Predicting Transfusion Requirements During Extracorporeal Membrane Oxygenation

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Objective: Patients requiring extracorporeal membrane oxygenation (ECMO) have a well-known bleeding risk and the potential for experiencing possibly fatal thromboembolic complications. Risk factors and predictors of transfusion requirements during ECMO support remain uncertain. The authors hypothesized that compromised organ function immediately before ECMO support will influence transfusion requirements.

Design: A prospective observational study.

Setting: A tertiary, single-institutional university hospital.

Participants: The study included 40 adult patients requiring ECMO for intractable cardiac and respiratory failure between July 2010 and December 2012. Blood samples were taken before initiation of ECMO (baseline), after 24 and 48 hours on ECMO, and 24 hours after termination of ECMO.

Interventions: None.

Measurements and Main Results: Independent of veno-arterial or veno-venous support, 26% of patients required ≥2 packed red blood cells per day (PRBC/d) and 74% of patients required <2 PRBC/d during ECMO. Requirements of ≥2 PRBC/d during ECMO support were associated with higher creatinine levels and lower prothrombin times (PT, %) at baseline and with impaired platelet function after 24 hours on ECMO. Platelet function, activated by thrombin receptor-activating peptide stimulation, decreased by 30% to 40% over time on ECMO. Receiver operating characteristic curve analysis showed cut-off values for creatinine of 1.49 mg/dL (sensitivity 70%, specificity 70%; area under the curve [AUC] 0.76, 95% confidence interval [CI] 0.58-0.94), for PT of 48% (sensitivity 80%, specificity 59%; AUC 0.69, 95% CI 0.50-0.87), and for thrombin receptor-activating peptide (TRAP) 32 U (sensitivity 90%, specificity 68%; AUC 0.76, 95% CI 0.59-0.93).

Conclusions: The results of this study demonstrated that increased creatinine levels and lower PT before ECMO and secondary impaired platelet function significantly increased transfusion requirement.

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KEY WORDS: extracorporeal membrane oxygenation (ECMO), transfusion requirement, creatinine, prothrombin time, thrombin receptor-activating peptide (TRAP), whole blood impedance aggregometry

BLEEDING AND THROMBOEMBOLIC complications in patients undergoing mechanical circulatory support, such as extracorporeal membrane oxygenation (ECMO), are common and associated with poor outcome.[1,2] In adults, 2 types of ECMO systems are used: veno-venous ECMO in respiratory failure and veno-arterial ECMO in heart failure.[3,4] On the one hand, ECMO systems are associated with inflammatory response and a hypercoagulable state. On the other hand, there is a high risk for bleeding complications, particularly in combination with increased anticoagulation and due to consumption of coagulation factors and platelets.[5] Unfractionated heparin (UFH) is the most commonly administered anticoagulant. The preferred method of anticoagulation monitoring during ECMO support is serial measurement of activated coagulation time (ACT).[6,7] Bleeding and thromboembolic complications often occur despite coagulant screening tests and ACT measurement values in the target range.[5]

The authors recently showed that short-term loss of high-molecular–weight von Willebrand factor (HMW vWF) bands occurs in all patients undergoing ECMO.[8] Moreover, in the same cohort the authors recently investigated various parameters of blood coagulation, platelet, and organ function. Primary endpoints were predictors of increased transfusion requirement during ECMO support. Blood loss was estimated indirectly by assessment of transfused packed red blood cells (PRBC).[9] In addition to various parameters of blood coagulation, the authors determined metabolic parameters and organ function, which also may influence transfusion requirement. Risk factors and predictors of transfusion requirement during ECMO support remain uncertain. The primary hypothesis was that compromised organ function immediately before ECMO support will influence transfusion requirement. The secondary hypothesis was that coagulation changes during support will influence transfusion requirement. The aim of this study was to present real-life laboratory results during ECMO support that may influence transfusion behavior of clinicians in such patients.

METHODS

This prospective, observational study was approved by the Ethics Committee of the Medical University of Innsbruck (UN4013;289/4.3). Because ECMO is an emergency intervention and the patients usually are sedated, unconscious, and already intubated before the decision for this intervention, written informed consent was obtained after recovery in survivors. The exclusion criterion was age <18 years.

In the interest of clarity and perspective, data on changes in vWF multimers, ristocetin cofactor activity, and factor VIII activity are presented separately.[8] Data that had not been the subject of the previous study article, but were recorded

simultaneously during the study period, make up the content of this article. Separate publication of these data was planned a priori.

ECMO Management

ECMO management during the study period was as follows: after induction of anesthesia with fentanyl, midazolam, and rocuronium and before insertion of the ECMO cannula, 100 IU/kg of UFH (Heparin Gilvasan; Gilvasan Pharma GmbH, Vienna, Austria) was routinely administered. During ECMO support, the ACT was maintained between 150 and 180 seconds (Hemochron Jr Signature, Accriva Diagnostics, San Diego, CA) by continuous administration of UFH. Details on ECMO support of these patients were described previously.[8]

Study Protocol

Blood sampling and recording of administered blood products were performed at the following 4 time points: before initiation of ECMO support (baseline, before administration of heparin and before insertion of cannulae), after 24 hours on ECMO support, after 48 hours on ECMO support, and 24 hours after ECMO termination. Blood samples were obtained through a 3-Fr radial catheter (Becton Dickinson, Swindon, UK) inserted for clinical indication. To diminish laboratory errors, more than 3 mL of blood were discarded. For analysis of coagulation parameters and rotational thromboelastometry (ROTEM; Tem International GmbH, Basel, Switzerland), samples were put in 3-mL tubes containing 0.3 mL (0.106 mol/L) of buffered (pH 5.5) sodium citrate (Sarstedt, Nümbrecht, Germany). For platelet function testing, samples were put in 2.7-mL tubes containing hirudin (Sarstedt, Nümbrecht, Germany).

Laboratory Analysis

Hemoglobin (reference range: 130-177 g/L), platelet count (150-380 g/L), and white blood cell count (4-10 g/L) were measured in 2.7-mL tubes containing 1.6 mg ethylenediaminetetraacetic (EDTA) acid/mL blood (Sarstedt, Nümbrecht, Germany) using the whole blood counter Sysmex XE-5000 (Sysmex, Norderstedt, Germany).

Prothrombin time (Thromborel S; reference range: 70%-130%), activated partial thromboplastin time (PTT) (Pathromtin SL; reference range: 26-37 s), antithrombin (Berichrom Antithrombin III; reference range: 79%-112%), fibrinogen concentration (Multifibren U; reference range: 210-400 mg/dL) and D-dimers (Innovance D-dimer test; reference range: 0-500 mg/L) were determined using assays from Siemens Healthcare, Marburg, Germany.

ROTEM was performed with the ROTEM Delta coagulation analyzer (Tem Innovations, Munich, Germany), which is based on the thromboelastograph system (Haemonetics, Braintree, MA). The measurement principle and interpretation of results are described in detail elsewhere.[10] In each case the ROTEM device was checked for proper function using a control sample (ROTROL; Tem Innovations, Munich, Germany) according to the manufacturer's recommendations. All tests were performed following the ROTEM manufacturer's specifications within 30 minutes after collecting the sample

using the STARTEM (calcium chloride), INTEM (ellagic acid, does not contain heparinase), EXTEM (tissue factor, heparinase), and FIBTEM (containing the platelet-blocking substance cytochalasin D and heparinase) assays (Tem Innovations, Munich, Germany). The following variables were determined for the INTEM and EXTEM assays: coagulation time (CT) (reference range EXTEM 38-79 sec, INTEM 100-240 sec), clot formation time (reference range EXTEM 34-179 sec, INTEM 30-110 sec), maximum clot firmness (MCF) (reference range EXTEM and INTEM 50-72 mm), α angle (reference range EXTEM 63°-83°, INTEM 70°-83°), and lysis index 30 minutes (LI30) and 60 minutes (LI60) (reference range ≥ 85% for both, describing the percentage of maximum clot strength present at 30 and 60 minutes). From the FIBTEM assay (allowing fibrinogen's contribution to clot strength to be measured), the MCF was recorded (FIBTEM MCF; reference range 9-25 mm).

Platelet function testing was performed using point-of-care whole blood impedance aggregometry (WBA) (Multiplate analyzer; Roche Diagnostics GmbH, Vienna, Austria). The Multiplate measurement principle and interpretation of results are described in detail elsewhere.[11] The following Multiplate tests were ordered from the manufacturer and performed according to the manufacturer's specifications: TRAPtest ([M-TRAP], containing thrombin receptor-activating peptide; reference range: 42-144 U), ASPItest ([M-AA], containing arachidonic acid; reference range: 75-136 U), and adenosine diphosphate (ADP) test ([M-ADP], containing ADP; reference range: 53-122 U).

Thrombin generation was determined as described previously.[12] Blood samples were centrifuged at 2,200 g over 15 minutes to obtain platelet-poor plasma. Platelet-poor plasma was stored in aliquots at −80°C for further analysis. Thrombograms were measured using the Calibrated Automated Thrombogram system (Thrombinoscope, Maastricht, Netherlands) with a fully automated, computer-controlled fluorometric microplate reader and specified software. Each thrombin generation was performed in duplicate, with the calibrator being added in a third well according to the manufacturer's recommendations (CL well). This process guaranteed correction for color of the tested plasma sample. The final concentrations of tissue factor and phospholipids were 5 pmol/L and 4 μmol/L, respectively. The following parameters were analyzed: lag time (min), time-to-peak (min), peak high (nmol), and endogenous thrombin potential (nmol × min).

Creatinine (CREP2 assay; reference range: 0.67-1.17 mg/dL), urea (UREAL; 18-55 mg/dL), bilirubin (BILT2; 0-1.28 mg/dL), glutamate oxalacetal transaminase (ASTEM; 10-50 U/L), glutamate pyruvate transaminase (ALTPM; 10-50 U/L), gamma glutamyl transferase (GGT-2; 20-71 U/L), lactate dehydrogenase (LDHI2; 100-250 U/L), and lactate (LACT2; 4.5-19.8 mg/dL) were measured using the tests from Roche (Mannheim, Germany) as previously mentioned.

Transfusion Practice and Definition of Increased Requirement of Packed Red Blood Cells Per Day on ECMO

Patients were treated according to clinical routine. Anesthesiologists, intensivists, and surgeons were blinded to the

laboratory results. According to clinical routine, PRBC depleted of leukocytes were transfused when hemoglobin was below 85 g/L. Apheresis platelet concentrates were administered when the platelet count was below 70 G/L.[1,5] Fresh frozen plasma (FFP) or prothrombin complex concentrate (PCC) was given in patients demonstrating reduced PT (<1.5 of the normal value). Fibrinogen concentrate was administered in the case of fibrinogen values lower than 250 mg/dL, or FIBTEM-MCF values lower than 12 mm.

The following administered blood products were recorded for every day on ECMO: units of PRBC, FFP, apheresis platelet concentrates, PCC (Prothromplex Total 600 IU; Baxter, Vienna, Austria), and fibrinogen concentrate (mg) (Haemo-complettan; CSL Behring, Vienna, Austria).

Increased transfusion requirement during ECMO was defined as a PRBC/d requirement ranging over the third quartile of the study population, which in this study referred to $\geq 2U$ PRBC/d. Patients receiving $<2U$ PRBC/d were pooled in the moderate transfusion requirement group. Comparable amounts of required PRBC/d on ECMO are reported in the literature.[1,13-16] Because the study start was predefined as ECMO support start, blood products given in surgical bleeding situations before ECMO start were not within the scope of this study and were not included in the analysis.

Statistical Analysis

The a priori power analysis resulting in a planned sample size of 40 patients was performed to address the research question investigated in the vWF study.[8] However, an a posteriori power analysis was conducted for this study using the areas under the curve (AUCs) of the receiver operating characteristic (ROC) curves. This analysis revealed that a sample size of 38 patients, in which one-fourth of all patients required excessive transfusion (ie, 10 v 28 patients, which matched the numbers observed in this study), allowed detection of a difference in the area under the ROC curve of 0.80 versus 0.50 (meaning no predictive power) on a significance level of $\alpha = 0.05$ with a power of 80%. Thus, in short, the authors were able to detect an AUC of at least 0.80 with a confidence of at least 80% in this study.

All patient characteristics and laboratory measurements were analyzed descriptively (absolute and relative frequency for qualitative, mean, and standard deviation [SD] for quantitative data) overall and stratified for time points. Student's t-test or Fisher's exact test (for dichotomous data) was performed to test for differences between groups.

To test for differences in means among the 4 time points, univariate one-way repeated-measures analysis of variance was performed for each coagulation and laboratory parameter. To account for possible sphericity violation among time points, p values were corrected according to the Greenhouse-Geisser method. If the p value of the within-subject effect time point was statistically significant ($p < 0.05$), pairwise comparisons (time points: baseline v 24 h on ECMO; baseline v 48 h on ECMO support; baseline v 24 h after ECMO support; and 24 v 48 h on ECMO support) were calculated with paired t tests as post hoc tests. Bonferroni correction for multiple testing was used.

Univariate analysis of coagulation and laboratory values comparing patients with moderate PRBC/d transfusion requirement and patients with increased PRBC/d requirement on ECMO was performed for baseline values and values after 24 hours on ECMO using Student's t-test. Because mean values for creatinine at baseline, PT at baseline, and M-TRAP after 24 hours on ECMO demonstrated associations with transfusion requirement in these univariate tests (significant at $p < 0.05$ for creatinine and M-TRAP; PT marginally non-significant [$p = 0.055$], but deemed clinically relevant), the authors further investigated the potential of these parameters to predict increased PRBC/d requirement on ECMO. The association among these 3 parameters and transfusion requirement was confirmed in a multivariate logistic regression model. Furthermore, the authors constructed ROC curves (univariately and multivariately for the probability score of the logistic regression) and calculated the AUC, specificity, positive predictive value, negative predictive value, and accuracy for various cut-off values, including the value for which the sum of sensitivity and specificity was highest. The 95% confidence interval (CI) was calculated for all these parameters according to the DeLong method for AUC and with bootstrapping on 2,000 replications for the other performance measures. A significance level of $\alpha = 0.05$ (2-sided) was used. Statistical analyses were performed using SPSS, version 22.0 (IBM Corp, Armonk, NY) and R, version 3.1.1 (R Foundation, Vienna, Austria).

RESULTS

Demographics and Procedural Data

Forty adult patients requiring ECMO support due to refractory cardiac and/or pulmonary failure were prospectively enrolled in this observational study. Two patients died within 24 hours after initiation of ECMO and were excluded from analysis. Thus, data from 38 patients were available for analysis (veno-arterial ECMO, n = 26; veno-venous ECMO, n = 12). Calculation of the requirement of the number of PRBC/d per patient during ECMO support demonstrated that 2 PRBC/d per patient fell over the 75th percentile and led the authors to define moderate (<2 PRBC/d, n = 28) and increased (>2 PRBC/d, n = 10) transfusion requirement groups. Patients with moderate and increased transfusion requirement showed no differences in demographics, antiplatelet therapy within 5 days before surgery, flow characteristics after 24 hours on ECMO, and type of ECMO support (Table 1). Because there were no differences between veno-arterial and veno-venous ECMO concerning cannula type, vascular access, pump type, or several laboratory values (data not shown), data were combined for analysis. For the sake of clarity and conciseness, only significant and clinically relevant data are detailed. Further information is included in Tables 2 to 4 and in Supplementary Tables 1 to 3.

Laboratory Data (Combined Data from Both Groups Over All Measurement Points)

Blood Cell Count and Coagulation Values

Platelet count (p = 0.001) changed over time. Compared with baseline, platelet count decreased after 24 hours

Table 1. Demographics and Procedural Data

	Moderate Transfusion Requirement (<2 Packed Red Blood Cells/d) n = 28 (74%)	Increased Transfusion Requirement (≥2 Packed Red Blood Cells/d) n = 10 (26%)	p Value
Demographics			
Age (yr)	52 ± 14	50 ± 15	0.679
Body weight (kg)	72 ± 18	77 ± 12	0.317
Body height (cm)	172 ± 9	170 ± 7	0.442
Body surface area	1.8 ± 0.2	1.9 ± 0.1	0.521
Antiplatelet therapy within 5 days before surgery			
	n = 10/28 (36%)	n = 3/10 (30%)	1.000
Flow characteristics after 24 h on ECMO			
Flow (L/min)	4.5 ± 0.9	4.2 ± 0.7	0.362
Rates per minute	3665 ± 519	3507 ± 532	0.425
Veno-venous ECMO (n = 12/32%)			
	n = 8/28	n = 4/10	0.694
Veno-arterial ECMO (n = 26/68%)			
	n = 20/28	n = 6/10	0.694

NOTE. Results of univariate analysis of demographics, antiplatelet therapy within 5 days before surgery, flow characteristics after 24 hours on extracorporeal membrane oxygenation, and kind of support comparing patients with moderate and increased transfusion requirement during extracorporeal membrane oxygenation using Student's *t*-test or Fisher's exact test (statistically significant: p < 0.05). Data are presented as mean ± SD or frequencies.

Abbreviation: ECMO, extracorporeal membrane oxygenation.

(p < 0.001) and 48 hours (p < 0.001) on ECMO. After termination of ECMO, values did not differ from baseline. Hemoglobin values did not change over time (p = 0.249). PT significantly changed over time (p < 0.0001), demonstrating higher values after 24 hours (p = 0.012), 48 hours on ECMO (p < 0.001), and after termination of ECMO (p < 0.004) than at baseline. PTT did not change significantly over time. Fibrinogen values increased over time (p = 0.012), but no differences between individual measurement points could be detected. D-dimers remained unchanged over time. ACT values did not differ between 24 hours and 48 hours on ECMO (see Table 2).

ROTEM Parameters

EXTEM-CT did not change over time (p = 0.111). EXTEM-MCF (p < 0.0001) changed over time. Compared with baseline, EXTEM-MCF was lower after 24 hours (p < 0.0001) and 48 hours (p < 0.0001) on ECMO. No hyperfibrinolysis was detected (see Supplementary Table 1).

INTEM-CT did not change over time (p = 0.677). INTEM-MCF changed significantly over time (p < 0.0001). INTEM-MCF was lower after 24 hours (p < 0.0001) and 48 hours (p < 0.0001) on ECMO. There were no signs of hyperfibrinolysis.

FIBTEM-MCF did not change over time (p = 0.168) (see Supplementary Table 1).

Multiplate Whole Blood Aggregometry

M-TRAP (p = 0.013), M-ADP (p = 0.008), and M-AA (p = 0.014) changed over time. Compared with baseline, values were lower after 24 hours on ECMO (M-TRAP: p = 0.004; M-ADP: p = 0.004; M-AA: p = 0.028). After 48 hours on ECMO, M-TRAP did not differ from baseline (p = 0.184); M-ADP (p = 0.016) and M-AA (p = 0.044) were still lower than baseline values. M-TRAP, M-ADP, and M-AA reached baseline values after weaning from ECMO (see Table 3).

Thrombin Generation

Peak high (p = 0.824) and endogenous thrombin potential (p = 0.903) did not change over time. Lag time (p = 0.012) and time-to-peak (p = 0.008) changed over time. Compared with baseline, lag time values were higher after ECMO support (p = 0.004). In contrast, time-to-peak values did not differ significantly in any pairwise comparison of measurement points (see Table 4).

Other Laboratory Results

L-lactate levels changed over time (p < 0.0001). Compared with baseline, values were lower after 24 hours (p < 0.0001) and 48 hours (p < 0.0001) on ECMO and remained lower after ECMO termination (p < 0.0001). Urea increased over time (p = < 0.001), demonstrating higher values after ECMO (p = 0.004) than at baseline. Glutamate oxalacetal transaminase changed over time (p = 0.027), without reaching significance for any pairwise comparison of measurement points. Creatinine, bilirubin, glutamate pyruvate transaminase, gamma glutamyl transferase, and lactate dehydrogenase did not change over time (see Supplementary Table 2).

Transfusion Requirements During ECMO

All patients required blood transfusions. PRBC calculated per day on ECMO ranged from 0.1 to 7.6 (median 1.30; interquartile range 1.0-2.0). Seventy-four percent (n = 28) needed <2 PRBC/d (moderate transfusion requirement). Increased transfusion requirement (≥2 PRBC/d) was seen in 26% (n = 10) of patients. Patients with increased PRBC/d requirement also received more platelet concentrates. In contrast, amounts of administered FFP, fibrinogen concentrates, and PCC were comparable between groups (Table 5).

Univariate Analysis Comparing Patients With Moderate and Increased Transfusion Requirement

After 24 hours on ECMO, hemoglobin was higher in patients with moderate transfusion requirement (mean ± SD),

Table 2. Blood Cell Count and Coagulation Values

Variables (Normal Range)	Baseline	24 h on ECMO	48 h on ECMO	24 h after ECMO	Overall p	p (Baseline v 24 h on ECMO)	p (Baseline v 48 h on ECMO)	p (Baseline v 24 h after ECMO)	p (24 h v 48 h on ECMO)
Platelet count (150-380 G/L)	134 ± 95	84 ± 57	73 ± 66	122 ± 112	0.001	<0.001	<0.001	1	0.296
White blood cells (4-10 G/L)	13.6 ± 7.8	10.3 ± 6.1	10.9 ± 5.2	13.6 ± 6.0	0.036	<0.001	0.148	1	1
Hemoglobin (130-177 g/L)	125 ± 14	94 ± 11	98 ± 10	106 ± 13	0.249	0.704	0.920	1	0.212
Prothrombin time (70%-130%)	49 ± 22	52 ± 17	63 ± 14	72 ± 18	<0.001	1	0.012	<0.001	0.004
Partial thromboplastin time (26-37 sec)	72 ± 58	62 ± 30	57 ± 20	46 ± 12	0.051	1	1	0.048	1
Fibrinogen (210-400 mg/dL)	377 ± 235	340 ± 171	402 ± 188	478 ± 198	0.012	0.736	1	0.124	0.120
D-dimer (0.0-500 µg/L)	6,650 ± 7,411	8,245 ± 9,360	9,102 ± 8,029	9,623 ± 7,626	0.243	1	0.780	0.804	0.836
Antithrombin (79%-112%)	61 ± 21	56 ± 20	57 ± 18	76 ± 21	<0.001	0.588	1	<0.001	1
Activated clotting time	—	182 ± 39	178 ± 16	—	0.870	—	—	—	—

NOTE. Data are presented as mean ± SD. Overall p values are from univariate one-way repeated-measures analysis of variance corrected according to the Greenhouse-Geisser method; post hoc p values are from paired t tests corrected for multiple testing according to the Bonferroni method; p values are considered significant when $p < 0.05$.

Abbreviation: ECMO, extracorporeal membrane oxygenation.

namely 96 ± 11g/dL compared with patients with increased transfusion requirement (88 ± 9 g/dL; p = 0.025). PTT after 24 hours on ECMO was lower in patients with moderate transfusion requirement (57 ± 18 s) than in patients with increased transfusion requirement (79 ± 47 s; p = 0.040). M-TRAP after 24 hours on ECMO was higher in patients with moderate transfusion requirement (44 ± 23 U) than in patients with increased transfusion requirement (24 ± 12 U; p = 0.002). Creatinine was lower in patients with moderate transfusion at baseline (1.2 ± 0.5 mg/dL) and after 24 hours on ECMO (1.3 ± 0.69 mg/dL) compared with patients with increased transfusion requirement at baseline (2.3 ± 1.5 mg/dL; p = 0.004) or after 24 hours on ECMO (2.2 ± 1.1 mg/dL; p > 0.002) (Table 6, see Supplementary Table 3).

ROC Analysis and Results of Multivariate Logistic Regression on Prediction of Increased Transfusion Requirement

Baseline creatinine, baseline PT, and M-TRAP after 24 hours on ECMO were used to investigate the potential to predict moderate and increased transfusion requirement. PT was included despite being marginally nonsignificant in a univariate Student's t-test, but deemed clinically relevant (see Table 6). PTT values after 24 hours on ECMO were excluded (heparin anticoagulation). Hemoglobin values after 24 hours on ECMO were excluded; all patients received PRBC (see Supplementary Table 3).

ROC analysis showed cut-off values to predict increased transfusion requirement for creatinine, namely 1.49 mg/dL (sensitivity 70%, specificity 70%; AUC 0.76, 95% CI 0.58-0.94), for prothrombin time 48% (sensitivity 80%, specificity 59.3%; AUC 0.69, 95% CI 0.50-0.87), and for M-TRAP 32 U (sensitivity 90%, specificity 68%; AUC 0.76, 95% CI 0.59-0.93) (Table 7, Fig 1). Combined multivariate ROC analysis of all 3 parameters showed an AUC of 0.9 (95% CI 0.79-1.00) (see Fig 1). Multivariate analysis demonstrated the 3 parameters together to have a highly significant combined effect on increased transfusion requirement (p < 0.001), with adjusted odds ratios for creatinine being 10.7 (95% CI 0.89-127.64; p = 0.061), for PT 0.93 (95% CI 0.86-0.99; p = 0.036), and for M-TRAP 0.90 (95% CI 0.80-1.00; p = 0.048).

DISCUSSION

In a prospective cohort of 40 adults treated at the authors' institution, predictors of transfusion requirement during ECMO support were investigated. Blood loss was estimated indirectly by assessing transfused PRBC. Independently of veno-arterial or veno-venous support, 26% of patients required ≥2 PRBC/d and 74% of patients required <2 PRBC/d during ECMO. Requirement of ≥2 PRBC/d during ECMO support was associated with higher creatinine levels and lower PT at baseline. Platelet function, measured using Multiplate WBA, decreased by 30% to 40% over time. This effect was more pronounced in patients receiving ≥2 PRBC/d than in patients administered <2 PRBC/d. The platelet count was cut nearly in half during ECMO support. Simultaneously, MCF measured using thromboelastometry decreased. Within 24 hours after weaning from ECMO, platelet count, platelet function, and

Table 3. Results of Multiplate Whole Blood Aggregometry

Variables (Normal Range)	Baseline	24 h on ECMO	48 h on ECMO	24 h after ECMO	Overall p	p (Baseline v 24 h on ECMO)	p (Baseline v 48 h on ECMO)	p (Baseline v 24 h after ECMO)	p (24 h v 48 h on ECMO)
Multiplate-TRAP (42-144 U)	57 ± 35	38 ± 21	37 ± 29	54 ± 33	0.013	0.004	0.184	0.868	1
Multiplate-ADP (53-122 U)	33 ± 32	16 ± 13	18 ± 17	30 ± 29	0.008	0.004	0.016	0.392	1
Multiplate-AA (75-136 U)	30 ± 32	15 ± 16	15 ± 15	36 ± 32	0.014	0.028	0.044	0.976	1

NOTE. Data are presented as mean ± SD. Overall p values are from univariate one-way repeated-measures analysis of variance corrected according to the Greenhouse-Geisser method; post hoc p values are from paired t tests corrected for multiple testing according to the Bonferroni method. p values are considered significant when $p < 0.05$.

Abbreviations: ECMO, extracorporeal membrane oxygenation; TRAP, thrombin receptor-activating peptide; ADP, adenosine diphosphate; AA, arachidonic acid.

MCF returned to baseline values. Initiation of clot formation (ROTEM CT) was not affected by ECMO support. Fibrinogen concentration and fibrin polymerization even enhanced during ECMO support. Hyperfibrinolysis did not occur. PT significantly changed over time, showing improvement after 24 and 48 hours and termination of ECMO support. PTT and thrombin generation (IIa) measured after the method of Hemker[17] did not change over time, although patients were anticoagulated with UFH. Previously demonstrated loss of HMW vWF multimer bands during ECMO support did not influence transfusion requirement.[8]

Reported numbers of daily required PRBC during ECMO in this study were independent of veno-arterial and veno-venous support. This finding was in accordance with published data.[13,14] Of note, in other studies,[13,14] numbers of transfused platelet concentrates were higher than at the authors' institution. The lower target platelet count at the authors' institution (70 G/L v 100G/L) may have contributed, at least partly, to this finding. Administration of blood products differed. In selected cases, the authors used fibrinogen concentrates and PCC. Administration of blood products did not result in any thromboembolic complication.

Depending on severity of disease, circulatory and respiratory restriction lead to renal and hepatic impairment and shock.[18-21] ECMO support is reserved for patients with end-stage cardiac and pulmonary failure. Increased creatinine plasma levels are an expression of renal disturbance in these severely diseased patients. Moreover, a high creatinine level is a predictor of bleeding (see Fig 1). Patients with a high PRBC requirement showed increased creatinine values (1.2 v 2.3 mg/dL) before ECMO support. This finding persisted in selected patients despite recovery of circulation and additional continuous veno-venous hemofiltration. Lower PT before ECMO likely reflected liver congestion due to low cardiac output and reduced synthetic capacity.[18,21] Continuously improving PT values during ECMO support supposedly indicated recovery.[22]

Platelet function and count are critically dependent on numerous factors, including severity of disease and treatment modality. Decreased adhesion and aggregation in patients with renal impairment are due to a disturbance in platelet α granules and an impaired synthesis of thromboxane A_2.[20] Enlargement of the liver and spleen due to venous hypertension influences platelet count via pooling. Patients in this study demonstrated a significant decrease in platelet function during ECMO. M-AA and M-ADP were cut nearly in half, and M-TRAP was reduced by 30%. Attenuation of platelet function after 24 hours on ECMO measured using M-TRAP was more pronounced in patients with increased transfusion requirement. TRAP is the most potent activator of platelets in vitro and it mimics in vivo thrombin effect. Thrombin induces activation of GPIIb-IIIa and is a powerful amplifier of platelet function.[23] The strong aggregation effect of M-TRAP is not sensitive to cyclooxygenase-1 inhibitors or ADP-receptor-blocking agents, such as clopidogrel.[24] Because thrombin is the most potent activator of platelets, a reduced TRAPtest finding may indicate an overall impaired platelet function.[25]

Activation of platelets is one of the primary events occurring after blood comes into contact with artificial surfaces in oxygenators, tubes, pumps, cannulae, and other equipment. Oxygenators have a large blood-contacting surface (up to 2-3 m^2) and are presumed to play a major role in platelet activation and

Table 4. Thrombin Generation in Platelet-Poor Plasma

Variables (Normal Range)	Baseline	24 h on ECMO	48 h on ECMO	24 h after ECMO	Overall p	p (Baseline v 24 h on ECMO)	p (Baseline v 48 h on ECMO)	p (Baseline v 24 h after ECMO)	p (24 h v 48 h on ECMO)
Peak high (nmol)	197 ± 88	184 ± 96	168 ± 92	193 ± 106	0.824	1	1	1	1
ETP (nmol × min)	996 ± 446	905 ± 417	900 ± 471	905 ± 494	0.903	0.780	1	1	1
Lag time (min)	3.6 ± 2.3	3.1 ± 1.1	3.7 ± 2.2	5.1 ± 2.4	0.012	1	0.436	0.004	0.692
Time-to-peak (min)	6.3 ± 3.2	5.7 ± 2.5	6.4 ± 2.5	7.8 ± 2.5	0.008	0.960	1	0.084	1

NOTE. Data are presented as mean ± SD. Overall p values are from univariate one-way repeated-measures analysis of variance corrected according to the Greenhouse-Geisser method; post hoc p values are from paired t tests corrected for multiple testing according to the Bonferroni method; p values are considered statistically significant when $p < 0.05$.

Abbreviations: ECMO, extracorporeal membrane oxygenation; ETP, endogenous thrombin potential.

Table 5. Comparison of Blood Products Required by Patients Undergoing Extracorporeal Membrane Oxygenation With Moderate and Increased Transfusion Requirement of Packed Red Blood Cells Per Day

	Patients with Moderate Transfusion Requirement (<2 Packed Red Blood Cells/d) n = 28 (74%)	Patients with Increased Transfusion Requirement (≥2 Packed Red Blood Cells/d) n = 10 (26%)	p Value
Packed red blood cells/d	1.1 ± 0.4	3.2 ± 1.8	0.006
Fresh frozen plasma/d	0.5 ± 1.0	0.6 ± 0.9	0.873
Apheresis platelet concentrates/d	0.4 ± 0.5	0.8 ± 0.8	0.002
Fibrinogen concentrate (mg/d)	230 ± 41	330 ± 66	0.607
Prothrombin complex concentrate (U/d)	15 ± 66	96 ± 206	0.250

NOTE. Data are presented as mean ± SD.

consumption during extracorporeal circulation.[26] Paul et al[26] quantified platelet adhesion to artificial surfaces by using Western blot antibodies directed against the platelet receptor GPIIb-IIIa (CD 41) and demonstrated the strong influence of coated surfaces. Their study was limited to 120 minutes. All oxygenators and tubes used in the study presented here had a commercially available albumin-heparin composition surface. Urlesberger et al[27] investigated the effect on coagulation of heparin-coated versus noncoated systems in pigs undergoing ECMO for 24 hours. Coated systems demonstrated delayed clotting activation, but after 6 hours no differences between groups were detectable. In the study presented here, platelet count continuously decreased in both groups during ECMO. In accordance with these experimental findings, patients demonstrated decreased platelet count during ECMO. Thrombocytopenia during ECMO was accompanied by a decrease in maximum clot firmness (MCF) and an increase in clot formation time. Remarkably, EXTEM MCF values remained within the normal (lower) range on ECMO, but INTEM MCF values were below the normal range during support. In contrast, FIBTEM MCF values and fibrinogen concentrations increased during ECMO and remained within the upper normal range after weaning from ECMO. Thromboelastometry is highly dependent on fibrinogen. The increase in fibrinogen and FIBTEM MCF during support is likely due to activation of the inflammatory system. Inflammation itself provokes activation and consumption of the clotting

Table 6. Results of Univariate Analysis (p < 0.05) of Coagulation and Laboratory Values Used to Investigate Discriminatory Power to Predict Moderate and Increased Transfusion Requirement During Extracorporeal Membrane Oxygenation

	Moderate Transfusion Requirement (<2 Packed Red Blood Cells/d) n = 28 (74%)	Increased Transfusion Requirement (≥2 Packed Red Blood Cells/d) n = 10 (26%)	p Value
Creatinine, mg/dL (baseline)	54 ± 22	39 ± 19	0.055
Prothrombin time, % (baseline)	1.2 ± 0.5	2.3 ± 1.5	0.004
Multiplate TRAP, U (24 h on ECMO)	44 ± 23	24 ± 12	0.002

NOTE. Data are presented as mean ± SD. Prothrombin time was included despite being marginally nonsignificant, but deemed clinically relevant; p values <0.05 are considered significant.

Abbreviations: TRAP, thrombin receptor-activating peptide; ECMO, extracorporeal membrane oxygenation.

Table 7. Sensitivity, Specificity, and Area Under the Curve for Selected Thresholds Predicting Requirement of ≥2 Packed Red Blood Cells/d During Extracorporeal Membrane Oxygenation Support

Variables	Threshold	TP	TN	FP	FN	Sensitivity (95% CI)	PPV (95% CI)	Specificity (95% CI)	NPV (95%CI)	AUC (95% CI)
Creatinine (mg/dL) at baseline	1.0	9	11	16	1	90% (70-100)	36% (28-46)	41% (22-59)	92% (75-100)	0.76 (0.58-0.94)
	1.3	8	15	12	2	80% (50-100)	40% (28-56)	56% (37-74)	88% (74-100)	
	1.6	5	21	6	5	50% (20-80)	46% (22-75)	78% (63-93)	81% (71-92)	
	1.49*	**7**	**19**	**8**	**3**	**70% (40-100)**	**47% (29-67)**	**70% (52-85)**	**86% (70-100)**	
Prothrombin time (%) at baseline	60.0	9	10	17	1	90% (70-100)	35% (27-44)	37% (19-56)	91% (71-100)	0.69 (0.50-0.87)
	50.0	8	15	12	2	80% (50-100)	40% (29-56)	56% (37-74)	88% (75-100)	
	40.0	5	19	8	5	50% (20-80)	39% (18-62)	70% (52-85)	79% (68-91)	
	48.0*	**8**	**16**	**11**	**2**	**80% (50-100)**	**42% (29-58)**	**59% (41-78)**	**89% (76-100)**	
Multiplate TRAP (U) after 24 hours on ECMO	32.0	9	17	8	1	90% (70-100)	53% (41-71)	68% (48-84)	94% (84-100)	0.76 (0.59-0.93)
	29.0	7	17	8	3	70% (40-100)	47% (29-67)	68% (48-84)	85% (72-100)	
	26.0	4	20	5	6	40% (10-70)	44% (17-75)	80% (64-92)	77% (68-88)	
	32.0*	**9**	**17**	**8**	**1**	**90% (70-100)**	**53% (41-71)**	**68% (48-84)**	**94% (84-100)**	

Abbreviations: TP, true-positive; TN, true-negative; FP, false-positive; FN, false-negative; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; TRAP, thrombin receptor-activating peptide.

*"Best" cut-off value (ie, sum of sensitivity and specificity is highest), bolded in these rows for emphasis.
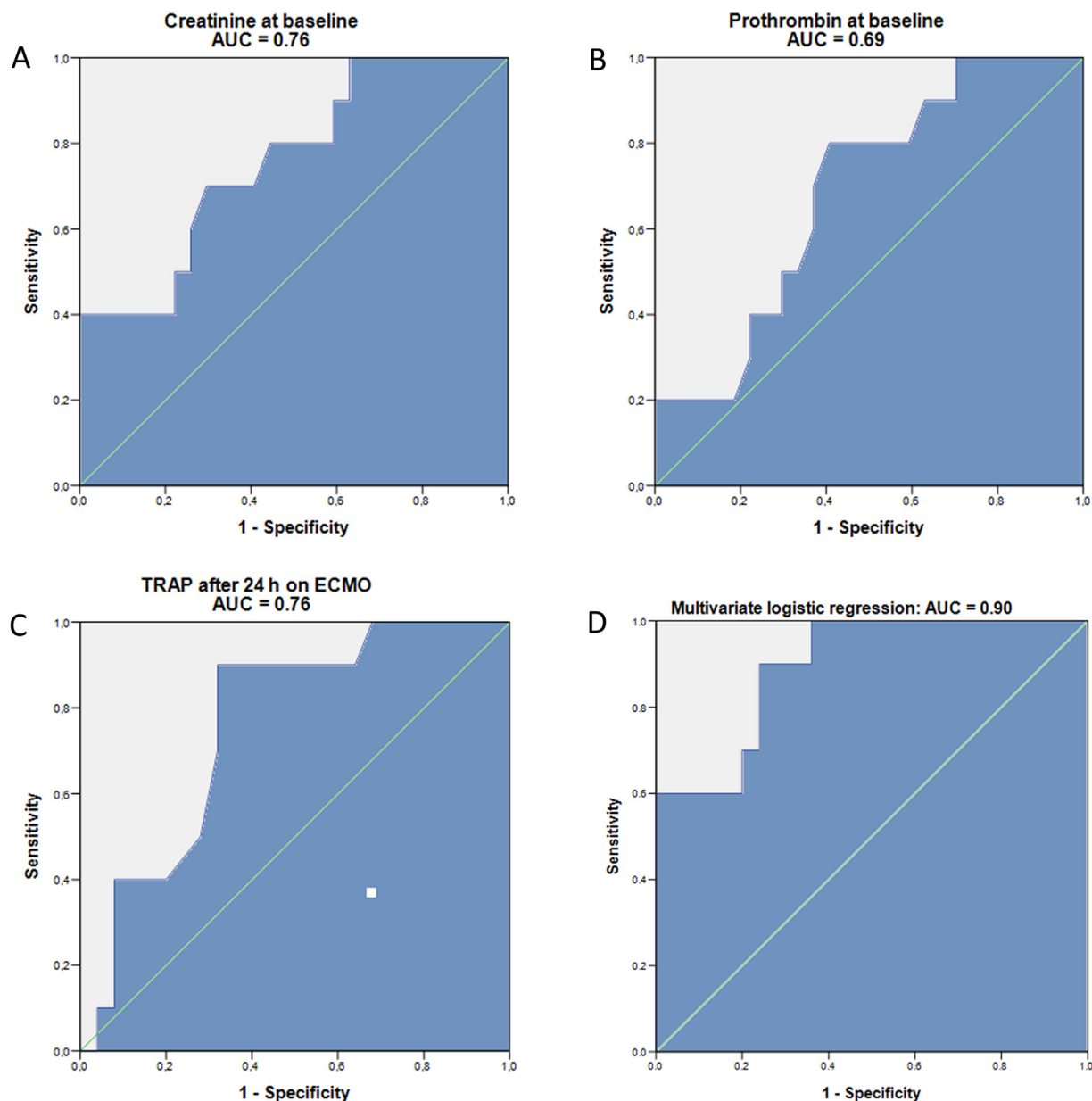
Fig 1. Area under the receiver operating characteristic (ROC) curve for creatinine at baseline (A), for prothrombin at baseline (B), for platelet function after 24 hours on extracorporeal membrane oxygenation (C) measured using thrombin receptor-activating peptide time (TRAP) and combined multivariate ROC analysis of all 3 parameters (D), predicting increased transfusion requirement during support by extracorporeal membrane oxygenation.

system.[28-30] Nair et al[31] reported similar results in platelet count and ROTEM values during ECMO support over time. After shifting from ECMO to a ventricular assist device, Huang et al[32] demonstrated improved coagulation in all patients. Platelet count and thromboelastographic maximal amplitude nearly doubled within 24 hours after bridging to the ventricular assist device, indicating that the ECMO system itself plays an important role in inducing coagulation disturbances.[32]

Despite anticoagulation with UFH, which maintained an ACT between 150 and 180 seconds, INTEM CT and PTT values did not change over time. PTT values were highest before ECMO, even before the first dose of UFH. PT values

were most compromised before ECMO. Combes et al[22] reported an association between PT <50% and poor outcome. In accordance therewith, the authors of the study presented here observed significant PT changes over time. PT and PTT continuously improved during ECMO, which may have been influenced by compromised liver function due to the underlying cardiopulmonary shock and following organ recovery.

Study Limitations

Decreased clot strength after 24 and 48 hours on ECMO also may have been affected by F:XIII deficiency, which was not determined in this study population. Certainly none of the patients

developed fibrinolysis, so there is no indirect evidence for a F:XIII deficiency. Hemolysis also may have influenced PRBC requirement. Because free hemoglobin was not measured during the study period, hemolysis during ECMO support cannot be excluded. As the authors recently demonstrated, ECMO induces short-term loss of HMW vWF multimers.[12] The effect was similar in patients with moderate and increased PRBC requirement; data were not included in the calculation for identification of predictors of PRBC requirement. Nevertheless, loss of HMW vWF multimers may have influenced blood loss and transfusion requirement. Anemia and thrombocytopenia during ECMO support may have influenced the results of WBA, although the authors considered its effects below threshold. Results of platelet function tests were judged clinically relevant, although some experts may not approve routine platelet function testing in ECMO patients. Thrombelastography/thrombelastometry considered imprecise and not dependable by many experts, even when quality controls are performed rigorously. Last but not least, the relatively small number of study participants may have led to underestimation and overestimation of at least some findings.

CONCLUSION

ECMO support improves outcome in patients with cardiac and pulmonary failure. It influences organ systems, inflammatory response, and clotting activity. This study's results demonstrated that increased creatinine levels and lower PT before ECMO and secondary impaired platelet function significantly increased transfusion requirement. The authors concluded that special attention to creatinine, PT, and platelet function is indicated. These parameters, preferably in combination, are excellent predictors of transfusion requirement.

High transfusion requirement increases morbidity and mortality in patients who undergo ECMO.[16] The data presented here indicated that timely administration of ECMO support may reduce transfusion requirement and consequently influence outcome.

APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1053/j.jvca.2016.01.009

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