Multi-modal characterization of the coagulopathy associated with extracorporeal membrane oxygenation

Tiago Granja*, Kurt Hohenstein*, Patricia Schüssel*, Claudius Fischer, Thomas Prüfer, David Schibilsky, Hans Peter Wendel, Karl Jaschonek, Lina Serna-Higuita, Christian Schlensak, Helene Häberle, Peter Rosenberger, Andreas Straub

* authors contributed equally

Online data supplement

Detailed methods description

Study population and protocol

The study population comprised 18 adult patients who were treated between January 2015 and February 2017 with veno-venous extracorporeal membrane oxygenation (vvECMO, group 1, 1n=10) or veno-arterial extracorporeal membrane oxygenation (vaECMO, group 2, n=8) at the Intensive Care Unit of the Department for Anesthesiology and Intensive Care Medicine of the University Hospital in Tübingen, Germany. Patients included in the study were≥18 and <85 years of age and vvECMO or vaECMO were indicated as a response to cardiac failure, lung failure, or a combination of these diseases. The following exclusion criteria were applied for study participation: hereditary coagulation and/or platelet disorders, refusal to receive blood transfusion, participation in other clinical research studies involving evaluation of investigational drugs or devices within 30 days, diagnosis of hepatitis B, hepatitis C, and HIV. All study procedures were approved by the ethics committee of the University of Tübingen, Germany (reference number: 535/2014BO1). Written informed consent for study participation was obtained from all patients or their legal representatives. The study presented herein is listed at the web resource “clinicaltrials.gov”

(https://clinicaltrials.gov/ct2/show/record/NCT02352805). Demographic characteristics of the patients enrolled in this study and indications for ECMO support are given in Table S1.

ECMO systems

Implantation and operation of ECMO devices was performed according to standard operating procedures established at the Department of Anesthesiology and Intensive Care Medicine (University Hospital Tübingen, Germany). ECMO circuits contained either the HLS set 7.0 (Maquet, Hechingen, Germany, inner coating: heparin-based) driven by a Cardiohelp system (Maquet) or the PLS set (Maquet, inner coating: heparin-based) driven by a Rotaflow system or contained an A.L.ONE ECMO oxygenator (inner coating: phosphorylcholine-based surface) and circuit from Eurosets (Medolla, Italy), which was driven by a CentriMAG pump (Thoratec, Zürich, Switzerland). Details on respective ECMO systems and corresponding canula sizes in enrolled patients are given in Table S3.

vvECMO was established via cannulation of a femoral vein for drainage of blood and cannulation of the right jugular vein or the right subclavian vein for blood return. VaECMO was established via canulation of a femoral vein and canulation of a femoral or subclavian artery. ECMO systems were primed using crystalloid solution.

Periprocedural anticoagulation and anticoagulation targets

Respective administration of anticoagulants and platelet inhibitors before, during, and after ECMO implantation is indicated in Table S2. Application of anticoagulants during ECMO was performed according to a SOP established at the Department of Anesthesiology and Intensive Care Medicine (Tübingen, Germany), the hospital where patients enrolled in this study were treated. According to the SOP an initial PTT of 60-80 seconds should be the objective. The initial target PTT should be individually adjusted and/or reduced in case of any contraindications for a target PTT of 60-80 seconds and/or when platelet inhibitors are

administered simultaneous to systemic anticoagulation. Specifically, the institutional SOP recommends a target PTT of 50-60 seconds whenever Multiplate® platelet aggregometry reveals areas under the curve in the ADPtest and Aspitest of ≤ 15 U. In cases of relevant bleeding, the SOP recommends a pause in anticoagulation treatment.

Blood sampling procedures

Blood sampling for analysis of study parameters was performed on each patient at four time points before and during the operation of the respective ECMO device: before ECMO implantation (time point 1), 1 hour (time point 2), 24 hours (time point 3), and 48 hours (time point 4) after ECMO implantation. At each time point the following blood samples were taken: tube 1 containing 2.7 ml of EDTA-anticoagulated blood (for whole blood count analysis); tube 2 containing 3 ml of citrated blood for coagulation analysis (International Normalized Ratio (INR), partial thromboplastin time (PTT), fibrinogen, and factor XIII (FXIII) levels); tube 3 containing 3 ml of citrated blood for ROTEM® analysis, flow cytometry, and vWF multimer analysis; tube 4 containing 2.7 ml of hirudinized blood for platelet aggregometry. Blood samples were primarily taken from an arterial catheter or, if the latter was not available, from a central venous line.

Analysis of conventional coagulation parameters

At each sampling time point whole blood count and coagulation analyses (PTT, INR, and levels of fibrinogen and FXIII) were measured in the clinical laboratory of the University Hospital in Tübingen, Germany according to standard methods.

Point of care tests

At each sampling time point the function of plasmatic coagulation and platelet aggregation were analyzed using a ROTEM® delta thromboelastometry analyzer (Instrumentation

laboratory Munich, Germany) and a Multiplate® platelet analyzer (Roche Diagnostics, Mannheim, Germany) according to the manufacturers' protocols.

Thromboelastometry assays

For ROTEM® analysis the INTEM, EXTEM, FIBTEM and HEPTEM assays were used. The following parameters, which are commonly employed, were used for interpretation of ROTEM® measurements: coagulation time (CT; unit: seconds), which represents the onset of coagulation, the clot amplitude 10 minutes after CT (a10; unit: millimeters), and the lysis index 30 minutes after CT (LI 30) (1).

The INTEM assay reagent induces contact activation and allows for fast assessment of clot formation and fibrin polymerization via the intrinsic coagulation pathway. The HEPTEM assay reagent also induces contact activation and contains a heparinase. Thereby, a direct comparison of the results from the HEPTEM and INTEM assays enables specific detection of a heparin effect. The EXTEM assay employs tissue factor (TF) activation for fast assessment of clot formation, fibrin polymerization, and fibrinolysis via the extrinsic pathway. The FIBTEM assay reagent consists of a combination of TF with a platelet inhibitor allowing to investigate the contribution of fibrinogen to the clot strength independent of platelet function. The contribution of platelets and fibrin polymerization to coagulation defects can be evaluated by comparing the clot amplitudes of the EXTEM and FIBTEM assays (1, 2).

Whole blood impedance platelet aggregometry assays

The Multiplate® aggregometer was originally developed for monitoring antiplatelet therapy, namely aspirin and P2Y12 inhibitors. However the Multiplate® can also be used in other clinical settings including the identification of patients with increased risk for perioperative bleeding (3). Platelet aggregation can be assessed within a few minutes using the Multiplate® analyzer. After adding specific platelet agonists, changes in electrical resistance are detected

in 1:1 saline-diluted and hirudin-anticoagulated whole blood samples.

Assay-specific agonists

In the present study, the agonists thrombin receptor-activating peptide (TRAP, used in the TRAPtest), arachidonic acid (used in the ASPItest), and adenosine diphosphate (ADP, used in the ADPtest) were used to induce platelet aggregation. Multiplate® results are depicted as the area under the curve (AUC).

Algorithm to characterize the ECMO-associated coagulopathy and to guide blood product administration in patients who suffer from bleeding during ECMO

To give recommendations for the therapy of bleeding complications in ECMO patients, we have included a treatment algorithm in Figure S4. This algorithm is based on a SOP established in the Department of Anesthesiology and Intensive Care Medicine at the University Hospital in Tübingen, Germany and has been modified for the treatment of ECMO patients. The original SOP is based on previously published recommendations for the perioperative treatment of coagulopathic patients (2). Clinical decision-making guided by this algorithm is based on measuring the effect of heparin, potential defects in the extrinsic and intrinsic coagulation pathways, fibrin polymerization, hyperfibrinolysis as well as potential defects in platelet aggregation using two POC tests, thrombelastometry (ROTEM®) and platelet aggregometry (Multiplate®). Most importantly, the results of these POC tests (CT, a10 values, and aggregometry tracings) present rapid and individualized information on the underlying factors of an ECMO-associated coagulopathy (EACP) within 10-15 minutes after blood sampling, thus allowing quick, scientifically guided therapy decisions.

Results of conventional tests (INR, PTT, fibrinogen, FXIII levels, and platelet counts) are included in this algorithm to further support the results of POC tests and for cases where POC measurements may not be available. In the latter case, it must be underscored that results of conventional tests are usually available approximately 45-60 minutes after blood sampling,

resulting in a significant delay of treatment decisions compared to POC measurements. For this reason the algorithm we present in figure S4 is primarily based on POC test results. Because testing for avWD may even take longer in the clinical setting it may not be practicable to wait for respective test results in a patient suffering from acute bleeding. We therefore recommend in our algorithm that empirical substitution of vWF preparations should be performed when bleeding persists despite algorithm-based blood product application.

As in all clinical decision-making processes, treatment decisions to combat ECMO-associated bleeding must be tailored to the specific clinical situation. Specifically, in situations of impaired coagulatory function clinicians must weigh the benefit of decreased heparin dosages and / or administering blood and coagulation products against the risk for ECMO/oxygenator thrombosis. This requires continuous monitoring of the ECMO driving pressure and possible thrombus formation inside the oxygenator. Also, it should be mandatory for a cardiotechnician or other qualified personnel to be on stand-by duty around the clock to ensure a rapid response to potential ECMO occlusion events.

Blood product application

Red cell concentrates, platelet concentrates and fresh frozen plasma (FFP) were obtained from the Institute for Clinical and Experimental Transfusion Medicine (IKET) of the University of Tübingen, Germany. Prothrombin complex concentrate (Beriplex®) and vWF/FVIII concentrate (Haemate®) were obtained from Behring (Marburg, Germany). Von Willebrand factor (vWF) concentrate (Willfact®) was obtained from LFB (Münster, Germany). Blood products were administered as indicated in Figure S3.

Flow Cytometry

Sample preparation

Incubation steps and flow cytometric measurements were performed as described previously (4, 5). Citrated blood samples were incubated at 37°C with respective antibodies as described

below. To determine platelet activation marker P-selectin expression and the release of platelet microparticles (PMP), an anti-human CD41-FITC (Beckman Coulter, Krefeld, Germany) antibody (ab) and an anti-human CD62P-PE ab (BD, Heidelberg, Germany) were added. In a separate assay platelets were detected using an anti-human CD41-APC ab (BioLegend, San Diego, USA). An anti-human PAC-1-FITC ab (BD, Heidelberg, Germany) was used to measure GP IIb/ IIIa activation. After an incubation period of 30 minutes, samples were fixed with CellFix® (BD Biosciences, Heidelberg, Germany).

Analysis

Samples were measured on a FACSCanto II flow cytometer (BD, Heidelberg, Germany), which was calibrated routinely using cytometer setup and tracking beads as recommended by the manufacturer. Each measurement was performed in duplicates. The BD FACSDiva software (Version 6, BD, Heidelberg, Germany) was used to perform calibration and acquisition procedures. Platelets, PMP, and fluorescence of platelet-bound antibodies were determined according to previously described principles (4).

Preparation of plasma samples

Platelet Poor Plasma (PPP) of citrate-anticoagulated blood was prepared by centrifuging blood samples at 1800 G for 20 minutes at room temperature. Aliquots of PPP samples were frozen and stored at -80°C for further analyses.

Analy**sis of vWF plasma levels, collagen binding activity, and vWF multimers**

vWF multimer analysis was performed in PPP samples using two-chamber vertical 1% SDS-agarose gel electrophoresis followed by western blotting using a commercially available enhanced chemiluminescence kit for detecting HRP-labeled antibodies (primary antibody: polyclonal rabbit anti-vWF Dako, Glostrup, Denmark; secondary antibody: goat

anti rabbit IgG-HRP, Santa Cruz Biotechnology, Heidelberg, Germany). Chemiluminescence imaging of labeled vWF multimer (PVDF) membranes was carried out using a Fusion SL system and was evaluated by densitometry. A pool of normal PPP from five healthy subjects was used as reference.

Plasma vWF levels and vWF-collagen binding activity (vWF:CBA) were measured in PPP samples in the clinical coagulation laboratory of the Medical Department II of the University Hospital Tübingen using enzyme-linked Immunosorbent assays (ELISA).

Von Willebrand Factor antigen (vWF:Ag) was determined with enzyme-linked immunoabsorbent assays (ELISA) using polyclonal antibody A0082 (Dako). A collagen binding activity assay of vWF (vWF:CBA) was performed using type III collagen-coated multititre plates (CovaLink™, Nunc®, Thermo-Scientific, Waltham, MA, USA). Collagen type III was obtained from SouthernBiotech, Birmingham, AL, USA.

Effects of heparin on the employed assays

Taking into account that bolus doses of up to 5000 units of unfractionated heparin (UFH) were administered during ECMO implantation in enrolled study patients, it can be expected that UFH maximum plasma levels of approximately 1 unit/ml were reached (6). As mentioned above, the INTEM assay is inhibited by heparin and coagulation entirely prevented at heparin concentrations of 2 IU/ml (7). EXTEM measurements are stable up to a heparin concentration of 2 IU/ml, at which point the CT is prolonged significantly. The FIBTEM assay is influenced by heparin concentrations greater than 4 IU/ml (7).

Multiplate® measurements are not affected by heparin concentrations below 20 U/ml (7).

The PTT (measured in our study using the Dade® Actin® FS reagent, Siemens, Marburg, Germany) is routinely used for heparin monitoring (8) and increases proportional to the heparin concentration. The INR (measured using the reagent Dade® Innovin®, Siemens, Marburg, Germany) is insensitive to heparin concentrations below 2 IU/ml. Fibrinogen level

measurements (using the reagent Dade® Thrombin, Siemens, Marburg, Germany) are insensitive to heparin concentrations below 0.6 IU/ml. Information on possible heparin effects on the assay used for the determination of factor XIII is not available.

The expression of platelet P-selectin, which we evaluated using flow cytometry, is induced by heparin. PMP formation and GP IIb/IIIa activation, however, are not influenced significantly by heparin concentrations up to 3 IU/ml (9). The effects of heparin anticoagulation on the quantitative analysis of vWF multimers, vWF:Ag analysis, and vWF:CBA assays have yet to be evaluated.

Statistical analysis

The normality of data distribution was evaluated using kurtosis, skewness, Q-Q plots and histograms. According to the distribution of the data, descriptive data are presented as medians, interquartile range, dot plots, and minimum and maximum as indicated.

Statistical testing was performed with the Statistical Analysis System R using the package nparLD that is available from the comprehensive R archive Network at http://CRAN.R- project.org/ package=nparLD. Coagulation, platelet and flow cytometry parameters were analyzed for potential effects of vvECMO and vaECMO therapy and for changes during the observation period. We used a nonparametric rank-based method for the relative treatment effect in the analysis of repeated measurements (10). The "nparLD" module was used for F2_LD_F1 design analysis at program R. The Wald-type statistic (WTS), the ANOVA-type statistic (ATS), and the modified ANOVA type statistic were calculated for testing group and time effects and their interaction. When statistically significant effects caused by vv/vaECMO treatment and/or time were determined, a multiple comparison analysis was performed for the respective parameter. Pairwise comparisons were adjusted for multiple testing according to Bonferroni corrections with a level of significance of $p \leq 0.05$.

References

1. Zaky A. Thromboelastometry Versus Rotational Thromboelastography in Cardiac Surgery. *Semin Cardiothorac Vasc Anesth* 2017;21(3):206-211.

2. Weber CF, Zacharowski K, Brun K, et al. [Basic algorithm for Point-of-Care based hemotherapy: perioperative treatment of coagulopathic patients]. *Anaesthesist* 2013;62(6):464-472.

3. Fries D, Streif W. Point-of-care testing in critically ill patients. *Semin Thromb Hemost* 2015;41(1):75-83.

4. Granja T, Schad J, Schüssel P, et al. Using six-colour flow cytometry to analyse the activation and interaction of platelets and leukocytes - A new assay suitable for bench and bedside conditions. *Thromb Res* 2015;136(4):786-796.

5. Köhler D, Straub A, Weissmuller T, et al. Phosphorylation of vasodilator-stimulated phosphoprotein prevents platelet-neutrophil complex formation and dampens myocardial ischemia-reperfusion injury. *Circulation* 2011;123(22):2579-2590.

6. Fujii Y, Takeuchi S, Koike T, et al. Heparin administration and monitoring for neuroangiography. *AJNR Am J Neuroradiol* 1994;15(1):51-54.

7. Gertler R, Wiesner G, Tassani-Prell P, et al. Are the point-of-care diagnostics MULTIPLATE and ROTEM valid in the setting of high concentrations of heparin and its reversal with protamine? *J Cardiothorac Vasc Anesth* 2011;25(6):981-986.

8. Winkler AM. Managing the Precarious Hemostatic Balance during Extracorporeal Life Support: Implications for Coagulation Laboratories. *Semin Thromb Hemost* 2017;43(3):291-299.

9. Straub A, Breuer M, Wendel HP, et al. Critical temperature ranges of hypothermia-induced platelet activation: possible implications for cooling patients in cardiac surgery. *Thromb Haemost* 2007;97(4):608-616.

10. Noguchi K, Gel YR, Brunner E, et al. nparLD: An R Software Package for the Nonparametric Analysis of Longitudinal Data in Factorial Experiments, Journal of Statistical Software, Articles. *Journal of Statistical Software* 2012;50(12):1-23.

Table S1

Group	No	Gender	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m^2)	bili (mg/dl)	SOFA	Diagnoses	Outcome
vvECMO	1	m	58	183	110	32.8	0.4	11	ARDS, pneumonia	Discharged
	2	f	55	158	60	24	1.1	16	ARDS after abd. surgery	Died in ICU
	3	m	22	168	73	25.9	2.6	13	ARDS, pneumonia	Discharged
	4	m	55	185	115	33.6	0.7	11	ARDS, pneumonia	Discharged
	5	m	63	170	76	31.1	1	14	ARDS, pneumonia	Died in ICU
	6	f	25	-	60	-	0.5	12	ARDS, urosepsis	Discharged
	7	m	67	187	87	24.9	1.3	17	ARDS, pneumonia	Died in ICU
	8	m	57	180	120	37	1.5	12	ARDS, pneumonia	Discharged
	9	m	70	185	100	29.2	0.2	12	ARDS, pneumonia	Discharged
	10	m	46	176	110	35.5	0.5	12	ARDS, pneumonia	Discharged
Median (IQR)	-	-	*56 (41-64)*	*180 (169-185)*	*93.5 (70-111)*	*31.1 (25-35)*	*0.85 (0.5-1.4)*	*12 (12-15)*	-	
vaECMO	1	m	28	184	70	20.7	0.9	9	Non compaction cardiomyopathy / CPR	Discharged
	2	f	35	175	90	29.4	0.8	12	Aspiration-induced ARDS, cardiocirculatory depression	Discharged
	3	m	55	170	120	41.5	5	15	Cardiopulmonary depression after cardiac surgery	Died in ICU
	4	m	76	170	78	27	1.1	4	pulmonary embolism, shock	Discharged
	5	m	73	162	72	27.4	0.6	7	cardiac shock, upper GI bleeding / CPR	Discharged
	6	m	63	176	100	32.3	1.2	8	DCM / cardiac shock	Died in ICU
	7	m	65	172	117	39.5	3.1	10	DCM / cardiac shock	Discharged
	8	m	64	175	85	27.8	0.7	12	STEMI / cardiac shock	Died in ICU
Median (IQR)	-	-	*63.5 (40-71)*	*174 (170-178)*	*87.5 (74-113)*	*28.6 (27-38)*	*1 (0.7-2.6)*	*9.5 (7-12)*	-	

Legend for table S1: Patients' characteristics per treatment group. Abbreviations: ARDS = Acute Respiratory Distress Syndrome, abd. = abdominal, BMI = body mass index, bili = total bilirubin value on admission before extracorporeal membrane oxygenation (upper reference limit = 1.1 mg/dl), CPR = cardiopulmonary resuscitation, DCM = dilated cardiomyopathy, "Discharged" indicates discharge from hospital, ECMO = extracorporeal membrane oxygenation, f = female, GI = gastrointestinal, ICU = Intensive Care Unit, m = male, SOFA = sequential organ failure assessment - value on admission before ECMO, STEMI = ST-elevation myocardial infarction.
Data given in column "outcome" indicate an in-hospital mortality of 30% for vvECMO patients and 37.5% for vaECMO patients.

Table S2

Group	No	Anticoagulants and platelet inhibitors before ECMO	UFH for ECMO implantation		24 hours after ECMO implantation	48 hours after ECMO implantation
			UFH bolus	UFH dose		
vvECMO	1	Enoxaparin 40mg once daily	+	5000 U	UFH: 13.6 U/kg/h	UFH: 18.2 U/kg/h
	2	-	+	1000 U	UFH: 16.7 U/kg/h	UFH: 16.7 U/kg/h
	3	-	+	5000 U	UFH: 9.6 U/kg/h	UFH: 15.1 U/kg/h
	4	-	+	5000 U	UFH: 13.9 U/kg/h	UFH: 16.5 U/kg/h
	5	-	-	-	UFH: 5.6 U/kg/h	UFH: 2.2 U/kg/h
	6	-	+	5000 U	UFH: 10 U/kg/h	UFH: 33.3 U/kg/h
	7	-	-	5.6 U/kg/h	Argatroban: 0.23 µg/kg/min ASA 300 mg once	Argatroban: 0.19 µg/kg/min ASA 100 mg/d
	8	Enoxaparin 40mg once daily	+	5000 U	UFH: 15 U/kg/h	UFH: 27 U/kg/h
	9	UFH (dose n.d.)	+	500 U bolus + 6 U/kg/h	UFH: 11 U/kg/h	UFH: 13 U/kg/h
	10	Phenprocoumon (INR target range: 2-3)	+	2000 U	UFH: 5.5 U/kg/h	UFH: 7.3 U/kg/h
vaECMO	1	ASA 100mg/d; Full heparinization during cardiac ablation directly before ECMO implantation	-	-	UFH: 17.1 U/kg/h	UFH: 15.7 U/kg/h
	2	-	+	n.d.	UFH: 6.7 U/kg/h	UFH: 7.8 U/kg/h
	3	UFH: 2.5 U/kg/h; pretreated with ASA 100mg/d	+	5000 U	UFH: 7.5 U/kg/h	UFH: 6.7 U/kg/h
	4	UFH: 7.7. U/kg/h; ASA 100 mg/d	-	-	UFH: 7.7 U/kg/h	UFH: 11.5 U/kg/h
	5	-	-	-	UFH: 8.3 U/kg/h ASA 100 mg once daily	UFH: 8.3 U/kg/h ASA 100 mg once daily
	6	UFH: 5 U/kg/h infusion; ASA 100 mg/d, Clopidogrel 75 mg/d	-	6 U/kg/h	UFH: 2.1 U/kg/h	-
	7	Apixaban 2.5 mg twice daily	-	-	-	UFH: 2.6 U/kg/h
	8	-	+	n.d.	UFH: 11.8 U/kg/h	UFH: 10 U/kg/h

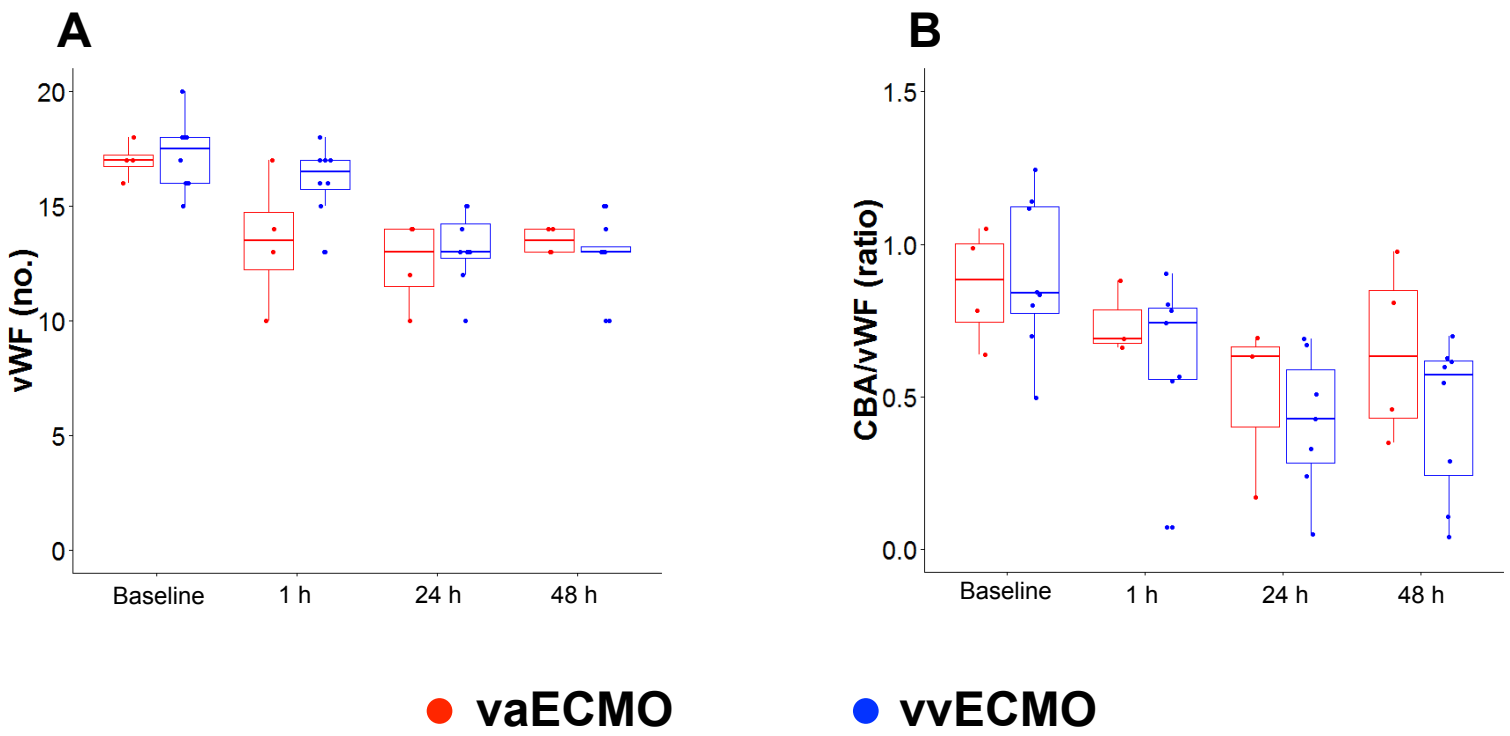
Legend for table S2: Application of anticoagulants and platelet inhibitors in enrolled patients. Anticoagulants and platelet inhibitors, which were administered within 24 hours before extracorporeal membrane oxygenation (ECMO), unfractionated heparin (UFH), which was administered directly during the ECMO implantation procedure as well as anticoagulants and platelet inhibitors, which were administered until 48 hours (h) after ECMO implantation are indicated.
Further abbreviations: ASA = acetylsalicylic acid, d = day, mg = milligram, µg/kg/min = microgram per kilogram bodyweight per minute, n.d. = not documented, no = number, U = Units, U/kg/h = Units per kilogram body weight per hour. UFH bolus administered = "+". UFH bolus not administered = "-".

Table S3

Group	No	ECMO system	Canula size (Fr)	
			venous	arterial
vvECMO	1	HLS set 7.0 / Cardiohelp	23	17
	2	PLS set / Rotaflow	21	19
	3	PLS set / Rotaflow	23	17
	4	PLS set / Rotaflow	23	17
	5	HLS set 7.0 / Cardiohelp	23	17
	6	PLS set / Rotaflow	21	17
	7	PLS set / Rotaflow	23	19
	8	A.L.ONE oxygenator + circuit, CentriMAG pump	25	19
	9	PLS set / Rotaflow	23	17
	10	HLS set 7.0 / Cardiohelp	25	19
vaECMO	1	PLS set / Rotaflow	25	19
	2	HLS set 7.0 / Cardiohelp	21	17
	3	PLS set / Rotaflow	25	21
	4	PLS set / Rotaflow	23	17
	5	PLS set / Rotaflow	23	17
	6	A.L. ONE oxygenator + circuit, CentriMAG pump	25	17
	7	HLS set 7.0 / Cardiohelp	25	19
	8	PLS set / Rotaflow	23	17

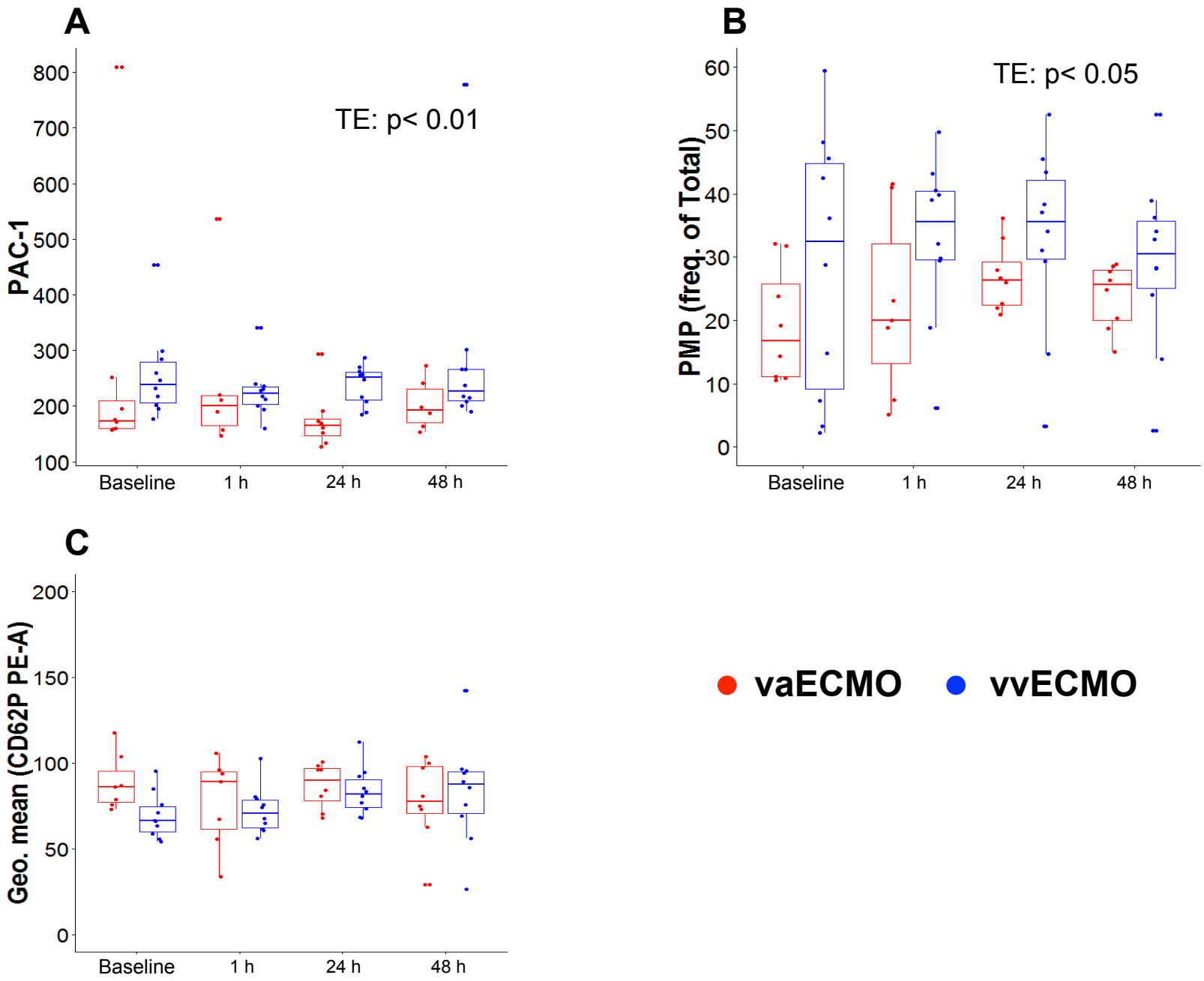
Legend for table S3: Extracorporeal membrane oxygenation (ECMO) system characteristics. The respective ECMO systems and corresponding venous and arterial cannula sizes are indicated. Abbreviation: Fr = French

Figure S1



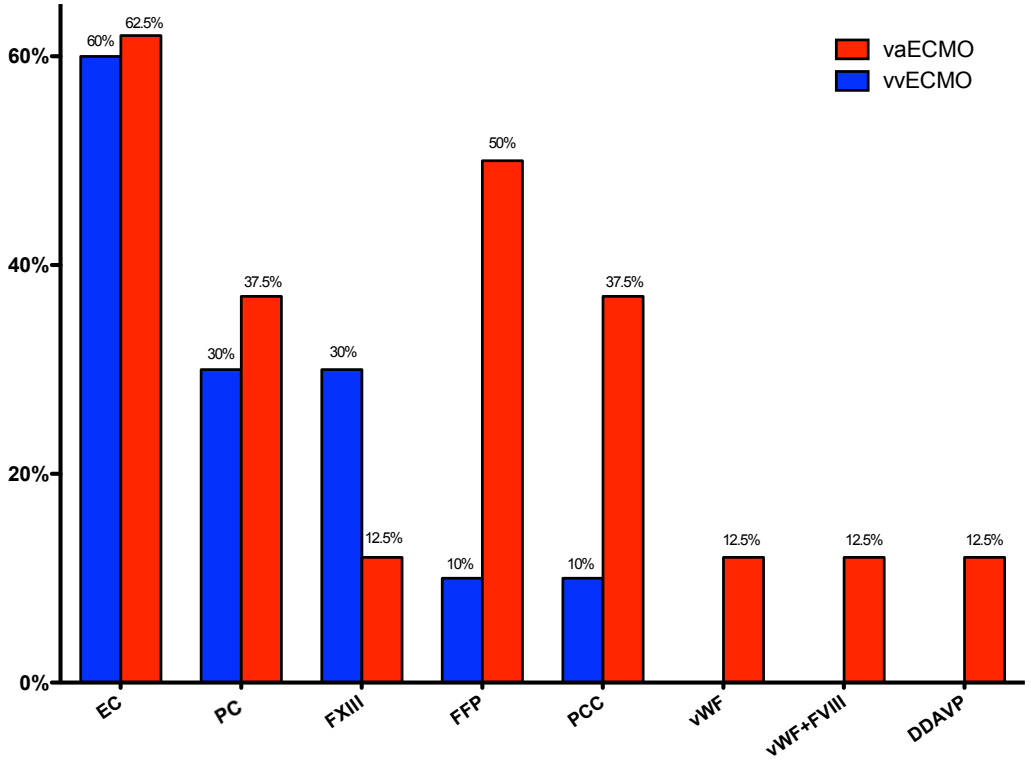
Legend for figure S1:
Numbers of von Willebrand factor (vWF) multimers (A) and the collagen binding activity / vWF ratio (B) were determined in patient blood samples using gel electrophoresis and Enzyme-linked Immunosorbent Assay respectively. Parameters were determined before (baseline), and 1 hour (h), 24 h, as well as 48 h after institution of veno-arterial (va) or veno-venous (vv) extracorporeal membrane oxygenation (ECMO).
Data are indicated as dot and box plots (indicating medians, interquartile ranges, and minimum and maximum of all data) of n=4 patients for vaECMO and n=8 patients for vvECMO.

Figure S2


A
PAC-1
800
700
600
500
400
300
200
100
TE: p< 0.01
Baseline
1 h
24 h
48 h
B
PMP (freq. of Total)
60
50
40
30
20
10
0
TE: p< 0.05
Baseline
1 h
24 h
48 h
C
Geo. mean (CD62P PE-A)
200
150
100
50
0
Baseline
1 h
24 h
48 h
vaECMO
vvECMO

Legend for figure S2:
Expression of activated GP IIb/IIIa on platelets (A), the release of platelet microparticles (PMP) (B) and the expression of the activation marker P-selectin on PMP (C) was analyzed in patient blood samples using flow cytometry before (baseline), and 1 hour (h), 24 h, as well as 48 h after institution of veno-arterial (va) or veno-venous (vv) extracorporeal membrane oxygenation (ECMO).
Each parameter was analyzed for potential effects of vvECMO and vaECMO therapy and for changes during the observation period using a rank-based nonparametric method for longitudinal data, “ANOVA-Type statistic”.
If significant differences between groups (vaECMO vs. vvECMO) were found, this is indicated in the figure as “therapy effect” (TE) together with the corresponding p-value.
Data are indicated as dot and box plots (indicating medians, interquartile ranges and minimum and maximum of all data of n=8 patients for vaECMO and n=10 patients for vvECMO.

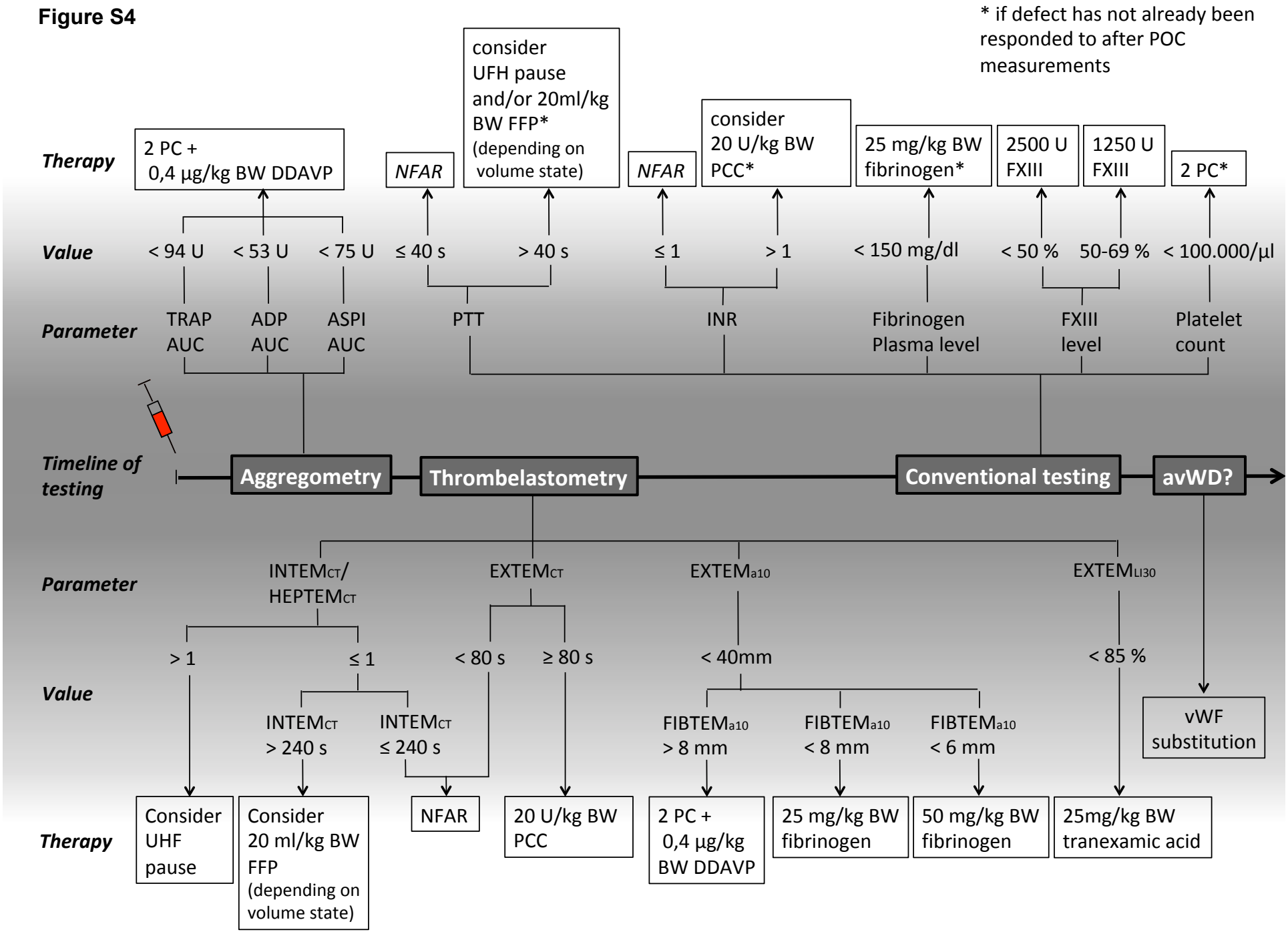
Figure S3



	vvECMO (n=10) median (p25-75)	vvECMO min-max	vaECMO (n=8) median (p25-75)	vaECMO min-max
EC (ml)	450 (0-1200)	0-1800	450 (0-1050)	0-2700
PC (ml)	0 (0-250)	0-600	0 (0-450)	(0-1500)
FXIII (IE)	0 (0-1250)	0-5000	0 (0-0)	0-250
FFP (ml)	0 (0-0)	0-600	450 (0-1500)	0-3000
PCC (IE)	0 (0-0)	0-1000	0 (0-300)	0-6000
vWF (IE)	0 (0-0)	0-0	0 (0-0)	0-2000
vWF+FVIII (IE)	0 (0-0)	0-0	0 (0-0)	0-6000
DDAVP (µg)	0 (0-0)	0-0	0 (0-0)	0-32

Legend for figure S3: Application of blood products and desmopressin in veno-venous extracorporeal membrane oxygenation (vvECMO) and veno-arterial extracorporeal membrane oxygenation (vaECMO) patients. The percentage of patients who received respective coagulation factor concentrates is indicated per group in graphs. The amounts of administered blood products are indicated in the table.
Abbreviations for respective blood products:
EC = erythrocyte concentrates,
PC = platelet concentrates,
FXIII = factor XIII,
FFP = fresh frozen plasma,
PCC = prothrombin complex concentrate,
vWF = von Willebrand factor,
vWF+FVIIII = von Willebrand factor + factor VIII,
DDAVP = desmopressin

Figure S4


* if defect has not already been responded to after POC measurements
Therapy
2 PC + 0,4 µg/kg BW DDAVP
NFAR
consider UFH pause and/or 20ml/kg BW FFP* (depending on volume state)
NFAR
consider 20 U/kg BW PCC*
25 mg/kg BW fibrinogen*
2500 U FXIII
1250 U FXIII
2 PC*
Value
< 94 U
< 53 U
< 75 U
≤ 40 s
> 40 s
≤ 1
> 1
< 150 mg/dl
< 50 %
50-69 %
< 100.000/µl
Parameter
TRAP AUC
ADP AUC
ASPI AUC
PTT
INR
Fibrinogen Plasma level
FXIII level
Platelet count
Timeline of testing
Aggregometry
Thrombelastometry
Conventional testing
avWD?
Parameter
INTEMCT/ HEPTEMCT
EXTEMCT
EXTEMa10
EXTEMLI30
Value
> 1
≤ 1
< 80 s
≥ 80 s
< 40mm
< 85 %
INTEMCT > 240 s
INTEMCT ≤ 240 s
FIBTEMa10 > 8 mm
FIBTEMa10 < 8 mm
FIBTEMa10 < 6 mm
vWF substitution
Therapy
Consider UHF pause
Consider 20 ml/kg BW FFP (depending on volume state)
NFAR
20 U/kg BW PCC
2 PC + 0,4 µg/kg BW DDAVP
25 mg/kg BW fibrinogen
50 mg/kg BW fibrinogen
25mg/kg BW tranexamic acid

Legend for figure S4: Algorithm to characterize underlying factors of the ECMO-associated coagulopathy and to guide blood product administration in patients who suffer from bleeding during ECMO

This algorithm is intended to give recommendations for the therapy of bleeding complications in ECMO patients. Clinical decision-making guided by this algorithm is based on measuring the effect of heparin, potential defects in the extrinsic and intrinsic coagulation pathways, fibrin polymerization, potential hyperfibrinolysis as well as potential defects in platelet aggregation using the point of care (POC) systems thrombelastometry (ROTEM® delta) and platelet aggregometry (Multiplate®). Recommendations for blood product application are given in response to test results as indicated.

After blood sampling POC testing is started immediately at the intensive care unit. Results of the POC tests are available approximately 10-15 minutes after blood sampling, thus allowing quick therapy decisions. In thrombelastometry clotting times (CT) of INTEM and HEPTEM are compared and indicate whether heparin contributes to the bleeding tendency or whether clotting factors in the intrinsic system are missing. Respective results recommend either a stop of heparinization or administration of fresh frozen plasma (FFP) as indicated. Depending on the EXTEM CT substitution of Prothrombin complex concentrate (PCC) is recommended. Evaluation of the clot amplitude at 10 minutes (a10) in the EXTEM assay in comparison with the FIBTEM a10 either indicates a defect in platelet function (followed by application of platelet concentrates (PC) and desmopressin (DDAVP)) or a defect in fibrin polymerization (followed by application of fibrinogen concentrate). The lysis index after 30 minutes (LI30) in the EXTEM assay indicates if tranexamic acid is indicated to treat potential hyperfibrinolysis. If platelet aggregometry detects decreases in the area under the curve (AUC) in any of the employed assays application of DDAVP and platelet concentrates (PC) is indicated.

Conventional coagulation tests are included in this algorithm to further support the results of POC tests and for cases where POC measurements may not be available. However, results of conventional tests take longer (approximately 45-60 minutes after blood sampling) until they are available. Increases of the Prothrombin Time (PTT) may be caused by anticoagulants and indicate the need to decrease heparinization. If PTT increases are caused by clotting factor depletion this may be treated with FFP. If the International Normalized Ratio (INR) is increased application of PCC is recommended. If the fibrinogen plasma level is decreased application of fibrinogen concentrate is recommended. If the plasma level of factor XIII is decreased application of factor XIII preparations is recommend. If the platelet count is decreased below 100.000/µl PC application is recommended.

If bleeding persists despite blood product application aquired von Willebrand disease (avWD) should be suspected and von Willbrand factor (vWF) substitution be performed. Also respective assays to diagnose avWD should be initiated.

As in all clinical decision-making processes, treatment decisions to combat ECMO-associated bleeding must be tailored to the specific clinical situation. Specifically, in situations of impaired coagulatory function clinicians must weigh the benefit of decreased heparin dosages and / or administering blood and coagulation products against the risk for ECMO/oxygenator thrombosis. This requires continuous monitoring of the ECMO driving pressure and possible thrombus formation inside the oxygenator. Also, it should be mandatory for a cardiotechnician or other qualified personnel to be on stand-by duty around the clock to ensure a rapid response to potential ECMO occlusion events. BW = bodyweight; NFAR = no further action required.