Intracranial haemorrhage and early mortality in patients receiving extracorporeal membrane oxygenation for severe respiratory failure

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Abstract

Background: Intracranial haemorrhage (ICH) is a serious complication in patients receiving veno-venous extracorporeal membrane oxygenation (VV-ECMO) and is associated with high mortality. It is unknown whether ICH may be a consequence of the ECMO or of an underlying disease.

Aims: We first aimed to assess the incidence of ICH at initiation and during the course of VV-ECMO and its associated mortality. The second aim was to identify clinical and laboratory measures that could predict the development of ICH in severe respiratory failure

Methods: Data were collected from a total number of 165 patients receiving VV-ECMO from Jan 2012 to Dec 2016 in a single tertiary centre and treated according to a single protocol. Only patients who had a brain CT within 24 h of initiation of ECMO (n=149) were included for analysis.

Results: The prevalence and incidence of ICH at initiation and during the course of VV-ECMO (at median 9 days) were 10.7% (16/149) and 5.2% (7/133), respectively. Thrombocytopenia and reduced creatinine clearance [CrCL] were independently associated with increased risk of ICH on admission; odds ratio [95% CI]: 22.6 [2.6- 99.5] and 10.8 [5.6-16.2]. Only 30-day (not 180-day) mortality was significantly higher in patients with ICH on admission versus those without (respectively, 37.5% (6/16) vs 16.4% (22/133); p=0.03 and 43.7% (7/16) vs 26.3% (35/133); p=0.15).

Conclusions: Reduced CrCL and thrombocytopenia were associated with ICH at initiation of VV-ECMO. The higher incidence of ICH at initiation suggests it is more closely related to the

severity of the underlying lung injury than to the VV-ECMO itself. ICH at VV-ECMO initiation was associated with early mortality.

Key words: Extracorporeal membrane oxygenation, respiratory failure, intracranial haemorrhage; cerebral ischaemic infarction, venous thrombosis, anticoagulation

Introduction

Veno-venous extracorporeal membrane oxygenation (VV-ECMO) is increasingly used to provide potentially life-saving pulmonary support to patients with severe respiratory failure. Despite increasing experience with VV-ECMO and recent technical improvements, the morbidity and mortality of patients receiving VV-ECMO remains high, but varies significantly between centres, patient subgroups and by underlying disease pathology [1-6]. The outcome of patients on VV-ECMO is influenced not only by factors independent of VV-ECMO (e.g., illness type and severity, other organ support) but also by complications arising from VV-ECMO itself. Mechanical complications of VV-ECMO have decreased with introduction of centrifugal pumps, low-resistance polymethylpentene membranes and modern heparin-coated surfaces [7]. Medical complications are now more common; [8] these include bleeding, or thromboembolism causing vascular and neurological complications and limb ischemia. Better understanding of factors contributing to VV-ECMO complications and affecting the outcome will inform development of safer care and improve outcomes.

The bleeding and thrombotic complications of VV-ECMO are now a leading cause of morbidity and mortality[9]. Intracranial haemorrhage (ICH) has been reported with an incidence ranging from 5% [10;11] up to 19%, [6;12;13] with a predominantly fatal outcome. Data from the 2013 Extracorporeal Life Support Organization (ELSO) registry showed that bleeding was the principal complication of VV-ECMO therapy. Specifically, an incidence of 3.9% for ICH, associated with a survival rate of only 20% [14]. This was confirmed in the August 2017 international ELSO summary, in which the VV-ECMO incidence of ICH was 3.6%, with a 21% survival rate [13]. A systematic review found comparable intracranial bleeding rates (5%) in patients with VV-ECMO [11]. However, these estimated incidences of ICH in VV-ECMO are based largely on retrospective studies and there is no prospective well designed

investigation assessing the true incidence of ICH, its timing and the coincident factors leading to its development. Notably, registry data do not distinguish between ICH developing during ECMO and ICH that may have begun prior to ECMO.

Venous thromboembolism (VTE) and ischaemic stroke are frequent thrombotic complications developing in patients receiving ECMO. In retrospective studies VTE was diagnosed in up to 10% of cases and in up to 18% when post de-cannulation venous Doppler ultrasound was routinely performed [5]. Summary report from the ELSO 2017[13] stated that the incidence of ischaemic stroke in patients receiving VV-ECMO was 1.7% with survival of 31.8%, which is equally as poor as for ICH.

The causes of bleeding and thrombosis in patients with VV-ECMO are likely multifactorial [12;15]. With regard to ICH, a number of factors can be identified. Firstly, patients requiring VV-ECMO support are critically ill with infection and systemic inflammation which promotes disseminated intravascular coagulation (DIC), thus causing both bleeding and thrombosis [16].

Secondly, to maintain circuit patency and minimize thromboembolic complications, anticoagulation is used, increasing the risk of bleeding. The optimal anticoagulation strategy for VV-ECMO is not known, but the frequency of ICH in these patients demonstrates that further insight into the delicate balance of haemostasis and thrombosis during VV-ECMO is essential to provide an optimised anticoagulation strategy for these critically ill patients.

Thirdly, forcing the blood at high shear through the VV-ECMO circuit results in acquired von Willebrand syndrome (AVWS), thrombocytopenia and platelet dysfunction due to loss of platelet surface receptors [12;15]. A recent study showed that loss of platelet surface receptors glycoprotein (GP)Ibα and GPVI in ECMO patients (for both veno-arterial [VA] and VV-ECMO)

may contribute to loss of platelet adhesion and activation and limit haemostatic plug formation under high or pathological shear conditions [17].

At the same time, a tendency to thrombosis may arise from exposure to the foreign surface of the ECMO circuit causing factor XII and contact pathway activation with subsequent thrombin generation. The effects of this phenomenon have not been studied in patients receiving VV-ECMO or VA-ECMO.

There is inconsistency in published data regarding the time at which ICH develops in patients receiving ECMO. Whilst some studies reported that ICH frequently occurred within the first 24 h or within a few days of ECMO initiation [18], others found that duration of ECMO was an independent predictor of ICH[19]. Furthermore, a variation in the incidence of ICH between centres may arise from different anticoagulation practice for patients receiving ECMO. Therefore, it would be valuable to assess the incidence of ICH and other bleeding and thrombotic complications within an individual ECMO centre in order to identify the factors contributing to their development and improve the subsequent outcome by addressing modifiable risk factors. Aims of this retrospective cohort study were to (i) assess the incidence of ICH at initiation and during the course of VV-ECMO and its associated mortality; (ii) identify clinical and laboratory measures that are predictive for development of ICH in patients with severe respiratory failure.

Patients and Methods

This is a retrospective single centre observational cohort study in a tertiary ECMO referral centre in UK. The study was approved by the Research Ethics Committee and the local

Research and Development Office (Reference number:17/LO/0808). Potential eligible patients for the study were identified from the Trust VV-ECMO data base. Patient demographic data, radiological, laboratory and clinical data were collected on all 165 consecutive patients aged ≥16 years old and who received VV-ECMO treatment for severe respiratory failure over a 5-year period (from Jan 2012 to Dec 2016). Patients received VV-ECMO when fulfilling the English national respiratory ECMO criteria: lung injury score of 3 or more; less than 7 days of conventional mechanical ventilation; and a cause of respiratory failure deemed reversible by the treating physician in patients with no limitation to on-going life-sustaining treatment. Only those patients who had brain computerised tomography (CT) performed within 24hrs of ECMO initiation (n= 149) were included for further analysis (patients without admission brain CT or with documented traumatic ICH were excluded).

It is our standard practice to give a 25-50 IU/kg bolus of unfractionated heparin (maximum 5000 units) pre-cannulation at the time of VV-ECMO initiation and then transfer the patient to the ECMO unit and perform non-contrast CT brain to exclude ICH before giving further doses of heparin. The initial dose of heparin is determined by a senior intensivist based on platelet count, coagulation times and perceived bleeding risk. If ICH is subsequently detected, no further heparin is given and it is usual practice to perform an interval CT to monitor progression and an early sedation hold to facilitate neurological assessment. Heparin is cautiously reintroduced if CT appearances improve, typically after more than 7-10 days. Patients who do not have ICH will receive unfractionated heparin (UFH) infusion to maintain the activated partial thromboplastin time (APTT) between 50-60 seconds or anti-Xa: 0.2-0.3 units/ml. All patients had APTT and anti-Xa measured initially and when judged to be discrepant, monitoring continued using the anti-Xa only. In patients with bleeding

complications, heparin is reduced (minor bleeding) or temporarily stopped (major bleeding/ICH). Baseline characteristics of the study included 149 patients are shown in table 1. Intracranial haemorrhage was defined as any visually detectable changes suggestive of bleeding in the CT head confirmed by two independent expert radiologists. Patients with admission CT appearances were in keeping with stroke with subsequent evidence of haemorrhagic transformation was not considered as ICH. Major bleeding [20] and clinical relevant non-major bleeding [21] were defined as per International society of Haemostasis and Thrombosis Subcommittee (ISTH SSC) recommendations. Thrombotic episodes were defined as objectively identified events identified by standard Doppler ultrasound or CT scans.

Relevant clinical information and the results of laboratory investigations at the time of initiation of VV-ECMO were obtained from ECMO referral form and the referring hospitals. Both clinical and laboratory data following the initiation of VV-ECMO were available and taken from patient records and the electronic data base.

Laboratory assays

Venous blood was collected into 0.109M trisodium citrate in the proportion 9:1 (Vacutainer Plus, Becton Dickinson, Franklyn Lakes USA), centrifuged at 2000g for 10 min at room temperature and processed within 1 h of collection. Anti-Xa using chromogenic Liquidanti-Xa assay (Werfen, Warrington, Cheshire, UK), and APTT using SynthASil (HemosIL®, Werfen, Warrington, Cheshire, UK) measurements were performed on the same sample using an ACL TOP 500 analyser (Werfen, Warrington, Cheshire, UK). The tests were performed

immediately after processing as part of patient management. Based on comparisons using an anti–factor Xa (anti-Xa) chromogenic assay within our hospital, an APTT of 50-60 seconds corresponds to the recommended anti-Xa therapeutic range of 0.2 to 0.3 IU/mL (an APTT 60-100 seconds corresponds to anti-Xa of 0.3-0.7 IU/mL). Clauss fibrinogen level was performed the using FIB-C XL kit (Werfen, Warrington, Cheshire, UK) on the ACL TOP 500. The intra- and inter-assay coefficients of variation (CV) were as follows: APTT 2.7% and 3.0%; anti-Xa 4.0% and 6.2%; Clauss fibrinogen 8.0% and 7.3% respectively. Creatinine was measured using a modified Jaffe method traceable to an Integrated Database Management System (IDMS) reference method (National Institute of Standards and Technology (NIST) reference material 967) on the Beckman AU680 analyser (Beckman Coulter, High Wycombe, UK). Creatinine clearance (CrCL) was calculated for each patient using Cockcroft-Gault formula[22].

Venous thromboembolism

Diagnosis of Venous thromboembolism (VTE) was based on duplex ultrasound scans (USS), CT scan or magnetic resonance angiography. Doppler USS of the lower limbs were performed to detect thrombotic episodes routinely in all patients who had a femoral catheter (125/149, 83.9%) at the time of removal. USS scans of the upper limbs and CT scans of the chest were performed only when clinically indicated.

Heparin induced thrombocytopenia

Heparin induced thrombocytopenia (HIT) was clinically suspected in patients treated with UFH who developed thrombocytopenia if the pattern of falling platelet count was suggestive of HIT with or without objectively proven thrombosis. Immediate HIT antibody

testing was performed on an ACL TOP500 analyser with Hemosil HIT- Ab (PF4-H) kit (Werfen UK). Positive tests were confirmed using an ELISA (HYPHEN BioMed, Neuville-sur-Oise, France).

Statistical analysis

Data analysis was performed using Stata version 14 and GraphPad Prism® version 7 (GraphPad Software, Inc. La Jolla, USA). Categorical data were presented as number and percentages and comparisons of these data done using the chi-squared or Fishers exact test. Numeric data were tested for normality using Shapiro-Wilk test. Data found to be normally distributed were presented as mean with their 95% confidence interval, while data found not to be normally distributed were presented as median with the interquartile range. Two sample independent t tests were used to compare normally distributed numeric data between groups, while the Wilcoxon rank-sum (Mann-Whitney) test was used for non-normal data. Univariate logistic regression was done to determine variables associated with ICH and those variables that were significant at 5% were included in a multivariable model to determine the variables that were independently associated with ICH. All statistical tests were 2 sided and significance was set at $p < 0.05$.

Results

Patients' demographic data and baseline characteristics of patients with and without ICH on admission are shown in Table 1. Primary respiratory infection, either bacterial or viral, was the main indication (60%) for VV-ECMO support, followed by adult respiratory distress syndrome (ARDS) secondary to systemic infection (14.8%) and acute interstitial lung disease (7.4%). The median (IQR) duration in VV-ECMO was 12.5 (4.6-15.8) days. There was no

relationship between the indication for VV-ECMO and the presence of ICH at the initiation of VV-ECMO, nor any significant difference in the age and sex of the patients and development of ICH. The prevalence of ICH at the initiation of ECMO was 10.7% (16/149) and the incidence of ICH during the course of VV-ECMO was 5.2 % (7/133). The median duration (IQR) of VV-ECMO in patients who did and did not develop ICH during the course of VV-ECMO were 9 (3-12) and 12.6 (4.8-15.8) days respectively.

ICH patient characteristics

The types of ICH on admission CT and during the course of VV-ECMO are shown in table 2. Intra-parenchymal haemorrhage was the main type of ICH seen both on admission CT and during the course of VV-ECMO. Univariate comparison of patients with and without ICH on admission CT revealed that baseline thrombocytopenia and reduced creatinine clearance [CrCL] were associated with ICH. The median and interquartile range (IQR) for platelets and mean (confidence interval) for CrCL (mL/min) in patients with ICH vs without ICH on admission were 107 $X10^9$/L (77-128) vs 180 $X10^9$/L (114-219) (p=0.001) and 50 mL/min (40-55 ml/min) vs 88 ml/min (84-94 ml/min) (p<0.0001) respectively (Figures 1 and 2). Multivariate logistic regression analysis confirmed that both thrombocytopenia and reduced CrCl were independently associated with ICH on admission. Odds ratios [95% confidence interval] for the presence of ICH with thrombocytopenia and reduced CrCl were 22.6 [2.6-99.5] and 10.8 [5.6-16.2] respectively.

Mortality

Thirty-day mortality in patients with ICH at the time of initiation of VV-ECMO was significantly higher than for patients without ICH [37.5% (6/16)] vs [16.5% (22/133); p =0.03]

but there was no difference in the mortality in these two groups at 180 days; 43.7 (7/16 vs 26.3% (35/133); p=0.15.

Thirty-day mortality of patients who developed ICH during the course of VV-ECMO was 28.6% (2/7) which did not change at 180 days. Details of the seven patients who developed ICH during the course of VV-ECMO are shown in table 3.

Considering all patients with ICH (on admission and during the course of VV-ECMO), thirty-day and 180-day mortality were 34.8% (8/23) and 39.1% (9/23) respectively. The corresponding figures for patients without ICH (either or admission or during the course of VV-ECMO) were 15.8% (20/126) and 26.2% (33/126). Therefore, 71.8% (107/149) of those receiving VV-ECMO survived at 6 months [(60.9%, 14/23 with ICH and 73.8%, 93/126 without ICH); p=0.21].

Thrombotic and ischaemic events

The prevalence of cerebral ischaemic infarction was 5.4% (8/149) at the initiation of VV-ECMO and the incidence of cerebral ischaemic infarction was 3.5% (5/141) during the course of ECMO. Although the incidence of cerebral ischaemic infarction was nearly half that of ICH at the initiation of VV-ECMO, 30-day mortality rate was equal in the two groups (3/8 patients who had cerebral ischaemic infarction died, 37.5%). This includes one of the two patients (2/8) with cerebral ischaemic infarction on admission CT who developed haemorrhagic transformation on repeat CT brain during the course of ECMO. These two patients were on anticoagulation with UFH, which was started after 10 days from the diagnosis of cerebral ischaemic infarction. There was no difference in the 30-day and 180-day mortality in the patients who had cerebral ischaemic infarction at the initiation of VV-

ECMO compared to those in whom cerebral ischaemic infarction did not occur. Univariate comparison of patients with and without cerebral ischaemic infarction on admission CT did not find any specific parameter associated with an increased risk of cerebral ischaemic events. Echocardiogram of the patients with ischaemic stroke on admission or during the course of VV-ECMO did not show evidence of patent foramen ovale.

Other bleeding and thrombotic events

The incidence of major (excluding ICH) and minor bleeding (as defined by ISTH SSC criteria for major and minor bleeding) were 4.7% (7/149) and 8% (12/149) respectively during the course of VV-ECMO. Three patients (2%, 3/149) were on therapeutic anticoagulation with UFH at the initiation of VV-ECMO due to previously diagnosed VTE or acute VTE. None of these patients had ICH on admission CT brain. The overall incidence of venous thrombotic events was 6.8% (10/146). Five out 146 patients (3.4%) developed cannula or line associated thrombosis, 3 patients (2%) developed deep vein thrombosis (DVT) and two patients (1.4%) developed pulmonary embolism (PE) during the course of VV-ECMO. All of the patients except one who developed thrombosis had had no heparin or had interruption of heparin due to bleeding complications. One patient developed heparin induced thrombocytopenia and was managed successfully by anticoagulation with argatroban. Figure 3 summarises all bleeding and thrombotic events at the initiation and during the course of VV-ECMO.

Discussion

We report the bleeding and thrombotic complications from a series of 149 consecutive patients with severe respiratory failure at the initiation and during the course of VV-ECMO

from a single tertiary centre representative of current VV-ECMO practice. Although there are other retrospective studies and large registry describing ICH in VV-ECMO cohort, this study describes both bleeding and thrombotic complications in the same cohort of patients, uniformly managed. Moreover, in this retrospective analysis we were able to demonstrate a prevalence of ICH of 10.7% (16/149) already present at the initiation of VV-ECMO compared to an incidence of ICH of 5.2% (7/133) during the course of VV-ECMO. Some of the other reported studies assessing the assessing the prevalence, risk factors and outcome associated with ICH in adult patients treated with ECMO summarised in Table 4.

In a retrospective study by Lockie et al, 2017, the prevalence of ICH on admission CT was 14% [6] compared to 10.7% in our study and both studies used similar clinical ECMO protocols. The most common pattern of ICH was subarachnoid haemorrhage (56%) in that study [6], compared to parenchymal haemorrhage (8/16, 50%) in our study. The frequent occurrence of ICH within the first 24 h or days of ECMO may indicate that the underlying disease necessitating VV-ECMO is a major risk factor for development of ICH. None of the patients who developed ICH at the initiation of VV-ECMO in this study was on anticoagulation or anti-platelet treatment except for the 25-50 IU/kg bolus of UFH (maximum 5000 units) given pre-cannulation (as based on platelet count, coagulation times and perceived bleeding risk) until CT brain was performed to exclude ICH.

In our study, only 30-day, but not 180-day, mortality of patients with ICH at the time of initiation of VV-ECMO was significantly higher compared to patients without ICH [37.5% vs 16.5%; p =0.03]. Overall 71.8 % (107/149) of those who received VV-ECMO treatment survived at 6 months with no significant difference between the patients with or without ICH (60.9%, 14/23 with ICH and 73.8%, 93/126 without ICH, p=0.21). Both 30-day and 180-

day survival in patients with all ICH patients [on admission and during VV-ECMO; 65.2% (15/23) and 60.9% (14/23)] and without ICH [84.1% (116/126 and 73.8% (93/126)] are much higher in our cohort of patients compared to other reported studies [10;11;23] except the more recent report from Lockie et al [6] in which 250 patients receiving VV-ECMO had a 6-month survival of 68.3% with ICH vs 76.0% without.

In the ELSO 2016 registry report, 10,601 adults had 58% survival to hospital discharge [24]. In our cohort 71.8% patients survived at 6 months. Importantly, we and the study by Lockie et al [6], performed CT brain scans routinely in all our patients within 24hrs of initiation of VV-ECMO. The discovery of ICH prompts the withholding of further anticoagulation pending neurological assessment and may contribute to an improved neurologically intact survival. In addition, it is our practice to monitor heparin anticoagulation using an anti-Xa assay rather than APTT which we have shown can underestimate heparin levels in these patients [25]. This may contribute to the lower incidence of major and minor bleeding (5% and 8% respectively) seen during VV-ECMO in our study compared to 13.5% and 10.5% cannula and surgical haemorrhage reported in the 2016 ELSO registry [24]. We did not see a significant difference in the coagulation profile of prothrombin time (PT), APTT or fibrinogen level in patients who had ICH on admission vs those who did not although there was a trend towards APTT prolongation. In another study, it was demonstrated that although pre-ECMO coagulopathy was frequent, it did not increase the occurrence of ICH during extracorporeal support [26]. We did not reproduce the previously reported association of low fibrinogen with ICH [27].

In the present study, multivariate comparison of patients with and without ICH on admission CT scan showed that a low platelet count (p=0.001) and reduced creatinine

clearance [CrCL] ($p<0.0001$) were independently associated with ICH on admission with odds ratios [95% CI] of 22.6 [2.6- 99.5] for thrombocytopenia and 10.8 [5.6-16.2] for reduced CrCl respectively. The larger CI for thrombocytopenia indicates uncertainty in the magnitude of the risk which may be due to small sample size. An association of thrombocytopenia with ICH is in keeping with previous reports that ICH in both VV and VA ECMO was associated with thrombocytopenia [12;23]. Platelet function is impaired in patients with renal failure even with normal platelet count [26;28] and the combination of thrombocytopenia and impaired renal function may act synergistically to increase the risk of bleeding, including ICH, in these patients. This highlights an important area for potential treatment strategies to prevent ICH development. For example, it may be reasonable to consider early renal replacement therapy and platelet transfusion at lower thresholds in patients with severe respiratory failure.

Of other studies assessing factors associated with ICH and survival, one study demonstrated ICH was independently associated with renal failure at intensive care unit admission and rapid partial pressure of carbon dioxide ($PaCO_2$) decrease at ECMO initiation, but not with haemostatic parameters [11]. Seven (70%) patients with ICH and one (33%) with ischemic stroke died compared to 40% of patients who did not experience a neurological events [11]. In another retrospective study of both VV and VA-ECMO, the mortality for patients with ICH was as high as 81% at 1 month and 85% at 6 months, respectively, compared to 28% and 33% in patients who did not develop ICH [23]. In this earlier study, ICH in adult ECMO patients was independently associated with pre-admission antithrombotic therapy and low platelet count [23].

Cerebral ischaemic infarction was present in 5.4% patients within 24 h of initiation of VV-ECMO compared to 3.5% during the course of ECMO. Of note, although the incidence of ICH (16/149) was twice that of cerebral ischaemic infarction (8/149), 30-day mortality between patients between the two groups was equal (37.5%). Univariate comparison of patients with and without cerebral ischaemic infarction on admission CT did not identify any specific parameter associated with increased risk of cerebral ischaemic events. The majority of deaths in patients with ICH and ischemic stroke detected on admission CT occurred within 30-days (6 ICH patients vs only one >30days and 3 ischemic stroke patients vs none >30-days) suggesting a direct impact on mortality. Indeed, 4 out of the 6 ICH-associated deaths occurred within 30 days as a result of progressive intracranial pressure related to ICH. If the patient with ICH survived the first 30 days, they also survived to 6 months, with no further impact on survival at 6 months. The incidence of major (excluding ICH) and minor bleeding were 5% and 8% respectively during the course of VV-ECMO. The overall incidence of venous thrombotic events during the course of VV-ECMO was 6.8% (3.4% cannula or line associated thrombosis and 2% DVT and 1.4% PE). The incidence of ICH and cerebral ischaemic infarctions (5.2% and 3.5 % respectively) in our cohort of patients is in keeping with a reported incidence of ICH and cerebral ischaemic infarctions during the VV-ECMO (3.9% and 2% respectively) in 2016 ELSO registry data [24].

In a retrospective study, after excluding those who had VTE at the initiation of ECMO, 29 cases (46.1%,29/63) were found to have VTE despite systemic anticoagulation with UFH monitored by APTT during VV-ECMO [29]. In another retrospective cohort study of 103 patients treated with VV-ECMO with 81 survivors, post-decannulation venous Doppler ultrasound showed that the prevalence of DVT in the cannulated vessel was 8.1/1,000

cannula days in patients who were screened [30]. In a systematic review and meta-analysis of 8 studies with 266 patients receiving VV-ECMO, approximately 10% of the patients had DVT [31]. In our cohort of patients, the incidence of VTE was 6.8% (10/146) with 3.4 % (5/149) cannula associated thrombosis during the course of VV-ECMO. However, the incidence of asymptomatic VTE is not known as we did not perform screening Doppler scans in our patients. The incidence of VTE is not reported in ELSO registry report [24].

The main limitation of our study is that it is a retrospective, observational single-centre study with a relatively small sample size and lacks a suitable control group with severe respiratory failure. We also recognise that the inclusion in the analysis of only those patients who had undergone cerebral CT scan introduces a possible source of bias because it may exclude those with a better prognosis leading to an over-estimation of the risk of ICH and of other morbidities. However, the number of patients excluded in this way was small (15/165, 9%) and similar to that reported in other studies. For example, the largest single centre study reported to date by Lockie et al, 2017[6] excluded similar proportion of patients (34/382, 8.9%) without admission CT of the head. In their study, ICH prevalence was 14% compared to 10.7% in our cohort. We have not assessed other parameters such as factor VIII level and von Willebrand factor (VWF) activity at the initiation of VV-ECMO or during the course of ECMO to investigate the influence on these factors, especially the potential effects of acquired von Willebrand Syndrome (AVWS) in development of bleeding or thrombotic complications and how they are affected by the ECMO circuit. Ideally patients should have the CT brain prior to starting the VV-ECMO to confirm ICH or ischemic changes are not related to ECMO, but imaging prior to starting EMCO is often not practical as patients are

referred from a wide area and cannot transfer to the ECMO Centre without initiating ECMO. Therefore, imaging is usually delayed for 12 -24 hours after commencing ECMO.

However, the study also has many strengths: investigating both bleeding and thrombotic complications in patients receiving VV-ECMO and the use of a standardized admission protocol that includes screening brain CT scan within 24 hours of initiation of VV-ECMO within a single institution. This approach provides a more accurate determination of the incidence of ICH than in previous publications. The avoidance of anticoagulation in cases of ICH also provides some insight into the outcomes associated with a screening CT and an anticoagulation omission policy and also monitoring of UFH anticoagulation with heparin anti-Xa levels rather than APTT. Although, there is a large registry reporting the bleeding and thrombotic events in VV-ECMO patients, this study provides information on a cohort of patients that have been managed uniformly.

In conclusion, although this is a retrospective cohort study, it highlights the complexity of issues related to both bleeding and thrombosis in critically ill patients requiring VV-ECMO support which carry high mortality rate. Reduced CrCL and low platelet count were associated with ICH at the initiation of VV-ECMO (odds ratios [95% CI] for the presence of ICH with thrombocytopenia and reduced CrCl were 22.6 [2.6- 99.5] and 10.8 [5.6-16.2] respectively). This may act synergistically to increase the risk of bleeding in patients with severe respiratory failure. The higher incidence of ICH at the initiation of VV-ECMO suggests that ICH is more likely related to the clinical severity of the underlying disease rather than the intervention of VV-ECMO. Presence of ICH at the initiation of VV-ECMO admission is associated with early mortality in patients with severe respiratory failure. The findings of this study highlight some important areas for potential treatment strategies to prevent ICH

development in patients requiring VV-ECMO. For example, it may be reasonable to consider early renal replacement therapy and platelet transfusion at lower thresholds in patients with severe respiratory failure.

Addendum

D. R.J. Arachchillage was responsible for design of the study, acquiring data, some of the statistical analysis, interpretation of the data and writing the first draft of the manuscript. M. Laffan, J. Pepper and B Patel critically evaluated the manuscript. M Passariello, TC Aw, L Owen and S Ledot collected the data. W Banya performed statistically analysis. All authors commented and approved the final manuscript.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Legends to figures

Figure 1. Platelet counts in patients with ICH vs without ICH on admission

Figure 2. Creatinine clearance (mL/min) in patients with ICH vs without ICH on admission

Figure 3. Summary of bleeding and thrombotic complications at the initiation and during the course of VV-ECMO

Table 1: Patients' demographic data and baseline characteristics of patients with and without ICH at the initiation of VV-ECMO

Characteristics	With ICH (n=16)	Without ICH (n=133)	P value
Age (years) mean (SD)	48.8 (13.4)	45.3 (14.8)	0.37
Sex Male N (%) Female	11 (68.75) 5 (31.25)	77 (58.78) 54 (41.22)	0.44
Blood pressure [median (IQR)] Systolic diastolic	 130 (110-160) 80 [65-100)	 130 (105-160) 78(60-100)	 0.52 0.71
Body mass index (BMI) (median (IQR)	24 (19-29)	23 (18-30)	0.60
Haemoglobin (g/L) median (IQR) Male (134 –166 g/L Female (115 – 151g/L)	98.0 (88.5-107.5)	100 (91-111)	0.43
Platelets (10^9/L) median (IQR) Male (136 – 343) x10^9/L Female (147 – 397) x10^9/L	107 (77-128)	180 (114-219)	**0.001**
PT (10.2-13.2s): Median (IQR)	14.65 (11.35, 16.85)	14.0 (12.0, 16.0)	0.67
APTT (26-36s): Median (IQR)	41.65 (30.70, 43.0)	33.7 (29.5, 41.0)	0.07
Fibrinogen (1.5-4.5g/L): Median (IQR)	4.3 (2.5-5.1)	4.8 (2.1-5.3)	0.74
CrCL (80 - 120 mL/min) mean (confidence interval)	50 (40-55)	88 (84-94)	**<0.0001**
C-reactive protein (<10 mg/L): Median (IQR)	212(158-267)	232(211-254)	0.72
Indication for the VV-ECMO (%) Primary respiratory infection (bacterial or viral) ARDS secondary to systemic infection Acute interstitial lung disease Aspiration pneumonitis Asthma Other	 11/16 (68.8%) 4/16 (25%) 0 1/16 (6.2%) 0 0	 80/133 (60.6%) 18/133 (13.6%) 6/133 (4.5%) 10/133 (7.6%) 6/133 (4.6%) 13/133 (9.8%)	 0.59 0.24 - 0.92 - -
Median duration of mechanical ventilatory support prior to ECMO in Days	3 (1-4.5)	2.8 (1-4)	0.76

ICH = Intracranial haemorrhage; VV-ECMO = Veno-venous extracorporeal membrane oxygenation; SD = Standard deviation; IQR= Interquartile Range

Table 2. Type of ICH on admission CT and during the course of VV-ECMO

Type of ICH (%)	On admission	During the course of VV-ECMO
Parenchymal	8/16 (50%)	5/7 (71.4%)
Subarachnoid Haemorrhage	4/16 (25%)	1/7 (14.3%)
Parenchymal and Subarachnoid Haemorrhage	2/16 (12.5%)	1/7 (14.3%)
>two types of ICH	2/16 (12.5%)	0/7

ICH = Intracranial haemorrhage; CT VV-ECMO = Veno-venous extracorporeal membrane oxygenation; SD = Standard deviation; IQR= Interquartile Range

Table 3: Details of the seven patients who developed intracranial haemorrhage during the course of VV-ECMO with laboratory data (median values) within 24hrs of development of ICH.

Patient	Age (years)	Sex	Duration of VV-ECMO to development of ICH (days)	PT (sec)	APTT (sec)	Fibrinogen (g/L)	Heparin anti-Xa (U/mL)	Platelets (10x109/L)	CrCl (mL/min)
1	57	F	12	13.2	62.5	2.2	0.21	99	60
2	69	M	3	14.6	49.3	5.2	0.26	76	103
3	39	M	7	16.5	37.4	3.7	0.16	31	80
4	59	M	15	14.1	38.1	4.3	0.17	70	73
5	62	M	9	10.5	29.4	4.2	0.14	124	66
6	67	F	12	12.7	31.1	4.6	0.21	119	85
7	61	M	4	17.2	40.2	5.2	0.16	43	76
Overall median	61		9	14.1	38.1	4.3	0.17	76	80

PT = Prothrombin time; APTT = activated partial thromboplastin time; CrCl = creatinine clearance; F= Female; M=Male, ICH= Intracranial haemorrhage

Table 4. Studies assessing the prevalence, risk factors and outcome associated with Intracranial Haemorrhage in adults patients treated with extra corporeal membrane oxygenation

Reference	**Aims/objectives of the study**	**Study design**	**Patients**	**Results/outcome**
Lockie et al, 2017 [6]	Assess the prevalence of ICH, survival, neurologic outcomes and factors associated with ICH	Single centre, retrospective, observational study	342: 250 VV-ECMO & 92 managed using conventional ventilation	The prevalence of ICH 16.4% in ECMO and 7.6% in conventionally managed patients (p = 0.04). Duration of ventilation (d) (OR, 1.13 [95% CI, 1.03-1.23]; p = 0.011) and admission fibrinogen (g/L) (OR, 0.73 [0.57-0.91]; p = 0.009) were independently associated with ICH. There was no difference in 6-month survival in patients with and without ICH (68.3% vs 76.0%; p = 0.35).Good neurologic function was observed in 92%.
Lorusso et al,2017[13]	Assess in-hospital CNS complications in adult patients undergoing VV-ECMO	Retrospective analysis of the data registry reported from 350 international ECMO centres during 1992-2015.	4,988 patient supported with VV-ECMO	Median age was 46 (IQR, 32-58). 426 neurologic complications were reported in 356 patients (7.1%), and included 42.5% ICH, 23.5 brain deaths, 19.9% stroke, and 14.1% seizure events.In-hospital mortality was significantly higher for those with CNS complications (75.8% vs 37.8%; p < 0.001); [79.6%, 68.2% and 50% with ICH, stroke and seizures respectively]. Pre-EMCO cardiac arrest, continuous veno-venous hemofiltration, and hyperbilirubinemia during ECMO were associated with increased odds of neurologic injury.
Fletcher Sandersjöö et al, 2017[24]	Identify predictors of ICH in ECMO	Single centre retrospective observational cohort study	253 patients (161 VV- CMO & 92 VA-ECMO, 42 required conversion from one system to another at least once)	21% had ICH during ECMO treatment. The mortality in ICH was 81% at 1 month and 85% at 6 months vs 28% and 33% in patients without ICH. Pre-admission antithrombotic therapy (p = 0.018), high pre-cannulation SOFA coagulation score (p = 0.015), low platelet count (p < 0.001), and spontaneous extracranial hemorrhage (p = 0.045) were predictors of ICH. Pre-admission antithrombotic therapy and low platelet count were independent risk factors associated with ICH.
Luyt al, 2016[11]	Assess the epidemiology, risk factors, and impact	Single centre, retrospective observational	135 VV-ECMO	ICH in 10 (7.5 %), ischemic stroke in 3 (2 %), or diffuse microbleeds in 2 (2 %), occurring after respective medians (IQR) of 3 (1-11), 21 (10-26), and 36 (8-63) days post-ECMO onset.

	of cerebral complications in VV-ECMO	study		ICH was independently associated with renal failure at ICU admission and rapid PaCO2 decrease at ECMO initiation, but not with age, comorbidities, or haemostasis disorders. 70 % ICH and 33 % with ischemic stroke died vs 40 % of patients without neurological events
Nasr et al, 2015[17]	Assess the rate and outcomes of neurologic complications of patients receiving ECMO	A large, multihospital database in USA where patients were identified using the ICD-9 procedure code for ECMO	23,951 patients (not possible to distinguish between VV-ECMO and VA-ECMO.	2,604 (10.9%) suffered neurologic complications of seizure (4.1%), stroke (4.1%), or ICH (3.6%). Patients with acute ischemic stroke had significantly higher rates of discharge to a long-term facility (12.2% vs. 6.8%, p<0.0001) and a longer mean length of stay (41.6 days vs. 31.9 days, p<0.0001) vs patients with neurologic complications. Patients with ICH also had significantly higher rates of discharge to a long-term facility (9.5% vs. 6.8%, p=0.007), mortality rates (59.7% vs. 50.0%, p<0.0001), and a longer mean length of stay (41.8 days vs. 31.9 days) vs patients without neurologic complications. There was no difference in the outcome in patients with seizure vs those without neurologic complications.
Aubron et al, 2013[7]	Assess the factors associated with outcome of patients undergoing ECMO and to compare VA-ECMO with VV-ECMO	Single centre prospective review of ECMO database and patients' medical records	151: 99 VA- ECMO (66.5%) and 52 VV-ECMO (33.5%)	Bleeding and infections were the frequent complications regardless of ECMO type and >70% of the ECMO episodes were successfully weaned in each ECMO group. The overall mortality was 37.3% (37.1% VA- ECMO vs 37.7%VV ECMO). Haemorrhagic events, assessed by the total of red blood cell units received during ECMO, were associated with hospital mortality for both ECMO types.
Kasirajan et al, 1999[12]	Assess the prevalence and risk factors associated with ICH	Single centre, retrospective observational study	74 VV-ECMO	ICH was present in 18.9% Female gender (P = 0.02, odds ratio 6.5), use of heparin (P = 0.05, odds ratio 8.5), creatinine greater than 2.6 mg/ dl (P = 0.009, odds ratio 6.5), need for dialysis (P = 0.03, odds ratio 4.3) and thrombocytopenia (P = 0.007, odds ratio 18.3) showed positive correlation

				with ICH. Female gender and thrombocytopenia were independent predictors of ICH. Patients with ICH had significant mortality (92.3% vs of 61% in those without ICH (P = 0.027).

ICH= Intracranial Haemmorhage; VV-ECMO= Veno-venous extracorporeal membrane oxygenation; VA-ECMO= Veno-arterial extracorporeal membrane oxygenation; OR= odds ratio; IQR= Interquartile range; PaCO2 = Partial pressure of carbon dioxide; ICD-9= International Classification of Diseases 9th Revision; SOFA=Sepsis-related Organ Failure Assessment;

Figure 1.

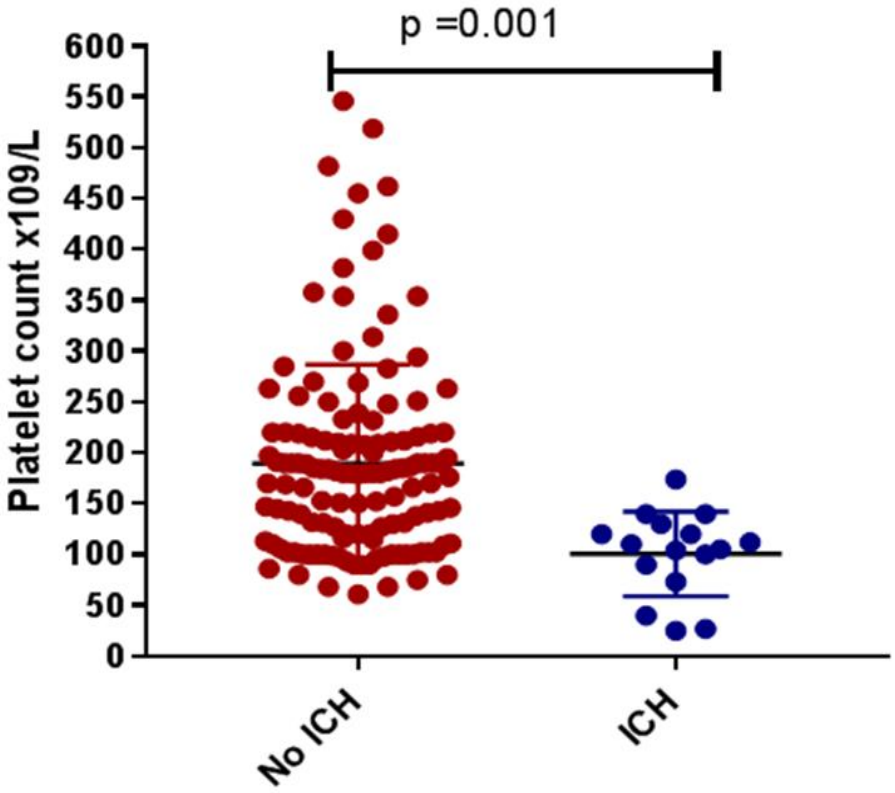

p =0.001
Platelet count x109/L
600
550
500
450
400
350
300
250
200
150
100
50
0
No ICH
ICH

Figure 2.

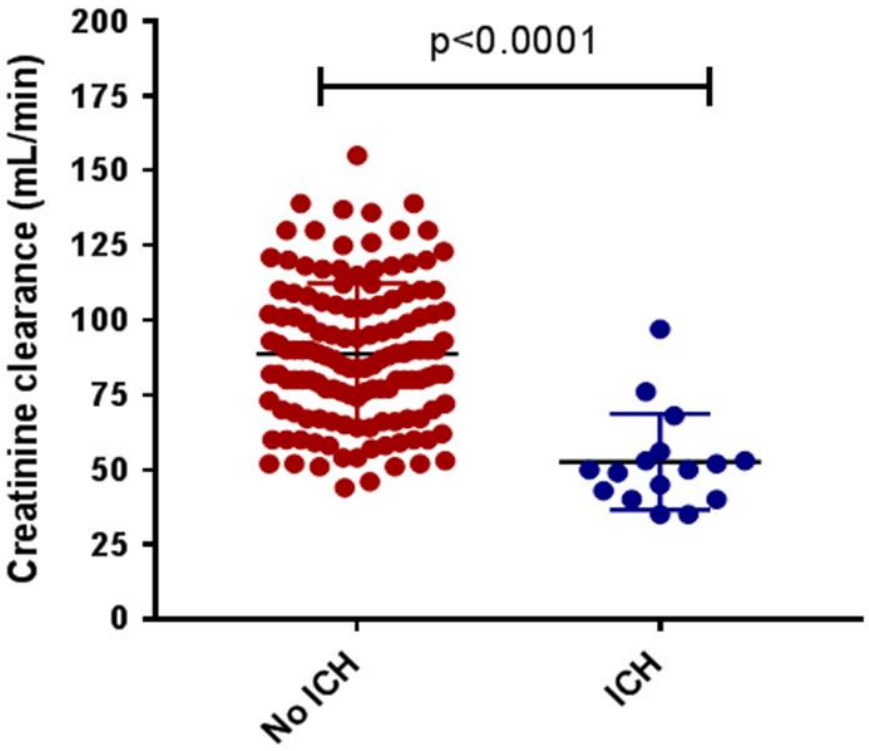
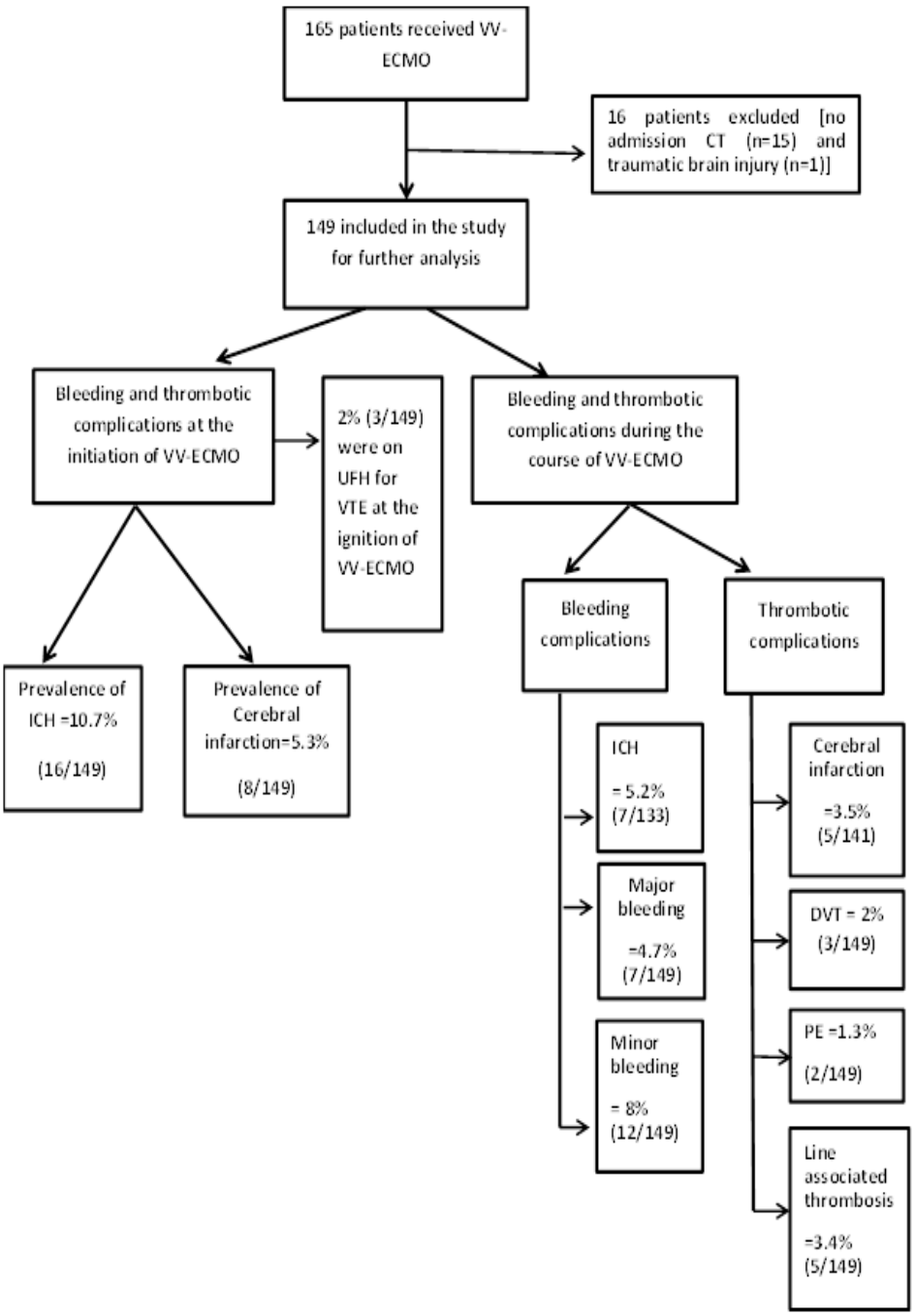

p<0.0001
Creatinine clearance (mL/min)
200
175
150
125
100
75
50
25
0
No ICH
ICH

Figure 3.


165 patients received VV-ECMO
16 patients excluded [no admission CT (n=15) and traumatic brain injury (n=1)]
149 included in the study for further analysis
Bleeding and thrombotic complications at the initiation of VV-ECMO
2% (3/149) were on UFH for VTE at the ignition of VV-ECMO
Bleeding and thrombotic complications during the course of VV-ECMO
Prevalence of ICH =10.7% (16/149)
Prevalence of Cerebral infarction=5.3% (8/149)
Bleeding complications
Thrombotic complications
ICH = 5.2% (7/133)
Major bleeding =4.7% (7/149)
Minor bleeding = 8% (12/149)
Cerebral infarction =3.5% (5/141)
DVT = 2% (3/149)
PE =1.3% (2/149)
Line associated thrombosis =3.4% (5/149)