrombosis despite standard intensity anticoagulation for VV-ECMO could be an indicator of a hyper-inflammatory response in these patients and unlike other studies (24),we did not alter the anticoagulation intensity in our cohort.

The 180-day probability of major bleeding was 32.8% (27.0%-38.1%) in this study which is lower than the rate reported in a previous study by Schmidt et al(24). However, Schmidt et al (24) included both VA- and VV-ECMO in their analysis and had increased the intensity of anticoagulation. Major bleeding also carried a significantly increased risk of death, with hazard ratios of 2.4 (1.3-4.4), 3.3 (1.6-6.7) and 3.7 (2.0-6.7) associated with intracranial, gastrointestinal, and pulmonary haemorrhages, respectively. Furthermore, bleeding was a documented cause of death in 20.7% of the overall study population (24/116) and 42.1% (24/57) of the patients who had major bleeding subsequently died. This is consistent with numerous previous studies in the context of VV-ECMO(7, 9, 23). As postulated by this and other groups, the strong relationship between major bleeding and mortality in the context of VV-ECMO may be related to the necessary temporary discontinuation of systemic heparin after the detection of haemorrhage, and resultant loss of off-target antiviral, anti-inflammatory and anti-complement effects of the drug(30-32) in addition to the direct effects of bleeding itself.

Of all recorded baseline characteristics, creatinine above normal and age >55 years were the only independent predictors of mortality in multivariate analysis, with significant hazard ratios of 1.91 (1.19-3.08) and 2.29 (1.33-3.93), respectively. These findings are consistent with numerous ECMO studies both in and outside the context of COVID-19(33-36)One recent meta-analysis reported older age and renal replacement therapy before ECMO initiation were predictive of in-hospital mortality (37). However, the meta-analysis also showed chronic lung disease and male sex to be mortality predictors, unlike this study.

The second cohort had a lower probability of 180-day survival than the first cohort (53.5% [45.9%-61.9%] versus 71.1% [64.4%-78.5%]) despite being more heavily treated with steroids and tocilizumab. This is consistent with the findings of other ECMO studies examining

mortality rate over the course of the pandemic(17, 38, 39). After the adoption of immunomodulatory therapies in the early hospital treatment of severe COVID-19, the patients who progressed to require VV-ECMO despite this treatment may have had more generally refractory disease than those previously accepted for ECMO, which might account for the higher mortality rate during second and subsequent waves. This study was conceived during the first wave and as a result no data were collected on COVID-19 strains. However, one large study demonstrated no crude difference between 90-day mortality rates in patients infected with the wild-type, alpha or delta variants of COVID-19;and the wild-type variant was dominant during the period associated with the greatest mortality (18). This study showed a trend towards more major bleeding events in Cohort 2 (p=0.084) compared to Cohort 1 which could have contributed to higher mortality in the second cohort in a multifactorial process. Greater steroid use and longer duration on VV-ECMO are likely to be contributory. Furthermore, there is a striking difference in the bleeding events after 50days (which was parallel until day 50) on ECMO between the two cohorts: Cohort 2 continued to have bleeding events until day 100 whilst Cohort 1 events plateaued. This coincides with the survival curve (Figure 1B). This could be due to longer duration on ECMO in Cohort 2 (19.5 [2-127] days vs 14 [2-153] days in Cohort 1, p=0.016) causing platelet dysfunction including acquired von Willebrand disease(40). Interestingly, a significantly higher proportion of patients in cohort 2 received platelet transfusion compared to cohort 1 (p=0.007) with no difference in the number red cell units or proportion of patients received FFP, cryoprecipitate, fibrinogen concentrate or tranexamic acids between the two cohorts.

Other causes for the higher mortality rate in Cohort 2 in this study could be the higher circuit thrombosis (28.1% versus 9.2%), which was strongly associated with mortality in multivariate analysis, though not a risk factor identified elsewhere(7, 23). The reasons why there was a

higher rate of circuit thrombosis was in the second cohort was not clear. Longer duration on VV-ECMO, unaltered anticoagulation, with hyper-inflammation (raised white cells and neutrophils) are likely contributory. Although there were several differences in the baseline laboratory parameters between the two cohorts, none of these parameters were associated with mortality in the multivariate analysis, suggesting the higher mortality in the second cohort is multifactorial. (**Table 2**). Reduced venous and overall thrombosis rate in the second cohort is most likely related to early initiation of thromboprophylaxis, including high intensity, early in the course of the disease prior to initiation of VV-ECMO, in accordance with evidence from the REMAP-CAP study(14, 15). This also may have been a contributing factor for higher major bleeding rate in Cohort 2. Both bleeding and thrombotic events were assessed within 24hrs of initiation of VV-ECMO by performing CT scans, especially of the brain.

The strengths of this study include its multicentre setting, large patient cohort, consistent treatment protocols, 180-day follow-up period, detailed data set generated using a pre-defined CRF, systematic imaging and documenting the bleeding and thrombotic events with robust statistical analysis. Its main limitations include the retrospective design with the lack of documented information regarding vaccination status and SARS-CoV-2 variant in the second cohort, the lack of documented information regarding vaccination status and SARS-CoV-2 variant in the second cohort. Additionally, this study did not have information on SAPS II score, SOFA score at the initiation of VV-ECMO, time from first symptoms to ICU admission, time from first symptoms to intubation, time from ICU admission to initiation of VV-ECMO, duration of non-invasive ventilation prior to intubation and ventilation parameters. However, patients were selected for VV-ECMO according to agreed national criteria in the UK. Furthermore, because we used Hemosisl AcuStarHIT-IgG rather than a functional assay such

as serotonin release assay (SRA), for the diagnosis of HIT, it is possible that we may have overestimated the incidence of HIT.

Conclusion

This multicentre study of 309 consecutive COVID-19 patients supported by VV-ECMO reports high incidence of thrombosis and major bleeding. Older age, renal impairment at the time initiation of VV-ECMO, arterial thrombosis, circuit thrombosis and major bleeding were independently associated with 180-day reduced survival. The cohort treated on or after 1st June 2020 had higher 180-day mortality, reflecting a concerning trend towards increasing mortality rates in COVID-19 patients supported by VV-ECMO over time (although this may be an artefact of improvements in early COVID-19 treatment preventing the need for VV-ECMO in responsive patients). Reduced overall thrombotic events are most likely due to early initiation of thromboprophylaxis prior to initiation of VV-ECMO in the second wave of the pandemic. This study further characterises the complex competing risks that affect long term outcome in COVID-19 patients supported by VV-ECMO. Prospective studies are required to determine optimal anticoagulant and haemostatic management of this patient group.

Contributors

DJA conceived the study, acquired the funding, involved in data collection, data verification, data analysis, figures, data interpretation, writing the original draft reviewing and editing the manuscript. AW contributed to the original draft of the manuscript. IR, ZO, and RS involved in data verification, data analysis, figures, data interpretation and reviewing and editing the manuscript. MG, GI, LC, LF, SL, RJ, AV and HY involved in data collection, data interpretation, reviewing and editing the manuscript. ML interpreted the data, reviewed, and

edited the manuscript. All authors interpreted the results, reviewed the manuscript, approved the final work, and agree to be accountable for the accuracy and integrity of the work.

Acknowledgments

Authors would like to thank all clinical and laboratory staff involved in care of the patients at participating ECMO centres. DJA funded by Medical Research Council UK (MR/V037633/1)

Declaration of interests

DJA received funding from Bayer plc to setup the multicentre database of the study as an investigator-initiated funding. Other authors have no conflict of interest to declare.

Funding

Bayer plc supported the study by providing the investigator-initiated funding (P87339) to setup the multicentre database of the study. The funder had no access to data and played no part in analysis or writing. The corresponding author is responsible for the study design, had full access to all the data in the study and had final responsibility for the decision to submit for publication. DJA is funded by MRC UK (MR/V037633/1)

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Legends to tables and Figures

Table 1. Baseline clinical characteristics of the whole cohort and the comparison of the first and the second wave cohorts

Table 2. Baseline (at the time of initiation of VV-ECMO) laboratory and observational characteristics of the whole cohort and the comparison of the first and the second wave cohorts

Table 3. Time-dependent effects of different outcomes on survival of the overall cohort

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Table 5. Comparison of the transfusion and haemostatic support between the first and second wave cohorts

Figure 1. Probability of 180-day survival (A) and major bleeding (B) in the overall cohort , comparison between of the survival (C) and the major bleeding (D) between first and second wave cohorts supported with VV-ECMO

Table 1. Baseline clinical characteristics of the whole cohort and the comparison of the first and the second wave cohorts

Characteristic	N =309	Cohort 1 (N=159)	Cohort 2 (N=150)	*p*-value
Gender				
Female	91 (29.5%)	37 (23.3%)	54 (36%)	**0.014**
Male	218 (70.6%)	122 (76.7%)	96 (64%)	
Age (years)				
<42	83 (26.9%)	41 (25.8%)	42 (28.0%)	
42-48	86 (27.8%)	43 (27.0%)	43 (28.7%)	0.91
49-55	76 (24.6%)	40 (25.2%)	36 (24.0%)	
>55	64 (20.7%)	35 (22.0%)	29 (19.3%)	
Ethnicity				
White	116 (37.5%)	57 (35.8%)	59 (39.3%)	
Mixed / Multiple Ethnic Groups	6 (1.9%)	3 (1.9%)	3 (2.0%)	
Asian / Asian British	72 (23.3%)	39 (24.5%)	33 (22.0%)	0.18
Black / African / Caribbean / Black British	19 (6.1%)	12 (7.5%)	7 (4.7%)	
Other ethnic group	9 (2.9%)	8 (5.0%)	1 (0.7%)	
Unknown	87 (28.2%)	40 (25.2%)	47 (31.3%)	
BMI (kg/m^2)				
18.6-24.9	45 (14.6%)	29 (18.2%)	16 (10.7%)	
25-29.9	97 (31.4%)	52 (32.7%)	45 (30.0%)	0.75
30-34.9	78 (25.2%)	41 (25.8%)	37 (24.7%)	
34.9-39.9	43 (13.9%)	21 (13.2%)	22 (14.7%)	
>39.9	46 (14.9%)	16 (10.1%)	30 (20.0%)	
Smoking				
Current smoker	3 (1.3%)	2 (1.3%)	1 (0.7%)	
Ex-smoker	32 (13.7%)	25 (15.7%)	7 (4.7%)	**<0.001**
No history of smoking	199 (85.0%)	82 (51.8%)	117 (78%)	
Missing	65	50	25	
History of lung disease				
No	252 (81.8%)	135 (84.9%)	117 (78.5.0%)	0.15
Yes	56 (18.2%)	24 (15.1%)	32 (21.5%)	
Missing	1		1	
History of diabetes				
No	234 (76.5%)	120 (75.4%)	114 (76.5%)	0.99
Yes	72 (23.5%)	37 (23.6%)	35 (23.5%)	
Missing	3	2	1	
Hypercholesterolaemia				
No	272 (88.0%)	141 (88.7%)	131 (87.3%)	0.72
Yes	37 (12.0%)	18 (11.3%)	19 (12.7%)	
Hypertension				
No	212 (69.1%)	115 (72.8%)	97 (65.1%)	0.15
Yes	95 (30.9%)	43 (27.2%)	52 (34.9%)	
Missing	2	1		

P values <0.05 are shown in bold

Table 2. Baseline (at the time of initiation of VV-ECMO) laboratory and observational characteristics of the whole cohort and the comparison of the first and the second wave cohorts

Laboratory parameter	**N =309 Median (IQR)**	**Cohort 1 Median (IQR)**	**Cohort 2 Median (IQR)**	***p*-value**
Haemoglobin (g/L)	105 (94,121)	106 (92,121)	103 (95,120)	0.31
White blood cells(10^9 /L)	11.7 (8.20, 15.8)	11.0 (7.80, 14.3)	12.4 (9.10, 19.4)	**0.002**
Neutrophils (10^9 /L)	10.2 (7.10, 14.1)	9.25 (6.38, 12.6)	11.3 (8.54, 17.8)	**<0.001**
Lymphocytes (10^9 /L)	0.70 (0.50, 1.10)	0.70 (0.50, 1.10)	0.68 (0.45, 1.05)	0.070
Platelets (10^9 /L)	246 (182, 324)	260 (194, 333)	232 (170, 304)	**0.016**
Prothrombin time (Seconds)	14.4 (13.2, 16.0)	14.3 (13.3, 15.7)	14.5 (13.1, 16.5)	0.14
Activated partial thromboplastin time (APTT)(Seconds)	40.1 (29.9, 66.3)	39.2 (29.9, 66.1)	41.8 (30.0, 68.3)	0.087
APTT ratio	1.45 (1.10, 2.93)	1.30 (1.10, 2.20)	2.00 (1.25, 3.85)	**0.003**
D-dimer (ng/mL)	1700 (340, 3450)	3070 (1320, 5170)	693 (5.60, 2700)	**0.008**
Fibrinogen (g/L)	5.80 (4.30, 7.40)	6.40 (4.90, 7.60)	5.00 (3.60, 6.90)	**<0.001**
Ferritin (µg/L)	1120 (528, 1990)	1290 (725, 2210)	945 (421, 1690)	0.28
Alanine aminotransferase (IU/L)	48 (32, 82)	45 (32, 73)	53 (34, 93)	0.17
Bilirubin (µmol/L)	12 (9, 21)	14 (9, 23)	12 (8, 20)	0.061
Creatinine (µmol/L)	71 (51, 126)	76 (54, 138)	69 (49, 115)	0.049
C-reactive protein (mg/L)	183 (92, 272)	231 (156, 294)	115 (58, 218)	**<0.001**
Lactate dehydrogenase (IU/L)	833 (579, 1110)	886 (571, 1180)	815 (602, 1030)	0.60
Troponin-I (ng/L)	50.0 (14.5, 169.0)	54.3 (17.7, 131)	47.3 (12.0, 202.5)	**0.016**
Fraction of inspired oxygen (FiO2)	60 (43, 70)	60 (40, 71)	60 (50, 70)	0.94
Oxygen saturation (SaO2)	96 (93, 97)	95 (93, 97)	96 (92, 98)	0.59
Heparin anti-Xa* (U/mL)	0.43 (0.20, 0.79)	0.33 (0.16, 0.55)	0.55 (0.30, 0.87)	**<0.001**
Average fraction of inspired oxygen (FiO2) %	50 (40, 65)	50 (40, 65)	50 (40, 69)	0.97
PaO2 (kPa)	9.7 (8.3, 12.0)	9.5 (8.2, 12.0)	9.7 (8.4, 12.2)	0.41
PaCO2 (kPa)	6.8 (5.9, 7.9)	7.0 (5.9, 7.9)	6.5 (5.4, 7.6)	0.13
Lactate (mmol/L)	1.5 (1.1, 2.0)	1.4 (1.1, 1.9)	1.7 (1.2, 2.4)	0.030
Bicarbonate (mmol/L)	27.3 (23.0, 31.8)	27.2 (23.9, 31.9)	27,7 (22.7, 31.5)	0.54

***** Median heparin anti-Xa levels presented here were at the time of initiation of ECMO therefore reflects higher intensity anticoagulation prior to start of VV-ECMO in patients in the second cohort. Once ECMO was initiated all patients in both cohorts received same intensity of anticoagulation with UFH.. P values <0.05 are shown in bold

Table 3. Time-dependent effects of different outcomes on survival of the overall cohort

Clinical outcome	Number of events	Unadjusted Hazard Ratio (95%CI)	P-value	Adjusted* Hazard Ratio (95%CI)	P-value
Arterial thrombosis	28	2.5 (1.3-5.0)	0.008	3.0 (1.5-5.9)	0.002
Venous thrombosis	110	1.1 (0.8-1.7)	0.57	1.1 (0.7-1.6)	0.72
Pulmonary embolism	90	1.2 (0.8-1.8)	0.43	1.1 (0.7-1.7)	0.64
Arterial or venous thrombosis	123	1.1 (0.8-1.7)	0.53	1.2 (0.8-1.8)	0.37
Major bleeding	93	4.4 (3.0-6.5)	<0.001	3.9 (2.6-5.8)	**<0.001**
Intracranial bleed	30	3.1 (1.7-5.5)	<0.001	2.4 (1.3-4.4)	**<0.001**
Gastrointestinal bleed	14	4.0 (1.9-8.2)	<0.001	3.3 (1.6-6.7)	**<0.001**
Pulmonary bleed	23	3.4 (1.9-6.0)	<0.001	3.7 (2.0-6.7)	**<0.001**
Heparin-induced thrombocytopaenia	31	1.5 (0.8-2.8)	0.19	1.4 (0.8-2.6)	0.26
Circuit thrombosis	46	3.4 (2.1-5.5)	<0.001	3.9 (2.4-6.3)	**<0.001**
Multi-organ failure	116	2.1 (1.5-3.1)	<0.001	1.7 (1.1-2.5)	**0.011**
Arterial or venous or circuit thrombosis	147	1.6 (1.1-2.3)	0.02	1.6 (1.1-2.3)	**0.017**
Non-major bleeding	108	1.2 (0.8-1.9)	0.32	1.2 (0.8-1.9)	0.35

*Adjusted models contained the factors patient age and creatinine at admission. P values <0.05 are shown in bold.

Table 4. Outcome probabilities in the overall cohort and the comparison between the two cohort

Outcome	Number of events	Overall Probability at 180 days % (95%CI)	Cohort 1 Probability at 180 days% (95%CI)	Cohort 2 Probability at 180 days% (95%CI)	P-value
Survival	193 (62.5%)	62.5 (57.3-68.1) %	71.1 (64.4-78.5) %	53.3(45.9-61.9) %	**0.003**
Arterial thrombosis	28 (9.1%)	10.9 (6.9-14.7%-)	8.1 (3.3%-12.6%)	14.1 (7.5-20.3) %	0.12
Venous thrombosis	110 (35.6%)	38.1(32.1-43.6) %	46.4(37.6-53.9%)	29.2 (21.0-36.6) %	**0.002**
Arterial or venous thrombosis	123 (39.8%)	43.5(37.2%-49.2) %	49.2(40.2-56.8) %	37.4 (28.3-45.4) %	**0.035**
Major bleeding	93 (30.1%)	32.8(27.0-38.1) %-	27.1(19.6 -33.9) %	39.2 (30.2-47.1) %	0.084
Heparin-induced thrombocytopaenia	31 (10.0%)	11.4(7.5-15.1) %	10.6 (5.3-15.5) %	12.3 (6.5-17.8) %	0.57
Circuit thrombosis	46 (14.9%)	18.1 (13.2-22.8%)	9.2 (4.3-13.9) %	28.1 (19.4-35.8) %	**<0.001**
Arterial or venous or circuit thrombosis	147 (47.6%)	52.2(45.5=57.9) %	52.5 (43.5-60.1) %	52.0 (42.3-60.0) %	0.49
Non-major bleeding	108 (35.0%)	38.8 (32.6-44.3) %	35.1 (26.7-42.6) %	42.9 (33.6-51.0) %	0.25

P values <0.05 are shown in bold

Table 5. Comparison of the transfusion and haemostatic support between the first and second wave cohorts

Transfusion and haemostatic support	Cohort 1	Cohort 2	P value
Red cell units (median, range)	1 (0-6)	1 (0-10)	0.86
Platelet units No Yes	 154 (97%) 5 (3%)	 129 (86%) 21 (14%)	**<0.001**
Fresh Frozen plasma (FFP) No Yes	 153 (96%) 6 (4%)	 142 (95%) 8 (5%)	0.51
Cryoprecipitate No Yes	 156 (98%) 3 (2%)	 143 (95%) 7 (5%)	0.17
Fibrinogen concentrates. No Yes	 157 (98.7%) 2(1.3%)	 147 (95.0%) 3(2%)	0.67
Tranexamic acid No Yes	 146 (92%) 13 (8%)	 129 (86%) 21 (14%)	0.10

P values <0.05 are shown in bold

Figure 1