The Coagulopathy of Extracorporeal Membrane Oxygenation

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Declaration

I, Andrew James Doyle, confirm that the work presented in this thesis is my own. When information has been derived from other sources, I confirm that this has been indicated in the thesis.

Contents

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Firstly, I am deeply grateful for the support and knowledge of my supervisors. Prof Beverley Hunt has been a mentor for the last 5 years and it has been a privilege to be under her guidance. Dr Karen Breen has been a calming and supportive influence during many a chaotic moment. Both have encouraged me to gain a deeper understanding of the field of haemostasis, thrombosis, and research methodology. I look forward to working with them both in the future.

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Following the unprecedented demands of the ECMO services during the COVID-19 pandemic that occurred during this study, I dedicate this thesis in the memory of those patients that lost their battle to the disease and to honour the healthcare workers who fought bravely and selflessly to help to others during the darkest of hours.

Abstract

Introduction

Extracorporeal membrane oxygenation (ECMO) is a supportive therapy for severe cardiac or respiratory failure associated with high rates of bleeding and thrombosis. Their development is associated with increased rates of mortality. Haemostatic and inflammatory changes are recognised to occur in patients with critical illness and because of the extracorporeal circuit. This thesis aims to assess bleeding and thrombosis during ECMO in more detail and assess specific temporal changes in haemostatic parameters.

Methods and materials

Three components were looked at in this thesis: 1) a single-centre registry review of patients receiving veno-venous ECMO for the development of bleeding and thrombosis and outcomes associated with the use of blood products transfusions, 2) haemostatic changes in an *ex vivo circuit* of ECMO, and 3) haemostatic changes in patients during veno-venous ECMO, focusing on thrombin generation, fibrinolysis and circulating histones.

Consent was obtained from patients receiving ECMO or nominated consultees with local ethics approval. For the *ex vivo* circuit, blood samples (whole blood with 3.2% trisodium citrate) were collected over a 24-hour period and compared to time-matched control samples. For patient samples, blood samples were taken at multiple time points prior to, during and after ECMO and correlated with bleeding and thrombotic events. ELISA-based techniques were used for thrombin generation markers, fibrinolytic assays and histone levels.

Results

A cohort of 365 patients with influenza, COVID-19 and bacterial pneumonia requiring ECMO were reviewed. Key findings demonstrated the underlying disease aetiology affected rates of

thrombosis, particularly COVID-19 infection. Small volume intracranial haemorrhage (ICH) and small volume pulmonary artery filling defects were seen frequently at initiation of ECMO. Increased mortality was seen in those who had progression of ICH and non-intracranial major haemorrhage as opposed to thrombotic events.

In reviewing blood transfusion-associated outcomes, patients receiving a 'restrictive' red cell transfusion approach (haemoglobin target 80-90g/L) had similar survival outcomes to those with a 'liberal approach (100-120g/L). Other blood product transfusions had high usage with major bleeding and an increased mortality rate was seen with platelet transfusions.

In the *ex vivo* circuit model, the key changes were an increase in plasminogen activator inhibitor-1 (PAI-1) and decrease in tissue plasminogen activator (tPA) after 24-hours. There was no increase in thrombin generation markers over 24 hours. However, circulating H3 histone levels were significantly increased because of the circuit.

In patients requiring ECMO, there were haemostatic changes in keeping with a reactive profile were seen prior to initiation. An early increase in thrombin generation was seen during ECMO followed by a subsequent increase in fibrinolysis. Both decreased following decannulation from ECMO. Low thrombin-activatable fibrinolysis inhibitor (TAFI) and elevated PAI-1 levels were seen in bleeding events.

Conclusions

The development of thrombosis and bleeding have temporal changes in patients during ECMO and this is reflected by changes in thrombin generation and fibrinolytic activation markers. Additionally, retrospective registry data suggests that more 'liberal' blood transfusion approaches may not necessarily improve survival outcomes and transfusion triggers during ECMO need to be investigated further.

Abbreviations

ACT	Activated Clotting Time
ADAMTS13	A Disintegrin and Metalloproteinase with a Thrombospondin 1 Motif-13
ADP	Adenosine Diphosphate
ALI	Acute Lung Injury
APACHE II	Acute Physiology And Chronic Health Evaluation II
APC	Activated Protein C
APTT	Activated Partial Thromboplastin Time
ARDS	Acute Respiratory Distress Syndrome
AT	Antithrombin
ATP	Adenosine Triphosphate
CaO2	Oxygen Content
Ca-DVT	Cannula-Associated Deep Vein Thrombosis
CPB	Cardiopulmonary Bypass
CO	Cardiac Output
COVID-19	SARS-CoV-2
CO_2	Carbon Dioxide
CRNMB	Clinically Relevant Non-major Bleeding
CRP	C Reactive Protein
CT	Computer Tomography
CTPA	Computer Tomographic Pulmonary Angiography
CV	Co-efficient of Variation
DAMP	Damage-Associated Molecular Pattern

DNA	Deoxyribonucleic Acid
DIC	Disseminated Intravascular Coagulation
DO_2	Oxygen Delivery
DTI	Direct Thrombin Inhibitor
DVT	Deep Vein Thrombosis
ECCO2R	Extracorporeal Carbon Dioxide Removal
ECBF	Extracorporeal Blood Flow
ECMO	Extracorporeal Membrane Oxygenation
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ELSO	Extracorporeal Life Support Organization
EPCR	Endothelial Protein C Receptor
ETP	Endogenous Thrombin Potential
F	[Procoagulant] Factor
FBC	Full Blood Count
FDP	Fibrin Degradation Product
FEU	Fibrinogen Equivalent Units
FFP	Fresh Frozen Plasma
GP	Glycoprotein
Hb	Haemoglobin
HIT	Heparin-Induced Thrombocytopenia
HLA	Human Leucocyte Antigen
HMWK	High Molecular Weight Kininogen

HMWM	High Molecular Weight Multimers
H3R8	Citrullinated Histone H3
ICH	Intracranial Haemorrhage
ICNARC	Intensive Care National Audit & Research Centre
ICU	Intensive Care Unit
ICAM	Intracellular Adhesion Molecules
IL	Interleukin
ISTH	International Society on Thrombosis and Haemostasis
LMWH	Low Molecular Weight Heparin
LOS	Length of Stay
MA	Maximal Amplitude
MAC	Membrane Attack Complex
MAHA	Microangiopathic Haemolytic Anaemia
MH	Major Haemorrhage
MMP	Matrix Metalloproteinase
NET	Neutrophil Extracellular Trap
NICMH	Non-Intracranial Major Haemorrhage
NO	Nitrous Oxide
NSE	Neuron-Specific Elonase
PAD4	Peptidylarginine Deiminase 4
PAI	Plasminogen Activator Inhibitor
PAP	Plasma-Antiplasmin Complex
PAR	Protease Activated Receptor

PBM	Patient Blood Management
pCO_2	Partial Pressure of Carbon Dioxide
PE	Pulmonary Embolism
PEX	Plasma Exchange
PFP	Plasma Free Plasma
PMN	Polymorphonuclear leucocytes
PMP	Platelet Microparticles
PolyP	Polyphosphate P
pO2	Partial Pressure of Oxygen
PPI	Proton Pump Inhibitor
PSGL-1	P-Selectin Glycoprotein Ligand-1
PT	Prothrombin Time
ROC	Receiver Operating Characteristic
RBC	Red Blood Cell
RRT	Renal Replacement Therapy
SaO_2	Oxygen Saturation
SAH	Subarachnoid Haemorrhage
SDH	Subdural Haemorrhage
SOFA	Severe Organ Failure Assessment
SRF	Severe Respiratory Failure
TAFI	Thrombin-Activatable Fibrinolysis Inhibitor
TAT	Thrombin-Antithrombin Complex
TEG	Thromboelastography

TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TGA	Thrombin Generation Assay
TGF	Tissue Growth Factor
TLR	Toll-Like Receptor
TNF	Tumour Necrosis Factor
tPA	Tissue Plasminogen Activator
TPO	Thrombopoietin
TRAP	Thrombin Receptor Activating Peptide
TT	Thrombin Time
UFH	Unfractionated Heparin
uPA	Urokinase Plasminogen Activator
uPAR	Urokinase-type Plasminogen Activator Receptor
US	Ultrasound
VA	Veno-Arterial
VAD	Ventricular Assist Devices
VCAM	Vascular Cell Adhesion Molecule
VEGF	Vascular Endothelial Growth Factor
VO2	Tissue Oxygen Consumption
VTE	Venous Thromboembolism
VV	Veno-Venous
vWF	von Willebrand Factor
WCC	White Cell Count

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Chapter 1 – Introduction

1.1 Overview of Haemostasis, Anticoagulants and Fibrinolysis

Haemostasis is the physiological mechanism to maintaining blood flow in the vasculature in a fluid state and ensuring vessel closure at a time of damage. There are multiple components to this system that occur at different time points. After a triggering event, typically injury to a vessel wall, there is vasoconstriction and formation of a platelet plug (primary haemostasis) followed by activation of coagulation factors leading to fibrin clot formation (secondary haemostasis). Subsequently, clot break down (fibrinolysis) occurs.

The mechanisms of haemostasis are closely related and interdependent. Haemostasis ensures that clot formation occurs at the site of vessel injury and blood flow is maintained in the rest of the circulation. When the balance between the procoagulant and anticoagulant mechanisms are disturbed, pathological clot formation (thrombosis) or excessive bleeding (haemorrhage) can occur.

1.1.1 The Blood Vessel and Endothelial Function

The blood vessel has developed to be in an 'inactive' state when intact ensuring that the clot formation does not happen inappropriately. There are three layers of vessel walls: the intima, media and adventitia. It is the intimal surface that comes in to contact with blood and is comprised on a monolayer of endothelial cells. Beneath this, the media is composed of smooth muscle, elastic fibres and connective tissues, of which the exposure of the latter is the main activator to haemostasis. The adventitia comprises of collagen and fibroblasts providing a flexible structure to the vessel.

The endothelium regulates various components of haemostasis depending upon vessel injury and other circulating stimuli by releasing various activators and inhibitors of haemostasis. In the non-active intact state, the endothelium acts a physical barrier between blood and thrombogenic substances in the subendothelial surface and releases various inhibitory molecules. These include tissue factor pathway inhibitor (TFPI), thrombomodulin, endothelial protein C receptor (EPCR), heparan sulphate and nitrous oxide[1].

When exposed to stimulatory molecules such as interleukin (IL)-1, tumour necrosis factor (TNF) and thrombin as a result of viral and bacterial infection or other inflammatory conditions, the endothelium becomes activated[1,2]. It produces leucocyte adhesion molecules such as e-selectin, Vascular Adhesion Molecules (VCAM-1), Intracellular Adhesion Molecules (ICAM)-1 to allow the localisation of cells such as neutrophils to the endothelial surfaces. It also becomes prothrombotic by reducing the production of nitrous oxide, prostacyclins and secretion of thrombomodulin and heparans.

1.1.2 Primary Haemostasis

Primary haemostasis is the process by which a platelet plug is formed at the site of vessel injury. The key components to this process are endothelial cells, platelets, von Willebrand factor (vWF) and collagen. This process can be considered in three stages: platelet adhesion, platelet activation and platelet plug formation. In the intact state, the endothelium acts as a barrier between circulating platelets in the blood, which are in close contact with its surface due to axial flow, and the subendothelial matrix, which consists of various proteins such collagen, laminin and fibronectin.

When a vessel is damaged, the barrier of the endothelium is lost. As a result, platelets can bind directly to the matrix or indirectly by vWF as part of platelet adhesion. Platelets can bind

directly to collagen by the glycoprotein (GP)-VI on their surface forming an immediate monolayer although this is insufficient to stop bleeding. vWF is a large multimeric protein that is released from the Weibel-Palade bodies in endothelial cells and α-granules in platelets. When released into blood, the circulating vWF multimers are coiled and do not bind to platelets in this state. vWF can bind to collagen that is exposed following vessel injury. Under shear stresses, vWF multimers unfurl exposing large numbers of A1 domains. These domains bind to the GPIIb/IIIa surface protein complex on platelets providing a 'capturing' mechanism for them at the site of vessel injury. A disintegrin and metalloproteinase with a thrombospondin 1 motif-13 (ADAMTS 13) can cleave vWF multimers to ensure the vWF chain length is not excessive.

Platelet activation then occurs following adhesion to the subendothelial matrix leading to structural changes and degranulation. Activated platelets change from a discoid shape to elongated cells with extensions to increase their surface area, known as pseudopods. Outside-in signalling subsequently occurs via various intracellular pathway leading to changes in platelet membrane structure and composition, granule release and further inside-out signalling. When activated, platelets express phosphatidylserine on their extracellular surface providing a suitable surface for procoagulant factor binding when they are no longer in contact with the site of vascular injury – a process known as flip-flop.

Platelets contain preformed granules that are released into the circulation upon activation. α-granules contain vWF, coagulation factors (fibrinogen, factors (F)V and FXIII), fibrinogen and adhesion molecules such as P-selectin, which can bind platelets securely to the endothelium and leucocytes by its ligand, PSGL-1. Dense granules are rich in ADP, ATP, calcium and serotonin, which act as agonists to other platelets in the circulation causing their activation

thereby propagating primary haemostasis. Platelet dense granules also release polyphosphate (PolyP). PolyP can activate several procoagulant factors of the intrinsic pathway. This pathway is increasingly felt to play a role in thrombosis particularly in inflammation and the innate immune system[3].

Platelets once activated at the site of vessel injury can then link together to form a platelet plug. This occurs using the GPIIb/IIIa receptor on platelets which binds to fibrinogen acting a cross-link between platelets. The process of primary haemostasis is sufficient to stop bleeding in small vessels typically in mucosal surfaces, however it is insufficient in larger vessels or more extensive injury. As a result, secondary haemostasis is required.

1.1.3 Secondary Haemostasis

Secondary haemostasis is the formation of an insoluble, cross linked fibrin plug mediated by coagulation factors in particular, thrombin. The cell-based model of haemostasis first described by Hoffman consists of three phases: initiation, amplification and propagation (Figure 1)[4].

Tissue factor (TF) is the principal initiator of secondary haemostasis. It is normally present in the subendothelial matrix after being secreted into this space by the endothelium. This process is upregulated when the endothelium is activated, such as during inflammation. TF is exposed to blood following vascular injury. Further TF is also produced from activated monocytes[5,6]. Circulating activated Factor VIIa (FVIIa), a serine protease, is present in low levels and binds to TF. The subsequent TF-FVIIa complex can bind to both Factor IX (FIX) and Factor X (FX) causing their activation. Activated FX (FXa) activates FV and cleaves prothrombin to thrombin. This results in the generation of small amount of thrombin. The thrombin

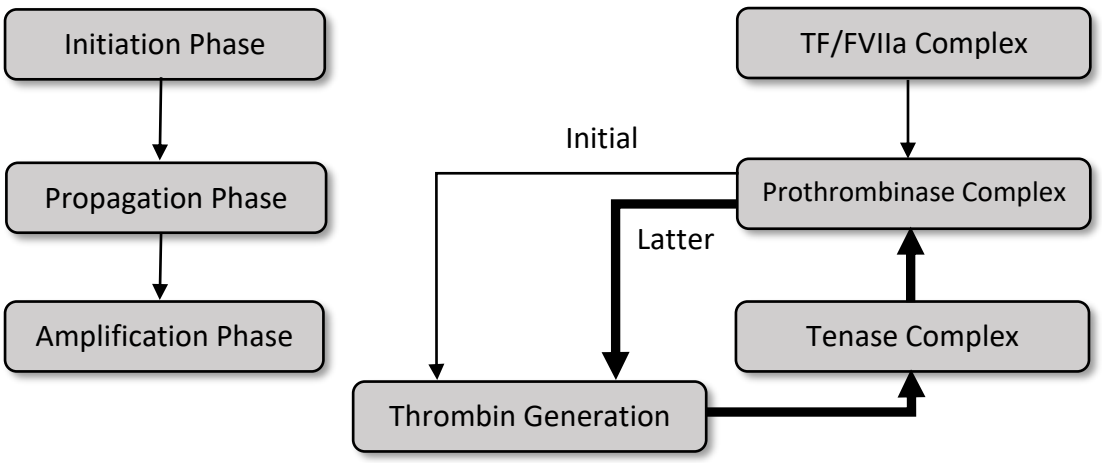
generated in this initiation phase in subsequently able to activate FV, FVIII and FXI leading to the amplification phase.

The amplification phase occurs through a positive feedback mechanism from the initial thrombin generation largely on the phospholipid surface of platelets. FXIa activates FIX to form Factor IXa. This then forms the tenase complex with co-factors calcium and FVIIIa acting as the main activator of Factor X to FXa.

Propagation occurs when the now increasing levels of FXa bind to FVa and calcium to form the prothrombinase complex, which catalyses large amounts of prothrombin to thrombin known as a 'thrombin burst'. Thrombin subsequently cleaves fibrinogen to form fibrin. The fibrin monomers polymerise to allow a scaffold for clot formation.

Thrombin also has other roles in haemostasis. It activates platelets via the protease activated receptors 1 and 4 (PAR1 and PAR4) causing the release of FV from alpha granules. It activates FV and FVIII to FVa and FVIIIa to further contribute to the amplification phase. It also activates FXIII to FXIIIa, which crosslinks fibrin multimer allowing multi-linked fibrin chains to form. These entrap red blood cells and platelets in the developing clot structure.

Figure 1: The Cell-Based Theory of Coagulation (Bold lines denote amplification phase)



The ‘Waterfall’ model of coagulation, which preceded the cell-based model, consists of the intrinsic (FXI and FXII, prekalikrein, high molecular weight kininogen) and extrinsic pathway (TF and FVII) activating the common pathway (FX with co-factor, FV) leading to subsequent thrombin generation[7]. The factors involved in the intrinsic pathway are activated in the presence of artificial substances, such as glass and kaolin, but are not felt to play a role in haemostasis under normal physiological condition. Increasingly, there is evidence that the intrinsic pathway may play a role in some forms of thrombotic diseases and are a link between the innate immune system, the complement system and coagulation[3,8,9]. The extrinsic, intrinsic and common pathways reflect the routine coagulation assays available in clinical care: the prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) respectively in additional to functional fibrinogen assays such as Clauss fibrinogen. Prolonged clotting times can indicate deficiencies of the procoagulant factors and can be used for therapeutic anticoagulant monitoring.

1.1.4 Natural Anticoagulants

To control and localise thrombin generation, several proteins can inhibit the activity of procoagulant factors. TFPI is released by activated endothelial cells. It is largely bound to the endothelium although about 20% circulates in plasma bound to lipoproteins. TFPI inhibits FXa in two ways – it inactivates FXa directly or inhibits the TF-FVIIa complex by forming a TF-FVIIa-FXa-TFPI complex[10].

Antithrombin is a circulating serine protease produced by the liver. It inactivates several serine proteases: FIXa, FXa, FXIa, FXIIa and thrombin. Heparan sulphate, a glycosaminoglycan secreted from endothelial cells, binds to the heparin-binding site on antithrombin causing a quaternary structural change. This leads to a change in the reactive site causing an increased

inhibitory effect of 2000-4000 times to thrombin and 500-1000 times to FXa[11]. Synthetic heparin molecules provide anticoagulant activity in a similar mechanism to heparans.

Thrombomodulin is a transmembrane protein found on endothelial cells. It acts a cofactor for thrombin leading to the activation of protein C. Activated Protein C (APC) binds with Protein S causing the proteolysis of FVa and FVIIIa, reducing the propagation and amplification phases of haemostasis. APC can also be bound to the EPCR, which downregulates inflammatory mediator release and vascular adhesion molecules on the endothelium resulting in an anti-inflammatory effect[12].

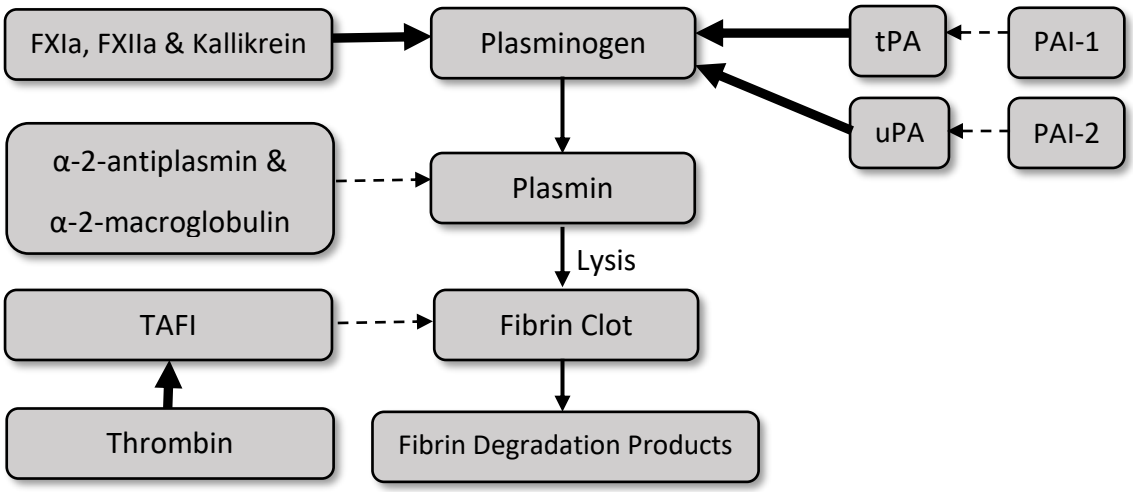
PAR-1 is activated by thrombin on various cells including the endothelium and blood vessel myocytes[13]. Its activation leads to the increased permeability of the endothelial barrier and increased expression of vascular endothelial growth factor (VEGF) in smooth muscle cells.

Alpha-2 macroglobulin is a large protease produced by the liver and has various targets. It reduces coagulation by inhibiting thrombin. It is found in higher levels in children reducing their thrombin generation potential and therefore thrombotic risk. It also decreases fibrinolytic activity by inhibiting plasmin.

1.1.5 Fibrinolysis

Fibrinolysis is the process of dissolution of a solid crosslinked fibrin clot. Several pro- and anti-fibrinolytic proteins are incorporated into the clot to allow this process to occur and regulate clot breakdown (Figure 2).

Figure 2: The Fibrinolytic System (Bold lines denote activation, dashed lines denote inhibition)



Plasminogen is a zymogen produced in the liver and released into the systemic circulation. Upon clot formation, circulating plasminogen is activated to plasmin by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Other molecules including kallikrein and FXII can cleave plasminogen. Plasmin is then able to cleave fibrinogen and fibrin leading to their degradation, producing various fibrinogen degradation products (FDPs) such as D-dimers. Cleavage of fibrin subsequently leads to the exposure of further lysine residues on the fibrin molecules to which plasminogen and tPA can bind further. This increases further plasmin formation and fibrin breakdown, augmenting the process of fibrinolysis. There are several proposed plasminogen/plasmin receptors to cell surfaces particularly to the endothelium and platelets including $PlgR_{KT}$, the S100A10-Annexin 2 complex and GPIIb/IIIa, which protect plasmin from degradation.

tPA is the main activator of plasminogen and is released principally by the endothelium following the stimulation by thrombin, histamine, adrenaline, vasopressin, hypoxia and shear stress[14,15]. uPA is secreted by endothelial cells, macrophages, monocytes and renal tubular cells activated from single chain uPA by plasmin, FXIIa and kallikrein. It has a lower affinity for plasminogen than tPA but is capable of plasminogen activation with or without the presence

of fibrin. In the subendothelial matrix, uPA is tethered by its interaction with urokinase-type plasminogen activator receptor (uPAR).

Anti-fibrinolytic proteins downregulate the degradation of the fibrin clot. Plasminogen activator inhibitor-1 (PAI-1) is released by endothelial cells, monocytes, hepatocytes and platelets from their α granules. PAI-1 binds directly to tPA forming a complex and inhibiting it from causing further plasminogen proteolysis, which is the predominant form of circulating tPA. Similarly, PAI-2 has an inhibitory effect on uPA and is secreted principally from monocytes and macrophages. Thrombin-activatable fibrinolysis inhibitor (TAFI) is activated by thrombin and thrombomodulin. TAFI cleaves exposed lysine residues from fibrin, which reduces plasmin binding and subsequently enhances its degradation.

Plasmin can be proteolysed mainly by two proteins – alpha-2 macroglobulin and alpha-2 antiplasmin. Like alpha-2 macroglobulin, alpha-2 antiplasmin is produced by the liver. Reduced levels in liver disease contribute to a bleeding phenotype.

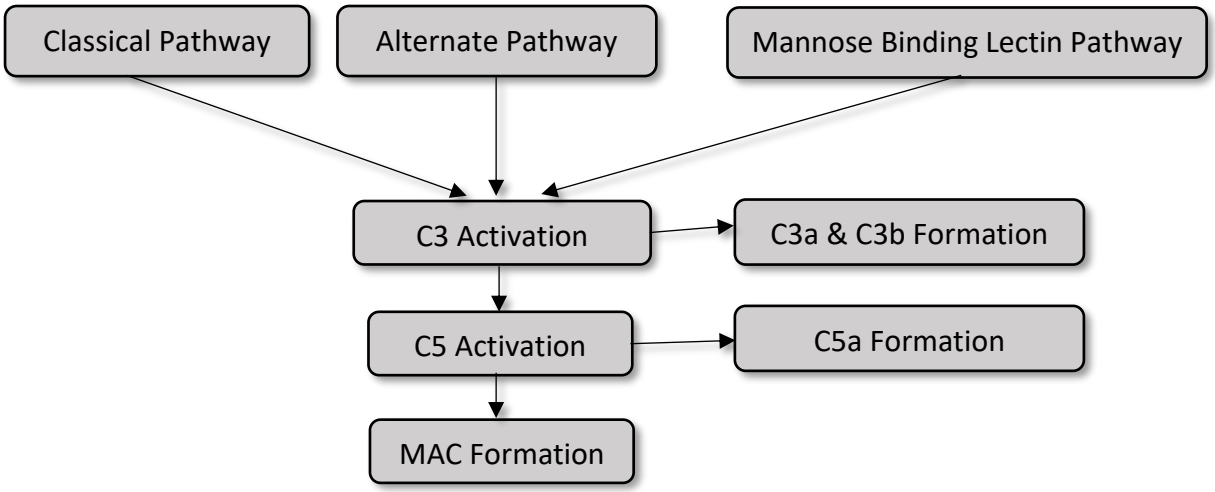
1.2 Overview of the Complement System

The complement system is key component of the immune system that interacts with both innate and adaptive immune response. The system has over 30 implicated proteins that interact with each other upon their activation by other complement proteins. The system is activated by one of three pathways: the classic, the alternative and lectin-binding pathway (Figure 3). The classical pathway is activated by an IgG or IgM immunoglobulin antibody – antigen complex. This leads to subsequent activation of C1q then C4 and C2. The alternative pathway is activated by foreign carbohydrates, lipids and proteins causing the activation of C3. The lectin-binding pathway is activated by mannose-binding lectins, which are pattern

recognition molecules normally expressed on the surface of microbial pathogens. These cause the activation of C4 and C2.

These pathways converge at C5, which then cause the formation of the membrane attack complex (MAC), a protein complex of C6, C7, C8 and C8. These downstream complement molecules disrupt the membrane of invading organisms. This can lead to its opsonisation by C3b, direct cell lysis by MAC and chemotaxis of neutrophils by C3a and C5a.

Figure 3: The Complement Cascade



There is increasing evidence of crosstalk between the coagulation and complement pathways that may be of particular importance in sepsis when the complement system is activated by invading pathogens. Subsequent activation of the coagulation and fibrinolytic systems may have a role in preventing the dissemination of infection in the bloodstream, a phenomenon known as 'immunohaemostasis'. Thrombin has been shown to activate C3 and C5 as have FX and FXI[16,17]. Reciprocally, C5a can induce endothelial tissue factor in a dose dependent manner and can induce the expression of PAI-1 from basophils, mast cells and macrophages[18–20].

1.3 Changes in Haemostasis during Critical Illness

1.3.1 Features of Bleeding in Critical Illness

The nature of bleeding in critically ill patients can be heterogenous in terms of timing and site of bleeding. Patients can be admitted to intensive care unit (ICU) with bleeding as the underlying main pathology, such as large intracranial haemorrhage causing significant neurological impairment or massive haemorrhage leaving to hypovolaemic shock. It can also develop as a secondary complication during the admission to ICU.

The main site of bleeding following admission to critical care is the gastrointestinal tract due to the development of stress-related mucosal injury. As a result of hypovolaemia and splanchnic vasoconstriction, the gastric blood supply decreases leading to the development of gastric erosions. Clinically relevant gastrointestinal bleeding has been described in between 3.3 to 9.0 % of critically ill patients[21,22]. Its development is associated with relative risk of death increased by 2.9-fold when compared to matched controls and an increased length of stay by 4-8 days[21]. Gastrointestinal bleeding is associated with the presence of coagulopathy such as those with liver disease or disseminated intravascular coagulation (DIC) and is reduced with the use of proton pump inhibitor (PPI) prophylaxis for stress ulceration[22]. However, the latter has been associated with increased rates of aspiration pneumonia[23,24].

Intracranial Haemorrhage (ICH) is not frequently seen as a complication of critical illness. It occurs spontaneously in <1% of patients with a high mortality rate in intensive care once diagnosed[25]. It is recognised in some patients in ICU such as those requiring mechanical ventilation with severe hypercarbia, prolonged thrombocytopenia, a preceding history of cancer (18% of patients with cancer in ICU) and impaired renal and hepatic function[25,26].

Due to the risk associated with venous thromboembolism (VTE) in critical care, the use of chemical thromboprophylaxis is now considered a standard of care. Major bleeding rates are described at between 5-6% in those on prophylactic dose anticoagulation in critical care[27]. As expected, this is higher than bleeding rates in medical in-patients on thromboprophylactic doses of low molecular weight heparins, with reported rates of major bleeding at around 0.5-1.7% of admissions in comparison, suggesting that critical illness increases bleeding risk[28,29].

1.3.2 Factors Contributing to Bleeding in Critical Illness

Bleeding in the setting of critical care can be attributed to various changes in the haemostatic pathways discussed in Section 1.1. These can include defects in platelet count and function and reduction in procoagulant factors and vWF. These are normally acquired defects that are a result of disease severity and organ failure or due to treatments given during critical illness. In addition, non-physiological factors including anticoagulation, antiplatelets and therapeutic interventions such as surgery and cannulation can contribute.

Thrombocytopenia

Platelets are small anucleate circulating blood cells that are essential for primary haemostasis, providing a phospholipid surface for secondary haemostasis to occur and are involved in the inflammatory response. They have a lifespan of 8-9 days, are produced predominantly by large multinucleated megakaryocytes in the bone marrow and destroyed by phagocytosis in the liver and spleen. Megakaryocytes are also present in the lung tissue producing platelets that have a distinct immunomodulatory profile compared to those from the bone marrow[30].

Normal platelet counts range from 150 to 400 x 10^9/L although a large physiological buffer exists so spontaneous bleeding does not occur until less than 10-20 x10^9/L, unless there is a concurrent defect in platelet function. Thrombocytopenia is the most common haemostatic

defect seen in critical care with its incidence being described as varying from 8 – 68% at the time of admission and a prevalence of 14 - 44% during the admission period in ICU[31]. Its presence is felt to be an indication of disease severity and organ decompensation, particularly in severe sepsis, as opposed to a primary haematological disorder. Those with platelet counts of less than 150 x 10^9/L have increased rates of bleeding and transfusion use[31]. The development of thrombocytopenia has been shown to be a marker of poor prognostic outcomes including higher mortality, increased duration of ICU stay and increased rates of organ support[31]. A delayed recovery of the platelet count also increases the risk of mortality in comparison to those without and with a temporary thrombocytopenia[32]. However, mechanisms for thrombocytopenia remain unclear in critically ill patients and are likely to be multifactorial with significant interpersonal variability.

The most probable cause of thrombocytopenia in critical care is from increased peripheral consumption and destruction of platelets. There are several molecules and pathways in the setting of sepsis and inflammation that may be implicated. DIC is a key component of this triggered by the activation of monocytes secreting tissue factor in the setting of sepsis. This leads to subsequent inappropriate, systemic activation of the coagulation system[33]. Additionally, neutrophil extracellular traps (NETs) and circulating endothelial microparticles are implicated in this process[34,35]. Thrombocytopenia is a defining feature of overt DIC along with prolongation of laboratory clotting times (PT and APTT), hypofibrinogenaemia and increased FDP[36]. However, non-overt DIC may not show all of these features but may still be a driver for thrombocytopenia in these patients[37].

Reduced bone marrow function and myelotoxicity may also lead to thrombocytopenia due to drugs, such as antimicrobials, or haematinic deficiencies accrued during prolonged critical

illness and malnutrition. During the inflammatory response in sepsis, IL-6 and C-reactive protein (CRP) levels are increased. Thrombopoietin (TPO), a glycoprotein hormone, is the key stimulant of megakaryocyte maturation and differentiation. Thrombopoietin levels increase with elevated levels of IL-6 and CRP in patients with severe sepsis, suggesting that a pro-inflammatory state itself does not suppress thrombopoiesis, unlike erythropoiesis[38]. A correlation was seen between increased TPO levels in patients with thrombocytopenia in this setting[38].

Haemophagocytosis is a syndrome that can occur in critical illness due to the unregulated production of cytokines, NK- and T-cell dysregulation and macrophage activation from various infectious, inflammatory and malignant conditions. Its development is rare with its true incidence being unknown but is invariably linked to critical illness when present. The state is defined by the presence of peripheral blood cytopenias, fever, hypofibrinogenaemia, liver dysfunction and extreme hyperferritinaemia. Bleeding in the setting of haemophagocytosis ranges from 16-23% but has been shown to be not increased in the presence of thrombocytopenia nor other coagulation defects[39]. Those with haemostatic defects due to haemophagocytosis however showed an increased ICU mortality rate of 33% in comparison to 18% without, which is suggestive that these changes are markers of severity in haemophagocytic syndrome.

ADAMTS13 plays a critical role in the regulation of vWF multimer length and primary haemostasis. It may also be implicated in thrombocytopenia. Reduced levels of ADAMTS13 may lead to increased levels of longer vWF multimers, which can increase platelet adhesion under sheer stress. vWF levels are increased during sepsis and inflammation. Moderately low ADAMTS13 levels are commonly seen is patients with sepsis[40]. Platelet counts are lower in

critically ill patients with lower ADAMTS13 level with other coagulation changes, suggestive of DIC. This also corresponds with lower survival rates from critical care[41]. Again, IL-6 is shown to be higher in this group suggesting a potential negative effect of inflammation on ADAMTS13 production or function[40,42].

Histones are a group of small, intranuclear proteins that are used for DNA packaging. When they are released into systemic circulation at times of cell damage, such as during critical illness and trauma, they become part of damage-associated molecular patterns (DAMP). The presence of extracellular histones in hyperinflammatory situations have been associated with endothelial dysfunction, organ failure and death[43]. They are cleared by APC. In a murine model, the introduction of intravascular histone induced platelet aggregation and a significant thrombocytopenia[44]. Elevated histone levels at admission to critical care have been associated to the subsequent development of thrombocytopenia with a correlation between the amount of circulating histone and degree of thrombocytopenia[45]. Elevated levels of serum histone H3 with thrombocytopenia in sepsis are correlated to the development of DIC and increased mortality rates[46,47].

Other conditions causing thrombocytopenia in critical care are microangiopathic haemolytic anaemia (MAHA) and heparin-induced thrombocytopenia (HIT). MAHA is a cause of thrombocytopenia is associated with a thrombotic phenotype with varying underlying cause. HIT, which has a prevalence of 5% in the intensive care setting, is also associated with thrombocytopenia occurring on exposure to heparins[48].

Platelet dysfunction

Other than thrombocytopenia, platelet function can be affected in the critical care setting. The contribution of this to bleeding in critical care is often problematic to assess due to the

difficulties in clinical testing and the multifactorial causes and dynamic changes that affect platelet function.

Platelet aggregation is reduced in those admitted to critical care with trauma and sepsis. This has been demonstrated by multiple activators including collagen, adrenaline, ristocetin, arachidonic acid and ADP to varying degrees by lumiaggregometry, flow cytometry and thromboelastography (TEG)[49–52]. Inhibition of platelet mitochondrial respiratory chain enzymes because of inflammatory processes seen in systemic inflammation may cause these aggregation changes, due to the inability to continue key platelet metabolic pathways. Similar to other platelet changes, these are associated with elevated levels of IL-6[53]. Platelet ballooning and dysfunction have been demonstrated in response to histone H4 exposure in major trauma, with histone release being a feature seen in other types of critical illness[54,55]. The use of continuous haemofiltration in renal failure also depresses platelet aggregation further. More profound defects (typically <60% of expected aggregation levels) were associated with a high rate of mortality. At present, it is unclear how acquired platelet function defects contribute to the development and severity of bleeding in critical care[56].

Bleeding related to uraemia in seen in those with both acute and chronic renal failure. Bleeding seen in this setting affects various components of coagulation although largely affects primary haemostasis[57]. There is a reduction in either vWF function or dysfunctional and reduced expression of platelet GP1b/IX receptors. Uraemic toxins, such as guanosuccinic acid and phenolic acids, cause increased levels of nitric oxide leading to vasodilation and reduced platelet aggregation. Anaemia is also seen in renal failure due to the reduction in erythropoietin synthesis. Anaemia can lead to a reduction in the normal axial flow of blood

where platelets localise towards the outer flow allowing for closer contact with endothelium at times of vessel injury normally.

Changes in Coagulation Factors

Coagulation factors can be altered due to various influences in the setting of critical care. Reduced coagulation factor levels can be due to a reduction in their synthesis, increased consumption or haemodilution.

The liver is the principal site of coagulation factor synthesis of both procoagulant (FII, V, X, VIII, IX, XI, XII and fibrinogen) and anticoagulant factors (protein C, protein S and antithrombin). Acute liver injury or liver cirrhosis can result in reduced levels in the majority of these factors. The rates of liver dysfunction as a complication of sepsis in critical care are unclear but vary from 1 – 35% of patients depending upon definitions[58,59]. FVIII and vWF levels however remain relatively preserved due to secretion from activated endothelium as acute phase proteins. Due to global reduction of both pro- and anticoagulation factors in liver dysfunction, prolonged routine laboratory coagulation tests such as PT and APTT are not representative of bleeding risk but are associated with adverse outcomes[60]. TEG is abnormal in patients with cirrhosis with reduced parameters of clot formation[61]. However, thrombin generation assays can be normal in patients with liver cirrhosis when using tissue factor and thrombomodulin. Recent data has suggested that less severe stages of cirrhosis (Child-Pugh stages A and B) have increased thrombin generation, particularly by endogenous thrombin potential[62,63]. These results largely supersede a previous belief of 'auto-anticoagulation' in patients with liver disease. These findings are similar regardless of the underlying aetiology of liver disease.

Consumption of coagulation factors during critical illness is typically seen in DIC with concurrent hypofibrinogenaemia and thrombocytopenia. This is seen as a prolongation of APTT and PT in routine laboratory testing. Reduced levels of coagulation factors II, V, VII, X and XII are seen in sepsis in comparison to controls contributing to these changes in APTT and PT[64].

Haemodilution is seen with the replacement of large volumes of exogenous crystalloid or colloid fluids, occurring in situations such as the use of cardiopulmonary bypass (CPB) and ECMO or following massive haemorrhage. It is typified by a significant reduction in haematocrit with expansion of the non-cellular portion of blood. This causes a global dilution of coagulation factors, fibrinogen and platelet counts increasing the risk of haemorrhage known as dilutional coagulopathy. The effects on coagulation parameters, such as clot firmness, clotting formation times and fibrinolysis, may vary according to the fluids given[65,66]. Outcomes following CPB are affected by the severity of haemoglobin reduction with increased rates of post-operative bleeding, blood component use and thromboembolic complications in those with severe haemodilution[67]. Changes in thromboelastography in the setting of haemodilution show a reduction in clotting time and clot firmness. In the elective surgical setting in patients who have dilutional coagulopathy, platelets and fibrinogen are reduced but do not vary between those with and without bleeding complications. However, PT and APTT correlate to bleeding severity with low levels of FV and FIX respectively[68]. The addition of fibrinogen and FXIII has been shown to improve the changes seen in thromboelastography[66]. The coagulation changes in this setting however are variable due to differences between *ex vivo* and *in vivo* analysis, pre-dilutional coagulation changes such as in the setting of elective versus emergency surgery and the lack of correlation between PT and APTT in comparison

with TEG to clinical outcomes. The role of FXIII is also not assessed with routine laboratory monitoring such as PT.

Changes in Fibrinolysis

Fibrinolytic activating factors are produced by the endothelium, plasminogen and alpha-2 antiplasmin by the liver and to a lesser extent, platelets produce PAI-1. These are all affected by critical illness. Endothelial activation leads to the release of tPA and PAI-1 early in septic shock, which is seen in both survivors and non-survivors at ICU admission[69]. PAI-1 and TAFI are also significantly increased in sepsis and septic shock[70,71]. Markers of fibrinolytic activity, plasmin-antiplasmin complexes and FDPs including D-dimers reflecting increased fibrinolytic activation, are also elevated in sepsis and are associated with illness severity. TEG shows a significant impairment of fibrinolysis by a higher maximal amplitude (MA) and reduced lysis at 30 minutes from MA (Ly30 time) in sepsis compared to normal controls[72]. These features are more common with more severe markers of critical illness and are related to increased rates of short-term mortality. Further systematic review has suggested that hypofibrinolysis due to elevated levels of PAI-1, as opposed to hyperfibrinolysis is related to adverse outcomes in septic shock[73]. Changes seen in TEG have not been correlated to one specific protein change and are likely to reflect a combination of both activation of fibrinolysis and subsequent consumption and inhibition of these proteins.

The addition of severe liver disease may also further impact the function of the fibrinolytic system. As the key site of synthesis for several fibrinolytic factors and its role in clearance of tPA, bleeding in liver disease has been at least in part attributed to abnormal fibrinolysis[74]. This has been further suggested by clinical responses in bleeding to anti-fibrinolytic agents[75].

1.3.3 Features of Thrombosis in Critical Illness

Thrombosis is typically a secondary event in critically ill patients although it can be the reason for ICU admission due to arterial or venous thrombosis leading to significant organ dysfunction. The presence of either symptomatic and asymptomatic deep vein thrombosis (DVT) in patients admitted to critical care is around 10%[76].

The risk of developing DVT is higher in critical illness than other hospitalised patient groups. The estimated incidence in hospitalised medical patients is approximately 5% of patients receiving chemical thromboprophylaxis and 10-15% in those without[28,77]. In contrast, a meta-analysis by Malato *et al* showed that the incidence of lower limb DVT detected principally by ultrasound screening following admission to ICU was 12.7% with chemical thromboprophylaxis. They found that the development of DVT was associated with duration of mechanical ventilation and patients developing DVT had an increased length of hospital stay (LOS) and higher hospital mortality rates[78]. In patients not receiving chemical or mechanical thromboprophylaxis, DVT rates as expected are high at 25-32%[76]. In critical care, their presentation is often largely silent and their detection by radiological testing with an absence of clinical manifestations is between 10-100% of patients who are diagnosed with a DVT[79].

The literature on the incidence of pulmonary embolism (PE) is less well described than DVT in the setting of critical care. However, it is of interest due to its greater impact upon morbidity and mortality. As a result, DVT is often used as a surrogate for PE risk in clinical studies in critical care. On post-mortem studies, McLeod *et al* showed 20-27% of critically ill patients had evidence of PE although mortality associated to this was difficult to extrapolate due to other confounding cardiopulmonary disease[80,81]. There are multiple risk factors for the

development of VTE and one or more are seen in critically ill patients. Therefore, admission to critical care itself should be considered a significant risk factor for VTE.

The presence of fibrin deposition and small volume thrombus has been long recognised as a component of acute respiratory distress syndrome (ARDS), an episode of acute respiratory failure due to pulmonary inflammation. Tomashefski *et al* showed near universal multifocal micro- and macrovascular thrombus in patients with ARDS regardless of underlying pathology correlating to acute endothelial damage in histological post-mortem studies[82]. Antemortem studies using balloon occlusive pulmonary angiography have demonstrated the presence of pulmonary vessel filling defects in patients with ARDS in critical care[83–85]. Their presence correlated with severity of ARDS, duration of intubation and development of DIC. These features are suggestive of extensive small volume pulmonary thrombi[83]. This mechanism, known as 'immunothrombosis', is proposed to be related to fibrin deposition at the sites of infection or inflammation in an exaggerated response of localisation of the invading pathogen or inflammatory process at the site of lung injury[86]. Immunothrombosis has been recognised as a key feature of ARDS in severe SARS-CoV-2 (COVID-19) pneumonia[87].

1.3.4 Factors Contributing to Thrombosis in Critical Illness

The thrombotic state seen in critical illness, like haemorrhage, is multifactorial. All components of Virchow's triad – venous stasis, vessel injury and hypercoagulability - are affected in intensive care contributing to the risk of developing VTE. Arterial thrombosis in comparison is again dependent on hypercoagulability but also the presence of high shear stress.

Hypercoagulability

At the onset on critical illness and sepsis, the immune system mediates the release of various molecules known as the acute phase response proteins. There are elevated levels on TNF-α, IL-1 and -6, Tissue Growth Factor (TGF)-β through various cells and tissues such as macrophages, neutrophils, platelets, hepatocytes and the endothelium. Haemostatic molecules released during these periods are FVIII, fibrinogen, vWF and monocyte-produced TF. As a result, TEG and thrombography in the setting of sepsis has shown attenuation of the initiation of coagulation but elevated levels of thrombin generation and clot formation[64,88]. This is correlated to increased markers of coagulation activation in sepsis such as soluble TF antigen, prothrombin fragment F1+2, fibrinopeptide A, and D-dimer[89].

NETs are a complex of chromatin fibres, DNA, histones with antimicrobial molecules, mainly myeloperoxidase and neutrophil elastase, which are extruded from neutrophils as a first line of defence to trap bacteria and other pathogens[90]. NETosis, the process of NET release, occurs at areas of inflammation allowing the adherence of platelets, red blood cells and other molecules including vWF, fibrin, FXII and high molecular weight kininogen (HMWK) therefore promoting thrombin generation and increasing coagulability[91,92]. This process of thrombus formation is recognised in immunothrombosis. NETosis have been implicated in the pathogenesis of various thrombotic conditions including sepsis, DIC, cancer-associated thrombosis, trauma and venous thromboembolism. The presence of NETs, circulating cell-free DNA and histones have been associated with poorer survival outcomes and an increased risk of multiorgan failure in severe sepsis[93,94].

HIT causes a procoagulant state typified by the development of antibodies to a heparin-PF4 complex. Although this occurs at significantly lower rates in critical care than the prevalence

of thrombocytopenia, the rates are higher than in non-critical care medical or surgical patients. HIT leads to a significantly increased rate of thrombosis. The incidence rate of thrombosis in approximately 60% of patients with HIT in critical care[95].

Stasis

Venous stasis is a key feature seen in critical care. Immobility of the patients is nearly universal in this setting. Hitos *et al* showed that popliteal vein blood flow drops by 40% after sitting for 100 mins in healthy volunteers[96]. Therefore, it is expected that lower limb venous blood flow is significantly reduced in critical care. LOS in critical care is typically days to weeks with mean durations of 19-26 day in previous studies observing rates of VTE. LOS has been identified as a risk factor for the development of DVT, PE and increased mortality rates in critical care[78,97,98]. The use of intermittent pneumatic compression during admission to the intensive care unit has not been shown to significantly reduce the incidence of VTE in combination with chemical thromboprophylaxis in comparison to the later alone. This suggests that other contributing factors of hypercoagulability and vessel injury, other than venous stasis alone, need to be addressed to reduce the development of VTE[99].

In-dwelling catheters are used extensively for drug administration and monitoring via centrally and peripherally inserted lines. Despite technological developments, they are prothrombotic surfaces and reduce surrounding blood flow particularly in vessel with reduced luminal flow. The surface of catheters can initiate coagulation through activation of the contact system. This effect has been shown to be reduced by the addition of corn trypsin inhibitor, a potent inhibitor of FXIIa[100,101]. Fibrin subsequently deposits around the line causing thrombus development within hours of insertion known as a fibrin sheath[102]. Blood flow in simulated *ex vivo* models suggest that flow may fall by up to 93%, particularly with

either larger lumen catheter or smaller blood vessels, such as superficial veins[103]. Risk factors for catheter-related thrombosis are the use of multi-lumen lines, peripheral inserted central catheters, line tips proximal to the superior vena cava and the use of femoral veins. The highest risk for development of thrombus around catheters in the first 10 days of placement[104]. Thrombus formation around the catheter is either intraluminal, causing occlusion and potential catheter failure, or mural, which are typically non-occlusive to the vessel due to the presence of the catheter.

Vessel Damage

Vessel injury can occur at sites of trauma or surgery in post-operative patients and at line insertion or procedural sites. Disturbance of the vascular endothelium leads to exposure of the subendothelial matrix activating normal haemostatic mechanisms to control bleeding at the site of vessel injury. However, in the setting of critical care, these may be confounded with other prothrombotic change to cause thrombosis at these sites.

Other critical illness related factors have been associated with the development of thrombosis such as the use of vasopressors, prolonged mechanical ventilation and neuromuscular paralysis[105,106]. Patient specific factors for thrombosis in the critical setting are increasing age, obesity, prior VTE and pregnancy, which are associated with hypercoagulable states.

1.4 The Function, Indications and Components of Extracorporeal Membrane Oxygenation

1.4.1 The Function of Extracorporeal Membrane Oxygenation

Severe cardiac and respiratory failure cause inadequate delivery of oxygen and other essential substances to body tissues and the failure to remove potential harmful metabolites such

carbon dioxide (CO_2). ECMO is used to substitute the function of the lung or the heart whilst allowing the organs to recover without potentially damaging therapies, such as high-pressure ventilation and vasopressors.

The two main forms of ECMO circuits are veno-venous (VV), to replace the function of the native lungs in respiratory failure or veno-arterial (VA) for cardiac failure. Both forms remove deoxygenated blood from the venous system that is then subsequently oxygenated outside of the body by the membrane oxygenator. In VV-ECMO, blood is returned into the venous system whereas in VA-ECMO, it is returned into the arterial system.

Oxygen delivery (DO_2) is dependent on the oxygen content (CaO_2) of arterial blood and cardiac output (CO), which at rest is around 5 litre per minute in an adult human.

$$DO_2 = CaO_2 \times CO.$$

CaO_2 includes the amount of oxygen bound to haemoglobin and the amount dissolved in plasma in relation to partial pressure of oxygen (PO_2).

$$CaO_2 = \text{haemoglobin (Hb)} \times \text{oxygen saturation } (SaO_2) \times \text{Hüfner's Constant (1.34)} + (pO_2 \times 0.03)$$

Therefore, tissue oxygen delivery can be affected by several factors including cardiac function, haemoglobin levels and blood oxygenation. Tissue oxygen consumption (VO_2) is affected by fever, drugs, hormones, paralysis and cooling in critical care. When the DO_2/VO_2 ratio falls below 2:1, there is inadequate oxygen delivery to tissues to maintain normal metabolic activities. Below this level, there is a change to anaerobic pathways that can only be tolerated for short periods, which is typically hours. ECMO can overcome these failures and various components of tissue oxygen delivery can be optimised by the circuit.

CO_2 removal by ECMO occurs via diffusion from circulating blood to the “sweep” gas. Its rate of removal is determined by the sweep flow of continuously flowing gas. If the main purpose of the membrane is to remove CO_2, high sweep to blood flow ratios can be used with than if for oxygenation.

1.4.2 Indications for Extracorporeal Membrane Oxygenation

ECMO is indicated when there is high risk of mortality despite other conventional therapies, typically mechanical ventilation and vasopressors. These indications include:

- Hypoxaemic respiratory failure
- Hypercapnic respiratory failure
- Refractory cardiogenic shock
- Cardiac arrest
- Following cardiopulmonary bypass surgery
- As a bridge to other long-term therapies, such as ventricular assist device and organ transplantation

1.4.3 Components of the Extracorporeal Membrane Oxygenation Circuit

The two key components to the circuit are the pump and the oxygenator membrane. These are configured differently as to whether respiratory support alone is required (VV ECMO) or cardiac support (VA ECMO). A schematic illustration of the circuit types is shown in Figure 4. The pump allows movement of blood from the recipient and through the membrane oxygenator (Figure 5). Nowadays, centrifugal pumps are the most used pump type although roller and peristaltic pumps have been used previously. Haemolysis is a recognised complication of the pump causing destruction to RBC as they pass through. The membrane oxygenator is a network of thousands of small hollow fibre tubes through which continually

flowing gas passes and the recipient's blood passes the other side of these fibres (Figure 5). The material of the fibres allows the diffusion of gases but not liquids. They consist of either solid silicone rubber, a microporous hollow-fibre, or a solid hollow-fibre. The flow rate is the rate at which the venous blood is passed through the oxygenator to allow for full oxygenation (SaO_2 greater than 95%). Therefore, increasing flow rate increases blood oxygenation. Sweep flow on the other hand is the rate of sweep gas flowing through the oxygenator and is indirectly proportional to the carbon dioxide level. Additionally, the membrane oxygenator can also act as a sieve to circulating thrombus. Oxygenator failure can occur when sufficient amounts of thrombus accumulate within the device.

Figure 4: Schematic Diagram of ECMO circuit (arrow lines indicate direction of blood flow)

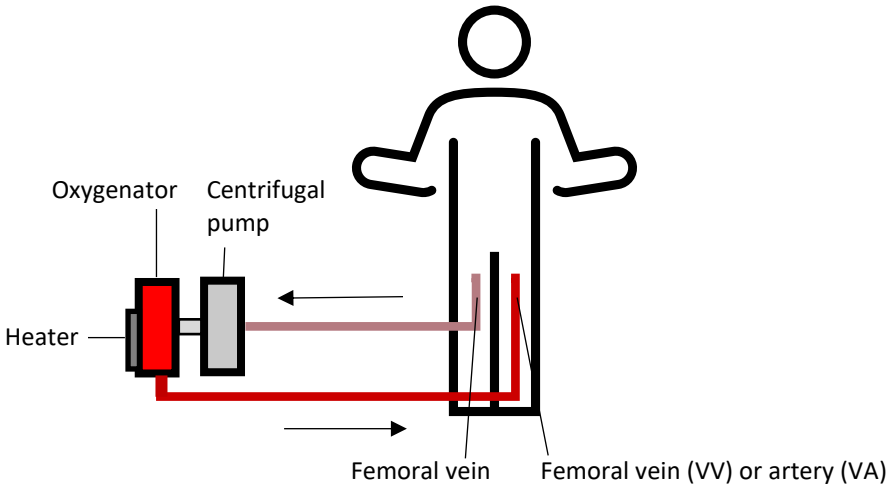
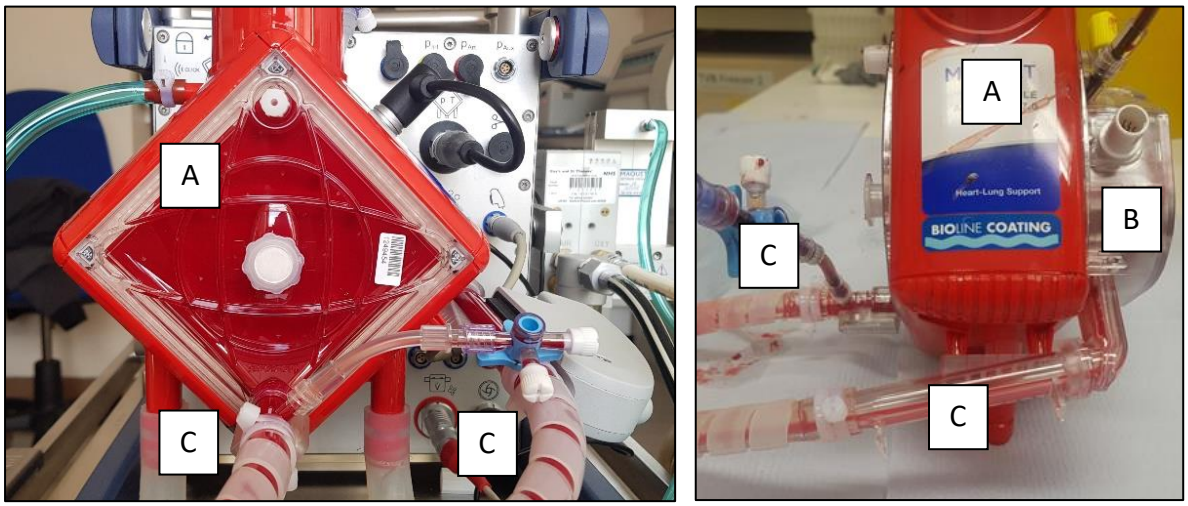


Figure 5: Components of ECMO Circuit (A) oxygenator membrane (B) pump (C) connectors and tubing



In addition to the oxygenator and pump, there are large bore cannulae that are typically sited in proximal vessels such as the common femoral veins, internal jugular veins and femoral arteries. These are the sites of removal and return of the recipient's bloods. Circuit tubing connects the various components of the circuits and is long enough for the pump and oxygenator to be stored next to the recipient. A heat exchanger is incorporated into the circuit to maintain a constant body temperature when blood leaves the recipient. Monitors are present to measure circuit blood flow and pressures and pre- and post-oxygenator oxyhaemoglobin levels.

1.4 History of Extracorporeal Membrane Oxygenation

The concept and development of an extracorporeal circuit was first established by surgeon Dr John Gibbon in the 1930s[107]. The initial development of a CPB circuit was used on a cat in 1939 but it was not until 1953 that CPB was first used on a human patient by Dr Gibbon for an atrial septal repair[108]. The initial device had a roller pump and passed deoxygenated blood over thin films exposed to oxygen.

The subsequent year in 1954, C Walton Lillehei advanced the CPB circuit using a bubble oxygenator with his colleague Richard De Wall, known as the De Wall bubble oxygenator[109]. They proceeded to develop this technology over the subsequent years. Issues with these early circuits were related to the presence of glass-blood interfaces causing damage to blood components and the use of bubble oxygenation producing haemolysis. This meant that its use was limited to short time periods in the operating theatre but not for longer term use in critical care.

The next key developments were a change from glass surfaces to silicone and the use of anticoagulation in conjunction with the circuit use. This allowed for significant improvements in the duration and versatility of extracorporeal circuits.

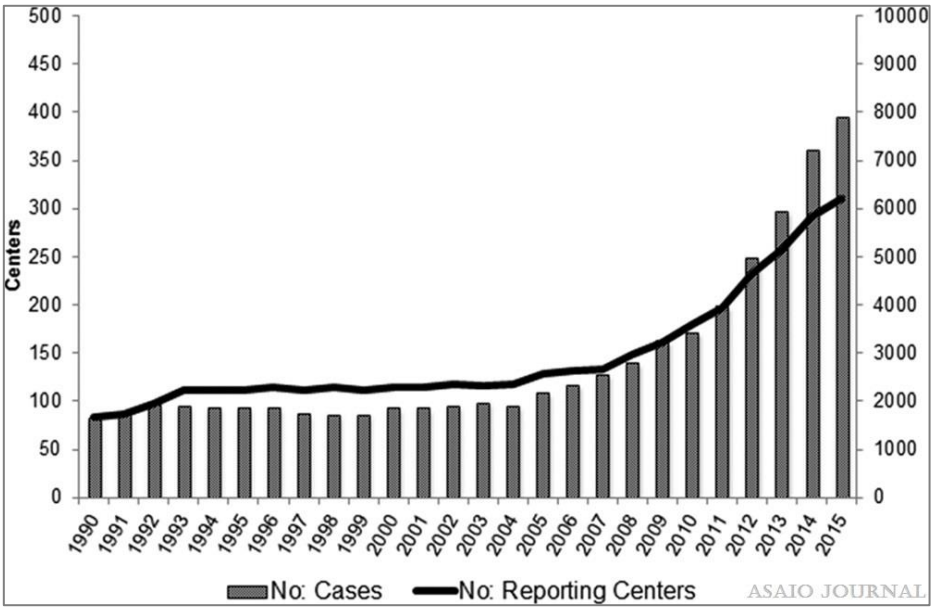
In 1972, Robert Bartlett and colleagues reported the first successful use of ECMO in a 2-year old child following cardiac surgery for 36 hours and subsequently described its use in a cohort of similar patients following low cardiac output after complex heart surgery[110]. Dr Hill also described the use of ECMO in an adult following road traffic accident for 75 hours with survival although subsequent use in adults showed low survival rates.

Further progression in circuit designs, increasing provision of service and clinical expertise in ECMO increased significantly over the 1970s and 1980s. It was predominantly used in the neonatal and paediatric settings with improved survival outcomes over 'standard' intensive care treatments. However, a randomised-control trial in the setting of severe respiratory failure (SRF) in adults in 1979 showed a 90% mortality so its use in this patient group was relatively abandoned[111]. The Extracorporeal Life Support Organisation (ELSO) was set up in 1989 to allow for harmonisation of practice, improved education to intensivists and development of patient registries of ECMO use.

In the 2000s, further developments were made in the membrane oxygenators to allow for separation of the liquid-gas interface and the use of centrifugal pumps leading to reduced rates of haemolysis. Devices became smaller and more user-friendly over this period but remained a 'niche' technology particularly in adults for procedures such as lung transplantation.

At the later part of 2000s, two further developments changed the current practice of ECMO use in SRF. The CESAR trial results were published in 2009; this was a randomised multicentre study in the United Kingdom[112]. It showed that patients transferred to a SRF Unit that could provide ECMO had improved survival than those with conventional treatment. The same year, the outbreak of the H1N1 influenza pandemic causing fulminant respiratory failure meant that the use of ECMO was sought as potential therapeutic option (Figure 6).

Figure 6: Extracorporeal Life Support Volume of Adult Patients and Number of Extracorporeal Life Support Organization Member Centres (Taken from Thiagarajan et al (2017) ASAIO)[113]



As a result of this, the global use of ECMO has increased year upon year to this present day. During the COVID-19 pandemic, the utilisation of ECMO has greatly increased. Subsequent improvements have included better vascular access techniques with a Seldinger approach

with small lumen sizes, development in the coatings of the circuit to largely include heparin-bonded materials to reduce haemostatic complications, improved oxygenation and increased membrane performance[114,115]. The indications for ECMO have also increased. In 2011, the first 2 cases of extracorporeal CPR were described. The use of 'awake' ECMO has been developed particularly by the Karolinska Institute, Sweden to allow for longer runs, bridging to lung or heart transplantation and to allow ambulation. The removal of extracorporeal carbon dioxide removal (ECCO2R) is also being established in those with exacerbations of chronic obstructive pulmonary disease[116].

1.6 Haemostatic Complications in Extracorporeal Membrane Oxygenation

1.6.1 Thrombosis During the Use of Extracorporeal Membrane Oxygenation

The development of thrombotic complications during the use of ECMO is common and can be either venous or arterial. Rates of reported ECMO-associated thrombosis vary according to centres, which may in part be due to different anticoagulation regimens used. VA-ECMO has been associated with higher rates of thrombosis and haemorrhagic complications and increased use of platelet and fresh frozen plasma (FFP) usage than VV-ECMO[117]. This may be attributable to multiple factors such as the use of antiplatelets, post-operative inflammatory states and underlying cardiovascular risk factors in patients receiving VA-ECMO.

The incidence of ECMO-related venous thromboembolism ranges from 18-85% in different centres with a predominance of cannula-associated deep vein thrombosis (Ca-DVT)[118,119]. The cannulae required for ECMO are large diameter to allow for the adequate blood flow to and from the patient, which significantly reduce the surrounding blood flow in the vessels.

Idenitifed risk factors for the development of Ca-DVT are increased age and obesity, which are also associated to VTE risk in non-ECMO patients[105,119].

The subsequent embolisation of DVT to form PE has not been consistently described following ECMO although it is not felt be a significant complication of the technology. ECMO itself has been used as a supportive therapy in patients with massive pulmonary emboli with cardiovascular compromise in combination with systemic or localised thrombolyis, or thrombectomy[120]. Similar to previous studies assessing for PE in critical care, patients with SRF have been identified at the ECMO initiation of having an incidence rate of 9-10%, comparable to those who had on-going invasive mechanical ventilation only[121]. Hartley *et al* showed that in patients with PE at the commencement of ECMO had no increase in mortality in comparison to those without. The location of the pulmonary artery filling defect at this time was predominantly peripheral in 72-100% of cases. As computer tomography (CT) is not used routinely to assess pulmonary vasculature following ECMO, it is unclear whether progressive PE is a concern although one centre reported no PE in the follow up of 48 patients with DVT following ECMO[119].

The presence of VTE has not clearly been identified with a worse survival outcome, particularly with Ca-DVT for which there is most data available. However, due to high rates of imaging in patients requiring ECMO, this may mean that there are high detection rates of thrombotic events and earlier introduction of therapeutic anticoagulation.

The main arterial ischaemic event seen with ECMO is ischaemic stroke although other sites such bowel and hepatic ischaemia are also recognised. Sutter *et al* described cerebral ischaemia occurring in 5% of patients requiring ECMO in meta-analysis of acute neurological complications and it was associated with a mortality rate of >50% if present[122]. Risk factors

identified were atrial fibrillation, the presence of coagulopathy, elevated lactic acid levels pre-ECMO, decreased cerebral perfusion due to poor cardiac output and the use of inotropes[122,123]. Rastan *et al* confirmed cerebral ischaemia as being the most common site of arterial events at the time of death following ECMO support[124].

1.6.2 Bleeding During the Use of Extracorporeal Membrane Oxygenation

Major bleeding is defined as bleeding in a critical site (intracranial, intraocular, and retroperitoneal) or with a haemoglobin fall of 2 g/L within 24 hours by the International Society on Thrombosis and Haemostasis (ISTH)[125]. Due to the higher use of blood products and greater frequency of anaemia in patients requiring ECMO, ELSO has defined major haemorrhage as intracranial haemorrhage or bleeding with 20 ml/kg of red cell concentrate within 24 hours.

ICH is a principal cause of mortality with regards to haemostatic complications during ECMO[122]. The incidence and subsequent survival outcomes may be related the use of screening imaging at the time of cannulation and subsequent cessation of anticoagulation. When cranial CT imaging is used at the initiation of ECMO in a 'pre-emptive' approach, there is a prevalence rate of ICH at 14%[126,127]. Subarachnoid (SAH) (57%) followed by petechial intraparenchymal (36%) haemorrhage are the most common types of bleeds at this time. This suggests that ICH may occur prior to the initiation of ECMO or at its initiation with anticoagulation administration. Survival rates using this approach are 69% with no difference in those without ICH requiring ECMO. In contrast, lower rates of ICH of between 3-7% have been described when a 'reactive' approach to imaging is used when a change in neurological status occurs. The time to detection of these events was 7 days from initiation to 22 days post decannulation[128]. More severe neurological sequelae and larger bleed volumes may happen

in these patients due to coagulopathy related to ECMO and exposure to anticoagulation leading to higher mortality rates of 32-100%[129].

Risk factors for ICH are antithrombotic therapy, higher Severe Organ Failure Assessment (SOFA) score prior to ECMO, thrombocytopenia, the use of renal replacement therapy (RRT), spontaneous extracranial haemorrhage and the amount of administered platelet and red cell tranfusions[130]. No correlation was identified between preceding APTT, PT, fibrinogen and platelet counts in the 4 days preceding ICH to those who did not. This suggests that other haemostatic factors that are not routinely monitored may play a role in the development of ICH.

Major bleeding at non-cranial sites is variable in prevalence and site. It may occur at multiple sites during the use of ECMO. These include at cannulae and surgical sites (93%), the naso- and oropharynx (64%) and lungs (57%), which are sites of vascular injury or inflammation in the setting of patients requiring ECMO[131]. An accurate prevalence of bleeding and subsequent mortality rates is often difficult to assess between studies due to a lack of standardisation of major bleeding in this cohort, small patient numbers and largely retrospective biased data.

1.7 Components of the Extracorporeal Membrane Oxygenation Circuit and Their Effects on Haemostasis

1.7.1 Biomaterial Surfaces

The surface of the ECMO circuit provides a large biomaterial surface, which is in contact with the recipients' blood. The interaction between biomaterial surfaces and blood has been extensively described by Vroman *et al* who reviewed the interaction principally between glass surfaces and protein adsorption over 30 years ago[132]. The subsequent 'Vroman effect' has

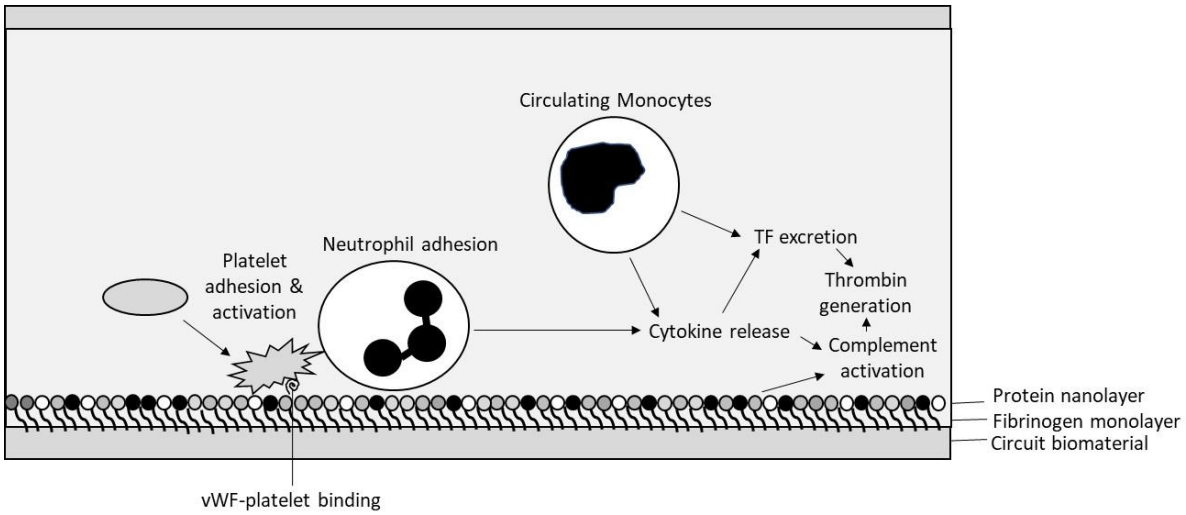
been described as the sequential adsorption of proteins to a biomaterial surface (Figure 7). Initial immediate adsorption of fibrinogen to the surfaces occurs within minutes of exposure[132]. Next, a variety of proteins then bind to the fibrinogen nanosurface. These include the coagulation factors of the contact activation pathway— HMWK and FXII–as well as high-density lipoprotein, albumin, immunoglobin (Ig) G, and complement component C3[133]. Passmore *et al* have shown that fibrinogen levels fall in an ovine lung injury model with the commencement of the ECMO circuit and recover to the baseline level within 48 hours suggestive of its adhesion to the biosurface and loss from circulation[134]. An acquired hypofibrinogenaemia is seen in children following CPB with increased haemorrhage rates and reduced clot strength[135].

After the formation of a protein-based layer on the surface the ECMO circuit subsequent cellular adhesion occurs. Platelets and polymorphonuclear leucocytes (PMN) interact with each other on the protein-covered biomaterial surface. Interaction with the complement system also contributes to their activation. This results in propagating further adhesion and PMN release of cytokines including IL-1β, -6, and -8 and TNF-α, potentially contributing further to a pro-inflammatory state[136] (Figure 7).

Different components of the ECMO circuit are designed to have varying properties depending on their use such as tubing, oxygenator membranes, and connectors. The membrane oxygenator is of importance as they have a large surface in contact with blood. They are made of materials such as polymethyl pentene and polypropylene, which are hydrophobic. This allows gas exchange to occur but inhibits blood from passing through the membrane pores. There is subsequent adsorption of the unwanted proteins, known as protein fouling, to the surfaces which undergo further conformational changes increasing their thrombogenicity.

The nature of protein adsorption depends on the biomaterial surface and alters further protein-cellular interactions and cellular adhesion. For example, HDL adsorption is higher to hydrophobic surfaces with reduced platelet adhesion but surfaces with higher fibrinogen adsorption have more platelet adhesion[133,137].

Figure 7: Schematic diagram of initial interactions between circuit biosurface and blood. Following the initial binding of fibrinogen to the circuit surface, further proteins form a nanolayer. This allows binding on platelets and white cells contributing to thrombin generation



To reduce protein fouling and the prothrombotic nature of the ECMO surfaces, small molecules can be incorporated in to the biosurface coating. Initially, molecules such as albumin and silicone have been used to act as a barrier to coagulation factors and complement but still cause a similar degree of thrombocytopenia[138]. Further development led to heparin-coating, which is widely used in commercially available ECMO circuits, that provides an additional localised anticoagulant effect. Heparin-coating decreases thrombin generation by binding to circulating antithrombin and demonstrates less platelet and leucocyte activation[139–141]. The development of this approach has reduced thrombosis and filter losses although they still occur[115]. Currently available ECMO circuits with different

coatings do not show any significant difference in haemostatic parameters indicating there is little evidence of heparin leaching off the surfaces[142].

Recent developments to further reduce protein fouling include the coating of high density hydrophilic molecules, which bind high amounts of water, or to include polymers with branch chains to stop conformational changes to the adsorbed proteins, known as polymer brushes[143,144]. Obstals *et al* have described the development of a polymer brush coated surface for ECMO, which significantly decreased coagulation, platelet, and leucocyte activation *ex vivo*[145]. At present, these are not used clinically due to difficulties in the polymerisation process.

1.7.2 Shear Stress in the ECMO Circuit

Shear stress is a form of frictional force that occurs to liquids as they pass perpendicularly to a lumen surface. The ECMO circuit has been shown to cause high flow shear stress, which is predominantly due to the centrifugal pump[146]. Shear stress can alter platelets, RBC and vWF in the patients' blood whilst passing through the circuit.

Platelets under normal circumstances approximate to the outer part of the vessel lumen with normal laminar flow but because of abnormal flow patterns with ECMO circuit, shear stress is the increased. Subsequently, platelets can adhere to the protein-coated monolayer of the biosurface of the ECMO circuit as well as reacting with other systemically activated coagulation factors and complement proteins.

Exposure to elevated shear flow from the ECMO circuit causes platelet receptor shedding of the key platelet adhesion glycoproteins, GPIbα and GPVI, and a loss of high molecular weight multimers of vWF (HMWM)[147]. This results in an impairment of the main mechanism of platelet adhesion. These changes persist despite platelet transfusions and the duration of the

ECMO circuit run. Subsequently, there are lower levels of platelet aggregation shown by light aggregometry using various agonists including ADP, ristocetin, collagen and epinephrine[51,147–150]. This may contribute to the increased levels of bleeding seen in these patients in addition to the use of anticoagulation and antiplatelet agents. Flow cytometry of platelets in those receiving ECMO showed severely reduced expression of membrane-bound p-selectin and CD63, which modulates platelet spreading[150].

Despite having reduced aggregation and expression of the key platelet adhesion and structural molecules, Cheung et al showed that there is a time-dependent platelet activation as measured by increased circulating matrix metalloproteinase (MMP)-2 and soluble p-selectin levels. This was not associated with significant activation of the endothelium[148]. It suggests that platelet adhesion and aggregation is defective in those receiving ECMO, but platelet activation occurs with the release of their granules.

In addition to platelet receptor shedding, the ECMO circuit can potentially induce the formation of thrombi and platelet microparticles. Although this has not been shown in ECMO, Danwanjee *et al* have demonstrated in animal models that CPB can contribute to the formation of microthrombi in the liquid phase and deposit in various organs shown at biopsy with indium radiolabelling. No significant macroscopic thrombi nor significant occlusive material within the bypass apparatus were seen[151]. These microthrombi may form around the biosurface then subsequently embolise systemically. It has been shown the microthrombi may cause subsequent neurological sequelae and memory impairment in those who have received CPB[152].

Circulating platelet microparticles (PMP) have been demonstrated to be increased in extracorporeal circulation[153]. PMP are small cell-derived particles typically 0.1-1 micro in size

that are produced from activated platelets in situations of shear stress[154]. They act as a prothrombotic surface and due to their size have been shown to be increased systemically at the time of extracorporeal circulation[151]. It is not clear whether PMP are important in the pathogenesis of ECMO coagulopathy and at present, their role in a prothrombotic phenotype is unknown.

vWF activity decreases with the duration of ECMO[155–157]. This is due to the loss of HMWM vWF from shear stresses in the circuit disrupting vWF molecules in the circuit. Their loss resolves with ECMO cessation. This effect is also seen with ventricular assist devices (VAD) [150,158]. Acquired von Willebrand dysfunction can be demonstrated *in vitro* by a significant reduction in ristocetin cofactor activity and collagen-binding ability. This has been correlated to increased bleeding, typically from the respiratory tract, mucosal surfaces and puncture sites[150]. Vincent *et al* have shown that devices with a high shear stress have increased levels of proteolysis without the presence of endothelium in an *in vitro* circuit. However, in the presence of devices with high pulsatility, this stimulates the release of vWF from the recipient's endothelium *in vivo*[159]. This is may be a reason why higher bleeding rates with centrifugal pumps are seen in comparison to roller pumps in patients requiring ECMO (26.1 vs 9.0 events/1000 treatment days respectively)[160]. Kalbhenn *et al* have demonstrated after the cessation of ECMO that vWF parameters largely normalise within a few hours and completely within 24-hours[149].

RBC breakdown (haemolysis) is routinely seen with ECMO circuits with 67% of patients showing increased levels of free circulating Hb[161]. This occurs with higher flow rates typically greater than 147ml/kg/min. The occurrence of significant haemolysis in ECMO is associated

with a higher use of blood products. Free plasma Hb levels of >50mg/dL after 24 hours have been shown to an independent predictor for mortality in ECMO[162].

Haemolysis causes prothrombotic changes that have been shown in other haemolytic conditions including sickle cell disease and autoimmune haemolytic anemia, which are also associated with the presence of free Hb and heme. Free Hb binds to nitric oxide (NO) further upsetting the haemostatic milieu by removing NO and therefore causing vasoconstriction and increasing platelet reactivity[163]**.** Meyer *et al* showed that free Hb increases platelet adhesion to fibrinogen and collagen-coated biosurfaces with vWF. They felt that this was a result of increased binding strength between the vWF A1-domain and the platelet GP1bα receptor[164]. Although there is a reduced level of HMWM vWF with ECMO as discussed previously, there are maintained levels of other multimer sizes that may contribute to platelet adhesion to biosurfaces.

In addition, RBC-derived microparticles can be formed due to ECMO-mediated haemolysis or from transfused blood products. RBC microparticles can increase thrombin generation as a result of phosphatidylserine exposure [165].

1.7.3 Thrombus Formation in the Extracorporeal Membrane Oxygenation Circuit

The presence of thrombosis has been demonstrated to occur at the venous inflow of the membrane oxygenator following their use, particularly in areas of increased turbulent flow when assessed using multi-dimensional CT[166,167]. This is seen universally in membrane oxygenators although the extent is variable between patients. There is an absence of thrombus formation in the arterial outflow component suggesting a low risk of thrombus embolisation to the recipient. Membrane oxygenators with a centrifugal pump have higher

rates of thrombosis than those with roller pumps although the duration of ECMO use and anticoagulation do not impact upon the rate and extent thrombus formation[160].

In the remaining components of the ECMO circuit, clots are mainly found at tubing connector junctions demonstrated by Hastings *et al*[167]. On review of 50 ECMO circuits used in children, they showed a lack of diffuse thrombus formation in the circuit tubing and instead it was focussed within these areas. On flow dynamic studies, the areas that corresponding to clot formation had significantly lower flow rates in comparison to other parts of the circuit. Histological analysis of these clots showed that they were fibrin- and red blood cell-rich with a lack of platelet deposition[167].

1.8 Anticoagulation and its Monitoring during Extracorporeal Membrane Oxygenation

Anticoagulation is used to reduce the risk of circuit occlusion due to thrombosis and prevent or treat systemic thrombosis in the patient. Unfractionated heparin (UFH) has been used as the mainstay of anticoagulation for ECMO since its conception as it is widely available and inexpensive. It contains heparin polysaccharides of various sizes that potentiate the action of antithrombin 10 000 - fold, which then inhibits the activity of FIIa, IXa, Xa, XIa and XIIa. The main side effect is an increased risk of haemorrhage. HIT can also occur as discussed previously. The incidence of HIT during ECMO use is unclear although it has been described at a rate of 0.36% of patients with similar survival rates to those who do not develop it[168]. Its diagnosis and detection can be made more difficult due to the presence of thrombocytopenia use with ECMO, the presence of heparin bound on to the biomaterial surfaces of the ECMO circuit and the lack of a validated risk assessment score for HIT in critical care and ECMO.

UFH is given as an initial bolus of 50-100 units/kg at the commencement of ECMO and then continued as a subsequent infusion[169]. Three current methods of monitoring UFH infusions can be used: Activated Clotting Time (ACT), APTT and anti-Xa. The ACT is a rapid, point-of care test using whole blood, which is cheap to use and requires minimal blood volume to perform. However, prolonged results can be due to multiple factors such as the anticoagulation itself, thrombocytopenia and critical-illness related coagulopathy. APTT is dependent on the intrinsic coagulation pathway and requires a plasma sample stored typically in citrate. It is also widely available in hospitals but as it is influenced by other components of the intrinsic pathway, it becomes less reliable in patients with critical illness. Anti-Xa levels measure the activity of heparin against FXa reflecting the degree of anticoagulation more accurately. Its use is becoming increasingly more widespread but can be affected by circulating free Hb and hyperbilirunbinaemia. There is heterogeneity in the use of these monitoring tests between centres although it appears that anti-Xa level monitoring increases the time in therapeutic range, decreased rates of circuit occlusion and reduces the risk of thrombotic and haemorrhagic events in single centre studies[170–173].

Direct thrombin inhibitors (DTI) can be used as an alternative to UFH. The most frequently described DTIs used during ECMO are argatroban and bivalirudin. These are largely reserved to cases developing HIT or showing heparin resistance during ECMO, due to the increased cost of the drugs, decreased availability and less clinical experience. Instead of binding to antithrombin, they inhibit thrombin directly and can be monitored with APTT or ACT. There are no current randomised controlled studies comparing the use UFH and DTI during the use of ECMO.

The utilisation of ECMO without anticoagulation has been explored with modern circuit designs. Chung *et al* have described the cessation of anticoagulation in a cohort of patients due to bleeding, coagulopathy or severe thrombocytopenia. There was an absence of subsequent thrombotic complications in this group suggesting that in a select number of patients anticoagulation may not be required for short periods[26].

Chapter 2 – Thrombotic and Haemorrhagic Complications during ECMO

2.1 Introduction

Bleeding and thrombosis are well-recognised complications during the use of ECMO. The timing, site and risk factors for these haemostatic complications are varied and have become more apparent with the increased utility of imaging during its use. Further evaluation of the nature of these events is required to optimise their screening and treatment. Studies describing the incidence of these key complications are shown in Table 1. Their development may be due to haemostatic changes in the patient due to their underlying illness, because of the ECMO circuit or both. Although approaches to ECMO circuit use are largely similarly, the disease aetiologies causing ARDS vary significantly with their own specific haemostatic profiles.

2.1.1 Pulmonary Embolism during Extracorporeal Membrane Oxygenation

Rates of symptomatic PE increase with the severity of illness. The PREVENT study showed an incidence of PE in 0.31% in acutely ill medical patients given low molecular weight heparin (LMWH) thromboprophylaxis over a 90-day period. In comparison, the incidence of symptomatic PE increases in critical illness to between 1.3-2.3% in the PROTECT study, a multicentre randomised control study in patients receiving either LMWH or UFH. Mechanical ventilation was required in approximately 90% of these patients with respiratory conditions and sepsis occurred in 45% and 15% of patients respectively. The study showed that the timing of PE was principally during critical care admission (70.1%) as opposed to during subsequent in-patient care[27]. Minet *et al* showed an incidence of PE at 18.7% during critical

care admission in a prospective observational cohort study[97]. 61% of these events were incidental and 33% were associated with a lower limb DVT. However, only 52% of patients received anticoagulation at either treatment dose or thromboprophylaxis. In post-mortem studies following critical illness, PE was described in 20-27% of patients by McLeod *et al*[80].

Hartley *et al* have shown an incidence of 10% of incidental and symptomatic PE at initiation of ECMO in patients with SRF[121]. These filling defects were predominantly in peripheral pulmonary vessels (72%). Those with PE had longer subsequent duration of ECMO but had no difference in short- and medium-term survival rates. Parzy *et al* demonstrated an incidence rate of incidental PE at 16.2% following decannulation from ECMO[174]. In both studies, most patients required ECMO for bacterial and viral infections leading to SRF.

With the onset of the COVID-19 pandemic, filling defects affecting smaller pulmonary vessels had been described particularly in critical care with an incidence rate of 15.7%[175]. In comparison, Avnon *et al* described a small case series with 25% of patients with H1N1 infection with SRF developing similar defects[176]. Small volume filling defects appeared to be more common in patients with severe viral pneumonias, particularly COVID-19 infection. This is felt to be mediated by immunothrombosis due to localised inflammation and coagulation activation as opposed to venous thromboembolism. Endothelial activation, circulating extracellular histones and neutrophil extracellular traps are key components in mediating immunothrombus formation[177]. A small observational study by Parzy *et al* has shown a 23% incidence of PE during ECMO in patients with COVID-19 infection[178]. Given the severity of respiratory disease in patients requiring ECMO, it is plausible that this process is more prevalent although further exploration of this is required.

2.1.2 Deep Vein Thrombosis during Extracorporeal Membrane Oxygenation

The rates of DVT during and after ECMO are variable depending upon imaging modality and the sites screened. As discussed in Chapter 1, the incidence of DVT following decannulation from ECMO varies from between 15-85%[118,119]. Imaging with CT venography may also show increased rates of DVT over ultrasound (US) Doppler due to proximal sites such as the inferior vena cava being imaged at the end of the cannulae as opposed to the proximal lower limb only that is normally assessed with US Doppler[174].

Risk factors for development of DVT have been shown to be a platelet count of >100x10^9/L, subtherapeutic anticoagulation levels by APTT and a smaller reduction of D-dimer levels after initiation of ECMO[174,179]. Therapeutic anticoagulation and blood product usage described by Parzy *et al* was not associated with a reduction in the incidence of DVT. These suggest that haemostatic changes related to ECMO, the underlying disease of the patient or abnormal blood flow around the cannula sites are contributing to these events.

In the setting of those requiring ECMO for COVID-19 pneumonia causing SRF, high rates of ECMO cannula-related DVT and other catheter-associated DVT have been seen. A small cohort study showed 100% of patients with COVID-19 infection had a VTE[178]. 77% of these events were isolated catheter-associated DVT and 8% were DVT and PE. These were controlled against patients with viral and bacterial pneumonias requiring ECMO with no difference in DVT rates, although cohort numbers were small.

2.1.3 Intracranial Haemorrhage during Extracorporeal Membrane Oxygenation

Intracranial haemorrhage is a potentially life-threatening complication seen with the use of ECMO. Retrospective cohort studies by Lockie *et al* and Arachchillage *et al* have shown that these are early events seen at the initiation of ECMO in 10-14% of patients if screening CT

imaging is used[126,127]. The rates of subsequent development of ICH during ECMO were lower at 2-5%. These events were principally SAH (56%) followed by intraparenchymal petechiael haemorrhage (39%)[126]. If detected earlier with anticoagulation strategies modified, survival rates at 6 months between those with and without ICH were similar (68 vs 76%). In contrast, Fletcher-Sandersjöö *et al* described mortality rates of 81% in patients with ICH during ECMO although the median day of detection was day 7 with preceding anticoagulation use in 13%[130]. In this cohort, there was a predominance of large volume intraparenchymal haemorrhage (76% with median haematoma volume 23mL) and lower rates of SAH (22%).

Further systematic review showed that the duration of ECMO, antithrombotic therapy, thrombocytopenia, hypofibrinogenaemia, renal impairment and replacement, cardiac arrest and hypercapnia at ECMO initiation with subsequent rapid correction of this were risk factors for ICH. This is in keeping with previous descriptions from cohort studies[122,129,180]. These features suggest that ICH may be related to the underlying critical illness causing cerebral injury and anticoagulation use as opposed to the longer-term use ECMO, due its early occurrence in the treatment course.

2.1.4 Major Haemorrhage during Extracorporeal Membrane Oxygenation

Major haemorrhage at non-intracranial sites is defined by ELSO as bleeding requiring 20ml/kg of red blood cell concentrate within 24 hours (equivalent to approximately 4U RBC in a 60-kilogram patient)[169]. A meta-analysis of 12 studies with 1042 patients by Vaquer *et al* showed an incidence of bleeding events in 29.3% of patients with 10% being considered as significant[181]. Zangrillo *et al* showed 7% of patients had gastrointestinal bleeding during ECMO in meta-analysis[182]. In a single centre observational cohort study by Kreyer *et al*, clinically relevant haemorrhagic events were seen in all patients. Clinically significant haemorrhage

occurred in 39% of patients; with survival from ICU being 36% in major bleeding compared to survival of 86% with mild bleeding[131]. The main sites of bleeding were puncture sites in both group (64% and 50%) and pulmonary haemorrhage (57%) and ICH (29%) in the severe haemorrhage group.

Risk factors for non-ICH major haemorrhage (NICMH) are less clearly described in the setting of ECMO in comparison to ICH. Lower FVII and FX and increased critical illness severity scores were associated with major haemorrhage[131]. Coagulopathic changes in routine coagulation tests before ECMO have been shown not to be associated with haemorrhage in children[183]. The cessation of ECMO has been associated with a response in bleeding[184]. These features suggest that NICMH may be related to coagulation defects induced by ECMO itself or anticoagulation received during treatment.

Table 1 Studies describing thrombotic and haemorrhagic complications during ECMO

Study	*Cohort Size*	*Events*	*Outcome(s)*	*Comments*
Hartley, J Thromb Hemost. 2019[121]	343 patients *290 - ECMO* *53 - no ECMO*	PE with and without ECMO in SRF	10% incidence 10% - ECMO 8% - no ECMO No ITU survival difference	Retrospective Non-randomised
Parzy, Crit Care Med. 2020[174]	103 patients	DVT and PE diagnosed by CT	DVT – 100% PE – 16%	Retrospective No comparison to US imaging
Cooper, Crit Care Med. 2015[118]	81 patients *72 (89%) scanned*	Proximal lower limb DVT diagnosed by US Doppler	DVT – 18%	Retrospective No control group IVC & distal lower limb not detected
Sutter, Crit Care Med. 2018[122]	Meta-analysis of 44 studies	Neurological events	All – 13% ICH – 5% Stroke – 5%	Timing of events not clearly described Variation in CT imaging practices
Lockie, Crit Care Med. 2017[126]	342 patients *250 – ECMO* *92 – no ECMO*	ICH at ECMO cannulation and during use	16% vs. 8% prevalence SAH – 56% Parenchymal – 39% Same survival outcomes	Retrospective Severity of ICH not specified
Arachillage, Semin Thromb Hemost. 2020.[127]	149 patients	ICH at ECMO cannulation and during use	11% at ECMO initiation 5% during ECMO	Retrospective No control group
Fletcher-Sandersjöö, J Intensive Care. 2017[130]	253 patients	ICH during ECMO use if clinical indication	21% ICH 81% vs 28% 1-month mortality in ICH or no ICH	Retrospective Imaging performed if clinical deterioration (not at initiation)
Kreyer, J Thorac Dis. 2017[131]	36 patients	Bleeding events during ECMO	74 haemorrhagic events 39% patients had severe haemorrhage	Non-ISTH or ELSO definitions of major bleeding

2.2 Aims

1. To assess the rates, natural history and routine laboratory markers of PE during ECMO
2. To assess the rates, natural history and routine laboratory markers of DVT during ECMO
3. To assess the rates, natural history and routine laboratory markers of ICH during ECMO
4. To assess the rates, natural history and routine laboratory markers of non-intracranial major bleeding at other sites during ECMO
5. To assess the nature of haemostatic complications during ECMO according to disease aetiology

2.3 Methods

2.3.1 Study Design

A retrospective review of patients requiring VV ECMO at St Thomas' Hospital was performed. Three studies groups were compared according to the underlying disease: bacterial, influenza and COVID-19 pneumonia. Patients with severe COVID-19 pneumonia requiring ECMO between 1st March and 31st May 2020 were compared to a cohort of patients with influenza pneumonia and bacterial pneumonia treated between 1st June 2012 and 31st May 2020. The data was extracted from a prospectively maintained ECMO registry and reviewed retrospectively.

The viral diagnosis was confirmed from polymerase chain reactions obtained by nasopharyngeal swab or bronchoalveolar lavage for both influenza and COVID-19. Bacterial pneumonia was identified by diagnosis from the Intensive Care National Audit & Research

Centre (ICNARC) registry and further reviewed for bacterial species by microscopy and culture samples from bodily fluids.

2.3.2 Ethical Approval

This study was registered as a service evaluation at Guy's and St Thomas' NHS Foundation Trust, London (Reference Number – 6741 and 10802) and ethical approval was not required in accordance with UK ethical guidelines (http://www.hra.nhs.uk, Accessed 31st May 2020).

2.3.3 Inclusion Criteria

Patients requiring in VV ECMO at Guy's and St Thomas NHS Foundation in the review period.

2.3.4 Exclusion Criteria

Patients were excluded if they were less than 16 years old and if the ECMO run was less than 24 hours. If a patient received more than one ECMO run, the initial run was included, and the subsequent ones were excluded. Patients without a diagnosis of COVID-19, influenza pneumonia or bacterial pneumonia were excluded.

2.3.5 Data Collection

Clinical data was collected from electronic records on Phillips ICIP Intellispace CCA (Amsterdam, The Netherlands) and iSOFT Clinical Manager System (DXC Technology, US). The electronic patients' records for identified patients were reviewed for clinical details. Disease severity was assessed using SOFA score and Acute Physiology And Chronic Health Evaluation II (APACHE II) score. Imaging reports were reviewed for radiologically proven thrombosis and intracranial haemorrhage. Electronic blood transfusion prescriptions were reviewed for major bleeding events (bleeding with ≥ 20ml/kg of red cell concentrate within 24 hours) in accordance with standardised ELSO definitions[16].

2.3.6 Details of Extracorporeal Membrane Oxygenation Circuit and Anticoagulation

For anticoagulation during ECMO, intravenous UFH with 50 IU/kg was given as a bolus at the time of cannulation with 2,500 units in the priming fluid for the circuit in keeping with ELSO guidelines unless contraindicated[12]. Prior to 1st November 2019, subsequent UFH infusions were monitored by activated partial thromboplastin time ratio (APTTr) with a target level of 1.5-2.0 if evidence of no thrombosis or haemorrhage and 2.0-2.5 if a thrombotic event. From 1st November 2019 to present, monitoring of UFH was using anti-Xa activity, targeting levels of 0.3 - 0.7 unit/mL if no evidence of thrombosis or haemorrhage were present and 0.6 - 1.0 unit/mL if there was a thrombotic event. UFH infusions were not given if there was evidence of major bleeding. Patients were also prescribed pharmacological thromboprophylaxis with subcutaneous LMWH unless contraindicated in critical care prior to ECMO use[19].

2.3.7 Outcome Variables

The clinical outcomes were the radiological diagnosis of PE, ICH, NICMH and Ca-DVT after decannulation from ECMO. Additional thrombotic events seen radiologically by CT or US imaging were recorded for all patients. NICMH was recorded during the use of ECMO and defined clinically at ≥4 units RBC transfusion per 24-hour period as adapted from ELSO[16]. The index date was the date on which VV ECMO was initiated. Data was censored on 26th June 2020. Patients with NICMH were matched by duration of ECMO and year of treatment to patients of ECMO without.

2.3.8 Imaging and Coagulation Testing

Patients requiring ECMO received a whole-body CT as soon as possible after cannulation (≤ 12 hours). The imaging is performed with either a Somatom Force 384 (2x192 sections,

Siemens Healthineer, Germany) or Brilliance iCT (256 sections, Philips Healthcare, Netherlands). CT Pulmonary Angiography (CTPA) was performed by bolus trigger at 110 Hounsfield Units. Ultrasound (US) Doppler scanning was performed within 24-72 hours of decannulation of the cannulas used for ECMO at both catheter insertion sites to assess for DVT. Additional imaging during ECMO was performed if there was clinical concern for bleeding or thrombosis. It is practice in the United Kingdom for Doppler ultrasound scanning not to fully evaluate the calf veins[17].

Testing for APTTr, PTr, D-dimers and Clauss fibrinogen were performed using Sysmex CS2100*i* (Norderstedt, Germany) as per manufacturer protocols considered as 'routine coagulation testing'. Full Blood Counts were performed using DxH 900 Hematology (Beckman Coulter, Brea, United States). Blood samples were taken after initiation of ECMO and following heparin boluses. The tests were performed within the first four hours of ECMO initiation and subsequently a minimum of once daily during ECMO.

2.3.9 Statistical Considerations

Statistical analysis was performed using SPSS Version 27.0 (IBM, New York, US). Null hypothesis significance testing was employed to examine variable distributions between the two groups. Data was analysed for distribution spread. Descriptive statistics were mean and standard deviation for parametric data and median and interquartile range (IQR) for non-parametric. Testing was performed depending on the normal distribution of the data. Unpaired student T-test and ANOVA testing was performed on parametric data. Mann-Whitney U test and Kruskal-Wallis testing was performed on non-parametric data. Chi-squared test for frequencies of categorical variables were performed. Fisher's exact test was used if group frequencies were less than 5. Survival analysis was shown using survival plots

and was analysed using the log-rank test. An α-significance level of 0.05 was used for all statistical tests.

2.4 Results

The number of identified patients was 365. This provided a total duration of ECMO support of 5347 days (14.6 years) with a median ECMO duration of 11 days (interquartile range, IQR 6 – 17 days).

2.4.1 Features of Pulmonary Embolism during Extracorporeal Membrane Oxygenation

44/365 (12%) of patients had an identifiable filling defect of CT imaging at the initiation of ECMO. Of these, the most proximal vessel affected was the main pulmonary artery in 8 patients (14% of filling defects), lobar artery in 7 patients (16%), segmental artery in 20 patients (45%) and subsegmental artery in 9 patients (20%). 9/30 (30%) patients with pulmonary artery filling defects had radiological features of right heart strain based upon available results (site of most proximal filling defect - 4 main artery, 2 lobar, 2 segmental and 1 subsegmental).

The coagulation parameters in those with and without filling defects are shown in Table 2. Statistically different features were a lower APACHE-2 score, higher platelet count and D-dimer level, lower haemoglobin and procalcitonin and shorter APTTr in those with a pulmonary artery filling defect in univariate analysis. In multivariate analysis, APACHE II score (OR – 0.97 p = 0.033, 95% CI 0.82 – 0.99) and D-dimer level (OR – 1.04, p<0.001, 95% CI 1.02 – 1.06) remained significant.

The Receiver Operating Characteristic (ROC) for D-dimer levels in all patients at the initiation of ECMO for the presence of PE was positive (Area Under Curve = 0.725), however there was bias due to two potential ties in the positive and negative state groups (Figure 8). Therefore, D-dimer levels was not considered specific for the detection of pulmonary artery filling defects at the time of ECMO initiation in this cohort.

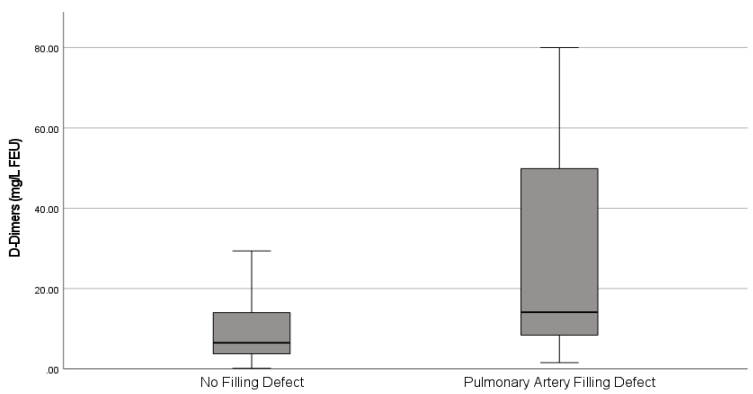
Three further PEs (two segmental and one subsegmental) were detected during ECMO that were not detected at the initial CT scan with a prevalence of 3/198 (2%) who had further interval imaging.

Survival to discharge from ICU was statistically different in those with PE at the initiation of ECMO. 31/46 patients with PE (67%) were alive to ICU discharge in comparison to those without PE was 261/322 (81%) (p=0.03). A survival plot for survival during ECMO is shown in Figure 9 (log rank test, degrees of freedom = 1, p = 0.06). If the size of filling defect is evaluated, the survival was 261/322 (81%) without a filling defect, 28/31 (74%) with a small vessel filling defects (segmental and subsegmental) with the highest mortality of 8/15 (53%) with large vessel filling defects (main artery and lobar). The survival plot for survival during ECMO according to size of PE in Figure 9 showed no statistical difference (log rank test, degrees of freedom = 2, p = 0.17).

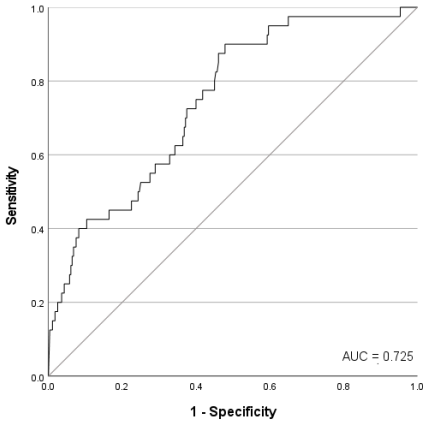
Table 2 Clinical and coagulation parameters in patients with PE at initiation of ECMO compared to those without ICH

Clinical Feature	*Pulmonary Embolism (n = 44)*	*No Pulmonary Embolism (n = 321)*	*p-value*
Median Age (years)	43.5	48.7	0.20
IQR	(34.2 – 56.5)	(38.8 – 57.1)	
Gender (Male)	31/44 (70.5%)	193/321 (60.1%)	0.20
SOFA Score	8	8	0.278
	(4 – 10)	(8 - 11)	
APACHE Score	17	18	**0.015**
	(12 – 20)	(15 - 21)	
Median Haemoglobin (g/L)	95.0	101.0	**0.034**
IQR	(87 – 103)	(91.0 – 113.0)	
Median Platelets (x10^9/L)	225	161	**0.043**
IQR	(112 – 312)	(91 – 247)	
Median PTr	1.1	1.1	0.767
IQR	(1.0 – 1.1)	(1.0 – 1.2)	
Median APTTr	1.3	1.6	**0.002**
IQR	(1.1 – 1.6)	(1.2 – 2.3)	
Median Fibrinogen (g/L)	5.4	5.5	0.813
IQR	(3.7 – 7.4)	(4.0 – 7.2)	
Median D-dimer (ng/mL FEU)	14.1	6.5	**<0.001**
IQR	(8.3 – 51.4)	(3.7 – 14.0)	
Median CRP (mg/L)	271.5	262.0	0.791
IQR	(191.3 – 326.8)	(155.0 – 338.0)	
Median Ferritin (µg/L)	2021	2514	0.734
IQR	(992 – 4847)	(982 - 4794)	
Median Procalcitonin (µg/L)	4.24	11.5	**0.007**
	(1.1 – 14.4)	(1.8 – 76.3)	
Median Creatinine (µmol/L)	112	133	0.121
IQR	(68-188)	(80 – 242)	
Median Troponin T (ng/L)	38	61	0.138
IQR	(23 – 113)	(28 – 160)	
Median Alanine Transferase (IU/L)	48	46	0.943
IQR	(23 - 99)	(26 – 83)	

Figure 8: D-dimer levels in pulmonary artery filling defects at initiation of ECMO (a) Box plot of D-dimers level in patients with and without filling defects (b) Receiver Operating Characteristic Curve for D-dimers level at the initiation of ECMO for the detection of Pulmonary Embolism

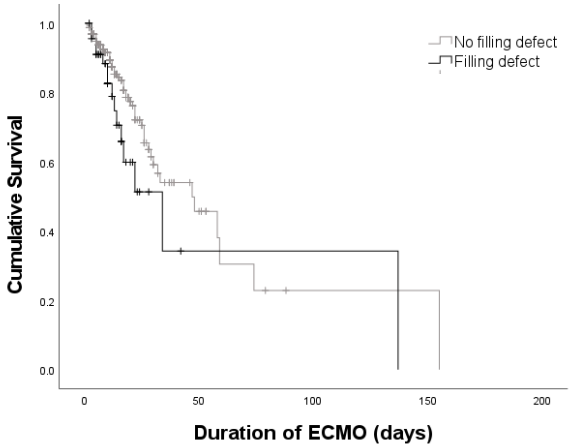


(a)

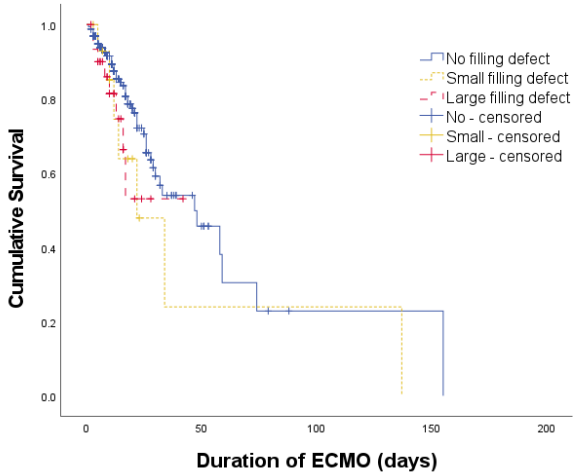


(b)

Figure 9: Kaplan-Meier Survival Plot of survival from Intensive Care with (a) the presence of pulmonary artery filling defects at initiation of ECMO to those without (b) the size of pulmonary artery filling defects to those without (large – main artery and lobar arteries, small – segmental and subsegmental vessels)



(a)



(b)

2.4.2 Features of Deep Vein Thrombosis during Extracorporeal Membrane Oxygenation

US Doppler screening was performed following decannulation in 280/365 of patients (77%). DVT was seen in 57/275 patients (21%). The clinical features of those with DVT in comparison to those without are shown in Table 3. Statistically significant findings were a lower disease severity score by APACHE II score and a pulmonary artery filling defect seen at ECMO initiation by univariate analysis. In binomial multivariate analysis, these features were not significant.

The proportion of DVT following decannulation was higher with a pulmonary artery filling defect (10/47, 21.3%) at initiation of ECMO than those without (18/217, 8.3%) (χ^2, p=0.04, df - 1). There was no difference in the proportion of patients with DVT who had a preceding ICH or NICMH (degrees of freedom – 1, p = 0.28 and 0.63 respectively).

Table 3 Clinical and coagulation parameters in patients with proximal lower limb DVT at decannulation from ECMO compared to those without DVT

	DVT at decannulation (n = 57)	*No DVT at decannulation (n = 218)*	*p-value*
Median Age (years)	4.5.5	46.6	0.56
IQR	(35.5 – 45.5)	(38.0 – 56.0)	
Gender (Male)	31/57 (54.4%)	135/218 (61.9%)	0.30
SOFA Score	6	9	0.06
	(5 - 10)	(5 – 11)	
APACHE II Score	16	18	**0.04**
	(13 – 19)	(15- 21)	
Duration of ECMO (days)	10	10	0.33
	(8 – 16)	(7 – 15)	
PE at ECMO initiation	10/57 (17.5%)	18/218 (8.3%)	**0.04**
ICH at ECMO initiation	8/57 (14.0%)	18/218 (8.3%)	0.28
Non-ICH MH during ECMO	7/57 (12.3%)	22/218 (10.1%)	0.81

2.4.3 Thrombosis at Other Sites during Extracorporeal Membrane Oxygenation

Thrombosis at other sites (non-pulmonary artery) were detected at the initiation of ECMO in 26/365 patients (7.1%). They occurred in 37 sites: cerebral (infarcts) – 10 (2.7%), splenic (infarcts) – 9 (2.5%), renal (infarcts) – 5 (1.4%), lower limb DVT – 4 (1.1%), inferior vena cava thrombus – 3 (0.8%), internal jugular vein thrombus – 3 (0.8%), hepatic (infarcts) – 2 (0.5%) and intestinal (infarct) – 1 (0.3%).

During ECMO, 188 patients had appropriate imaging to be able to detect further thrombotic events. 20/188 patients (10.6% of those imaged and 5.4% of all patients) had identified thrombosis at 21 sites: upper limb arterial thrombus - 7, pulmonary embolism – 3 new events with 2 events showing extension, lower limb arterial thrombus - 3, internal jugular vein thrombus - 2, splenic (infarct) - 1, upper limb DVT - 1, inferior vena cava thrombus – 1 and renal (infarct) - 1.

2.4.4 Features of Intracranial Haemorrhage during Extracorporeal Membrane Oxygenation

The incidence of ICH was 40/365 (11%) in all patients at the initiation of ECMO. The most common type of bleeding was SAH occurring in 31/40 (78%) of cases. Intraparenchymal haemorrhage occurred (in the absence of SAH) in 13% and subdural haemorrhage (SDH) occurred in 10%. The Modified Fisher Scale of SAH in these patients were: Grade I - 18, Grade II - 0, Grade III – 13 and Grade IV - 0. Intraparenchymal involvement was in 7/31 (23%) patients with SAH. There was an absence of intraventricular haemorrhage and no midline shift identified at the initiation of ECMO. The clinical features and routine laboratory testing for patients with and without ICH are shown in Table 4. Statistically significant finding were a lower haemoglobin and higher D-dimer level with ICH by univariate analysis.

If patients with an ICH diagnosed at initiation of ECMO were excluded, 198 patients had further CT imaging, which were performed due to a clinical change or monitoring of previous events, as opposed to asymptomatic screening. 4/198 (2%) of these patients were shown to develop an ICH whilst receiving ECMO, although this consisted of 4/325 (1%) of the remaining total patient cohort with no previous ICH. There was no variation in the incidence of ICH according to infectious aetiology as discussed further below.

The survival to discharge from ICU was not different in those with ICH at the initiation of ECMO. 27/40 patients with ICH (68%) were alive to ICU discharge in comparison to those without ICH was 261/325 (80%) (p=0.06). A survival plot for ICU discharge in shown in Figure 10 (p=0.27).

Table 4 Clinical and coagulation parameters in patients with ICH at initiation of ECMO compared to those without ICH

Clinical Parameter	*No ICH (n=325)*	*ICH (n=40)*	*p-value*
Survival to ICU Discharge	261/325 (80%)	27/40 (68%)	0.06
Median Age (years)	45.7	46.5	0.27
IQR	(35.5 – 55.2)	(41.1 – 51.8)	
Gender (Male)	202/325 (62%)	23/40 (58%)	0.57
Median APACHE II Score	17	16.5	0.99
IQR	(13 – 21)	(12.5 – 20.25)	
Median SOFA Score	8	6	0.63
IQR	(5 – 11.25)	(4 – 11.75)	
Median Haemoglobin (g/L)	99.5	90.0	**0.001**
IQR	(92.8 – 112.0)	(84.5 – 101.8)	
Median Platelet Count (x10^9/L)	175	245	0.90
IQR	(101 - 279)	(211 - 310)	
Median PTr	1.1	1.0	0.33
IQR	(1.0 – 1.3)	(1.0 – 1.1)	
Median APTTr	1.6	1.25	0.79
IQR	(1.2 – 2.2)	(1.1 – 1.7)	
Median Fibrinogen (g/L)	5.40	7.05	0.63
IQR	(3.95 – 7.78)	(5.23 – 8.58)	
Median D-dimer (ng/mL FEU)	6.56	9.81	**0.04**
IQR	(3.19 – 16.34)	(6.58 – 23.91)	
Median CRP (mg/L)	125	164	0.57
IQR	(63 - 253)	(67 - 285)	
Median Ferritin (µg/L)	44.0	39.5	0.71
IQR	(22.8 – 150.8)	(15.8 – 93.3)	
Median Procalcitonin (µg/L)	61.5	44.0	0.54
IQR	(26.8 – 103.0)	(27.3 – 62.5)	

6/40 (15%) patients with an initial ICH showed progression of ICH during ECMO. All of these were SAH at initiation of ECMO. The median day of detection for progression was 3.5 days (range 2 – 7 days). 4/6 of these patients (67%) died during admission to ICU if there was progression of ICH whereas 10/34 (29%) died during ICU admission if there was no progression (degrees of freedom - 1, p = 0.041). Routine coagulation tests in those with ICH progression and those without showed no statistical differences (Table 5). There was no difference in the use of RRT, tranexamic acid and UFH infusions between these two groups.

Figure 10: Kaplan-Meier Survival Plot of survival from Intensive Care in patients with Intracranial Haemorrhage to those without at initiation of ECMO

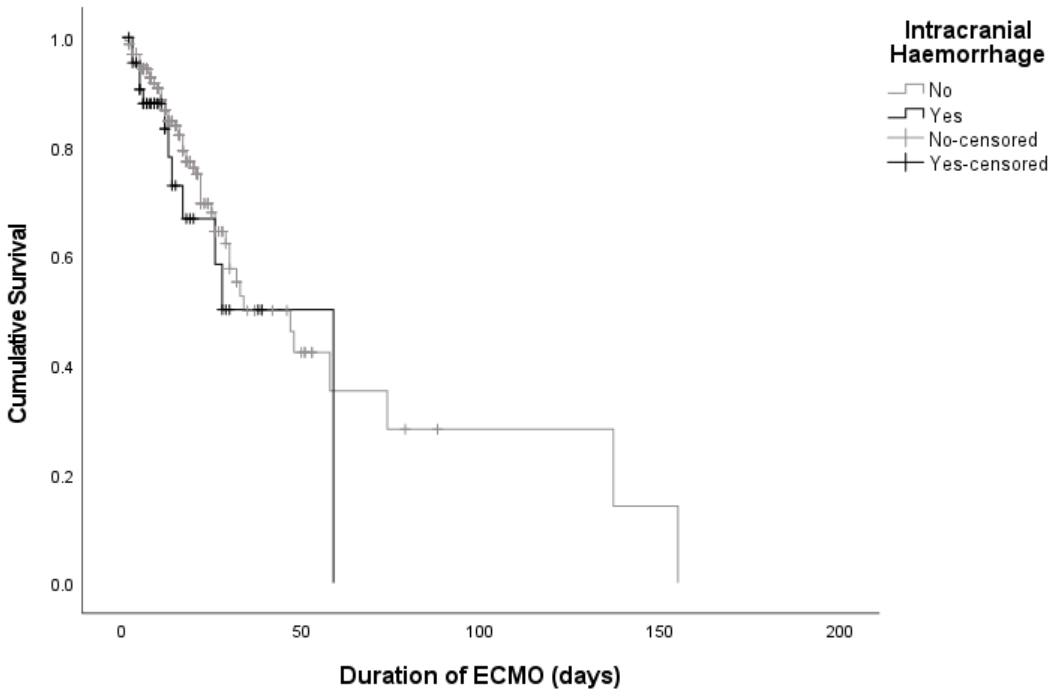


Table 5 Clinical and coagulation parameters and anticoagulation use in patients with Intracranial Haemorrhage at initiation of ECMO with and without progressive Intracranial Haemorrhage in first 3 days following diagnosis

Clinical Parameter	*Non-Progression (n=34)*	*Progression (n=6)*	*p-value*
Survival to ICU Discharge	24/34 (71%)	2/6 (33%)	**0.015**
Haemoglobin (g/L)	91.5	84.6	0.65
IQR	(82.1 – 99.4)	(82.9 – 91.1)	
Platelet Count (x10^9/L)	202	148	0.84
IQR	(83 – 253)	(93 – 237)	
PTr	1.1	1.0	0.73
IQR	(1.0 – 1.2)	(1.0 – 1.1)	
APTTr	1.1	1.4	0.12
IQR	(1.0 – 1.4)	(1.2 – 2.1)	
Fibrinogen (g/L)	4.5	4.7	0.83
IQR	(3.5 – 5.5)	(3.6 – 5.3)	
D-dimers (ng/mL FEU)	22.1	24.1	0.70
	(9.5 – 32.5)	(14.3 – 54.9)	
UFH Use following ICH	6/34 (18%)	2/6 (33%)	0.58
Mean hourly use			
Renal Replacement Therapy	22 (65%)	3/6 (50%)	0.65
Tranexamic Acid Used	4 (12%)	1 (17%)	1.0

2.4.5 Features of Major Haemorrhage at Non-Intracranial Site during Extracorporeal Membrane Oxygenation

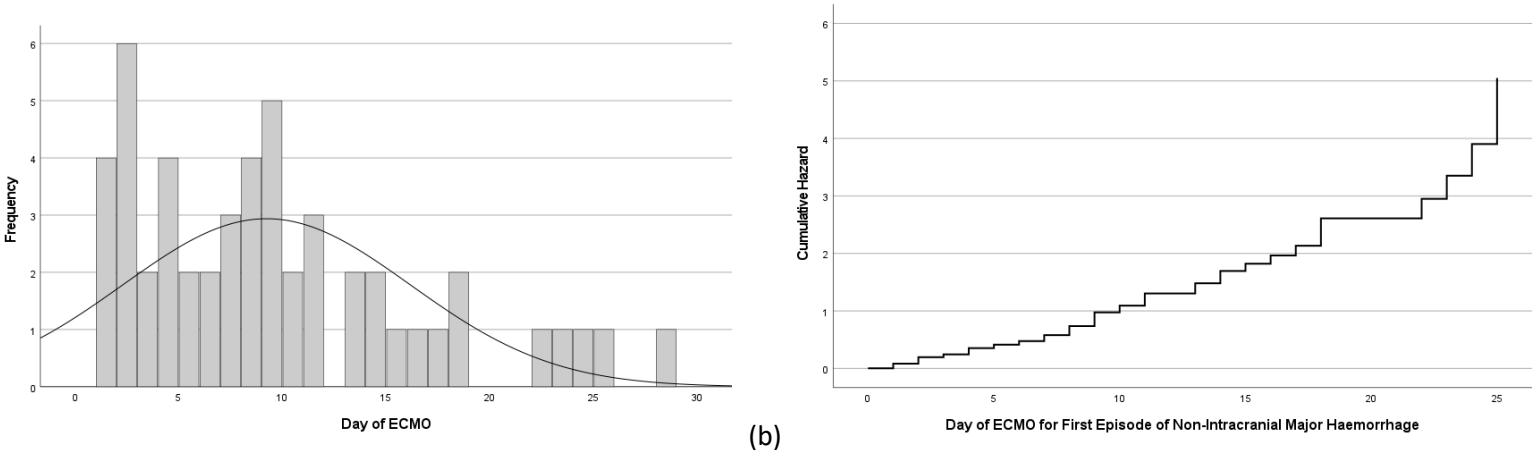
In accordance with the ELSO definition of major haemorrhage (MH) of >4 units or 20ml/kg of RBC transfusion in 24 hours, 59 episodes of MH were defined at non-intracranial sites. These occurred in 51/365 patients (14%) of patients. 44 (12%) patients having 1 episode, 6 (2%)

patients having 2 episodes and 1 (0.3%) patient having 3 episodes of NICMH. 314/365 (86%) did not have an episode of NICMH. The event rate for first episode of NICMH by day of ECMO is shown in Figure 11. The median time to the first episode of NICHMH was 8 days (IQR 4 - 13.5days), for the second episode was 14.5 days (IQR 13.75 – 30.5 days). The median duration of ECMO was longer in patients with NICMH and those without (17 days vs. 10 days respectively, $p<0.001$). The clinical features of the patients with NICMH to those without are shown in Table 6.

The sites of NICMH were pulmonary (lower respiratory tract and pleural) in 16 episodes, 15 abdominal (lower gastrointestinal and retroperitoneal), 9 upper gastrointestinal, 6 upper respiratory tract including tracheostomy-related, 5 pelvic, 3 cardiac, 2 musculoskeletal, 2 line-related and 1 unknown. For the treatment of these events, 32 patients (63%) had invasive procedures (8 required surgery, 12 had endoscopic procedures, 11 required drains, 6 underwent embolisation or other interventional radiological procedures) and 19 (37%) had medical management alone.

Survival from ICU was higher in those without than those with NICMH. A survival plot comparing those with one or more episode of major haemorrhage to those without is shown in Figure 12 ($p = 0.25$).

Figure 11: Rates of Non-Intracranial Major Haemorrhage (a) Histogram of all events per day during ECMO (b) Cumulative Hazard Plot of the first episode of non-intracranial major haemorrhage by day of ECMO



(a) (b)

Figure 12: Kaplan-Meier Survival Plot of survival from Intensive Care of patients with Non-Intracranial Major Haemorrhage during ECMO to those without.

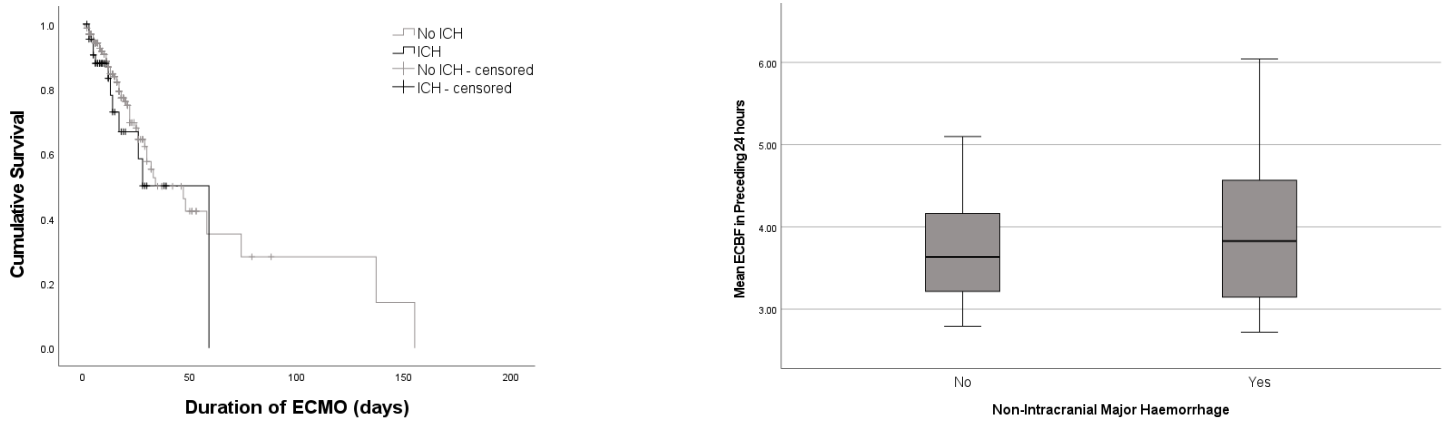


Figure 13: Box plot of extracorporeal blood flow rates in patients with Non-Intracranial Major Haemorrhage during ECMO to those without

Table 6 Clinical Details of Patients with Non-Intracranial Major Haemorrhage during ECMO

Clinical Parameter	*Patients without Major Haemorrhage (n = 316)*	*Patients with Major Haemorrhage (n = 51)*	*p-value*
Median Duration of ECMO	10 days	17 days	**<0.001**
IQR	(6.5-16)	(11-25)	
Survival to discharge from ICU	256/314 (82%)	32/51 (63%)	**0.002**
Median Age (years)	47.0	56.2	0.067
IQR	(37.6 – 55.6)	(41.9 – 63.7)	
Gender (Male)	191 (61%)	34 (67%)	0.43
Median SOFA Score	8	8	0.93
(IQR)	(5 – 11)	(5 – 12)	
Median APACHE II Score	17	19	0.12
(IQR)	(14 - 21)	(16 - 21)	

Table 7 Extracorporeal circuit blood flow rates in patients preceding first major haemorrhagic event in comparison to matched-controls without major bleeding

	Patients with Major Haemorrhage	*Patients without Major Haemorrhage*	*p-value*
Age (years)	51.6	51.7	0.91
Duration of ECMO (days)	19.2	19.1	0.94
Mean flow rate over	4.01 Litres/min	3.75 Litres/min	0.09
preceding 24 hours (IQR)	(3.14 – 4.82)	(3.21 – 4.17)	
Mean flow rate over	4.03 Litres/min	3.80 Litres/min	0.18
preceding 48 hours (IQR)	(3.16 – 4.72)	(3.25 – 4.36)	
Mean flow rate over	4.02 Litres/min	3.85 Litres/min	0.29
preceding 72 hours (IQR)	(3.30 – 4.59)	(3.25 – 4.57)	

Extracorporeal blood flow (ECBF) rates were compared in those with NICMH to those without by matching for age, year of treatment and duration of ECMO use. ECBF rates were not statistically difference in the preceding 24 hours, 48 hours and 72 hours in those suffering from non-ICH MH (Table 7) although a trend to significance was noted within the preceding 24 hours to be higher in those with NICMH (Figure 13, p=0.09).

2.4.6 Incidence and Characteristics of Thrombotic and Haemorrhagic Events According to Disease Aetiology during Extracorporeal Membrane Oxygenation

Of the 365 patients reviewed in the study cohort, there were the following number of each diagnosis: bacterial pneumonia (n=234), influenza pneumonia (n=80) and COVID-19 pneumonia (n=51). Within the influenza group, there were 43 cases of H1N1 influenza, 31 of other influenza A infections and 6 cases of influenza B. Within the bacterial group, a species was identified in 124/234 (53%) of cases by sputum or bronchoalveolar lavage microscopy and culture or pneumococcal and legionella urinary antigen testing.

The clinical features of patients requiring VV ECMO for bacterial, influenza and COVID-19 pneumonia are shown in Table 8. The gender, duration of ECMO, survival to ICU discharge, haemoglobin levels and serological markers of renal, cardiac and hepatic impairment (creatinine, Troponin T and alanine transferase) were not significantly different between infectious aetiology. Procalcitonin levels were significantly higher in patients with bacterial pneumonia and CRP was higher in COVID-19 pneumonia.

The incidence of thromboembolic and haemorrhagic complications associated with use of ECMO are shown in Table 9. High rates of thrombotic events were seen with COVID-19 in comparison to bacterial and influenza pneumonia. There was no difference in major haemorrhage between the three conditions.

Pulmonary arterial filling defects were present on CTPA in 21/234 (9%) with bacterial pneumonia, 6/80 (8%) with influenza and 17/51 (33%) with COVID-19 after initiating ECMO (df – 2, p<0.001). Of these, segmental and subsegmental filling defects in the absence of preceding DVT were seen in 13/234 (6%) with bacterial pneumonia, 4/81 (5%) with influenza and 14/51 (27%) with COVID-19 (df – 2, p<0.001). The incidence of larger vessel pulmonary artery filling defects was 3%, 3% and 10% respectively at initiation of ECMO (df – 2, p=0.08).

MH did not vary between infectious aetiology (Table 9). The incidence of ICH was 23/234(10%) with bacterial pneumonia, 9/80 (11%) with influenza and 8/51(16%) with COVID-19. The prevalence of NICMH was 30/234 (13%), 14/80 (18%) and 7/51 (14%) respectively.

At decannulation from ECMO, Ca-DVT seen by US Doppler was present in 57/274 (21%) of survivors. The incidence of Ca-DVT was significant elevated in COVID-19 (53%) in comparison to bacterial (12%) and influenza pneumonias (25%, df – 2, p<0.001).

Table 8 Clinical characteristics of patients at initiation of VV ECMO according to diagnosis

(values indicate interquartile range)

Characteristics	*Bacterial (n=234)*	*Influenza (n=85)*	*COVID-19 (n=51)*	*p-value*
Median Age (years)	49.2 (37.7 – 57.9)	48.3 (38.7 – 57.3)	46.2 (35.6 – 53.2)	0.52
Gender (Male)	140 (59.8%)	47 (55.3%)	38 (74.5%)	0.12
Median SOFA Score	9 (5 - 12)	9 (5 - 11)	6 (4 - 10)	**0.015**
Median APACHE II Score	19 (16 - 22)	17 (15 – 21)	14 (11 - 17)	**<0.001**
Survival from ICU	184 (79%)	66 (78%)	37 (74%)	0.40
Median ECMO Duration (days)	9 (6-14)	13 (10 - 19)	13 (8 - 21)	0.65
Median CRP (mg/L)	261 (160-332)	225 (146 - 326)	320 (225 - 362)	**0.011**
Median Procalcitonin (µg/L)	17.3 (2.1-87.2)	6.4 (1.4 – 72.4)	3.5 (1.1 – 8.5)	**<0.001**
Median Ferritin (µg/L)	2674 (845-5303)	2543 (985 - 4809)	1903 (1117 - 4159)	0.24
Median Haemoglobin (g/L)	99.5 (91.0-112.0)	102.5 (90.3–118.8)	99.0 (88.0 – 106.0)	0.52
Median Creatinine (µmol/L)	124 (80-223)	152 (97 - 261)	86 (53 – 203)	0.46
Median Troponin T (ng/L)	80 (38-176)	58 (22 - 164)	31 (13 - 61)	0.15
Median Bilirubin (µmol/L)	16 (9-32)	12 (8 - 20)	14 (9 - 20)	0.21
Median ALT (IU/L)	44 (24-92)	43 (28 - 81)	52 (30 - 77)	0.36

Table 9 Incidence of haemostatic complications at initiation and termination of ECMO according to infections aetiology

Type of Complication	*Bacterial (n=234)*	*Influenza (n=80)*	*COVID-19 (n=51)*	*Significance (p-value)*
Total pulmonary arterial filling defects seen at initiation of ECMO	21/234 (9%) *3 - central* *5 - lobar* *10 - segmental* *3 - subsegmental*	6/80 (8%) *2 - central* *0 - lobar* *4 - segmental* *0 – subsegmental*	17/51 (33%) *3 - central* *2 - lobar* *6 - segmental* *6 – subsegmental*	**<0.001**
Small vessel arterial filling defect[1]	13/234 (6%)	4/80 (5%)	12/51 (24%)	**<0.001**
Large vessel arterial filling defect[2]	8/234 (3%)	2/80 (3%)	5/51 (10%)	0.08
Intracranial haemorrhage present at initiation of ECMO	23/234 (10%) *16 - Subarachnoid* *4 - Parenchymal* *3 – Subdural*	9/80 (11%) *8 - Subarachnoid* *1 - Parenchymal*	8/51 (16%) *7 - Subarachnoid* *1 - Subdural*	0.43
Non-Intracranial Major Haemorrhage during ECMO	30/234 (13%)	14/80 (18%)	7/51 (14%)	0.58
Deep vein thrombosis seen on US Doppler at decannulation	22/177 (12%)	15/60 (25%)	20/38 (53%)	**<0.001**

[1] Defined as lobar or main arterial filling defects as the most proximal vessel

[2] Defined as segmental or subsegmental arterial filling defects as the most proximal vessel

2.4.7 Routine Coagulation Testing According to Infectious Aetiology during Extracorporeal Membrane Oxygenation

Hyperfibrinogenaemia and significantly elevated D-dimer levels were seen across all three disease aetiologies, which are shown in Table 10. In patients with COVID-19 pneumonia at initiation of ECMO, there were shorter APTTr and PTr, higher platelet counts and higher levels of fibrinogen than in influenza and bacterial pneumonias. There were no patients with DIC according to the 'Overt DIC' ISTH criteria compared to 6% in patients with influenza and 12% of patients with bacterial pneumonia. D-dimer levels were significantly elevated in all diseases but were more significantly elevated in COVID-19 pneumonia ($p<0.001$).

The routine laboratory parameters according to infectious aetiology with and without pulmonary artery filling defects are shown in Table 11. Patients with bacterial pneumonia and COVID-19 pneumonia had significantly higher D-dimer levels than those without. There was no statistically significant difference in D-dimer levels in patients with influenza pneumonia with and without pulmonary artery filling defects. Fibrinogen levels in patients with bacterial pneumonia were lower in those with filling defect that those without.

Table 10 Routine coagulation parameters according to infectious aetiology at the initiation of ECMO

Characteristics	*Bacterial (n=234)*	*Influenza (n=85)*	*COVID-19 (n=51)*	*p-value*
Median Admission APTTr (normal range 0.8-1.2)	2.19 (1.93-2.49)	2.65 (2.13-3.16)	1.61 (1.35-1.89)	**0.012**
Median Admission PTr (normal range 0.8-1.2)	1.22 (1.18-1.26)	1.17 (1.09-1.25)	1.10 (1.05-1.13)	0.026
Mean Admission Platelets (normal range 150 – 400 x 10^9/L)	182 (165-198)	171 (151-192)	254 (224-285)	**<0.001**
Mean Admission Fibrinogen (normal range 1.7-3.9 g/L)	5.65 (5.3-6.0)	4.99 (4.5-5.4)	7.2 (6.5-7.8)	**<0.001**
Mean Admission D-dimer (normal range 0 – 0.55 mg/L FEU)	12.4 (10.2-14.7)	13.3 (9.9-16.6)	24.0 (16.8-31.2)	**<0.001**
Presence of Overt DIC (ISTH Score ≥5)	23 (11.7%)	5 (6.4%)	0	**0.022**

Table 11 Coagulation parameters in patients with and without filling defects on CT Pulmonary Angiography at initiation of ECMO with severe ARDS due to bacterial, Influenza & COVID-19 infections

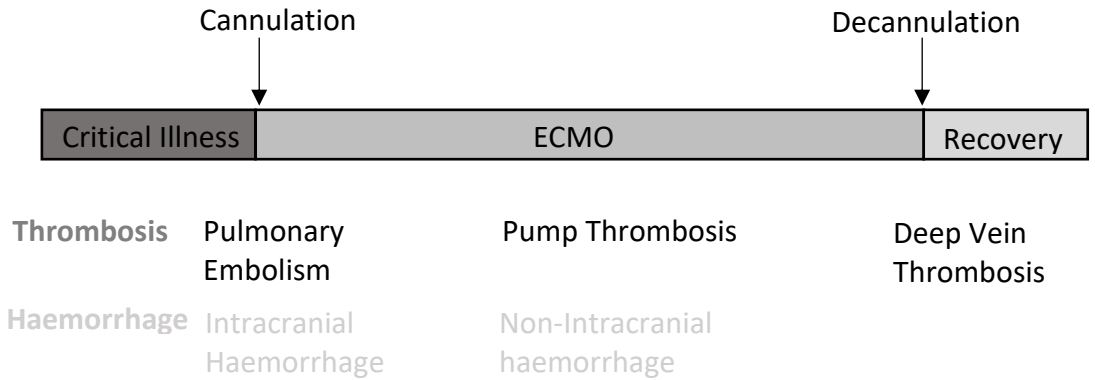
Coagulation Parameter	*Bacterial*		*Influenza*		*COVID-19*	
	Arterial filling defect (n=21)	No filling defect (n=213)	Arterial filling defect (n=6)	No filling defect (n=72)	Arterial filling defect (n=19)	No filling defect (n=39)
Median Platelets (x109/L) (IQR)	213 (61.5-264)	152 (82.5-257)	183 (169-344)	252 (169-344)	252 (169-344)	223 (170-310)
Median PTr (IQR)	1.1 (1.05-1.3)	1.1 (1.0-1.3)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.1)
Median D-dimer (mg/L FEU) (IQR)	12.4 (8.2-44.6)	6.0 (4.0-11.8)	16.2 (9.5-21.5)	6.9 (3.5-18.1)*	33.3 (7.0-69.1)	7.2 (3.2-28.3) *
Median Fibrinogen (g/L) (IQR)	4.6 (2.8-6.6)	5.5 (4-7.3) *	4.4 (3.8-6.6)	5.0 (3.4-6.4)	7.4 (5.3-8.6)	6.9 (5.5-9.0)

* Difference between those with and without filling defects by disease aetiology with $p < 0.05$

2.5 Discussion

This cohort of patients with SRF requiring VV ECMO demonstrates that the development of thrombotic and haemorrhagic complications vary at differing time points. This is shown schematically in Figure 14. The development of thrombosis was influenced by various factors particularly the underlying disease aetiology as described in section 2.4.6.

Figure 14: Schematic representation of thrombotic and major haemorrhagic complications over time during the use of ECMO



Distinct events were seen throughout the use of ECMO. ICH and pulmonary artery filling defects were typically seen at the initiation of ECMO. NICMH was seen throughout the use of ECMO although further ICH events were limited. DVT was seen at the end of ECMO in 21% of patients surviving to decannulation with the use of routine US doppler imaging. Thrombosis at other sites were infrequent during ECMO.

Thrombosis in the pulmonary vasculature is seen at the initiation of ECMO with low rates of symptomatic events occurring subsequently during ECMO. The nature of these thrombi is yet to be delineated but it seems likely that 'immunothrombosis' is a key component of this. Immunothrombosis is the development of localised fibrin deposition at sites of significant inflammation typified by activation of platelets, endothelial cells, neutrophils and the

coagulation system[86]. Features of severe inflammatory patterns of lung disease seen at the commencement of ECMO, elevated serological infection markers such as CRP and procalcitonin, small volume thrombi on CT imaging and the lack of associated DVT to cause embolisation are in keeping with this process in the patient cohort. There was an association with preceding PE at the initiation of ECMO with those subsequently developing DVT after the use of ECMO suggestive that these patients may have an increased prothrombotic tendency. However, at present, there are no standardised clinical definitions or markers of immunothrombosis in the antemortem setting[185]. Of note, the rate of DVT was not higher in those with preceding major haemorrhage, for whom systemic anticoagulation is typically held, suggesting that anticoagulation may not fully protect against the development of this complication.

ICH is an early event in the treatment period of ECMO, typically seen within the first 12 hours of commencement seen on screening CT scans in this cohort. These rates were similar to previous levels described by Lockie *et al* and Arachchillage *et al*[126,127]. However, rates were significantly higher than previously described in patients not requiring ECMO with critical illness at 0.4%[25]. The incidence of ICH at initiation of ECMO does not vary between disease aetiologies suggesting these events may be attributed to the severity of organ failure and preceding brain injury, the use of heparin boluses at the time of ECMO initiation or due to changes in coagulation that did not vary between conditions[186]. Hunsicker *et al* have recently described that platelet counts >100x^9/L are associated with lower rates of ICH[187]. Their study did not demonstrate differences in critical illness severity scores or markers of injury to other organs with ICH. In this cohort and those described by others, the most common type of ICH was SAH, which outside of ECMO and critical care, are mainly caused by aneurysm rupture. However, SAH seen are typically small with an absence of intraventricular bleeding in ECMO

if screened at initiation such as in this cohort. SAH was instead associated with intraparenchymal bleeding in 23% of patients. ICH detected at initiation with subsequent changes to clinical management showed similar survival rates to discharge from ICU to those without ICH. These finding are similar to those described previously by Lockie *et al*[126].

However, a small number of patients who had progressive ICH despite these measures demonstrated a lower rate of survival. These results however conflict with the meta-analysis by Sutter *et al* showing high rates of mortality with ICH[122]. This may be because the timing of diagnosis and subsequent management of ICH were not reviewed as part of the analysis for its impact upon survival. This cohort demonstrates that the short-term mortality was largely in those that had extension of ICH within the first few days of ECMO, which was not reviewed in the meta-analysis. These results show there are no clear defining features of the patients who had ICH extension by clinical features, routine laboratory tests nor treatments given. The survival outcomes of these patients were in keeping with lower survival rates described by Sutter *et al* and at centres performing cranial imaging at time of developing neurological complications as opposed to universal screening[122,129,188]. This may therefore reflect a similar group of patients rather than the small asymptomatic ICH detected in this cohort. Routine haemostatic markers were similar between those with and without ICH progression and were therefore not of clinical use in screening for patients that may be at increased risk of mortality.

Previously described risk factors associated with the development of ICH include RRT and severe hypercarbia, which may indicate cerebral hypoxia and injury as being key changes to cause these events. Markers of cerebral injury, such as S100B and MMP-9 are predictive of haemorrhagic transformation of ischaemic stroke[189,190]. S100B has also been demonstrated as a marker of ICH in patients requiring ECMO and therefore these markers may be more

useful in predicting those at risk of ICH progression than routine coagulation parameters[191,192].

NICMH is recognised as a significant complication of ECMO. However, its development is described in a limited nature with predominantly retrospective single-centre cohort studies. Although these data are of a similar study design and retrospective in data collection, this is the largest cohort describing this type of complication during ECMO and uses a recognised standardised definition by ELSO[169]. The development of NICMH is associated with a significantly prolonged duration of ECMO similar to previous findings[193]. Patients that have prolonged treatment duration may have an increased period in which these haemorrhagic events can occur and may be prone to susceptible bleeding events such as stress ulceration and surgical intervention.

However, these results do not show a significant difference between ECBF in those with and with major haemorrhagic events. It has been demonstrated that ECMO reduces vWF activity and the presence of high molecular weight multimers in comparison to control[155]. However, it is unclear the levels that these changes become significant to cause bleeding during ECMO and the changes in ECBF rates required for a fall in vWF activity to occur. Therefore, further evaluation of ECBF and vWF changes is needed with correlation to haemorrhage. Accrued vWF dysfunction and platelet GP damage may be relevant in these patients, particularly with higher flow rates causing shear stress in the days after starting ECMO[149,193]. This is suggested in this cohort by a predominance of bleeding at mucosal sites such as the respiratory and gastrointestinal tracts, which is frequently seen in defects of primary haemostasis. Mazzeffi *et al* described rates of 25% of serious bleeding during ECMO that was predominantly from the gastrointestinal tract (36%), chest (28%) and upper respiratory tract (16%) [194]. Similarly,

there were higher rates of in-hospital mortality at 33% with major bleeding during VV ECMO. With VA ECMO, serious bleeding occurred in 69% with a mortality rate of 59%. Kreyer *et al* also showed a predominance of bleeding from the respiratory tract with high rates of bleeding from cannulae sites. They included both non-major and major haemorrhage were include[131].

This analysis focuses upon infectious causes to SRF due to their predominance in being the main indications for VV ECMO in around 30% of cases[195]. When reviewing those with infectious pathologies, one of the key features was variability in the rates in thrombosis depending on the type of pathogen. High rates of pulmonary artery filling defects are seen in COVID-19 pneumonia followed by influenza then bacterial pneumonia. Radiological review of the filling defect sizes showed a high rate of small vessel involvement in the segmental and subsegmental pulmonary arteries. Although routine US Doppler scanning was not applied prior to ECMO in this cohort, there was an absence of preceding DVT in these patients to suggest embolization, although this cannot be excluded. This has similarly been shown by Le Berre *et al* who demonstrated higher levels of pulmonary perfusion defects with the exclusion of PE in COVID-19 (60%) in comparison to other disease processes (27%)[196].

Patients with SRF requiring ECMO have extensive pulmonary changes that support the hypothesis that these changes represent immunothrombosis, as discussed above. As a syndrome, ARDS is heterogeneous depending on the underlying aetiology and the inflammatory response of the patient[197]. COVID-19 has been demonstrated to cause platelet, endothelial and complement activation as well as provoking a significant acute phase response[198–200].The features of the latter have been demonstrated in COVID-19 with elevated levels of fibrinogen, CRP and ferritin levels, in keeping with a significant acute phase response that is seen in patients without DIC[201]. In contrast, the incidence of DIC in patients with

bacterial pneumonia was 12% at the initiation of ECMO, which is similar to previously describes rates of DIC in bacterial-related sepsis[202,203].

The imaging approach utilised in this cohort was whole-body CT imaging at the time of ECMO initiation, which in part was to screen for bleeding and thrombotic complications to guide subsequent anticoagulation and blood product support. At present, there is no standardisation of imaging practice in ECMO. *Sutter et al* suggest that cranial CT is performed if indicated by a deterioration in neurological status[122]. With an incidence of ICH at 11% and at 12% for pulmonary artery filling defects at initiation in this cohort, it seems appropriate that the approach of CT screening would favour earlier detection to improve survival outcomes. Imaging is also used at this time for other indications such as cannulae site location and assessment of lung injury. Due to the lower detection rates of ICH and other sites of thrombosis and haemorrhage during ECMO, it seems appropriate to reserve imaging for a change in clinical status although further studies are required to assess this. Similarly, screening US Doppler scans after decannulation identified an incidence of 21% in this cohort and significant rates in other centres[118,119]. As the diagnosis of DVT guides subsequent continued anticoagulation use, if DVT is detected to reduce the risk of complications such as post-thrombotic syndrome and PE, it appears rational from this data to use US Doppler at this time point.

The key limitations of this analysis are the retrospective nature and limitation to a single centre. This invariably introduces bias into the outcomes reported and the practice of one centre with a lack of alternatives for comparison. For example, detection of thrombosis during ECMO may be different if routine imaging was used, rather than based on clinical changes in the cohort described above. Similarly, anticoagulation use at a single centre may not reflect

outcomes at other centres with other anticoagulation regimens. As such, either multicentre observational cohort studies or randomised-cohort studies would be optimal in this situation. We have aimed to limit the confounding effect of multiple disease aetiologies by reviewing three infectious disease processes, which are common indications for ECMO and subsequently performed subgroup analysis to review the complications.

2.6 Conclusions

This retrospective review has identified that there are different temporal patterns of haemorrhagic and thrombotic events during ECMO. These are particularly more prevalent for thrombotic events with COVID-19 infection than bacterial pneumonia. Major bleeding, both intracranial and non-intracranial, remained similar regardless of the underlying disease aetiology. Routine coagulation testing was not related to the development of these events and D-dimer levels, although elevated were not specific in this cohort of critically ill patients requiring ECMO. This suggests that further haemostatic parameters and markers of organ injury are required to understand and predict these complications. Universal screening imaging appears to be an acceptable approach for these events if available due to high prevalence rates and impact upon subsequent anticoagulation use.

Chapter 3 – Blood Component Use during Extracorporeal Membrane Oxygenation

3.1 Introduction

The utilisation of blood components during ECMO is variable between patients but in general, the requirements are higher than other patients in critical care[204–206]. At present, there is an absence of prospective trial data to guide the use of different blood components and therefore their use has been extrapolated from other studies in critical care or based upon consensus expert opinion. A recent meta-analysis has largely highlighted that current evidence has been based upon single centre studies[207].

The indications for transfusion of blood components during ECMO can be considered in two broad categories: 1) as prophylaxis to maintain clinically appropriate target levels in the setting of anaemia, thrombocytopenia or coagulopathy, or 2) for treatment during episodes of major bleeding to achieve haemostasis and maintain tissue oxygen delivery. The transfusion targets for the former are poorly defined although guidance is given by ELSO based on consensus opinion and retrospective data[208] (Table 12). The Canadian Extracorporeal Life Support program recently suggested more restrictive approaches to transfusion threshold (Hb <70-50g/L, platelets <50 x10^9/L and no routine use of Fresh Frozen Plasma (FFP) in non-bleeding patients)[209]. The latter is based upon evidence from major studies outside the use of ECMO in the setting of critical care and high-risk cardiothoracic surgery[210,211]. However as discussed in Chapter 1, the nature of bleeding is complex during the use of ECMO and therefore transfusion protocols in this setting of haemorrhage may have

different requirements. At present, large-scale prospective randomised studies are lacking on patient blood management during ECMO.

Recent studies have demonstrated the association of poorer survival outcomes with high transfusion rates use during ECMO shown in Table 13. Guimbretière *et al* showed that blood transfusion rates were higher in adults requiring VA in comparison to VV ECMO and those defined as being 'high users' of transfusion (19 units RBC, 5 units platelets and/or 12 units FFP) had a risk of mortality in excess of 80%[212].

Table 12 Blood Transfusion Triggers during ECMO (Adapted from ELSO Anticoagulation Guidelines 2014 and ELSO Red Book)

Blood Component	*Transfusion Trigger(s)* [169,213]
Red Blood Cell Concentrate	Haematocrit >35-40% or haemoglobin 120-140g/L
Platelets	Platelet count <100x109/L
Fresh Frozen Plasma	PTr >1.5-2.0 and/or if there is significant bleeding
Cryoprecipitate	Fibrinogen <100-150g/L

Table 13 Studies describing the Use of Transfusion in Extracorporeal Membrane Oxygenation

Study	*Cohort Size*	*Events*	*Outcome(s)*	*Comments*
Guimbretiere, Perfusion. 2019[212]	509 (VA ECMO 81%)	VA vs VV for: *30-day survival* *6-month survival* *FFP Use* *Platelet Use*	 58 vs 71% 41 vs 51% 61 vs 32% 62 vs 34%	Retrospective Single centre High mortality in high transfused group (80%)
Cashen, Ped Crit Care Med. 2020[214]	511 children	Daily platelet transfusion volume: *Mortality* *Thrombosis* *Haemorrhage*	All p ≤ 0.01 OR 1.05 OR 1.01 OR 1.01	Retrospective Multicentre
Henriquez-Henriquez, Int J Art Organs. 2014[215]	98 adults (VA ECMO 92%)	Daily blood product use: *RBC* *Platelets* *FFP* *Cryoprecipitate*	 39.5ml/kg 34.3ml/kg 12.9ml/kg 1.4ml/kg	Single centre Retrospective
Smith, Perfusion. 2012[216]	484 ECMO runs in children	Characteristics of survivors and non-survivors	RBC use: 16 vs 27 ml/kg/d Platelet use: 17 vs 23 ml/kg/d Haemorrhage: 27 vs 49%	Single centre Retrospective
Swol, Perfusion. 2018[217]	81 adults	Risk of ICU death: *Haematocrit* *Units of RBC/day*	56% ICU survival >31% - RR 1.73 RR 4.4	Single centre Retrospective
Esper, Vox Sang. 2018[218]	676 adults	Risk of mortality according to blood product use	Increased 90-day (OR 1.05) and 1-year mortality (OR 1.05) with platelet transfusion	Large single centre series Retrospective
Hughes, Membranes. 2021[219]	54 publications (2 prospective and 2 multi-centre) 3808 patients	RBC transfusion per patient per day	2.6 /day VA: 3.9/day VV: 1.2/day Wide variation in use: 0.15 - 17.8/ /day	Systematic Review Unable to assess affect on mortality

3.1.1 Red Blood Cell Transfusion during Extracorporeal Membrane Oxygenation

Red cell transfusion has been used as a strategy to maximise oxygen delivery during periods of SRF. Matucci *et al* surveyed 447 clinicians globally for their use RBC transfusion triggers during ECMO[220]. They highlighted that there was an absence of pre-defined haemoglobin triggers for 54% of clinicians and that centres with higher number of ECMO runs per annum had lower transfusion triggers (84g/L in centres with > 24 runs per year and 94g/L in centres with <12 runs per year). Clinicians also used haemoglobin transfusion triggers that were higher than other patients in critical care not requiring ECMO.

Current guidelines suggest targeting a haemoglobin level greater than 150 g/L although in clinical practice, practitioners use significantly lower thresholds as illustrated by the findings of Martucci *et al*[220,221]. This threshold suggested by ELSO is proposed as an optimal cut-off balancing the risks of transfusion and increased circuit flow rates to maintain adequate oxygen delivery[222]. Patients receiving ECMO require higher usage of RBC transfusions[204]. The intended benefit of transfusion is to improve DO_2 at lower rates of ECBF[223]. High rates of ECBF can result in excessive negative pressure on the drainage side of the circuit causing haemolysis. In extreme cases, this can cause pump-related cavitation causing significant negative pressure in the access line, and subsequent failure of forward flow potentially causing cardiac arrest. Practically, ECBF is frequently limited by the size and position of the drainage cannula and the patient's intravascular volume[224].

In contrast, there is an increasing awareness to limit the use of transfusion particularly to achieve physiologically 'normal' levels of haemoglobin or coagulation profiles. The complications of transfusion include haemolytic reactions, blood group incompatibility, volume overload, transfusion-associated acute lung injury, immunomodulation, human

leukocyte antigen (HLA) and RBC antigen sensitisation, and transfusion-transmitted infection[223]. Therefore, patient blood management (PBM) strategies have been devised to apply an evidence base to blood transfusion use. However, this has not been done in the setting of ECMO although retrospective studies have shown that this approach can reduce blood utilisation without affecting recovery[225,226].

Several large, randomised control trials (TRIC, TRICS-III, and TITRe2) have consistently demonstrated that a 'restrictive' RBC transfusion strategy, typically maintaining haemoglobin concentration between 70 and 90 g/L is non-inferior to a 'liberal' transfusion strategy maintaining haemoglobin between 100 and 120 g/L in critically ill adults[227–229]. However, patients receiving ECMO were not included in these studies. Retrospective studies have suggested that lower haematocrit and haemoglobin concentration may provide acceptable clinical outcomes during ECMO[217,230]. High volumes of RBC transfusion during ECMO have been associated with increased mortality and morbidity including thrombosis, acute renal impairment, and sepsis, although the causality of this relationship is unclear as major haemorrhage may be a significant confounder[217,231]. The utilisation of 'restrictive' RBC transfusion practice with a lower haemoglobin level trigger for transfusion is therefore a key clinical quandary over recommended 'liberal' ones with a higher trigger.

3.1.2 Cryoprecipitate, Fresh Frozen Plasma and Platelet Transfusion Use during Extracorporeal Membrane Oxygenation

The development of coagulopathy and thrombocytopenia is common during ECMO use in both asymptomatic patients and patients with haemorrhage. The EOLIA study demonstrated that thrombocytopenia of varying levels was more common in patients using ECMO than in those having alternative respiratory support methods for SRF[204].

The description of non-RBC transfusions (i.e. platelets, FFP and cryoprecipitate) in the setting of ECMO is poorly defined and again largely based upon retrospective single centre data in adults[214,215,232]. Transfusion rates of these products were high but variable between these groups. In both paediatric and adult cohorts, transfusion requirements increased with cardiac indications for ECMO. Additional identified risk factors for transfusion are thrombocytopenia, older age in children, bleeding, recent antiplatelet use and longer duration of ECMO. As with RBC transfusion, the transfusion of platelets, cryoprecipitate and FFP can be associated with transfusion-related reactions.

A large multi-centre review of platelet transfusion use during ECMO in paediatric patients showed high rates of transfusion (>96%) with increased platelet transfusion volumes associated with increased mortality rates and both higher rates of thrombosis and bleeding particularly in the neonatal population[214]. Similarly, Karam *et al* showed high use of non-RBC transfusions during ECMO in children with their use related to bleeding or predefined transfusion targets[233]. Both studies failed to show a relationship between preceding platelet counts and bleeding although recognised discrepancies between blood sampling times and subsequent time to transfusion. Esper *et al* demonstrated a high rate of mortality at 90-day and 1-year in adult patients requiring platelet transfusion during VA ECMO (odds ratio 1.05) but not in VV ECMO nor with other non-RBC blood components[218]. However, this did not take in to account major haemorrhage, which is a significant determinant of platelet use and survival during ECMO[234–236].

The current data suggests that the use of non-RBC transfusions during ECMO may have adverse outcomes, including mortality, despite the intention to reduce haemostatic complications and improve survival rates with current transfusion practices. Due to this, a

retrospective cohort of adult patients requiring VV ECMO at Guy's & St Thomas' NHS Foundation Trust was reviewed for transfusion practice, the factors relating to transfusion use and the subsequent impact on clinical outcomes.

3.2 Aims

1) To compare the survival outcomes of patients with 'liberal' versus 'restrictive' red blood cell transfusion approaches during ECMO

2) To assess the survival outcomes of patients receiving platelet, FFP and cryoprecipitate/fibrinogen concentrate transfusions during ECMO

3.3 Methods

3.3.1 Study Design

A retrospective review of patients requiring VV ECMO was performed for the use of RBC, platelet, FFP, fibrinogen concentrate and cryoprecipitate transfusions. The period of review was for 7 years between 1st January 2011 and 31st December 2017 for initiation of ECMO. For RBC transfusion, there was a change in haemoglobin transfusion triggers in January 2014 and therefore a retrospective cohort study was performed between these two groups. For non-RBC transfusions, the groups were compared to those who required and did not require transfusion of these blood components as a retrospective cohort study.

3.3.2 Ethical Approval

Approval by an ethics committee was not required in accordance with NHS Health Research Agency (http://www.hra-decisiontools.org.uk/ethics/). The study was registered and approved as a clinical audit (reference number 10129) with the Trust Research department.

3.3.3 Inclusion Criteria

Patients requiring in VV ECMO at Guy's and St Thomas NHS Foundation Trust in the review period.

3.3.4 Exclusion Criteria

Patients were excluded if they were less than 16 years old and if the ECMO run was less than 24 hours. If a patient received more than one ECMO run, the initial run was included, and the subsequent ones were excluded. Patients that required plasma exchange (PEX) were not included in the analysis of FFP use on survival outcomes.

3.3.5 Data Collection

Subject clinical details were extracted using R Version 3.5.1 (R Foundation for Statistical Computing, Auckland, New Zealand; http://www.r-project.org/) from electronic records on Phillips ICIP Intellispace CCA (Amsterdam, The Netherlands). Clinical details including gender, age at initiation of ECMO, cause of respiratory failure, APACHE II score at initiation, duration of ECMO, and the use of RRT were recorded. Transfusions were identified from the patients' electronic health record which records units transfused by pack serial number to prevent double counting of transfusions.

3.3.6 Details of Extracorporeal Membrane Oxygenation Circuit

All patients reviewed in this study received VV ECMO. The oxygenator circuit used at was the Cardiohelp system (Getinge, Gothenburg, Sweden) and BE HLS 7.0 oxygenator (Getinge). Cannulation was routinely performed using bifemoral Biomedicus cannulae (Medtronic, Dublin, Ireland). Anticoagulation at the time of the study was with an IV UFH with 50 IU/kg as a bolus at the time of cannulation with 2,500 units in the priming fluid for the circuit. A

subsequent UFH infusion was monitored by the APTTr targeting a level of 1.5–2.0—unless there was clinical or radiological evidence of bleeding. This was also guided by a preceding history bleeding or thrombotic complications and whole-body CT performed at the time of cannulation.

3.3.7 Transfusion Indications and Targets

RBC transfusion were given due to either anaemia or bleeding with 1 unit having an approximate volume of 350ml. Between January 2011 and December 2013, Hb targets in the patient with stable parameters were of 100–120 g/L. Between January 2014 to December 2017, Hb targets were 80–90 g/L but for stable patients and particularly in those where the concerns about HLA sensitization were important (as a limitation to potential lung transplantation if anti-HLA antibodies developed causing acute graft rejection), a transfusion target of 70 g/L or less is used. For clinically unstable patients with difficult oxygenation, higher RBC transfusion triggers were defined by the attending physicians. For patients with active bleeding, a pragmatic target Hb of 80–90 g/L was used.

Patients given platelet transfusions when platelet counts were < 50 x 10^9/L if there were no signs of bleeding. Patients with active bleeding when platelets were < 80-100 x 10^9/L. A single platelet unit has an approximate volume of 200ml.

Patients who were not bleeding were transfused FFP when PTr was greater than 1.5. Patients were given FFP with active bleeding when APTTr or PTr was greater than 1.5. A single FFP unit has an approximate volume of 350ml and was given at a dose of 15-20ml/kg. Adult patients will therefore receive typically 2-4 units of FFP for one transfusion episode.

Patients were transfused cryoprecipitate or fibrinogen concentrate without bleeding when Clauss fibrinogen levels were <1.5 g/L. Patients were given these products with active

bleeding when fibrinogen levels were <2.0 g/L. A single cryoprecipitate unit has an approximate volume of 300ml and is given at a dose of 15ml/kg. Adult patients will therefore receive typically 2-3 units of one transfusion episode. Fibrinogen concentrate (Riastap, CSL Behring, USA) was given as a 1g infusion with 50ml volume used in patients with fluid overload.

3.3.8 Outcome Variables

When assessing the use of a 'restrictive' versus 'liberal' RBC transfusion practice, the primary outcomes were survival to decannulation, survival to discharge from the ICU and survival at 6 months after discharge. Patients with incomplete follow-up at 6 months were not included in the outcome frequencies at that time point. The data was collected prospectively from entry to a local registry and reviewed retrospectively for the purpose of this study.

The primary outcome was transfusion rates of platelet, FFP and cryoprecipitate in survivors and non-survivors from ECMO decannulation, discharge from ICU and follow-up at 6-months. The secondary outcomes were 1) the use of these products with the presence or absence of Ca-DVT at the time of ECMO decannulation, 2) those with and without ≥1 episode of MH during ECMO 3) the corresponding parameter (platelet count, PTr and fibrinogen level respectively) at which the transfusion was given and 4) the day of ECMO use that transfusion was received. Bleeding assessment was performed qualitatively during ECMO by the clinical care team and recorded contemporaneously in the electronic medical record. MH for the purpose of this study was defined as the transfusion of ≥ 4 units of RBC transfusion within a 24-hour period with clinical evidence of bleeding. Large volume RBC transfusion not due to bleeding were not included in this analysis.

3.3.9 Laboratory Blood Testing Values and Assays

A full blood count (FBC) is routinely performed once daily for patients receiving ECMO and additionally if there are other clinical indications such as bleeding, preoperatively or following circuit change. All FBC results from venous samples were included in this study to give a median Hb concentration and haematocrit during ECMO for further statistical analysis. Results were reviewed for outliers, and these were then reviewed manually with clinical records for errors. FBC testing was performed using DxH 900 Hematology (Beckman Coulter, Brea, CA) to provide haemoglobin concentration and haematocrit results at our centre. Testing for APTT, PT, D-dimer and fibrinogen were performed using Sysmex CS2100*i* (Norderstedt, Germany) as per manufacturer protocols.

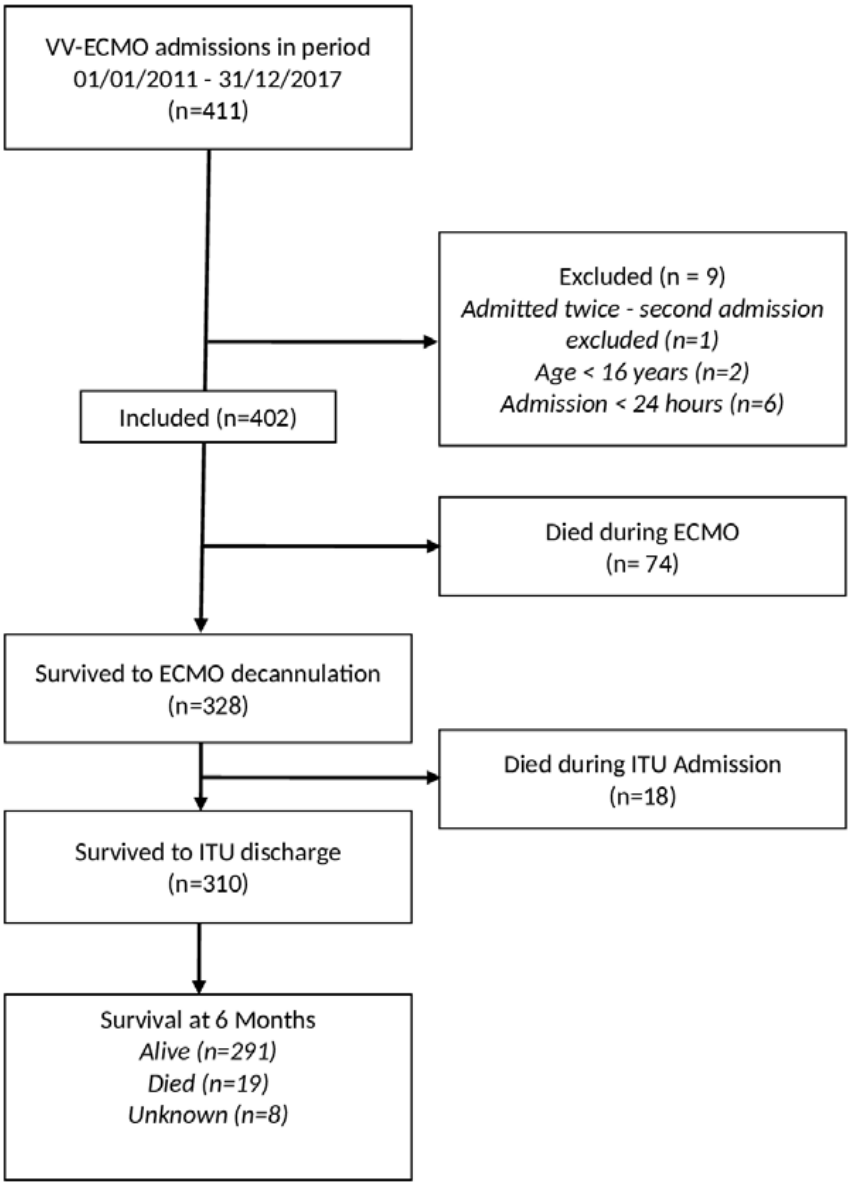
3.3.10 Statistical Considerations

Statistical analysis was performed using R Statistics Version 3.5.1 (R Foundation for Statistical Computing) and SPSS version 27 (IBM, USA). Descriptive analysis of continuous variables was given as medians and interquartile ranges. Categorical variables were given as numbers of individuals/events and percentages. Null hypothesis significance testing was employed to examine variable distributions between the two time periods. Mann-Whitney U tests were performed to assess continuous variables, and chi-square goodness-of-fit tests were performed to assess categorical variables. Logistic regression was performed to examine independent variables associated with the development of the investigated outcomes. The regression model covariates were selected with consideration for plausible and known predictors of these outcomes. A significance level of alpha equals to 0.05 was used for all statistical tests.

3.4 Results

During the study period, 411 patients received VV ECMO for SRF. Nine patients were excluded from the analysis: six patients survived less than 24 hours and two patients were under 16 years old. One patient was placed on ECMO twice, their second run was not included in the analysis. Figure 15 shows the study cohort and their outcomes. In total 402 patients were reviewed with a total of 5438 days (14.9 years) of ECMO. Nine patients were excluded for analysis of FFP usage due to the indication of PEX during ECMO.

Figure 15 Flow Diagram with Patient Outcomes during use of ECMO between 2011 - 2017



3.4.1 'Liberal' versus 'Restrictive' Red Blood Cell Transfusion Practice during Extracorporeal Membrane Oxygenation

Ninety-nine patients were treated between 2011 and 2013 and 303 patients between 2014 and 2017. The characteristics of the groups are shown in Table 14. The characteristics between the groups are similar with no statistically significant differences in age, APACHE score at admission, the use of RRT, and survival outcomes to decannulation, discharge from ICU and survival at 6 months. The most common reason for patients to require VV ECMO was bacterial pneumonia and thereafter viral pneumonia. The occurrence rate of ICH was 16 patients (16.2%) receiving VV ECMO between 2011 and 2013 and 41 (13.5%) between 2014 and 2017. Major haemorrhage occurred in 21 (21.2%) and 50 (16.5%) respectively with no statistically significant difference with both types of bleeding events in the two time periods.

After the introduction of 'restrictive' transfusion practice in 2014, the median Hb concentration decreased from 97 to 87 g/L ($p < 0.001$) shown in Figure 16(a). The average number of units transfused to patients when a restrictive approach was used fell from 0.66 RBC units per ECMO day to 0.44 ($p < 0.001$). Thereafter, the median flow rate on ECMO between the groups as ECBF is a key determinant of systemic oxygenation. The mean ECBF during the first 7 days of those receiving ECMO was higher in the initial group at 4.0 L/min (range, 3.4–4.5 L/min) receiving a 'liberal' transfusion approach and lower at 3.5 L/min (3.2–4.2 L/min) ($p < 0.05$) in the 'restrictive' transfusion period (Figure 16(b)).

Multiple logistic regression analysis for all patients in the study showed that APACHE II score, age at cannulation, and number of RBC were factors independently associated with survival to decannulation, whereas the period that they received ECMO was not (Table 15).

The number of RBC units transfused in patients who had survived or died to ECMO decannulation in the two time periods is shown in Figure 17. The rates of transfusion among survivors were similar in the two groups (p=0.38). Patients in the later period with a 'restrictive' approach showed that those patients who died received more RBC transfusions compared with those who survived.

Figure 16 Comparison of haemoglobin and extracorporeal membrane oxygenation (ECMO) blood flow rates between 'liberal' and 'restrictive' transfusion practices. (a) Median haemoglobin concentration; (b) Extracorporeal blood flow during the first 7 days during VV ECMO between 2011 and 2013 and 2014–2017

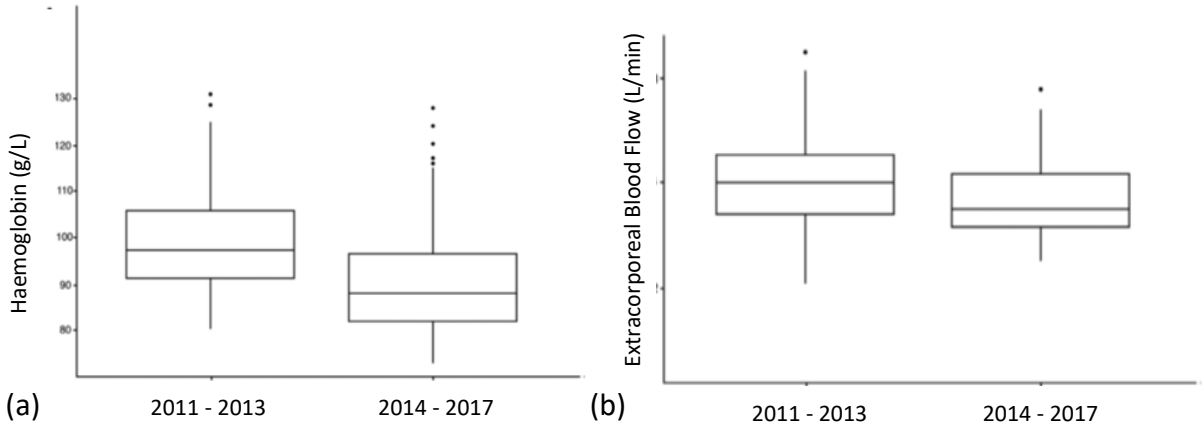


Table 14 Clinical features of patients receiving ECMO comparing patients between 2011-2013 ('liberal' RBC transfusion approach) to 2014-2017 ('restrictive' RBC transfusion approach)

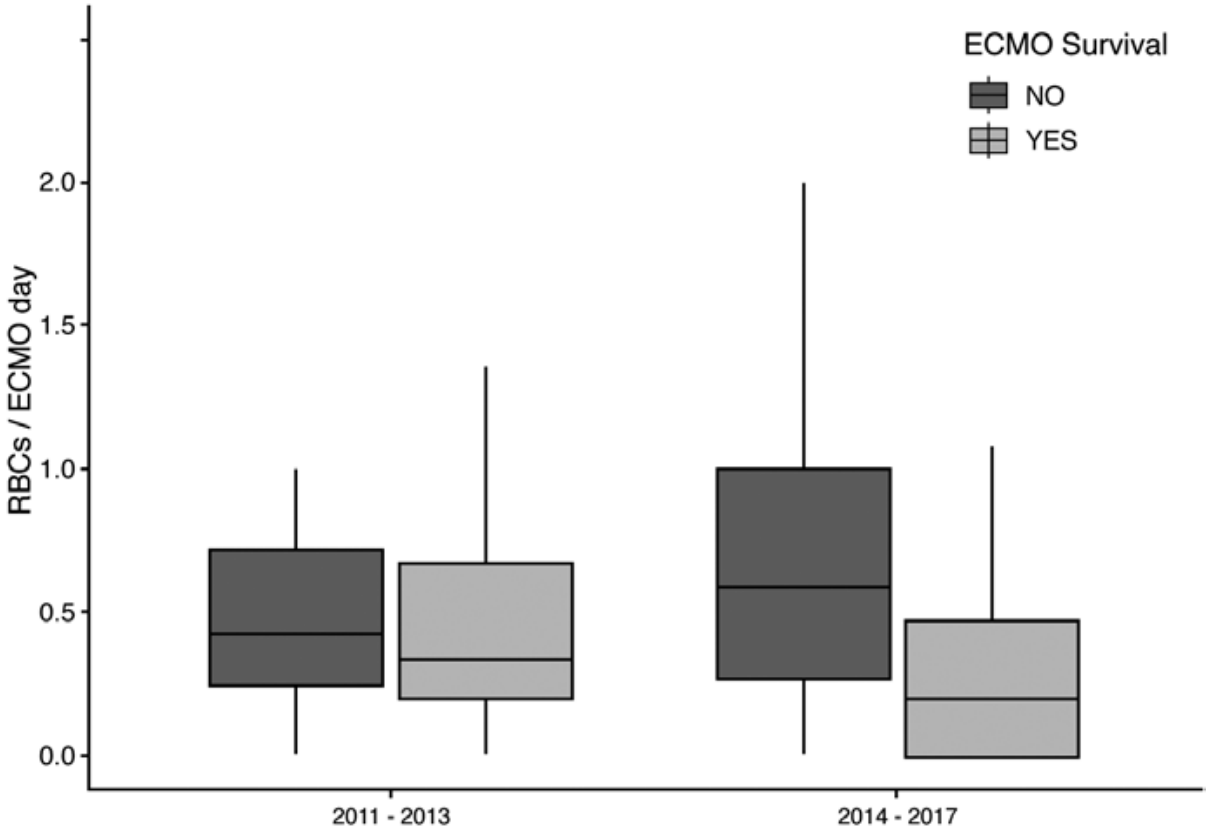
Clinical Features	*Total*	*Liberal*	*Restrictive*	*p-value*
Number of patients	402	99	303	-
Gender (Male)	227 (56.5%)	49 (49.5%)	178 (58.7%)	0.135
Median Age (IQR)	45 (34 – 56)	46 (35 – 55)	45 (34 – 56)	0.822
Median APACHE II Score (IQR)	18 (15 – 21)	18 (15 – 21)	18 (15 – 21)	0.817
Median duration of ECMO, days (, IQR)	8 (5 – 14)	9 (5.5 – 14)	8 (5 – 14)	0.84
Use of RRT	227 (56.5%)	51 (51.5%)	176 (58.1%)	0.304
ECMO Survival	328 (77.1%)	78 (78.8%)	250 (82.5%)	0.497
ICU Survival	310 (77.1%)	73 (73.7%)	237 (78.2%)	0.433
6-Month Survival	291 (73.9%)	70 (72.9%)	221 (74.2%)	0.914
Primary respiratory diagnosis				
Pneumonia – Bacterial	163	47 (47.5%)	116 (38.3%)	0.335
Pneumonia – Viral	71	18 (18.2%)	53 (17.5%)	
Extra-pulmonary ARDS	37	8 (8.1%)	29 (9.6%)	
Asthma	29	8 (8.1%)	21 (6.9%)	
Other causes	102	18 (18.2%)	84 (27.7%)	
Median Haemoglobin during ECMO g/L (IQR)	89.5 (83 – 99)	97.0 (91.3 – 106.0)	87.0 (82 – 92.3)	**<0.001**
Haematocrit % (IQR)	28.2 (25.8 – 30.7)	30.3 (28.1 – 33.1)	27.1 (25.2 – 30.0)	**<0.001**
Median ECMO blood flow L/min (IQR)	3.67 (3.17 – 4.28)	4.01 (3.36 – 4.50)	3.51 (3.16 – 4.17)	**0.001**
Intracranial Haemorrhage	57 (14.2%)	16 (16.2%)	41 (13.5%)	0.627
Major Haemorrhage	71 (17.7%)	21 (21.2%)	50 (16.5%)	0.36

Table 15 Multiple Logistic Regression Showing the Odds Ratio and Confidence Intervals for Variables for Mortality While Receiving Extracorporeal Membrane Oxygenation

Characteristics	*Odds ratio*	*Confidence Intervals (95%)*
Sex (Female)	0.78	0.44 – 1.37
Age	**1.04***	1.02 – 1.06
APACHE II Score	**1.09***	1.03 – 1.16
Duration of ECMO	0.99	0.97 – 1.01
Median haemoglobin	0.99	0.96 – 1.02
Admission after 2014	0.88	0.43 – 1.83
Number of RBC Transfusions	**1.07***	1.03 – 1.11

* p-value < 0.05

Figure 17 Rate of red blood cell transfusion and survival to decannulation from extracorporeal membrane oxygenation (ECMO) over the two study time periods



3.4.2 Platelet Transfusion during Extracorporeal Membrane Oxygenation

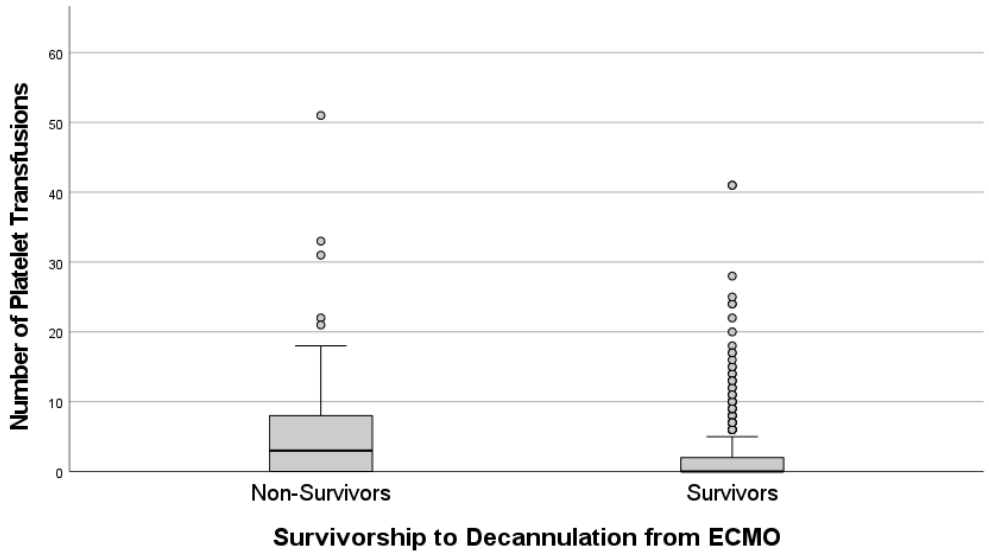
A total of 1261 units of platelets were transfused over the study period during ECMO. 163/402 patients (40.5%) received platelet transfusions at any time point and it was used on 753/5438 (13.8%) days of ECMO. The median number of platelet transfusions transfused during ECMO, if used, was 5 units per run. The median platelet count prior to transfusion was 49 $x10^9$/L (IQR 36-67 $x10^9$/L). The median time from the preceding platelet count to transfusion was 3.9 hours (IQR 2.5 - 6.1 hours). Table 16 shows the comparison of platelet transfusion rates against survival outcomes, MH and development of Ca-DVT following decannulation from ECMO.

Table 16 Clinical Outcomes according to the use of platelet transfusion during ECMO

Clinical Outcome	*Platelet Transfusion (n=163)*	*No Platelet Transfusion (n=239)*	*p-value*
Median Duration of ECMO	10 days	7 days	**0.001**
Survival to Decannulation	116 (71.2%)	212 (88.7%)	**<0.001**
Survival to Discharge from ICU	107 (65.6%)	203 (84.9%)	**<0.001**
Survival at 6 months	100 (61.3%)	191 (79.9%)	**<0.001**
Major Haemorrhage	49 (30.1 %)	20 (8.4%)	**<0.001**
Deep Vein Thrombosis	22/105 (20.9%)	32/186 (17.2%)	0.42

Figure 18 shows the amounts of platelets used between survivors to non-survivors of ECMO decannulation. There was a mean use of 2.5 platelet transfusion in survivors in comparison to 6.0 platelet transfusions in non-survivors ($p<0.001$).

Figure 18 The Number of Platelet Transfusions between Survivors and Non-Survivors to decannulation from ECMO



3.4.3 Fresh Frozen Plasma use during Extracorporeal Membrane Oxygenation

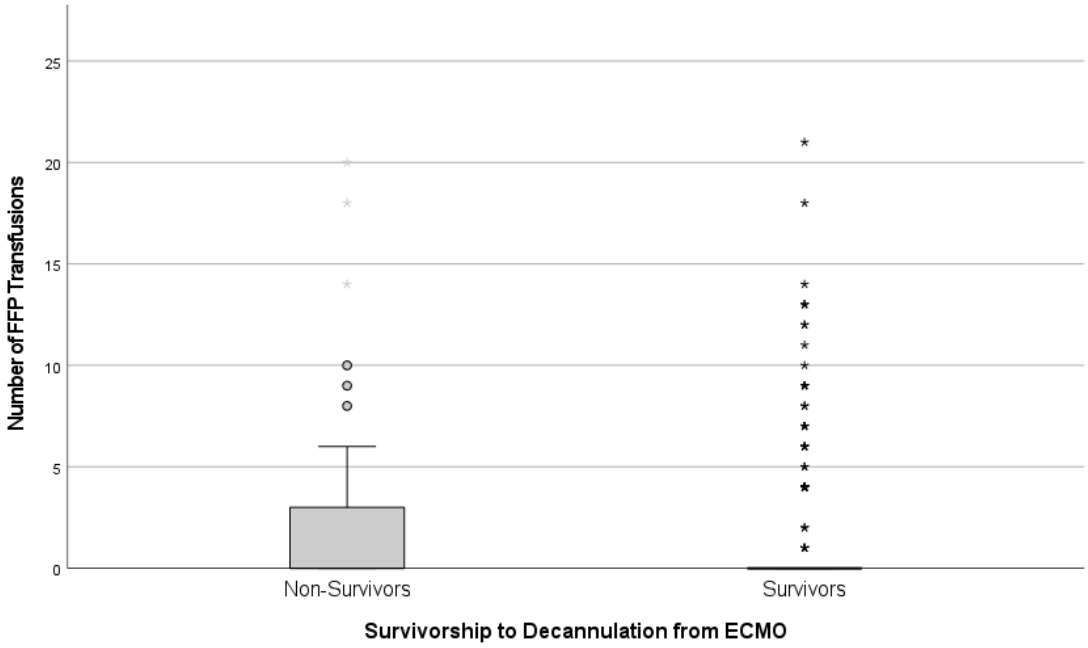
A total of 772 units of FFP were transfused over the study period during ECMO. Nine patients (2.2%) received PEX requiring a total of 338 units of FFP. This was 46.8% of the total use of FFP in patients requiring ECMO. The indications for PEX were for pulmonary vasculitis (8 cases) and dermatomyositis (1 case). The remaining 393 patients that did not require PEX, 61 patients (15.5%) received 434 units of FFP and they were used on 81/5352 (1.5%) total ECMO days The median number of FFP transfusions during ECMO if used was 6 units per run. The median PTr prior to transfusion was 1.4 (IQR 1.2-1.7) prior to FFP transfusion. The median time from the preceding PTr test to transfusion was 4.4 hours (IQR 3.0 – 6.6 hours). Table 17 shows the comparison of FFP transfusion rates against survival outcomes, MH, and development of Ca-DVT following decannulation from ECMO.T

Figure 19 shows the amounts of FFP used between survivors to non-survivors of ECMO decannulation. There was a mean use of 0.9 FFP transfusions in survivors in comparison to 1.9 FFP transfusions in non-survivors (p=0.017).

Table 17 Clinical Outcomes according to the use of FFP transfusion during ECMO

Clinical Outcome	*FFP Transfusion (n=61)*	*No FFP Transfusion (n=332)*	*p-value*
Median Duration of ECMO	8 days	8 days	0.36
Survival to Decannulation	41 (67.2%)	287 (86.4%)	**<0.001**
Survival to Discharge from ICU	37 (55.2%)	273 (82.2%)	**<0.001**
Survival at 6 months	32 (52.5%)	259 (78.0%)	**<0.001**
Major Haemorrhage	29 (47.5%)	41 (12.3%)	**<0.001**
Deep Vein Thrombosis	6/34 (17.6%)	48/257 (18.7%)	0.9

Figure 19 The Number of Fresh Frozen Plasma Transfusions between Survivors and Non-Survivors to decannulation from ECMO



3.4.4 Fibrinogen Replacement use during Extracorporeal Membrane Oxygenation

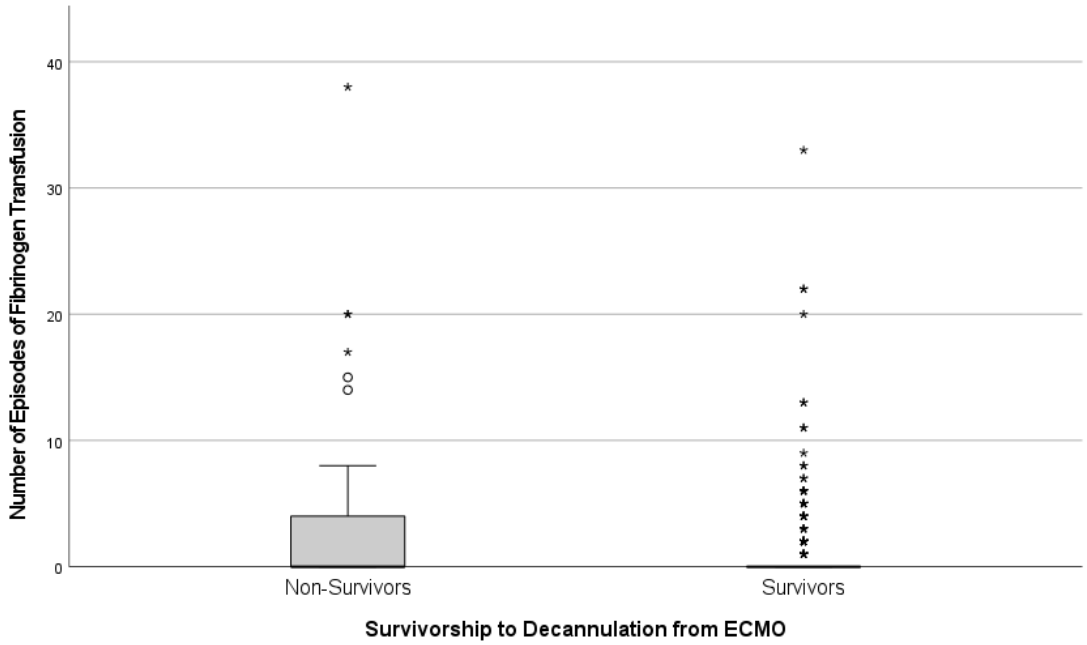
A total of 490 units of cryoprecipitate and 70 vials of fibrinogen concentrate (both will be referred subsequently to as 'fibrinogen replacement') were transfused over the study period during ECMO. 100/402 patients (24.9%) received fibrinogen replacement at any time point and it was used on 215/5438 (3.9%) total ECMO days. The median number of units of fibrinogen replacement for those who were transfused was 4 units per ECMO run. The median fibrinogen level prior to transfusion was 1.3g/L (IQR 1.0-1.7g/L). The median time from fibrinogen level to transfusion was 4.1 hours (IQR 2.8-8.7 hours). Table 18 shows the comparison of fibrinogen replacement against survival outcomes, MH, and development of Ca-DVT following decannulation from ECMO.

Table 18 Clinical Outcomes according to the use of Cryoprecipitate and Fibrinogen concentrate transfusion during ECMO

Clinical Outcome	*Fibrinogen Replacement (n=100)*	*No Fibrinogen Replacement (n=302)*	*p-value*
Median Duration of ECMO	12 days	8 days	**<0.001**
Survival to Decannulation	68 (68.0%)	260 (86.1%)	**<0.001**
Survival to Discharge from ICU	63 (63.0%)	247 (81.8%)	**<0.001**
Survival at 6 months	58 (58.0%)	233 (77.2%)	**<0.001**
Major Haemorrhage	38 (38.0%)	32 (10.6%)	**<0.001**
Deep Vein Thrombosis	18/62 (29.0%)	36/229 (15.7%)	**0.02**

Figure 20 shows the amounts of fibrinogen replacement used between survivors to non-survivors of patients to ECMO decannulation. There was a mean use of 1.1 fibrinogen transfusions in survivors in comparison to 3.0 fibrinogen transfusions in non-survivors ($p<0.001$).

Figure 20 The Number of Fibrinogen Replacement Transfusions between Survivors and Non-Survivors to decannulation from ECMO



Binary logistic regression was performed on all patients in the study for the duration of ECMO, the use of platelet, FFP and fibrinogen replacement transfusions during ECMO, the presence of MH during ECMO and the use of PEX during ECMO. For survival outcomes, the use of platelet transfusions was statistically significant with a reduced survival to ECMO decannulation ($p = 0.006$, OR 0.43, 95% CI 0.24 – 0.79), discharge from ICU ($p = 0.006$, OR 0.46, 95% CI 0.27 – 0.80) and survival at 6-months ($p = 0.016$, OR 0.52, 95% CI 0.30 – 0.88). Furthermore, the number of platelet transfusions was also found to be statistically significant ($p = 0.018$, OR 0.95, 95% CI – 0.92 – 0.99) for decreased survival to decannulation. This was not significant for other blood transfusion products or the presence of MH in this multivariate

analysis. Duration of ECMO was found to be significant to survival at 6 months (p = 0.003, OR 0.98, 95% CI 0.96 – 0.99) but not for the other shorter-term survival outcomes.

The use of fibrinogen replacement as a risk factor for the development of lower limb Ca-DVT at decannulation sites was not found to be statistically significant (p = 0.054, OR 2.13 CI 95% 0.99 – 4.60) using multivariable regression. Additionally, this was not associated with the total amount of fibrinogen used (p = 0.93) or the mean use of fibrinogen replacement per day (p = 0.50)

3.5 Discussion

3.5.1 'Liberal' versus 'Restrictive' Red Blood Cell Transfusion Practice during Extracorporeal Membrane Oxygenation

This is a large single-centre retrospective study examining RBC transfusion practice in adults receiving VV ECMO for SRF. A restrictive approach was historically adopted on the basis of the known risks of RBC transfusions and the increasing number of studies demonstrating RBC transfusions linked with significantly increased mortality and morbidity in critical illness[219,227,229,237]. However, this practice had not been reviewed and these studies did not include patients using ECMO. These data demonstrate that a 'restrictive' approach to transfusion in ECMO is not associated with an inferior survival outcome to decannulation from ECMO, survival to ICU discharge and survival at 6 months. Additionally, mortality rates are comparable to those of the ELSO registry and national and international studies[204,238]. The average number of units transfused to patients with a restrictive approach fell by 0.2 RBC units per ECMO day equating to approximately 1-2 units per ECMO run. This has the potential to generate a significant cost saving and minimise unnecessary blood component use.

Despite a lower transfusion threshold, the average ECBF in the first 7 days was 500 mL/min higher in those patients where a 'liberal' transfusion practice was used. This contradicts modelling to suggests that an elevated Hb increases DO_2 during ECMO[239].The reason for this is unclear and further analysis to include DO_2 and VO_2 pre- and post-transfusion may assist in understanding this. At this centre, it is clinical practice to provide the fractional delivery (Fd) of oxygen at 100% in the ECMO circuits and with an aim to maintain blood flows between 3 - 4.5 L / min to target an SaO_2 > 88%. Weaning is commenced with an improved clinical state to allow local 'weaning' protocols from ECMO to be used. For this, ECBF is generally maintained >3L / min to reduce the risk of thrombosis and circuit loss, which are associated with low ECBF rates[240]. The discrepancy in this practice and previous modelling by Spinelli and Bartlett may account for some of the differences observed between the expected and observed flow rates with differing RBC transfusion practices[224].

These results are consistent with two other retrospective studies of ECMO that have shown non-inferiority survival for patients with lower Hb concentration and haematocrit targets with a small proportion being maintained with an average haematocrit above the targeted 40% during treatment[217,230]. There was a higher use of RBC transfusion in non-survivors at all survival time points following a change to a restrictive transfusion approach. This is consistent with previous studies in critical care[241]. Although age and severity of illness at presentation were associated with an increased risk of mortality prior to decannulation, the mean Hb level during ECMO was not. Associated events such as bleeding and increased efforts to improve cardiovascular stability in those not responding to ECMO support may have contributed to a higher use of RBC transfusions in non-survivors.

This retrospective study suggests that 'restrictive' transfusion practice (Hb concentration target 80–90 g/L) may have comparable survival rates to a 'liberal' transfusion strategy (target

100–120 g/L) with regards to decannulation from ECMO, discharge from ICU and survival at 6 months. This is yet to be fully evaluated in prospective multi-centre randomised studies, in addition to other areas such as duration of RBC storage that may also be worth exploring[242].

3.5.2 Transfusion of Platelets during Extracorporeal Membrane Oxygenation

The use of non-RBC transfusions during ECMO appears to be variable. Platelet transfusions were used in the largest number of patients and for more days of ECMO, followed by fibrinogen replacement then FFP. All blood product types were used more frequently in those who had major haemorrhage in comparison to those who did not. These results suggest platelet transfusions were given more extensively for a 'prophylactic' intent as there were higher numbers of transfusions and preceding platelet counts showed severe thrombocytopenia.

Short- and longer-term survivorship from ECMO was affected by the transfusion of platelets on multivariate analysis. The odds of this were higher in the short-term. This was independent of those with MH, which is a significant potential confounder for increased mortality as shown in Chapter 2 and other cohorts[131,182]. Kreyer *et al* demonstrated higher critical illness severity scores in those experiencing MH[131].

Other recent studies have highlighted that mortality rates may be related to the use of platelet transfusions in both adult and paediatric cohorts requiring ECMO. This cohort has similar rates of platelet transfusions for adult patients to other centres such Guimbretriere *et al* who described 34% of patients requiring them during VV ECMO[212]. Esper *et al* did not demonstrate an association with platelet transfusion and mortality during VV ECMO but did with VA ECMO[218]. However, the number of patients included was smaller and did not control for the presence of major haemorrhage. Aubron *et al* showed platelet transfusions were

associated with non-survivorship from ECMO in a 5-year retrospective review from VV ECMO[243]. Rates of platelet transfusion in this cohort were lower than in these paediatric cohorts[214,233,234]. Platelet transfusion triggers are typically higher in children due to the concerns of neonatal intracranial haemorrhage and an underdeveloped haemostatic system. However, survival outcomes in neonates and children requiring platelet transfusions during ECMO are also reduced[214,233,244]. Other previously identified risk factors for platelet transfusions are antiplatelet use and Hb level falls of >55 g/L[232]. It appears that further understanding of the role of platelet transfusion during ECMO is required due to its potential association with mortality.

The use of platelet transfusion has shown conflicting outcomes in other indications for transfusion. The PlaNET2 and PATCH studies showed adverse survival outcomes in those with lower platelet transfusion thresholds in neonates and adults taking antiplatelets following cerebral haemorrhage respectively[245,246]. There have also been further studies suggesting either lower or non-superior survivorship in those requiring platelet transfusions in the setting of traumatic brain injury, following cardiac surgery, patients with cancer and after haemopoietic stem cell transplantation[247–250]. Additionally, the use of prophylactic platelet transfusion prior to invasive procedure increases the short-term risk of thrombosis[251]. However, these retrospective studies are likely underrepresenting key confounders such as disease severity and haemorrhagic events.

The haemostatic function of transfused platelets in ECMO as a therapeutic option is still to be fully evaluated. In bleeding patients undergoing CPB, the extracorporeal circuit increases platelet aggregation by TRAP and arachidonic acid following platelet transfusion[252]. Similarly in those with severe thrombocytopenia, platelet transfusion increased platelet aggregation

to various activators[253]. However in major trauma, the use of platelet transfusion with large volume RBC transfusion has not shown increased platelet aggregation but attenuated fibrinolysis on TEG[254]. Increased levels of PAI-1 and decreased in tPA were seen that may cause this effect, likely attributable to release of PAI-1 following platelet activation. Previous groups have also described platelet activation as a marker of disease severity in sepsis[255,256]. This raises the concern of platelet activation during ECMO, particularly in those without bleeding, severe thrombocytopenia and inflammatory conditions, and may be a potential mechanism leading to the increased mortality seen in relation to platelet transfusion. The paradox of platelet activation and reduced platelet adhesion due to the shedding of key adhesion molecules such as GPIbα as a result of mechanical circuit may mean that transfused platelets may not correct coagulopathic changes and cause potential harm[147].

3.5.3 Transfusion of Fresh Frozen Plasma during Extracorporeal Membrane Oxygenation

FFP was used at higher rates in those with major haemorrhage than other blood components. Risk stratification tools to assess bleeding risk during ECMO have been developed, which correlate to the use of plasma products, but have been not widely adopted[257]. The average PTr prior to administration did not meet a transfusion trigger of >1.5 suggesting an empirical use in MH may have been employed. The use of FFP transfusion was lower in our cohort than that described by others[215]. Historically, FFP use has been associated with inappropriate use and demonstrates less correction in coagulation time in those with lower PTr or APTTr[258]. In addition, the use of prophylactic FFP use has not been associated with reduced rates of RBC use during critical care admission[259]. A recent randomised control study by McMichael *et al* showed that the prophylactic use of FFP did not alter the lifespan of an ECMO circuit and did

not increase the rate of thrombosis[260]. Additionally, a ratio of RBC:FFP during ECMO >1.0 is associated with a higher rate of in-hospital mortality during major haemorrhage[261]. This data supports the suggestion that its use should be mainly used during bleeding. It is probable that those with procoagulant deficiencies also have concurrent deficiencies in natural anticoagulants[262].

In previous retrospective studies, higher rates of FFP use was seen in patients developing DVT after cardiac surgery, liver transplantation and pelvic surgery for cancer[263–265]. This was not demonstrated in this cohort and may be attributable to different transfusion thresholds.

3.5.4 Transfusion of Fibrinogen Replacement Products during Extracorporeal Membrane Oxygenation

The use of fibrinogen replacement may also have an impact upon short-term survival and the development of post-decannulation DVT although this was not shown to be statistically significant on multivariate analysis. Plasma fibrinogen levels have not been correlated with the development of DVT although a smaller reduction in D-dimer levels after decannulation was associated with DVT[179]. In Chapter 2, the results showed higher rates of Ca-DVT following decannulation in patients with COVID-19 pneumonia, which had higher levels of fibrinogen in comparison to bacterial and viral pneumonias. Furthermore, cryoprecipitate also contains other procoagulant factors such as FVIII and vWF that may cause an additive effect to fibrinogen in this risk of thrombus formation.

In other clinical scenarios, there is a suggestion that fibrinogen replacement increases the risk of thrombosis. Shams *et al* showed that fibrinogen replacement increased clot stability during cardiopulmonary bypass[252]. Emuakhagbon *et al* showed that the use of intra-operative FFP and cryoprecipitate during liver transplantation was a risk factor for VTE post-operatively[265].

Exploration of thrombotic events therefore warrants further evaluation in the setting of fibrinogen replacement during ECMO.

Lower transfusion thresholds for non-RBC transfusions have been adopted during ECMO use due to concerns from clinicians that the additional use of anticoagulation may result in major bleeding. Chung *et al* have shown retrospectively that there is no increased risk of thrombosis or circuit occlusion if anticoagulation is held due to bleeding, severe thrombocytopenia or peri-operatively[266]. Similarly, Kurihara et al have shown that VV ECMO can be used without anticoagulation without adverse outcomes in a retrospective matched cohort[267]. Therefore, a more 'permissive' approach to transfusion triggers could be explored in patients featuring coagulopathy by laboratory parameters only.

3.5.5 Limitations

This study is a single-centre retrospective analysis and is therefore prone to bias and physician selection, which may not have been identifiable. Furthermore, this study did not include VA ECMO, which have been shown to use larger amounts of RBC transfusion[207,219,231]. The indications and appropriateness for transfusion were not assessed individually in this cohort and this is recognised as a serious limitation of this study. Also, the use of transfusion in clinically relevant non-major bleeding (CRNMB) has not been described with a focus on major haemorrhage in the cohort due its impact upon survival. CRNMB has not been defined by ELSO in the setting of ECMO and other definitions by ISTH may be difficult to adopt in this setting[169,268]. Analysis of the APACHE II score as a potential confounder may be inaccurate as they were calculated using data collected from this centre once patients have been retrieved from another hospital. Therefore, most patients had already been placed on ECMO before the initial APACHE II score was calculated impacting upon recorded parameters.

With regards to RBC transfusions, there is also substantial physiologic rationale to support a higher transfusion threshold in patients with SRF, who in the majority have approached or exceeded a critical threshold of DO_2. Hb concentrations do not inform a clinician of a patient's DO_2, and a once- or twice-daily measurement has limited utility. Another limitation was the inability to differentiate if survival outcomes with respect to Hb concentrations were confounded by MH in individual cases, although these rates were not statistically different between the two time periods. With regards to non-RBC transfusions, transfusion thresholds did not change over the review period although clinician experience may have changed transfusion practice over this period.

3.6 Conclusions

This retrospective single centre cohort corresponds with other similar studies suggesting that a more 'restrictive' approach to blood component transfusion could be considered during ECMO due to either similar survival rates or potential increases in mortality with 'liberal' use. This was principally seen with RBC and platelet transfusions, which had the highest transfusion rates over the review period. Prospective multicentre studies for transfusion triggers to identify when to use blood components should be used 1) during major haemorrhage, 2) as prophylaxis to prevent bleeding and additionally 3) anticoagulation targets with the aim to reduce the bleeding events, are needed. With the second point, it may also be considered that changes in transfusion triggers are required throughout the circuit run. Further consideration for other methods of measuring haemostasis and anticoagulant activity, such as TEG, vWF and antithrombin (AT) assays, during ECMO should be considered to optimise the balance between thrombosis, haemorrhage, and risk of mortality.

Chapter 4 – Methods for Assessing Haemostatic Changes in *Ex Vivo* Circuits and Patients During Extracorporeal Membrane Oxygenation

4. 1 Study Outline

Blood samples from patients in a small prospective observational study during ECMO were compared to samples from *ex vivo* circuits run over 24-hours (design and set-up described in Chapter 5). Similar markers of thrombin generation, fibrinolysis and circulating histones were reviewed in both groups.

4.2 Study Design for Assessing Haemostatic Changes in Patients

Consecutive patients were enrolled prospectively at a single centre observational study at St Thomas' Hospital, London between March 2020 and February 2021 (Coagulopathy of Patients Receiving Extracorporeal Membrane Oxygenation – CYCLE Study). Blood sampling was taken prior to, during and at cessation of ECMO use.

4.3 Ethical Approval and Consent

Ethical approval for collection of patient blood samples and clinical information was reviewed and approved by the London NHS REC (Reference Number – 19/LO/1016) and King's College London as part of the CYCLE Study. Consent was gained from the patient or a nominated professional and personal consultee prior to starting ECMO. If capacity was regained, retrospective consent from the patient was sought.

4.4 Inclusion and Exclusion Criteria for Patients

The inclusion criteria for patient recruitment were:

- Patients receiving ECMO
- Aged >18 years

The exclusion criteria for recruitment were:

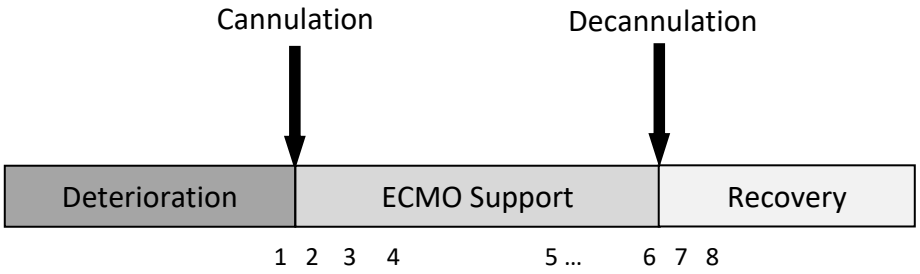
- Patients with a known inherited bleeding disorder
- Plasma exchange within previous 24 hours

4.5 Blood Sampling from Patients

Blood samples were taken at the following time points for each patient (Figure 21):

- Prior to starting ECMO (1), 1 hour after starting ECMO (2), 1 day after starting ECMO (3), 2 days after starting ECMO (4) and once weekly after starting ECMO (5)
- Prior to stopping ECMO (6), 1 hour after stopping ECMO (7) and 1 day after ECMO (8)
- During an episode of major haemorrhage (MH - defined as >20ml/kg RBC transfusion used within a 24-hour period)[169]

Figure 21**:** Timepoints for blood samples from patients receiving ECMO



Samples were collected by arterial lines used as part of routine clinical care into 3.2% sodium citrate (11 ml collected) and EDTA (3ml) vacutainers. They were immediately spun at 3000 rpm at room temperature for 15 minutes with aliquots of platelet-poor plasma removed. This platelet-poor plasma was then spun again at 3000 rpm for a further 15 minutes and aliquots of this platelet free plasma (PFP) were used for subsequent analysis. Samples were stored at -80°C until further analysis described below. Details of samples collected on listed in Appendix.

4.6 Data Collection for Patients

Patient information was collected prospectively from electronic care records. Information collected included demographics and clinical details of the patients (age, gender, cause of SRF and critical illness severity score). Routine laboratory parameters were collected from FBC (Hb, platelet and white cell count (WCC) [Unicell DxH 800, Beckman Coulter, High Wycombe, UK]), coagulation times (APTTr and PTr) and anti-Xa levels (CS-2100i, Sysmex, Milton Keynes, UK). Information on clinical care and ECMO support were collected daily (ECBF rates, anticoagulation type and dosing and blood products transfused). Imaging was reviewed throughout the period of ECMO use and the day following decannulation for each patient (CT imaging of the whole body at initiation of ECMO, US Doppler scans following decannulation and any other additional images). Clinical features and complications are described in more detail Appendix.

4.7 Measurement of Haemostatic Factors using Enzyme-Linked Immunosorbent Assays

These assays were performed on samples from patients and the *ex vivo* circuit with results described in Chapters 6, 7 and 8. The assays performed were for D-dimer, TAT (Thrombin-Antithrombin complex), PAP (Plasmin-Antiplasmin Complex), PF1+2 (Prothrombin Fragments 1+2), PAI-1, TAFI, tPA, uPA, Histone H3.1 and Histone H3R8.

4.7.1 Principles of Enzyme-Linked Immunosorbent Assays

In the first stage of the enzyme-linked immunosorbent assays (ELISA), sample plasma is added to the wells of a pre-coated plate containing antibodies binding to the protein/complex of interest (a monoclonal antibody for an epitope of the protein). Following an initial incubation during which any of the protein/complex in the sample binds to the antibody coating the well, a wash step removes any unbound material. In the second stage, a second antibody to the specific protein/complex is then added, which binds to any protein/complex immobilised in the first step, with a subsequent wash step. The second antibody is linked to a fluorescent enzyme. In the third stage, a substrate is added which undergoes a reaction with the fluorescent enzyme. This reaction is subsequently stopped chemically, and the resulting colour intensity measured spectrophotochemically. The measured colour intensity is proportional to the amount of protein/complex in the sample.

4.7.2 Details of D-Dimer ELISA

Materials and Reagents: High-sensitivity D-dimer kit (Hyphen Biomed, Neuville-sur-Oise, France) consisting of

- Antibody coated microwells

- D-Dimer Standards
- Low and High controls
- Stop Solution (containing sulphuric acid)
- Wash Solution
- Specimen Diluent
- TMB Substrate with hydrogen peroxide
- Conjugate

The kit was brought to room temperature prior to use. Citrated plasma samples, standards and controls were diluted with diluent solution to 1:50. 200 µL of samples, standards, and control were added to the wells and left to incubate for 1 hour at room temperature. Following incubation, plates were washed 5 times using 300 µL wash solution (20-fold diluted with distilled water), after which 200µL conjugate was added to each well. Following a further 1-hour incubation at room temperature, plates were washed again 5 times using 300 µL wash solution. 200 µL substrate was added to each well, followed by a 5-minute incubation at room temperature. 50 µL Stop solution was then added to each well, and plates read at 450nm.

4.7.3 Details of Thrombin-Antithrombin ELISA

Materials and Reagents: TAT kit (Sysmex, Milton Keynes, UK) consisting of

- Antibody coated microwells
- TAT Standards
- Control plasma
- Stop Solution (containing sulphuric acid)
- Wash Solution
- Working Chromogen solution
- Substrate with hydrogen peroxide

50 µL of TAT Buffer solution were added to each well followed by 50 µL of undiluted citrated plasma, standards and controls, and left to incubate for 15 minutes at 37°C. 300 µL Wash Solution was added to the wells for 3 washes after which 100 µL conjugate was added to each well. Following a further 15 minutes incubation at 37°C, plates were washed again 3 times as described above. 100 µL Working Chromogen solution was added to each well, followed by a 30-minute incubation at room temperature avoiding light. 100 µL Stop solution was then added to each well, and plates were read at 492nm.

4.7.4 Details of Plasmin-Antiplasmin ELISA

Materials and Reagents: PAP Complex kit (Immuno Diagnostic Systems Ltd (IDS), Tyne & Wear, UK) consisting of

- Antibody coated microwells
- PAP Standards
- Control plasma
- Stop Solution containing sulphuric acid
- Wash Solution
- Buffer Solution
- Conjugate Diluent
- TMB Substrate
- Conjugate

The kit was brought to room temperature prior to use. 50 µL Buffer Solution were added to each well followed by 50 µL of undiluted citrated plasma, standards and controls for 15 minutes at room temperature, after which plates were washed 3 times using 300 µL Wash Solution. 100 µL conjugate was added to each well. Following a further 15-minute incubation at room temperature, plates were washed again 3 times as described above. 100 µL substrate

was added to each well, followed by a 15-minute incubation. 100 µL Stop solution was then added to each well, and plates read at 450 nm.

4.7.5 Details of Prothrombin Fragments 1+2 ELISA

Materials and Reagents: PF1+2 kit (Sysmex, Milton Keynes, UK) consisting of

- Antibody coated microwells
- PF1+2 Standards
- Control plasma
- Stop Solution containing sulphuric acid
- Wash Solution
- Sample Buffer
- Conjugate Buffer
- TMB Substrate
- TMB Conjugate

1900 mL distilled water added to the 100 mL Wash Solution Concentrate to make a 2-litre solution. Standards and controls were reconstituted with 1ml of distilled water and incubated for 15 minutes at 22°C. 50 µL of Sample Buffer were added to each well followed by 50 µL of citrated plasma, standard and controls whilst being agitated and left to incubate for 30-minutes at 37°C. Following incubation, plates were washed 2 times using 300 µL wash solution, after which 100 µL conjugate was added to each well. Following a further 15-minute incubation at 37°C, plates were washed again 3 times as described above. 100 µL Chromogen Buffer/Substrate solution was added to each well, followed by a 15-minute incubation at room temperature avoid light. 100 µL Stop solution was then added to each well, and plates read at 450 nm.

4.7.6 Details of Plasminogen Activator Inhibitor-1 ELISA

Materials and Reagents: PAI-1 Kit (Diagnostica Stago UK Ltd, Theale, UK) consisting of

- Antibody coated microwells
- PAI-1 Standards
- Stop Solution containing sulphuric acid
- PET Buffer
- OPD Substrate Concentrate
- Hydrogen peroxide
- Conjugate

100 µL PET buffer was added to each well with 1-minute of gentle agitation. 20 µL of citrated plasma, standards and control (of a known value of the standard) were added undiluted to the plate. Following this, 50µL conjugate was added to each well for 2 hours with agitation. Wells were then washed used PET buffer 4 times. 300 µL substrate concentrate, 3mL water and 300 µL of hydrogen peroxide were mixed and agitated for 15 minutes. 200 µL substrate was added to each well, followed by a 15-minute incubation at room temperature on a plate shaker. 50 µL Stop solution was then added to each well, and plates read at 492 nm.

4.7.7 Details of Thrombin-Activatable Fibrinolytic Inhibitor Antigen ELISA

Materials and Reagents: TAFI kit (Invitech, Huntingdon, UK) consisting of

- Antibody coated microwells
- TAFI Calibrator
- Low and High controls
- Stop Solution with sulphuric acid
- PET Wash Solution

- Sample Diluent
- Conjugate Diluent
- TMB Substrate with hydrogen peroxide
- Conjugate

TAFI Calibrator and controls were each reconstituted with 500 µL distilled water and the conjugate was reconstituted with conjugate diluent. Samples and controls were diluted using sample diluent, to make a 1/50 dilution. 200 µL of diluted citrate plasma, standards and controls were added to the plate and left to incubate for 2 hours at 37°C. Following incubation, plates were washed 5 times using 300µL wash solution, after which 200 µL conjugate was added to each well. Following a further 1 hour at 37°C incubation, plates were washed again 5 times as described above. 200 µL substrate was added to each well, followed by a 5-minute incubation at room temperature. 50 µL Stop solution was then added to each well, and plates read at 450nm.

4.7.8 Details of Tissue Plasminogen Activator ELISA

Materials and Reagents: tPA antigen kits (R&D Systems, Minneapolis, USA) consisting of

- Antibody coated microwells
- tPA Standards
- Stop Solution containing sulphuric acid
- Wash Buffer Concentrate
- Assay Diluent
- Calibrator Diluent
- Conjugate
- Colour Reagents A and B

Plasma samples were diluted with Calibrator diluent to a ratio of 1:5. 480 mL distilled water were added to 20mL Wash Buffer Concentrate to prepare a volume of 500 mL Wash Buffer solution. Colour Reagents were mixed in equal amounts to make the substrate solution. 80 mL distilled water were added to 20mL Calibrator diluent to prepare a volume of 100 mL Calibrator diluent solution. 50 µL Assay diluent were added to each well, after 50µL citrated plasma, standards and control (of a known value of the standard) were added to the wells and left to incubate for 2 hours at room temperature agitated. Following incubation, plates were washed 4 times using 400 µL Wash Buffer solution, after which 200 µL conjugate was added to each well. Following a further 2 hours incubation at room temperature agitated, plates were washed again 4 times as described above. 200 µL substrate was added to each well, followed by a 30-minute incubation at room temperature avoiding light. 50 µL Stop solution was then added to each well, and plates read at 450nm.

4.7.9 Details of Urokinase Plasminogen Activator ELISA

Materials and Reagents: uPA antigen kit (Bio-Techne, R&D Systems, Minneapolis, USA) consisting of

- Antibody coated microwells
- uPA Standards
- Stop Solution containing sulphuric acid
- Wash Buffer Concentrate
- Assay Diluent
- Calibrator Diluent
- Conjugate
- Colour Reagents A and B

Plasma samples were diluted with Calibrator diluent to a ratio of 1:4. 480mL distilled water were added to 20 mL Wash Buffer Concentrate to prepare a volume of 500 mL Wash Buffer solution. Colour Reagents were mixed in equal amounts to make the substrate solution. A 60mL distilled water were added to 20 mL Calibrator diluent to prepare a volume of 80 mL Calibrator diluent solution. 100 µL Assay diluent were added to each well, after 50 µL citrated plasma, standards and control (of a known value of the standard) were added to the wells and left to incubate for 2 hours at room temperature agitated. Following incubation, plates were washed 4 times using 400 µL Wash Buffer solution, after which 200 µL conjugate was added to each well. Following a further 2 hours incubation at room temperature agitate, plates were washed again 4 times as described above. 200 µL substrate was added to each well, followed by a 30-minute incubation at room temperature avoiding light. 50µL Stop solution was then added to each well, and plates read at 450nm.

4.7.10 Details of Histone H3.1 ELISA

Materials and Reagents: H3.1 kit (Volition, Isnes, Belgium) consisting of

- Antibody coated microwells
- H3.1 Standards
- Low and High controls
- Stop Solution containing hydrochloric acid
- Wash Buffer
- Assay Buffer
- TMB Substrate
- Conjugate

1mL distilled water was added to reconstitute each standard and control bottle. 50 mL distilled water added to the 450 mL Wash Buffer Concentrate to achieve a 10-fold dilution.

The kit was brought to room temperature prior to use. Wells were washed with 200 µL Wash Buffer 3 times. 80 µL Assay Buffer were added to each sample. 20 µL of EDTA plasma, standards and controls were added to the wells and left to incubate for 150 minutes at room temperature agitated. Following incubation, plates were washed 3 times using 200 µL diluted wash solution, after which 100 µL conjugate was added to each well. Following a further 90 minutes incubation at room temperature agitated, plates were washed again 3 times as described above. 100 µL substrate was added to each well, followed by a 20-minute incubation avoiding light. 100 µL Stop solution was then added to each well, and plates read at 450nm.

4.7.11 Details of Citrullinated Histone H3R8 ELISA

Materials and Reagents: H3R8 kit (Volition, Isnes, Belgium) consisting of

- Antibody coated microwells
- H3.1 Standards
- Low and High controls
- Stop Solution containing hydrochloric acid
- Wash Buffer
- Assay Buffer
- TMB Substrate
- Conjugate

50mL distilled water was added to the 450 mL Wash Buffer Concentrate to achieve a 10-fold dilution. The kit was brought to room temperature prior to use. 80 µL Assay Buffer were added to each sample. 20µL of EDTA plasma, standards and controls were added to the wells and left to incubate for 2.5 hours at room temperature agitated. Following incubation, plates were washed 3 times using 200 µL diluted wash solution, after which 100 µL conjugate was

added to each well. Following a further 1.5 hours incubation at room temperature agitated, plates were washed again 3 times as described above. 100 µL substrate was added to each well, followed by a 20-minute incubation avoiding light. 100 µL Stop solution was then added to each well, and plates read at 450nm.

4.7.12 Result Interpretation of ELISA Assays

The mean of each duplicate absorbance value was taken. A standard curve was generated by plotting the absorbance value (y-axis) versus its corresponding protein/complex concentration (x-axis).

The obtained intra- and inter-assay co-efficient of variation (CV) for these assays were – D-dimers: 3.0% and 4.0%, PF 1+2: 6.0% and 9.0%, TAT: 5.0% and 8.0%, tPA 5.5% and 5.3%, uPA2% and 6.8%, PAP 4.2% and 7.3%, PAI-1 3.3% and 2.9%, TAFI 5.9% and 8.4%, H3.1: 7.6% and 11.8% and H3R8: 4.5% and 8.2%.

4.7.13 Calculation of Results

A curve fit was applied to the dilution and standard and the amount of each protein/complex in the plasma samples was determined. The results were multiplied by any dilution factors used in the assay to obtain the concentration of protein/complex in the neat sample.

4.7.14 Details of Clauss Fibrinogen Assay

Class fibrinogen levels were based on a clotting-based assay. The principle of the assay is that diluted platelet-poor plasma is activated by high concentration of thrombin to allow fibrin-rich clot formation. Optical densimetry is used to measure the end point of clot formation once it has exceeded a threshold. A reference sample is used at varying dilutions to form a calibration curve, to which the concentration of test plasma levels is deduced.

They were performed using the ACL TOP 350 analyser (Werfen, Warrington, UK) using HemosIL reagents (Werfen) according to manufacturer's protocols. Assays were performed in duplicate. The intra-assay CV was 3.5% and inter-assay CV was 4.2%.

4.8 Statistical Analysis

Statistical analysis was performed using SPSS version 27 (IBM, USA). Descriptive analysis of continuous variables was given as medians and interquartile ranges. Categorical variables were given as numbers of individuals/events and percentages. Null hypothesis significance testing was employed to examine variable distributions between the two time periods. Mann-Whitney U tests for unrelated and Wilcoxon U tests for related samples were performed to assess continuous variables. A significance level of alpha equals to 0.05 was used for all statistical tests.

Chapter 5 - *Ex vivo* Circuits to Assess Haemostatic Changes during ECMO

5.1 Introduction

Changes in haemostasis can be considered as attributable to either the circuit or the patient during ECMO with an interplay between the two. To understand this further, ensuring homogeneity in both these components is needed. Clinical studies can be impacted by a variety of diseases causing cardiorespiratory failure and their associated haemostatic changes, similar to those described in Chapter 2. Consequently, a specific disease cohort or large sample size are needed to overcome these confounders. Animal models can also be used to assess disease processes and circuit function although these need large animal models to allow the use of the circuits, identification of species with similar haemostatic systems to humans to replicate changes during ECMO, and the institutional set-up and cost considerations to perform these studies. Therefore, *ex vivo* models can be considered as a plausible and attractive alternative to assess the circuit-attributable changes of ECMO.

Malfertheiner *et al* have described various *ex vivo* circuit models for studying changes during ECMO. These studies have focussed on the assessment of oxygenator performance, pump performance, haemostasis and drug pharmacokinetics[269]. Haemostatic and anticoagulation management is not standardised between centres using ECMO in clinical practice. Therefore, *ex vivo* models assessing these areas can be heterogenous. The cornerstones of these circuit designs consisted of a pump, membrane oxygenator, tubing and a flexible reservoir for blood sampling. Some models included additional pump drivers for additives and drug administration[270,271].

Various components must therefore be considered during the design and use of these circuits to achieve the study outcome, whilst also being functional: anticoagulation, the size and type of pump, the size and volume of the circuit, the type of priming fluid and subsequent haemodilution, source and volume of blood, and blood flow rates. Table 19 summarises published *ex vivo* circuit models focusing on haemostatic and platelet-related changes. There is variety in the use of heparin for these models although a dose of 1 IU/mL was frequently used, although circuits using citrate alone have been described[269,271]. Anticoagulation strategies frequently depends on whether the development of thrombus formation was an outcome of the study. There was a median duration of 6-hours for the run time and circuit occlusion was described in a small number of these studies[272]. Paediatric circuits have lower circulating volumes so haemodilution becomes less significant in comparison to adult circuits.

Table 19 Studies describes haemostatic changes in ex vivo ECMO circuits and components of their design

Study	*Measure(s)*	*Anticoagulation*	*Circuit & Pump*	*Blood source*	*Priming fluid and haemodilution*	*Outcome(s)*
Ki et al. Int Care Med Exp. 2019[273] N = 10	Free Hb vWF Multimers Platelet aggregation ROTEM Coagulation assays Flow cytometry	UFH 400IU and calcium chloride (3.33mg/ml) at time of blood addition to circuit	PLS (Maquet, Germany) Rotaflow centrigual pump BIOLINE coating	Donated human whole blood from top-bottom bags with CPD	0.9% sodium chloride No pre-haemodilution level for comparison Post haemodilution Hb 98 – 102 g/L	Assessment of high flow (4L/min) (n = 5) to low flow (1.5L/min) rates over 6-hours (n = 5)
Teligui. Perfusion. 2016.[274] N = 10	CD40L C3a B-thromboglobulin TF, FVIIa & FXII PF1+2	UFH 400IU (1IU/ml) at time with ACT maintained ≥ 500s	Roller pump (COBE Perfusion system, USA) Paediatric oxygenator (Affinity)	Donated human whole blood 450-480ml with 66.5ml CPD	175ml 1.4% sodium bicarbonate and 325ml 0.9% sodium bicarbonate Haemodilution 39±3%	Changes over 2-hours
Meyer. J Thromb	TEG Flow cytometry Thrombogram	UFH 1IU/ml at time of blood collection	COBE CV Roller Pump (Sorin)	Donated human whole blood	No priming fluid No discussion of haemodilution	Assessment of low (0.3ml/min), medium

Haem. 2020.[272]* N = 15	Electron microscopy of oxygenator		KIDS D100 Oxygenator (Sorin)	400±50ml with UFH in collection bag		(0.5ml/min) and high (0.7ml/min) flow rates Assessment of thrombus in oxygenator after 6-hours
Meyer. Pediatr Crit Care Med. 2012.[275]* N = 20	Platelet count Free Hb Flow cytometry	UFH 4IU/ml	Jostra Rotaflow vs Jostra rotator (Maquet) pump Quadrox D (Maquet) vs silicone membrane (Medtronic, USA) oxygenator	Porcine whole blood No discussion of volume or additives	No discussion of priming fluid or haemodilution	Assessment of 4 combinations of centrifugal and rotator pumps, and polymethylpentene and silicone oygenators after 6-hours
Van Pouche. Art Organs. 2016.[276] N = 3	Platelet count Platelet aggregation ROTEM	Not discussed	Not discussed	Donated human whole blood	Not discussed	Changes with hypothermia and rewarming after 6-hours

Bleilevens. ASAIO J. 2014.[271]* N = 8	Platelet counts Free Hb Coagulation times TEG Flow cytometry VWF and IL-β	Citrate sodium hydrogen phosphate (5.66mg/mL)	ID-Pediatric Oxygenator (Maquet) and centrifugal pump (MEDOS, Germany)	Porcine whole blood	No priming fluid Isovolaemic fluid replacement after blood sampling	Comparison of unmodified *ex vivo* model vs modified with CO_2-enhanced gas and additive nutrient solution over 12-hours
Winnersbach. Perfusion. 2021.[270]* N = 5	Platelet count Free Hb Flow cytometry ROTEM MPO-DNA Electron microscopy	No additional UFH with continuous protamine infusion for first 2 hours	Delta Stream DP-II pump & uncoated hilite 800 LT oxygenation (Xenos AG, Germany)	Donated human whole blood 75ml per circuit	3.75IU/mL UFH at sample collection with 75ml crystalloid Post haemodilution Hb 70-80 g/L	Comparison between platelet-rich and platelet poor circuits over 6-hours

5.2 Aims

- To assess the feasibility of an *ex vivo* circuit of ECMO without unfractionated heparin
- To assess haemostatic parameters and blood gas parameters in an *ex vivo* circuit

5.3 Methods

5.3.1 Study Design

An *ex vivo* circuit was designed and performed in six circuits at the same flow rates using donated human whole blood. Serial blood samples were taken to assess for changes in haemostatic parameters. Blood samples were compared to controls that were constantly rotated but not used in the circuit but from the same donor.

5.3.2 Ethical Approval

The study was reviewed and approved by the local Research Departments at Guy's & St Thomas' NHS Foundation Trust and King's College London. Approval by an ethics committee was not required in accordance with NHS Health Research Agency (http://www.hra-decisiontools.org.uk/ethics/) with subsequent confirmation.

5.3.3 Blood Donation

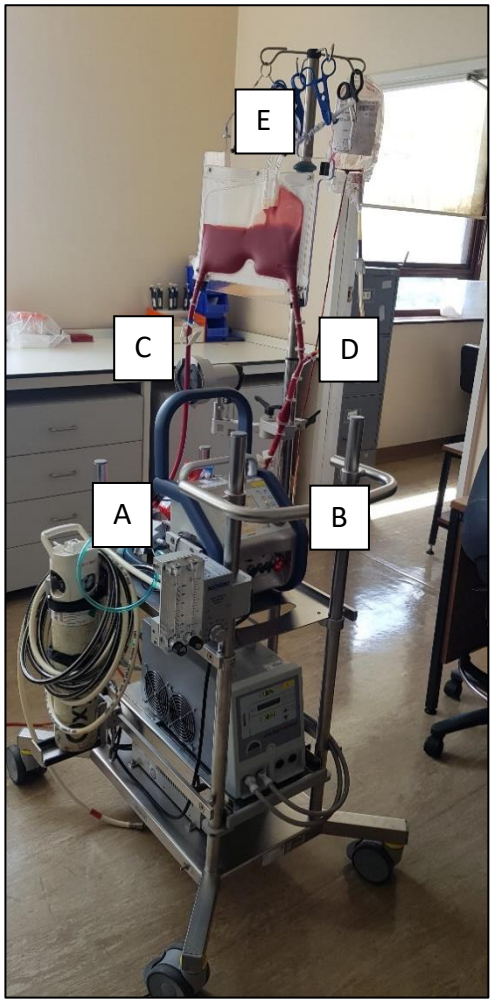
450ml whole blood were venesected from adult donors >18 years as part of disease treatment for hereditary haemochromatosis at Guy's & St Thomas NHS Foundation Trust. The blood is routinely discarded as part of clinical care. Donations were taken in a sealed venesection bag containing 50ml of 3.2% trisodium citrate. Donors were excluded if there was a medical history of an inherited coagulation disorder or the use of anticoagulant or antiplatelet drugs

in the preceding 5 days. The time from blood donation to circuit commencement was <1 hour in all circuit runs. A 15ml aliquot was taken at this time as a baseline control sample.

5.3.4 Circuit Design

An adult HLS 7L Cardiohelp system (Maquet, Germany) and centrifugal pump were attached to a flexible plastic reservoir bag using BIOLINE-coated tubing (Figure 22). The returning tubing was reduced to 90cm in length and the leaving tubing was reduced to 70cm in length to give a functioning circulating volume of 600ml without cavitation occurring in the reservoir bag. The circuit was primed with 1000ml Plasma Lyte 148 (Baxter Healthcare Ltd, Thetford, UK) and tested for 5 minutes to assess circuit function and allow for heating of the priming fluid. 800ml of priming fluid was subsequently removed as the whole blood was added to the circuit simultaneously. Through the study period, a flow rate of 4L/min and temperature of 37°C via an in-built heater within the pump were maintained. Each circuit was run for 24-hours. A total of six circuits were used with the same design and were gifted from Maquet.

Figure 22 Ex vivo circuit model set-up A) Membrane Oxygenator B) Centrifugal Pump C) Returning Tubing D) Leaving Tubing E) Reservoir Bag



5.3.5 Control Samples

The circuit was run at a low flow rate (0.5ml/min) for 1 minute after the addition of blood. Six aliquots of 15ml were taken from the circuit into 20ml plastic containers without additional additives. One was centrifuged immediately to function as a post-haemodilution control sample. The remaining five aliquots were constantly rotated using an SRT6 analogue tube roller (Stuart, Stone, UK) for the duration of the *ex vivo* circuit run to function as controls maintained at 37°C.

5.3.6 Blood Sampling

15 mL blood samples were taken from an outlet in the oxygenator after 1-hour, 4-hours, 8-hours, 12-hours, and 24-hours. A 15 mL aliquoted control sample was taken from the tube rotator at the same time point. For both the circuit sample and control sample, 2.5 mL were taken for FBC in an EDTA vacuntainer and 1.5mL blood gas analysis in a syringe. The remaining 11 mL samples were centrifuged at 17°C at 3000 rpm for 15 minutes on two occasions to obtain platelet-poor plasma. After the first centrifugal spin, 5mm of plasma above the buffy coat were discarded.

5.3.7 Outcome Variables

Serial time samples were compared for changes from the initial post-haemodilution sample in 1) the circuit samples and 2) the control samples. Circuit samples were compared to time-matched control samples from the same donor sample.

5.3.8 Laboratory Testing and Assays

Samples taken for FBC testing were processed within 30 minutes for testing. This was performed on pocH-100i Automated Hematology Analyzer with pocH-pack 65 regents (Sysmex, Kobe, Japan) in accordance with manufacturers' guidance. Blood gas parameters were analysed using the Cobas b 221 system (Roche, Rotkreuz, Switzerland) in accordance with manufacturers' guidance. Coagulation times (APTT and PT) were performed using ACL Top 300 (Werfen, Warrington, UK). Other haemostatic assays performed are described in Chapter 4.

5.3.9 Statistical Considerations

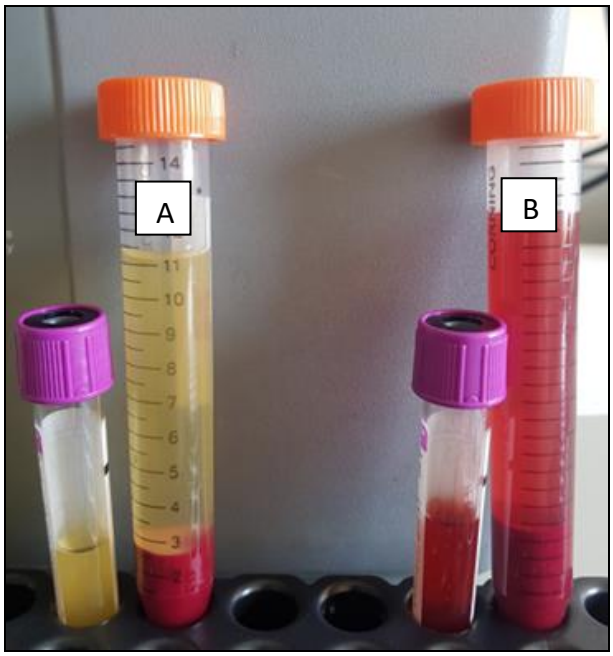
Statistical analysis was performed using SPSS version 27 (IBM, USA). Descriptive analysis of continuous variables was given as medians and interquartile ranges. Categorical variables

were given as numbers of individuals/events and percentages. Null hypothesis significance testing was employed to examine variable distributions between the two time periods. Wilcoxon Rank Sum tests were performed to assess continuous variables due to small sample size and related samples. A significance level of alpha equals to 0.05 was used for all statistical tests.

5.4 Results

No circuit occlusion or thrombus deposition was seen macroscopically after 24-hours in the six circuits. Gross haemolysis was seen at the end of the 24-hour period in the circuit samples in comparison to the control (Figure 23).

Figure 23 *Macroscopic appearance after 24-hour in (A) Control sample and (B) Circuit sample*



Full Blood Count

There was significant haemodilution seen after the circuit was perfused with Plasma Lyte showing a median haematocrit decrease from 0.40 L/L to 0.19 L/L (-53%) (Figure 24(a)). There was no subsequent change following this in either the circuit or control samples after 24-

hours. There was a similar degree of dilution in median white cell counts (WCC) of $4.3x10^9$/L to $2.2x10^9$/L (-49%) however this continued to decline in the circuit samples by from $2.2x10^9$/L to 1.4 $x10^9$/L (-36%) after 1-hour but remained stable at 2.1 $x10^9$/L in the control samples (Figure 24(b)). There were no subsequent changes in WCC and neutrophils over 24-hours in both circuit and control samples (Figure 24(b & d)). Platelet counts showed a dilution with a median decline from $226x10^9$/L to $104x10^9$/L (-54%), however there were no further changes over the subsequent 24-hours (Figure 24(c)). There was an increase in mean corpuscular volume (MCV) in both control (median 98 fL) and circuit samples (median 101 fL) over 24-hours however this increase was greater in circuit sample (94 fL at initiation). There was no significant change in red cell distribution width (Figure 24(e & f)).

Coagulation Times

Routine coagulation times (activated partial thromboplastin time (APTT) and prothrombin time (PT)) were attempted, however as expected after haemodilution, both parameters were longer than the detectable end point of the automated tests in all samples (> 300 seconds).

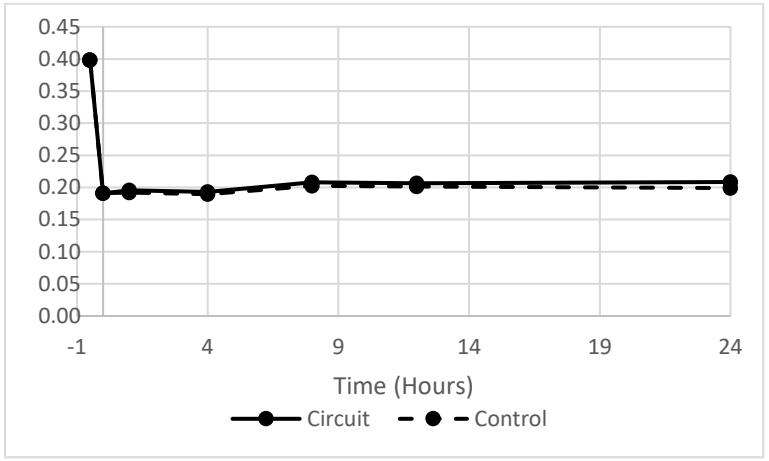
Blood Gas Parameters

pH in the control samples decreased showing a progressive acidosis over the 24-hour period from 7.33 to 7.12 with an increase pCO2 from 2.7 to 4.1 kPa (Figure 25(a & c)). In comparison, the median pH was similar in the circuit samples at 7.39 and the pCO2 decreased to 1.0 kPa. There was a decrease in bicarbonate levels in both circuit and control samples over 24-hours from 10.7 mEq/L to 4.4 and 4.5 mEq/L respectively (Figure 25(d)). Potassium levels showed a significant increase in the circuit samples after 24-hours from 3.9 to 8.5 mmol/L whereas these remained stable in the control samples at 3.9 mmol/L at 24-hours (Figure 25(e)).

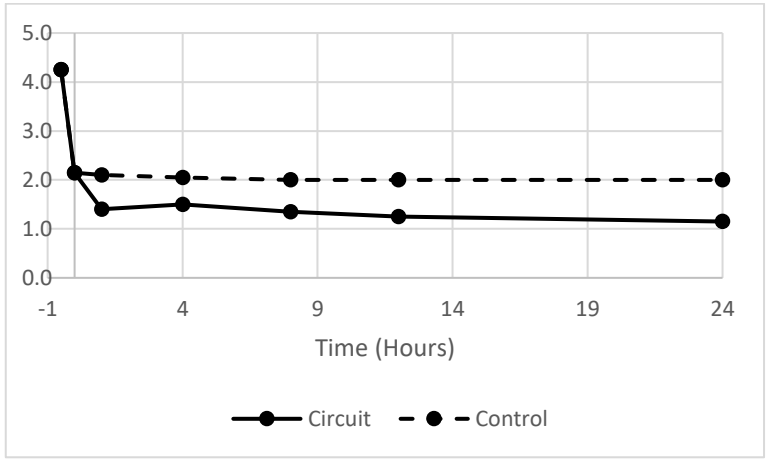
Glucose levels decreased over the study period from 2.7 mmol/L but decreased more rapidly in the circuit samples after 4-hours to 1.7 but after 12-hours in control samples to 1.8 (Figure 25(f)). Glucose levels decreased to 0.6 and 0.9 mmol/L in the circuit and control samples respectively after 24-hours. Lactate levels increased from 1.3 at initiation but increased earlier in the circuit samples at 4-hours to 3.8 mmol/L in comparison to the controls after 12-hours of 3.2 mmol/L (Figure 25(g)). Lactate levels increased to 6.5 and 4.9 mmol/L in the circuit and control samples respectively at 24-hours.

Figure 24 *Median changes in full blood count parameters in ex vivo circuit versus control samples over 24-hours (a) Haematocrit (L/L) (b) White Cell Count (x10^6/L) (c) Platelets (x10^9/L) (d) Neutrophils (x10^6/L) (e) Mean corpuscular volume (fL) (f) Red Cell Distribution Width (%)*

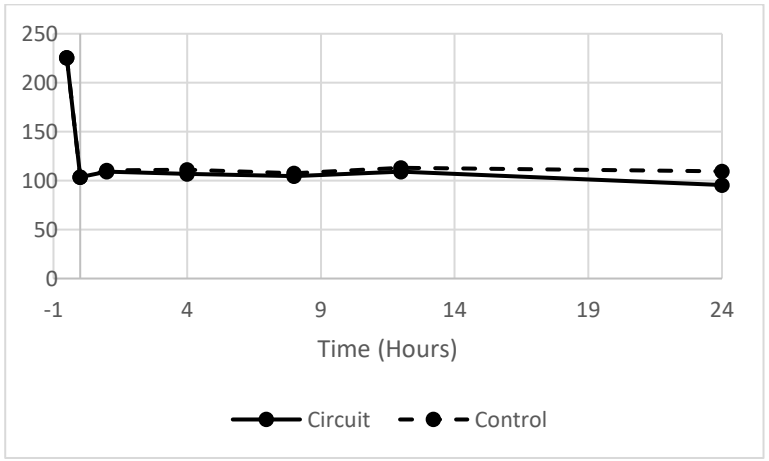
(a)



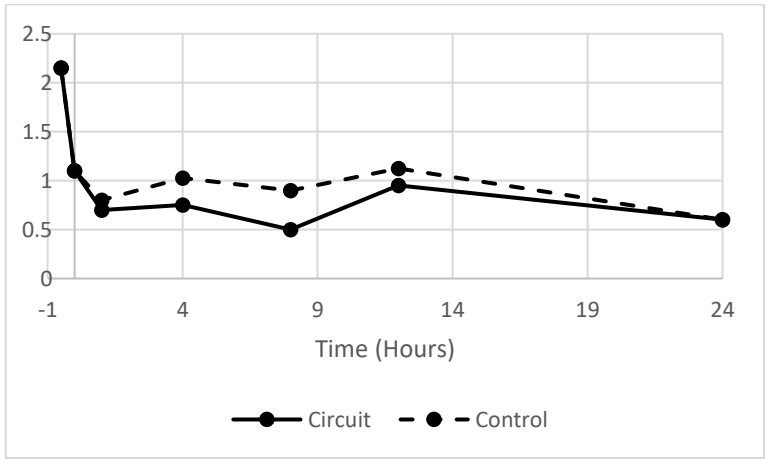
(b)



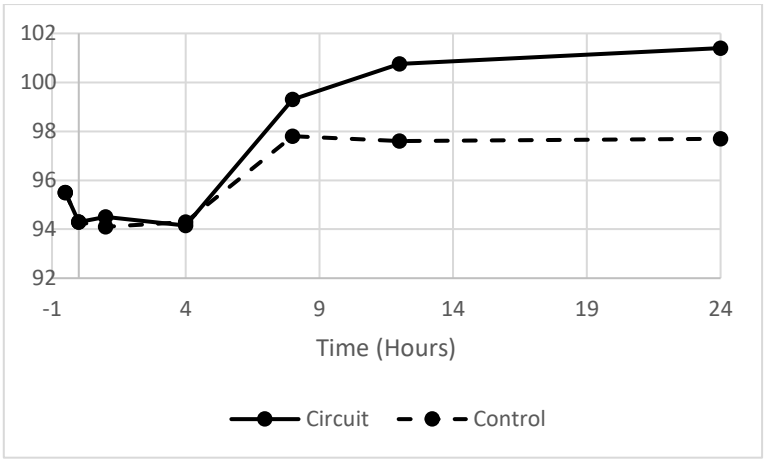
(c)



(d)



(e)


102
100
98
96
94
92
-1
4
9
14
19
24
Time (Hours)
Circuit
Control

(f)

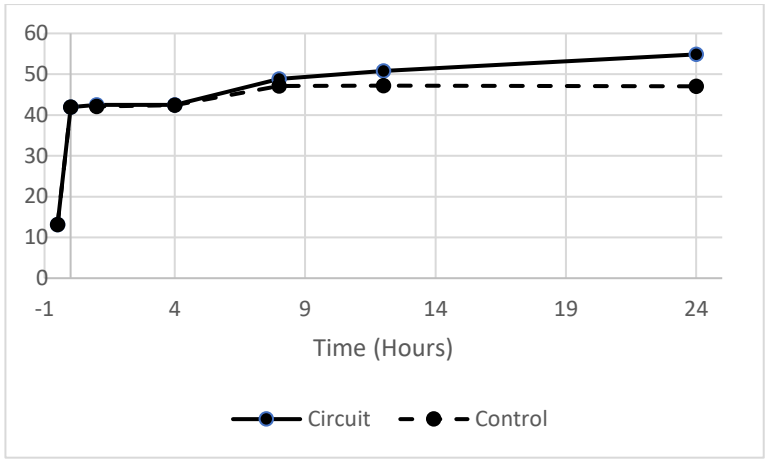
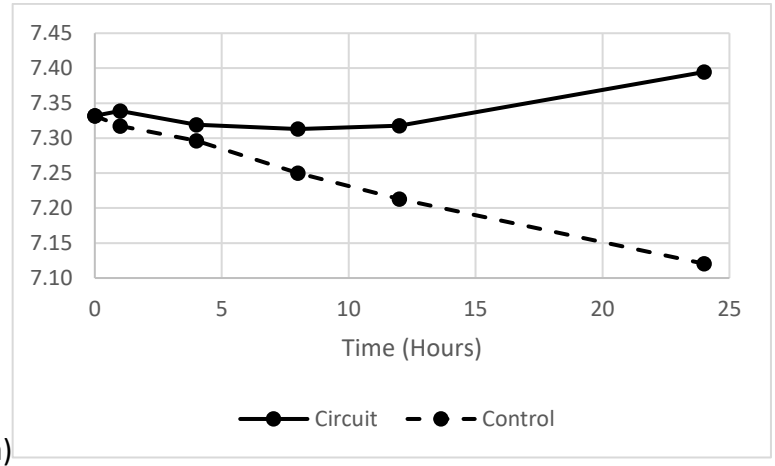
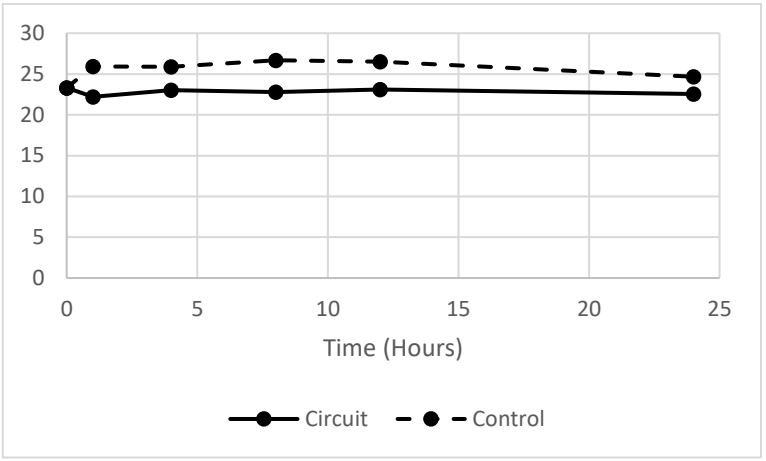

60
50
40
30
20
10
0
-1
4
9
14
19
24
Time (Hours)
Circuit
Control

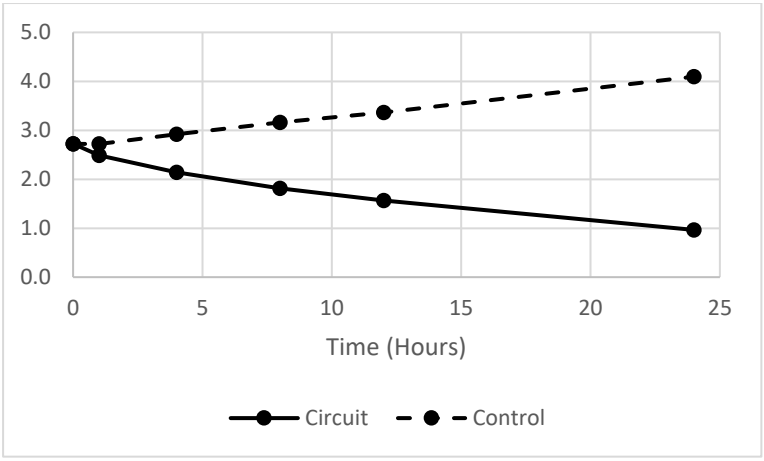
Figure 25 Changes in blood gas parameters in ex vivo circuit versus control samples over 24-hours (a) pH (b) pO2 (kPa) (c) pCO2 (kPa) (d) Bicarbonate (mEq/L) (e) Potassium (mmol/L) (f) Glucose (mmol/L) (g) Lactate (mmol/L)



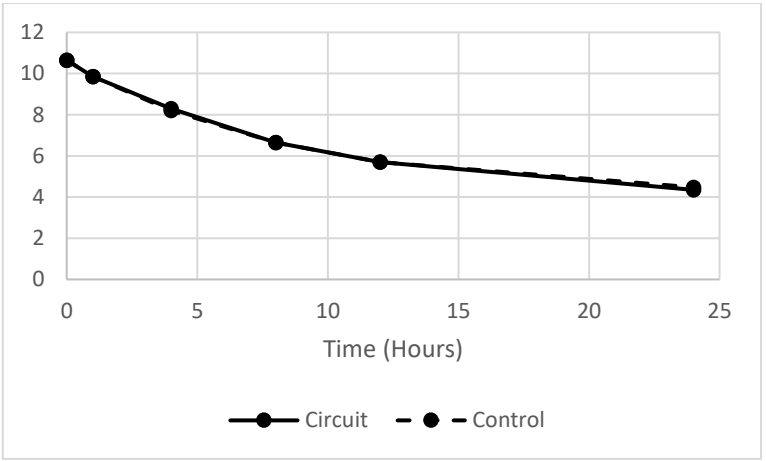
(a)



(b)

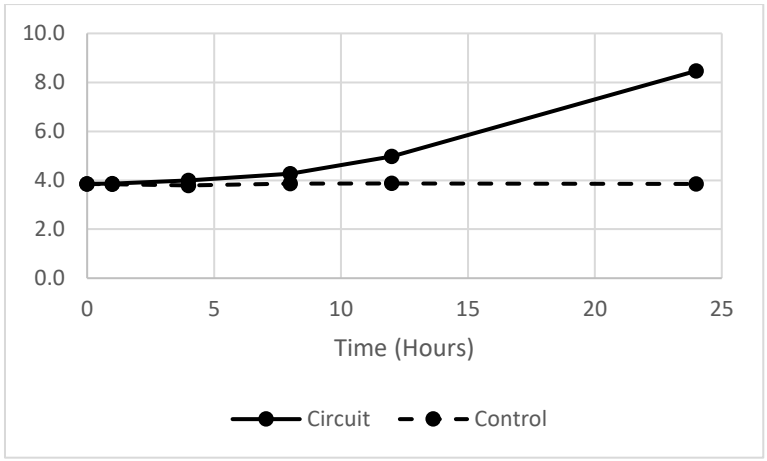


(c)



(d)

(e)


10.0
8.0
6.0
4.0
2.0
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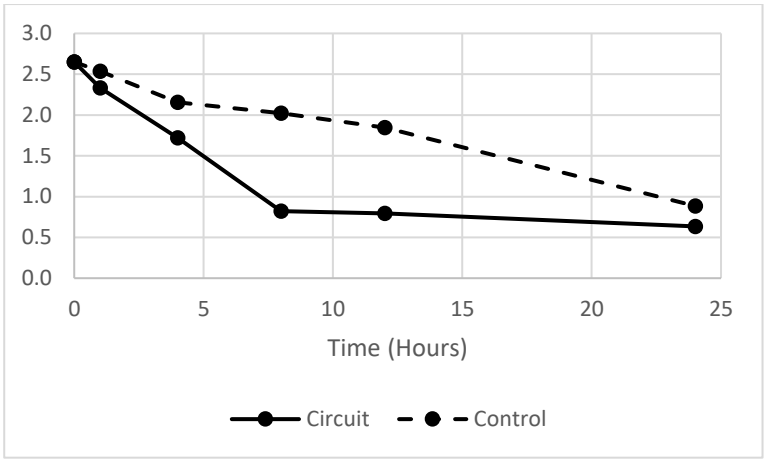
0
5
10
15
20
25
Time (Hours)
Circuit
Control

(f)


3.0
2.5
2.0
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1.0
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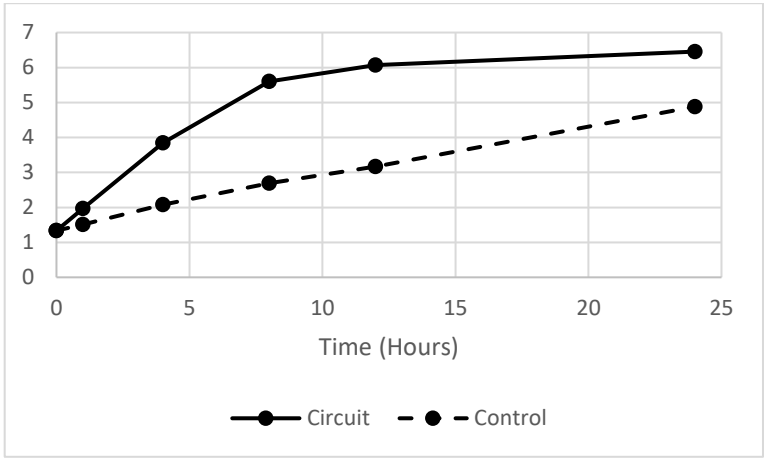
25
Time (Hours)
Circuit
Control

(g)


7
6
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3
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20
25
Time (Hours)
Circuit
Control

5.5 Discussion

This study was to investigate haematological changes in a non-heparinised *ex vivo* circuit. All circuits functioned over a 24-hour period without circuit occlusion occurring.

The salient changes in the full blood counts were a haemodilution of approximately 50%, a WCC decline during the first hour of the circuit run and an increase in the red cell MCV. This has reflected the concern of other *ex vivo* models of ECMO that also demonstrated significant haemodilution[270,273]. This was seen in a significant prolongation in both APTT and PT following haemodilution. Similarly, these issues are seen in CPB and dilutional coagulopathy of trauma[274,277,278].

In addition to haemodilution as affecting coagulation testing, leaching of surface-bound heparin may have added to this effect[274]. This has previously been recognised by Bleilevens *et al* with prolonged of APTT and TEG by INTEM and reversal with heparinase and subsequent investigations by their group using uncoated surfaces that did not demonstrate this effect[270,271].

The predominant changes in the blood gas parameters were a fall in pCO_2 in the circuit samples with a preserved pH. In comparison, control samples showed a rise in pCO2 and subsequent acidosis over 24-hours. Although this circuit design did not use supplemental oxygen or carbon dioxide, it is probable that carbon dioxide was removed due to blood flow through the membrane oxygenator[279]. Similar designs have shown alkalosis when additional carbon dioxide was not added[271]. In samples with normothermia, acidosis has been shown not to affect coagulation profiles in whole blood with relation to the acidosis seen in control samples when compared to the circuit samples.[280].

Although free Hb was not measured in this study, the significant levels of hyperkalaemia is attributable to intracellular release by haemolysis[271–273]. This is not reflected by Hb and derived haematocrit levels seen in this study, which remained stable. This may be due to the lack of clearance of circulating free Hb in the circuit by adequate level of haptoglobin and subsequent hepatic clearance that would be expected to occur physiologically, and therefore detected by optical methods of assessing Hb[281]. Additionally, the high flow rate of 4L/minute may cause additional RBC damage and haemolysis given the circulating volume was considerably lower in this model, more than that occurring in patients. This means that the blood in the circuit had increased exposure to the effects of the pump and oxygenator.

A more rapid decline in glucose and an associated increase in lactate levels suggest that RBC from the circuits may have an increased stress and therefore energy requirements than those in the control samples. Bleilevens *et al* have demonstrated that additional nutrient solution (phosphate-adenine-glucose-guanosine-saline-mannitol) to the circuit priming fluid may optimise RBC survival[271].

The limitation of this study was principally of the haemodilution described above that may affect the interpretation of testing and the rheological properties of blood changing axial flow and cellular adhesion to the circuit surfaces. The use of quantitive based assays such as ELISA may therefore be preferred to functional/clotting-based assays. The use of paediatric circuits may ameliorate the problem of haemodilution although membrane surface areas would be smaller. This circuit model also used sodium citrate for its anticoagulant effect, which has been described previously, although heparin is typically used for patient care during ECMO but is also increasing in haemodialysis[271]. Further modifications to explore *ex vivo* circuits models can be considered, such as the addition of calcium to whole blood in the primed

circuit, adding prothrombotic stimulants such as thromboplastin or the use of uncoated circuits, particularly if the purpose of the study is to activate the coagulation pathway to achieve pump or membrane occlusion[282]. There was also no additive oxygen or carbon dioxide to this circuit. Due to the short duration of the study using normal whole blood, it was felt appropriate to not incorporate supplemental oxygen. Additionally, providing carbon dioxide would require including further components potentially increasing the complexity the circuit.

5.6 Conclusions

Ex vivo circuits can be an option when investigating specific components of ECMO, in this case haemostatic changes. As highlighted by the circuit designed for this study, non-physiological changes can occur and limitations may affect extrapolation to clinical studies, particularly with haemodilution, when using adult sized components.

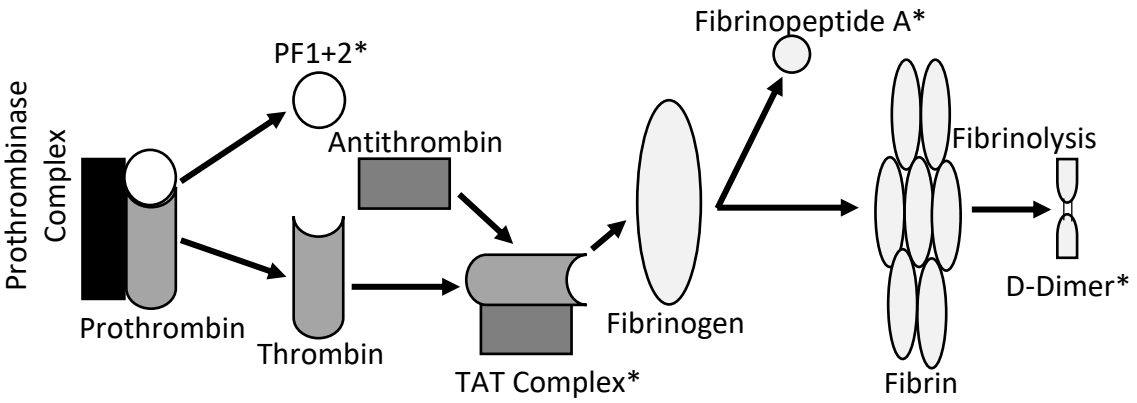
Chapter 6 – Thrombin Generation during ECMO

6.1 Introduction

Thrombin is a serine protease formed by the cleavage of prothrombin. Its generation is essential in achieving secondary haemostasis cleaving fibrinogen to fibrin, allowing fibrin multimers to form at the site of vessel injury. Thrombin has other haemostatic and inflammatory roles including the activation of platelets by binding to PAR1 and PAR4, activating FXIII, binding to thrombomodulin to APC, inhibiting fibrinolysis by activating TAFI, and activating white cells and endothelium. Inadequate generation with low thrombin concentrations cause the formation of low density, thin fibrin strands increasing the risk of bleeding[283]. However, excessive thrombin generation is associated with thrombosis[284].

Thrombin generation can be assessed using several *in vivo* markers and functional assays[285]. Prothrombin fragments 1+2 (PF1+2) and Thrombin-Antithrombin complexes (TAT) are markers of thrombin generation. Both have short half-lives (90 minutes and 10 minutes respectively)[286,287]. Fibrinopeptide A can be used as a marker of thrombin activity as a cleavage product of its substrate, fibrinogen. D-dimer levels reflect both activation of coagulation and fibrinolysis, of which thrombin generation is a component. D-dimer as a marker, has a longer half-life of 7-8 hours. Figure 26 demonstrates the assays available to assess thrombin generation. Global thrombin generation can be assessed *ex vivo* using a calibrated automated thrombogram or thrombin generation assays (TGA) by measuring the cleavage of a fluorogenic substrate measuring several parameters including endogenous thrombin potential (ETP) and peak thrombin generation.

*Figure 26 Illustration of markers of thrombin generation and activation. Markers denoted with **



Thrombin generation during ECMO has been shown to be increased due to both the procoagulant state of the critically ill patient and by synthetic extracorporeal circuits[288]. These changes have been evaluated in CPB more extensively[287]. Patients requiring CPB typically do not have the extensive proinflammatory state as those requiring ECMO. Thrombin generation increases during the use of CPB with an initial decrease in haemostatic factors, predominantly of fibrinogen, due to haemodilution[286,287]. Haemodilution can distort interpretation of TGA during the initiation period[287]. During CPB, lower levels of thrombin generation and hypofibrinogenaemia have been associated with increased rates of post-operative bleeding and higher blood product usage whereas higher concentrations have been associated with post-operative thrombotic complications such as graft occlusion[135,289–291]. Bursts of thrombin generation during major cardiac surgery are temporally related to surgical incision and the commencement of the extracorporeal circuit, and remain elevated in the post-operative period for several days[286,287,292–294]. This occurs in the presence of standard anticoagulation during the period of extracorporeal circuit use and thrombin generation is not related to the amount of anticoagulant used[295]. Some suggest this is as result of tissue factor induced-activation from blood vessel injury although further data is conflicting suggesting that contact

pathway activation from the artificial surface of the circuit or tissue factor from circulating activated monocytes are drivers[5,292,296].

During ECMO, thrombin generation is increased as described in Table 20. Hundalani *et al* showed that in neonates <30 days older have a significant increase in thrombin generation markers after 5 days of ECMO compared to baseline although was not significantly increased in babies >30 days old. These results were replicated by Arnold *et al* in another small cohort of neonates requiring ECMO in spite of decreased concentrations of procoagulant factors, predominantly FV, FVII and fibrinogen over the first 6-hours of ECMO use[297]. Similarly, Hékiman *et al* showed increases from Day 1 to Day 7 of ECMO use predominantly in PF1+2, fibrin monomers and D-dimer levels in both patients with COVID-19 pneumonia and other causes of SRF[298]. They also described 3 patients with mortality due to major haemorrhage, who had elevated levels of thrombin generation without over-anticoagulation.

Studies describing the changes of thrombin generation are largely limited to ECMO initiation, but its role at later time points including following decannulation have not been described. In addition, the role of thrombin generation during bleeding and thrombotic events during ECMO events have been poorly described[298].

Table 20 Studies describing thrombin generation markers during use of extracorporeal circuits

Study	*Cohort Size*	*Events*	*Outcome(s)*
Mazzeffi. Anesth Analg. 2019.[299]	20 adult patients requiring VA ECMO in comparison to controls	Changes in thrombin generation with and without thrombomodulin (TM)	Increased peak thrombin generation and endogenous thrombin potential. Resistance to TM with ECMO
Hundalani. Ped Crit Care. 2014.[288]	29 neonates requiring ECMO	Changes of TAT, PF1+2 and D-dimer on Day 1 to Day 5 in <30 days neonates and >30 days babies	Increased levels in all assays from day 1 to 5 in <30 days (18.7 to 55.6 µg/L, 2,038 vs 377.5 pmol/L and 1.45 vs 2.96 µmol/L respectively). These changes were not seen in >30 days. Markers did not correlate to heparin dose or anti-Xa activity.
Hékimian. Coag Fibrinol. 2021.[298]	20 adult patients with COVID-19 infection and 10 without requiring ECMO	Changes in PF1+2, TAT, fibrin monomers and D-dimers at Day 1 and Day between to both groups	There were higher levels of PF1+2 and TAT prior to ECMO in both groups. There was a significant increase in PF1+2 and D-dimer from Day 1 to 7 in both groups
Arnold. Int Care Med. 2001[297]	10 neonates with severe hypoxaemia	Changes in D-dimer, PF1+2 and TAT prior to ECMO, after 6- and 24-hours	Significant increase in D-dimer, PF1+2 and TAT after 6 hours.
Larsson. Sci Transl Med. 2014[300]	Lapine model using ECMO	Thrombin generation by TGA with and without 3F7, a FXIIa inhibitor	Thrombin generation and events of thrombosis and haemorrhage were lower with the use of 3F7

6.2 Aims

1) To assess the changes in thrombin generation markers over 24-hours in an *ex vivo* ECMO circuit
2) To assess the changes in thrombin generation markers in adult patients prior to, during and following decannulation from ECMO
3) To assess whether there is a relationship between changes in thrombin generation markers in thrombotic and haemorrhagic events during ECMO

6.3 Methods

The methods described pertain to both samples collected from an *ex vivo* ECMO circuit and serial samples collected from patients. The details of the design and set-up of the *ex vivo* circuit have previously been described in Chapter 5. The methods for performing ELISA to assess markers of thrombin generation during ECMO are described in Chapter 4. Anti-Xa levels were obtained from clinical samples analysed using Sysmex CS2100*i* (Norderstedt, Germany) according to manufacturer's protocol.

6.3.1 Outcome Variables

The primary outcome for both the *ex vivo* circuits and patients were the changes in markers of thrombin generation from the baseline level prior to starting ECMO. Secondary outcomes for the patient samples were the changes in markers of thrombin generation in those developing thrombosis, haemorrhage and membrane occlusion to those not developing these complications and correlation between anti-Xa levels and markers of thrombin generation in patients using ECMO.

6.4 Results

6.4.1 Thrombin Generation in an *ex vivo* model of ECMO

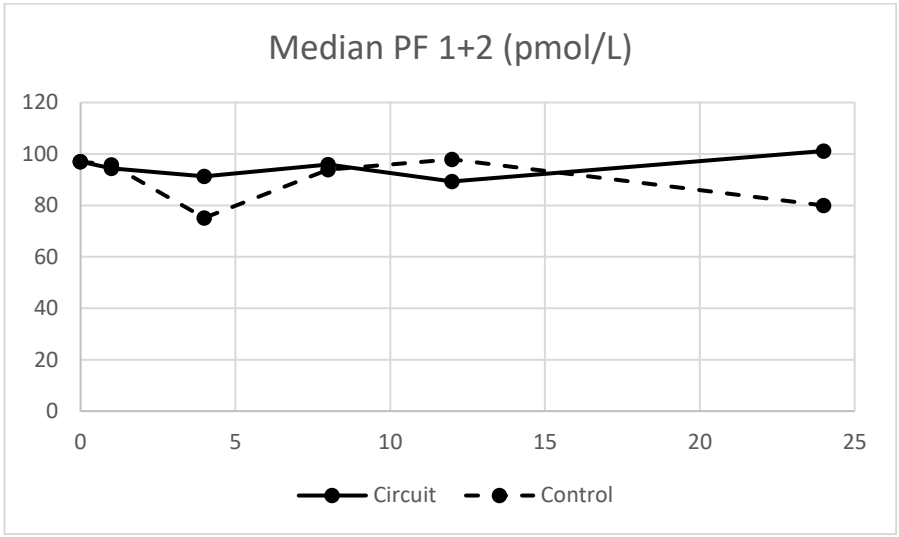
Prothrombin Fragments 1 + 2

Following the initiation of the *ex vivo* circuit, there was no change in PF 1+2 levels (reference range 200-1200 pmol/L) from initiation of the circuit (median 97 pmol/L) to 24-hours (median 101 pmol/L, p = 0.5) shown in Figure 27(a). There was a small decrease seen in the control samples over the same period (97 pmol/L to 80 pmol/L respectively, p = 0.043). There was no statistically significant difference between controls and circuit samples at 24-hours (p = 0.92).

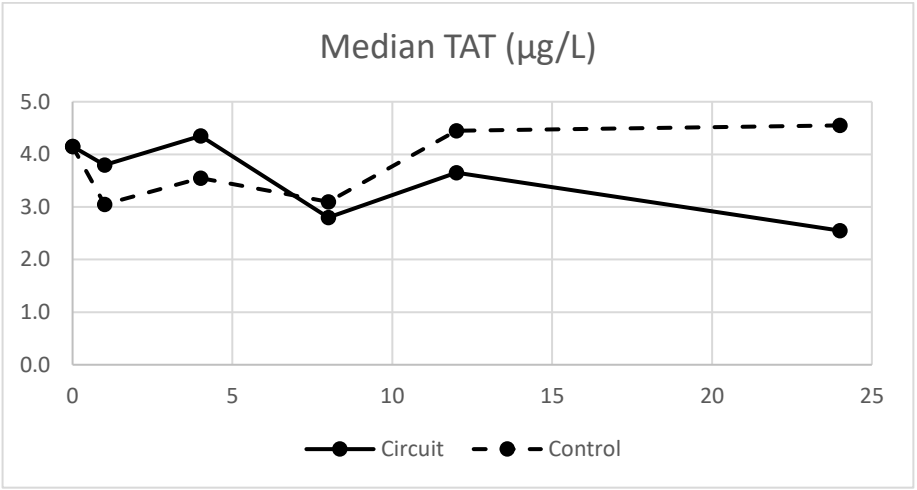
Thrombin-Antithrombin Complexes

Following the initiation of the *ex vivo* circuit, there was no change in TAT levels (reference range 0.8-3.8µg/L) from initiation of the circuit (median 4.2 µg/L) to 24-hours after (2.6 µg/L, p=0.3) shown in Figure 27(b). There was no change seen in the control samples over the same period (4.2 µg/L to 4.6 ng/mL respectively, p = 0.92). There was no statistically significant difference between controls and circuit samples at 24-hours (p = 0.92).

Figure 27 Changes in (a) PF1+2 and (b) TAT over 24-hours in an ex vivo extracorporeal model



(a)



(b)

These results for D-dimer levels are shown in Section 7.4.1. There was no significant change over the 24-hour duration in the circuits and no difference in comparison between the *ex vivo* circuit and control sample at 24-hours.

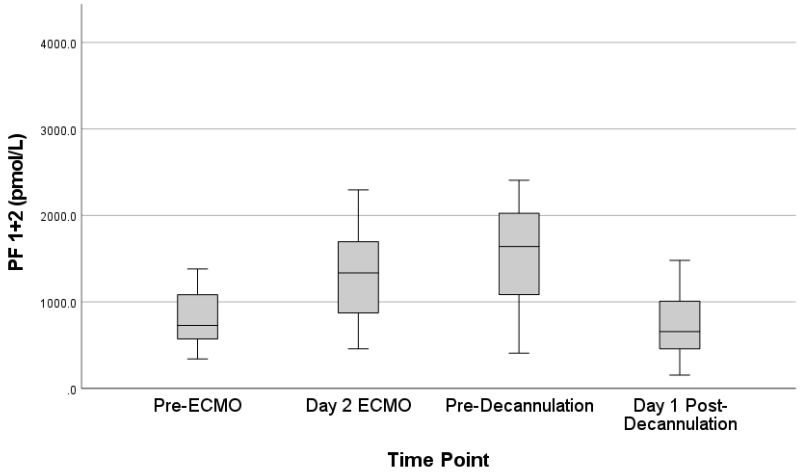
6.4.2 Changes in thrombin generation in adult patients receiving ECMO

Seventeen participants were reviewed in this analysis.

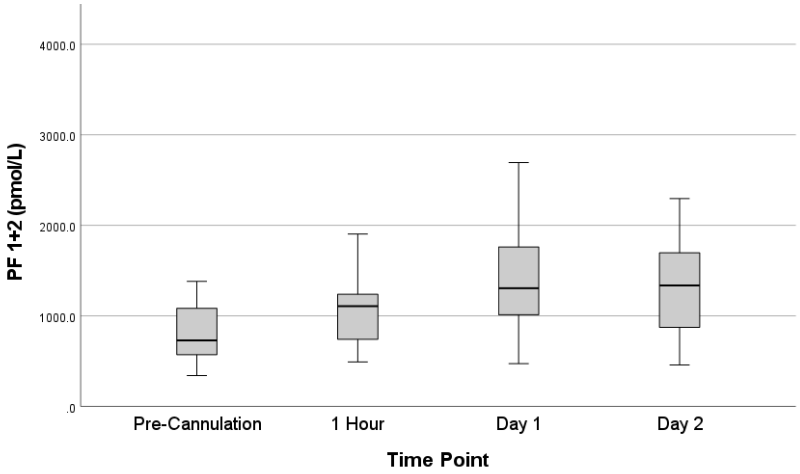
Temporal Changes in Prothrombin Fragments 1+2

The normal reference range for PF1+2 derived from 38 control samples was 200 – 1200 pmol/L. PF1+2 levels were within normal ranges prior to ECMO (median 729 pmol/L, IQR 530 – 1150 pmol/L) (Figure 28(a)). There was a subsequent increased in PF1+2 levels after 1 hour, Day 1 and Day 2 of ECMO in comparison to pre-ECMO (median PF1+2 after 1-hour: 1107 pmol/L, IQR 735 – 1250 pmol/L, $p = 0.039$, median on day 1: 1305 pmol/L, IQR 955 – 1859 pmol/L, $p = 0.034$ and median on Day 2: 1337 pmol/L, IQR 857 – 1706 pmol/L, $p = 0.020$) (Figure 28(b)). There were no subsequent increases from Day 1 to Day 2 and Day 7 (median on Day 7: 1325 pmol/L, IQR 504 – 2195 pmol/L, $p = 0.58$) (Figure 28(c)). At the time of decannulation, PF1+2 levels were elevated with a decrease 1 day after, which returned to normal ranges (median prior to decannulation: 1453 pmol/L and 1 day following decannulation: 658 pmol/L, $p < 0.001$) shown in Figure 28(d).

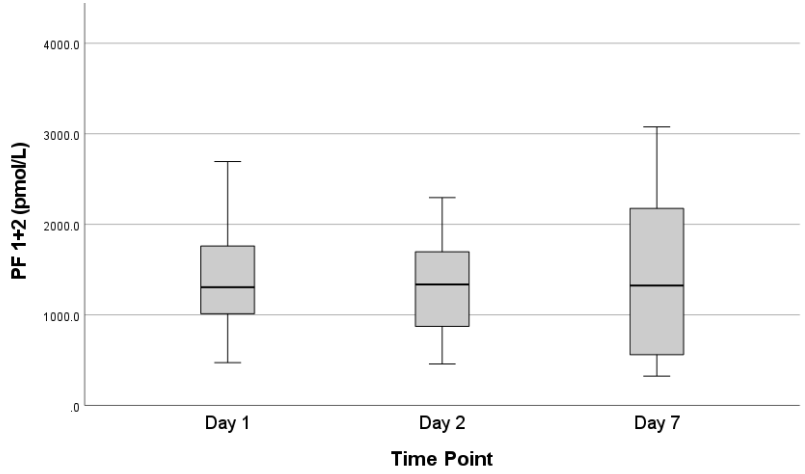
Figure 28 Box plots for changes over time in PF1+2 levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



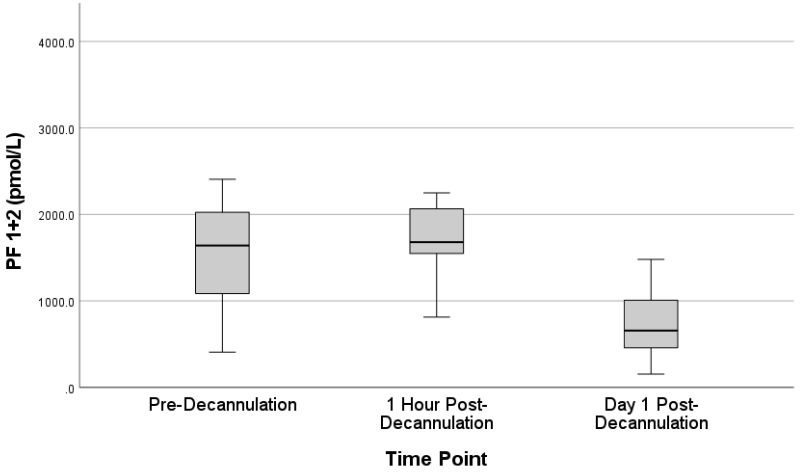
(a)



(b)



(c)



(d)

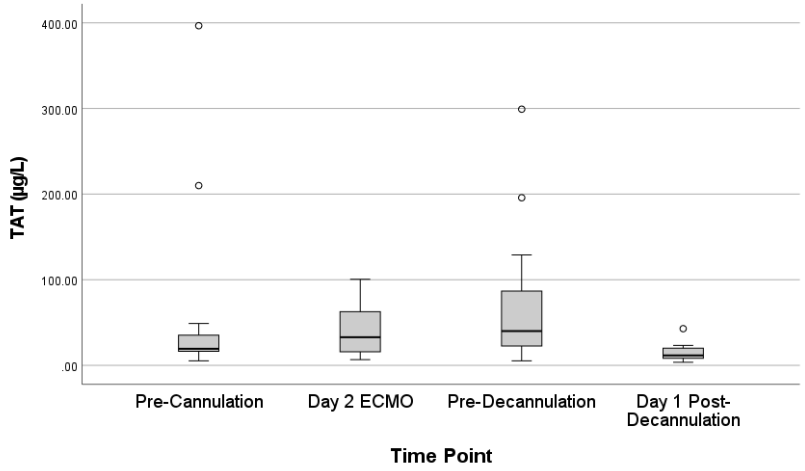
Temporal change in Thrombin-Antithrombin Complexes

The normal reference ranges for TAT levels were 0.8 – 3.8μg/L. TAT levels were elevated above reference ranges prior to ECMO (median 19.5μg/L, IQR 16.5 – 35.7μg/L) (Figure 29(a)). There was a subsequent non-statistically significant increase in TAT levels 1 hour, Day 1 and Day 2 of ECMO in comparison to pre-ECMO (median TAT after 1 hour: 30.7μg/L, IQR 12.2-64.1μg/L, p = 0.44, median on day 1: 36.9μg/L, IQR 10.9 – 62.0μg/L, p = 0.68 and median on Day 2: 32.9μg/L, IQR 13.8 – 64.3, p = 0.36) (Figure 29(b)). There were no subsequent increases from Day 1 to Day 2 and Day 7 (median on Day 7: 38.3μg/L, p = 0.48) (Figure 29(c)). At the time of decannulation, TAT levels were elevated with a decrease 1-hour and 1-day afterwards (median prior to decannulation: 40.1μg/L, median 1-hour following: 20.8, p = 0.10, and 1-day following decannulation: 11.7 μg/L, p < 0.001) (Figure 29(d)).

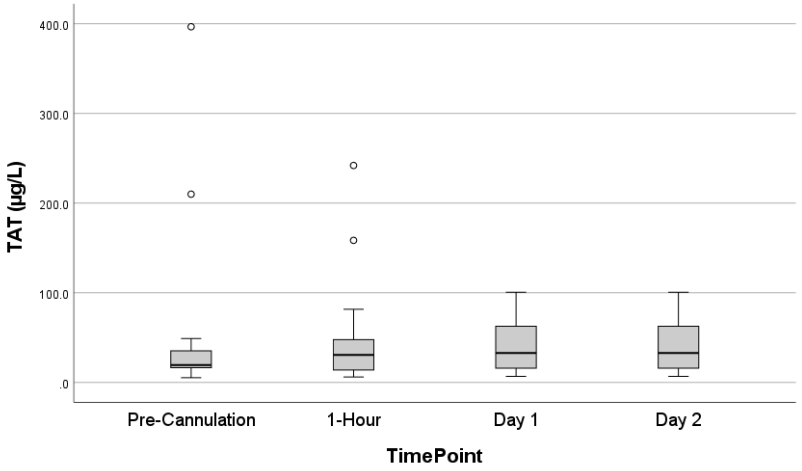
Correlation between D-Dimers and other Markers of Thrombin Generation and Plasmin Activation

There was a positive correlation between D-dimer levels and PF1+2 (Pearson rank, r = +0.67, p <0.001) and D-dimer levels and TAT (r = +0.57, p<0.001) in samples taken after 1 day of ECMO as shown in Figure 30(a) and (b). There was also a positive correlation between D-dimer levels and PAP, a marker of fibrinolysis, (r = +0.77, p <0.001) shown in Figure 30(c) and discussed further in Chapter 7. These all remained positively correlated regardless of the period that the samples were taken at.

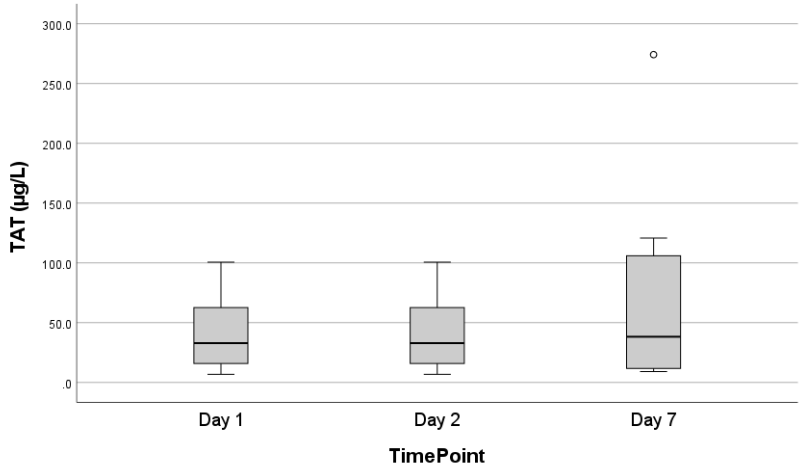
Figure 29 Box plots for changes over time in TAT levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



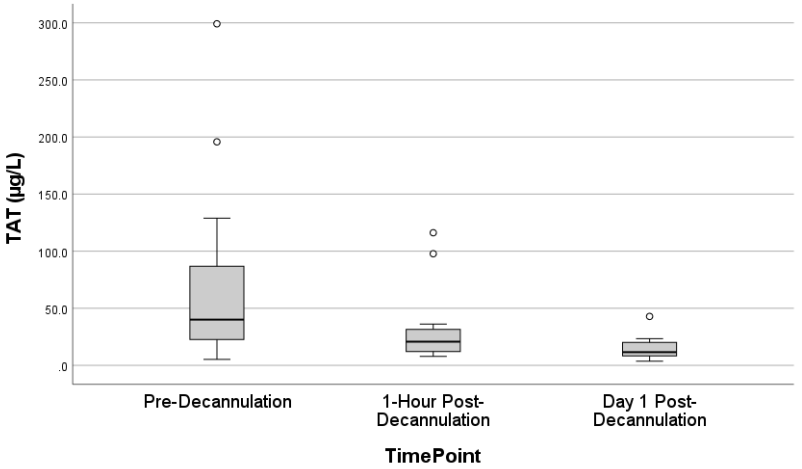
(a)



(b)

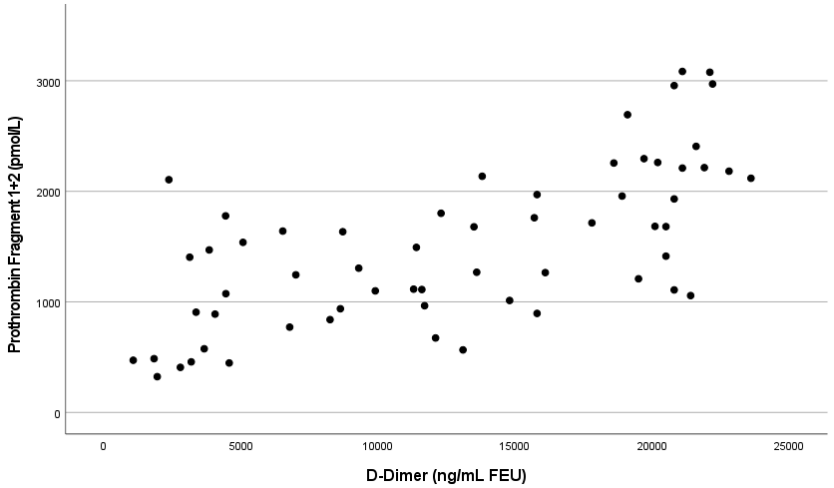


(c)

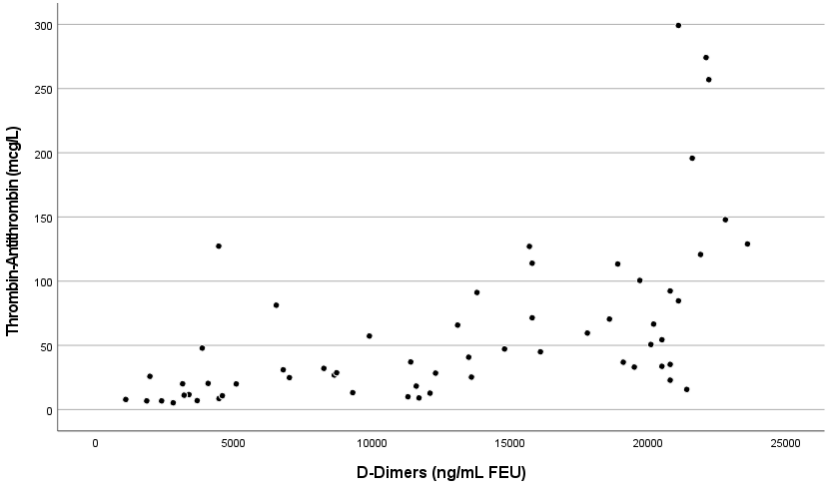


(d)

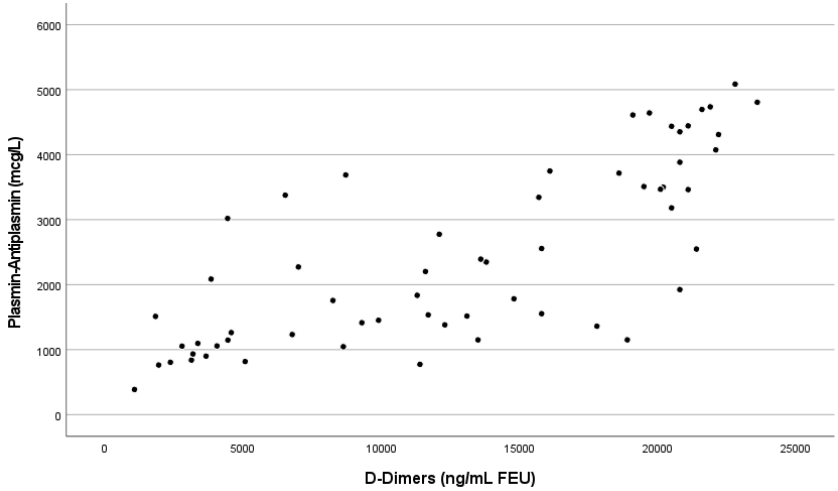
Figure 30 Correlation between (a) D-dimer and PF1+2 (b) D-dimer and TAT (c) D-dimer and PAP



(a)



(b)



(c)

6.4.3 Markers of Thrombin Generation during haemorrhagic and thrombotic complications of Extracorporeal Membrane Oxygenation

Pulmonary Embolism

Blood samples reviewed were those taken prior to initiation or 1-hour after initiating ECMO. PF1+2 (median 749 vs 729 pmol/L, p = 0.77) and TAT levels (median 19.5 vs 26.1 µg/L, p = 0.77) were not statistically different in those without PE (n = 13) to those with PE (n = 4) before starting ECMO. There was no difference after 1-hour after starting ECMO (PF1+2 1142 vs 1145 pmol/L, p = 0.41) (TAT 22.0 vs 37.6 µg/L, p = 0.79)

Deep Vein Thrombosis

Blood samples reviewed were those taken prior to decannulation from ECMO. PF1+2 (median 1681 vs 1085 pmol/L, p = 0.45), and TAT levels (median 54.4 vs 22.6 µg/L, p = 0.45) were not statistically different in those with DVT (n = 7) to those without DVT (n = 13) prior to ceasing ECMO.

Intracranial Haemorrhage

Blood samples reviewed were those taken prior to initiation or 1-hour after initiating ECMO. PF1+2 (median 781 vs 691 pmol/L, p = 0.32), and TAT levels (median 17.7 vs 21.2 µg/L, p = 0.95) were not statistically different in those with ICH (n = 4) to those without ICH (n = 13) before starting ECMO. There was no difference in these levels 1-hour after starting ECMO (PF1+2 1079 vs 1373 pmol/L, p = 0.20) (TAT 29.4 vs 49.6 µg/L, p = 0.55). There was a larger increase in PF1+2 in those with intracranial haemorrhage (+614 pmol/L) from preceding ECMO to 1-hour after ECMO in comparison to those without ICH (+224 pmol/L, p = 0.031).

This amount of change was not seen with TAT levels (1.0 without ICH vs 23.3 μg/L with ICH, p=0.37).

Non-Intracranial Haemorrhage

There were 3 patients with NICMH with 6 relevant blood samples to assess. In those with NICMH, PF1+2 levels (n = 3, median 1253 pmol/L, p = 0.79) to those who did not have bleeding (n = 14, median 1260 pmol/L). TAT levels were similar in those with NICMH during these episodes (median 51.4 μg/L, p = 0.44) to those who did not (median 34.9 μg/L).

There were 10 patients that had clinically relevant non-major bleeding (CRNMB) with 12 relevant blood samples to assess. At the time of CRNMB, levels of PF1+2 were non-significantly higher in than those that had bleeding (median 1484 pmol/L, p = 0.33) than those without bleeding (median 1197 pmol/L). There were higher TAT levels in those CRNMB than those without (81.5 vs 25.3 μg/L, p = 0.004).

Circuit Change

There were 5 patients that had 6 circuit changes during their ECMO runs although samples were limited to 4 patients. There was no identified difference in PF1+2 and TAT levels in those who had circuit changes and those that did not on matched days (p = 0.12 and 0.31 respectively). There were non-significantly higher levels of PF1+2 (2158 vs 1240 pmol/L, p=0.11) and TAT (119.6 vs 40.2 μg/L, p = 0.14) in samples preceding a circuit change to those who did not. There was a decrease in level of PF1+2 (2160 to 1057 pmol/L, p = 0.11) and TAT (119.6 to 75.1 μg/L, p=0.29) pre- and 1-day post-circuit change although this was not statistically significant due to small patient numbers.

6.4.4 Markers of Thrombin Generation in comparison to Unfractionated Heparin use

PF1+2 and TAT levels were compared to concurrent anti-Xa levels to monitor UFH infusion levels. Samples were excluded from the first day of ECMO due to boluses of UFH given around the time of cannulation and following decannulation. The total number of samples available for assessment was 56. There was a positive correlation between unfractionated heparin dosing and anti-Xa levels during ECMO use (Pearson r rank = +0.58, $p < 0.001$) shown in Figure 31. There was no correlation between TAT (Pearson rank = -0.095, $p = 0.49$) and PF1+2 (Pearson r rank = -0.096, $p = 0.48$) with UFH dosing (Figure 32(a) and (b)). There was no correlation found between TAT (Pearson rank = -0.2, $p = 0.14$) and PF1+2 (Pearson r rank = -0.11, $p = 0.43$) with anti-Xa levels (Figure 32(c) and (d).

When comparison was made between day 2 of ECMO (as an earlier time point in ECMO use), and prior to decannulation (as a later time point), there was a difference in PF1+2 levels between those that had no anticoagulation, low levels of UFH (anti-Xa <0.5 IU/mL) and high levels of UFH (anti-Xa ≥0.5 IU/mL) (Kruskall-Wallis test, $p = 0.038$) although this was not seen in TAT level ($p = 0.21$) as shown in Figure 33(a) and (b). There was no difference in either PF1+2 ($p = 0.89$) and TAT ($p = 0.93$) levels prior to decannulation according to the level of UFH.

Figure 31 Correlation of Anti-Xa levels to unfractionated heparin dosing during ECMO

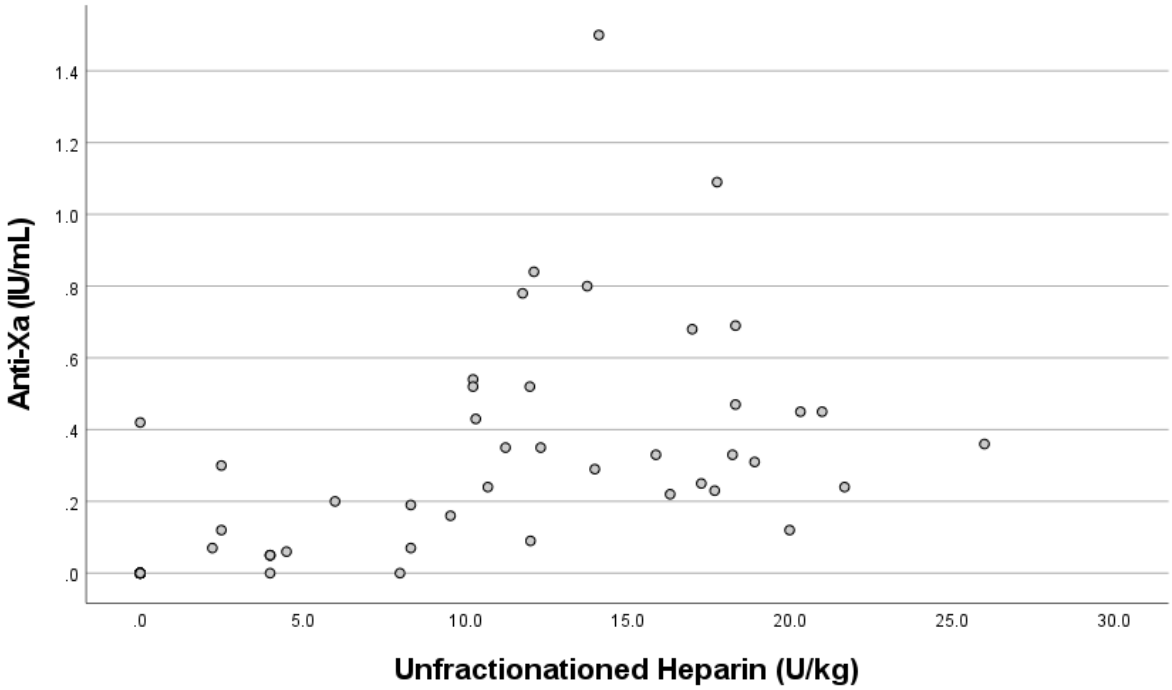
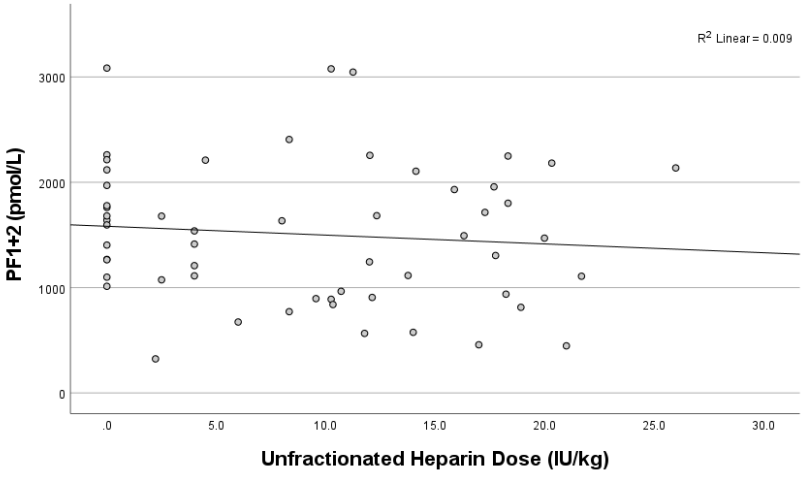
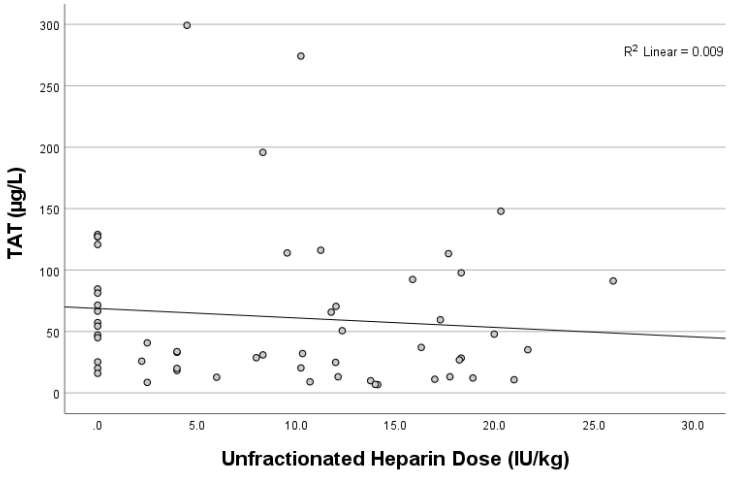


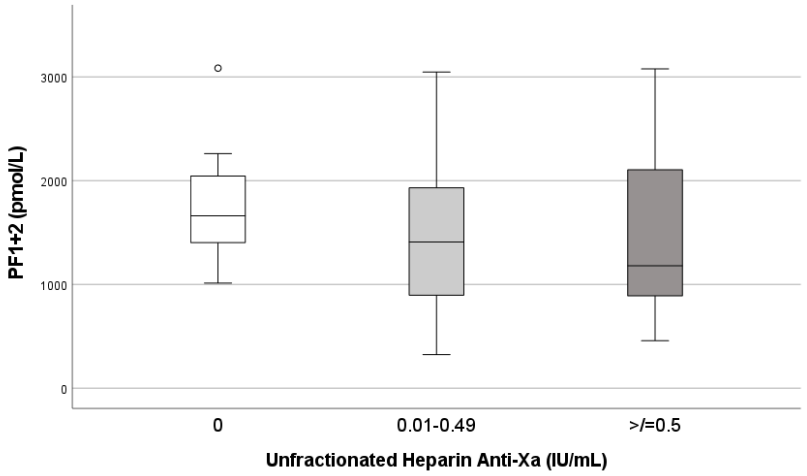
Figure 32 Comparison of unfractionated heparin dose (IU/kg) to (a)PF1+2 (b)TAT & intensity of anticoagulation by anti-Xa to (c)PF1+2 (d) TAT



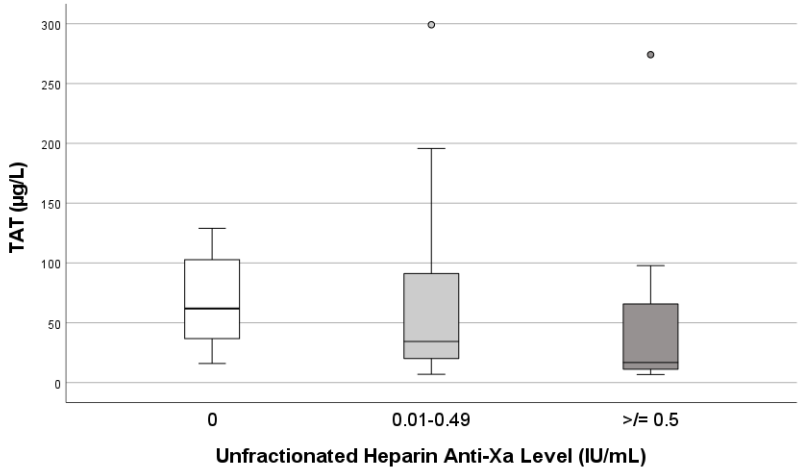
(a)



(b)

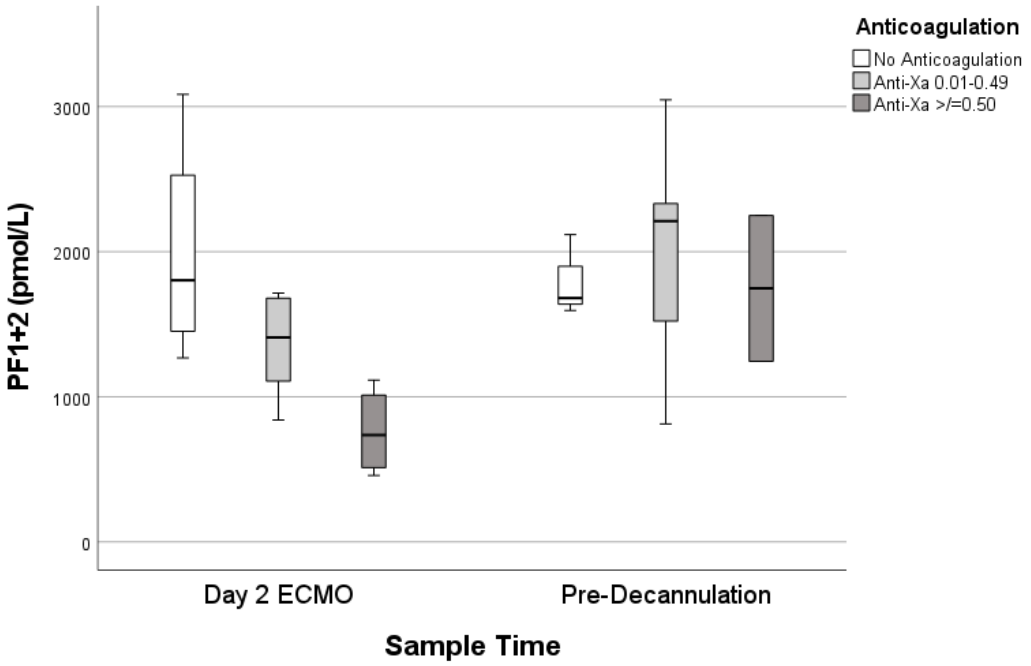


(c)

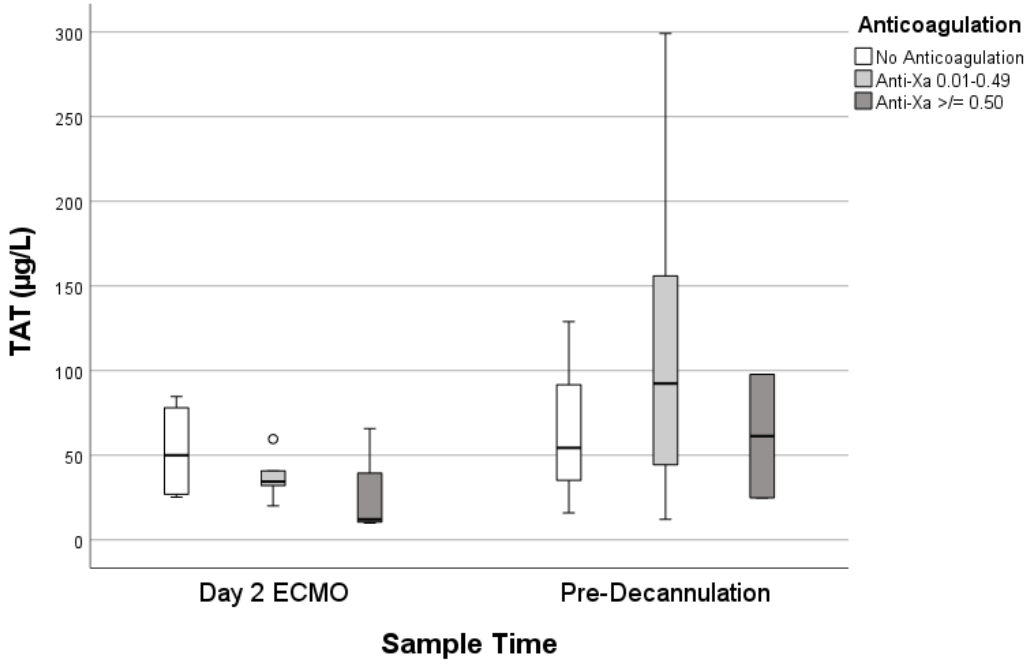


(d)

Figure 33 Intensity of anticoagulation by unfractionated heparin according to anti-Xa on Day 2 of ECMO and prior to decannulation (a) PF1+2 (b) TAT



(a)



(b)

6.5 Discussion

Changes in thrombin generation have been described during ECMO, predominantly in the neonatal population and during the first few days of its use. The purpose of this study was to assess markers of thrombin generation throughout the entirety of ECMO use and to see if there was a correlation between bleeding and thrombotic events and with anticoagulation.

The *ex vivo* circuit showed there were relatively static markers of thrombin generation over a 24-hour period. This circuit design used citrate as opposed to heparin as an anticoagulant. However, haemodialysis circuits can routinely use citrate unlike ECMO. Wiegele *et al* described the absence of thrombin generation using tissue factor activated TGA during haemodialysis in critical care using citrate, despite the endogenous thrombin potential being higher in these patients than normal controls[301].

In comparison to the *ex vivo* model of ECMO, there were elevated levels of PF1+2 prior to cannulation in patients with SRF. There were subsequent increases during the first 2 days of ECMO. These levels remained elevated but did not continue to increase at day 7 of ECMO. TAT complex levels did not change to a similar extent over the first 2 days. An acquired antithrombin deficiency is recognised in the early stages of ECMO so may therefore impact upon TAT level interpretation[298]. These increases in thrombin generation are similar to the result described by other similar studies[288,297]. There was subsequently a significant decrease following decannulation to normal ranges in both PF1+2 and TAT. This suggests that both removal of the extracorporeal circuit and recovery from critical illness both contribute to thrombin generation, as has been demonstrated in severe sepsis previously[302].

The role of D-dimer levels reflects both coagulation and fibrinolytic activity. In the setting of ECMO, there was a positive correlation of thrombin generation markers as shown in CPB.

However, there was a stronger positive correlation with PAP complexes suggesting that the role of D-dimer should not only be considered as marker of thrombotic generation solely during ECMO, but additionally indicative of fibrinolytic activity.

There was no clear relationship with PF1+2, TAT and D-dimer levels in this cohort relating to thrombotic complications. However, in Chapter 2 with a large patient cohort there was an association of D-dimer levels with pulmonary emboli at the time of cannulation. This may be because of a small cohort size with PE in this cohort. With respect to circuit changes, there was a small number of events that failed to show a difference in markers of thrombin generation around circuit change in comparison to patients that did not require one. However, if considered there was a decrease in these markers following decannulation, it seems plausible that these may be significant if a larger number of samples around the time of circuit changes were reviewed. D-dimer levels have been shown to correlate to clot size within the membrane oxygenator and as an early marker to predict subsequent circuit change[303,304]. As D-dimer has been shown to be non-specific during ECMO for coagulation and fibrinolysis, both PF1+2 and PAP could be explored further as being a more specific marker of this.

In bleeding events, there was a larger increase in PF1+2 levels prior to and 1-hour cannulation in those with ICH in comparison to those without. There was a trend-to-significant increase in D-dimer levels in those with ICH than those without, but this was not seen in PAP levels (discussed further in Chapter 7). Similarly, higher levels of D-dimer and fibrin monomers have seen in those with progressive traumatic brain injury and associated subsequent trauma-related coagulopathy[305–307]. Furthermore, this was described in Chapter 2 with higher D-dimer levels in those with ICH than those without in a large retrospective patient group

shortly after ECMO cannulation. This suggests that there are similarities in the coagulation of ICH during ECMO to that of trauma. Higher levels of TAT complexes were seen in those with CRNMB. This may be a compensatory response to achieve haemostasis and has been described in CPB with higher TAT levels being related to increased levels of post-operative blood loss[308].

There was a correlation between with anti-Xa and UFH doses during ECMO after cannulation. However, there was no relationship seen between TAT and PF1+2 and unfractionated heparin dosing. PF1+2 levels correlated with intensity of anticoagulation or no anticoagulation in early ECMO use but this was not seen in prior to decannulation. In CPB, there is also a poor relationship between both anticoagulation and thrombin generation markers with progressively higher levels throughout the use of the extracorporeal circuit[295,309]. Of concern in Chapter 2, bleeding was demonstrated to have an impact upon survival during ECMO use and therefore fastidious anticoagulation to reduce thrombin generation may not be appropriate, although this requires further evaluation in prospective studies.

There were several limitations to this study. Firstly, there was the small patient cohort and subsequent small number of clinical events. However, the purpose of this study was for hypothesis generation to further assess potential haemostatic changes that may require further evaluation. Despite these small numbers, significant changes could be seen. Secondly, this study used two specific markers of thrombin generation, PF1+2 and TAT, alongside D-dimer levels, which are widely used in clinical practice although they are non-specific. The rationale for this as part of the study design was mainly due to the ability of sampling and processing in the early time points of ECMO use, which occurred at other hospital sites prior to transfer to the study centre. TGA would provide further details of changes in coagulation

during ECMO, which were not performed as part of this study due to the ability to analyse early sample within an appropriate period. Correlation between PF1+2 and results from TGA have been described so it was felt appropriate to explore this in this study[285].

6.6 Conclusions

Thrombin generation appears to change dynamically in patients requiring ECMO for SRF, particularly when using PF1+2 as a marker, although these were not seen in an *ex vivo* extracorporeal model using citrate anticoagulation. Different *ex vivo* model designs using heparin may be therefore required if thrombin generation from the circuit is to be fully evaluated. Additionally, this pilot study suggests there are increases in these thrombin generation markers during bleeding events although large patient cohorts are needed to explore this further. The intensity of anticoagulation appears to have a poor correlation with thrombin generation particularly in the latter days of ECMO use. Given more significant findings found with relation to PF1+2, this may be a more suitable marker for further analysis than TAT.

Chapter 7 – Fibrinolysis during ECMO

7.1 Introduction

The physiological role of the fibrinolytic system is to breakdown fibrin clot to prevents its excessive accumulation. This is regulated by pro-fibrinolytic (tPA and uPA) and anti-fibrinolytic enzymes (PAI-1, α-2-antiplasmin, α-2-macroglobulin and TAFI) ultimately regulating plasmin activity, which breaks down cross-linked fibrin multimers (Figure 2). Primary fibrinolysis is independent of other components of haemostasis whereas secondary fibrinolysis occurs because of haemostatic activation and thrombin generation.

Dysregulation of the fibrinolytic system can lead to bleeding or predispose to thrombosis depending on the overall balance of fibrinolytic proteins. Hyperfibrinolysis is an over-activation of the fibrinolytic pathway associated with bleeding. Hypofibrinolysis or 'fibrinolytic shutdown' is an under-activation of the pathway, which occurs in various disease states and may be associated with thrombosis, fibrin deposition and multi-organ injury in critical illness. Defects in the pathway can be assessed by 1) antigen and activity levels of the component enzymes of the fibrinolytic system, 2) complexes and markers of recent fibrinolytic activity such as plasmin-antiplasmin (PAP) complexes and D-dimers, and 3) functional assessments of fibrinolytic activity such as clot lysis time by TEG.

Acquired fibrinolytic defects are recognised as a component of several disease states with organ injury and ischaemia. Trauma is one such condition in which both hyperfibrinolysis and fibrinolytic shutdown occur. Early after injury, there is stimulation of tPA release with patients having markedly increased levels associated with increased mortality. Later, as part of the inflammatory response increased levels of PAI-1 result in fibrinolytic shutdown[310]. Defects of

fibrinolysis are also seen in sepsis and acute lung injury (ALI). Fibrin deposition is recognised as a pathological pattern in ALI, due to immunothrombosis. Both PAI-1 and uPA secreted from the pneumocytes and macrophages play roles in the development of localised fibrinolytic dysregulation[311–313]. Furthermore, TNFα, IL-1 and IL-6, as key mediators of an the inflammatory state can upregulate PAI-1 levels in endothelial injury[314–316]. This suggests that preceding lung injury and sepsis prior to ECMO may play a role in subsequent fibrinolytic changes during its use.

The changes in fibrinolysis attributable to ECMO circuits are less well described (Table 21). Hundalini *et al* showed that PAP complexes were increased in infants with bleeding complications after five days of use[288]. As expected, PAP levels correlated to D-dimer levels as well as TAT complexes, suggesting that part of the increment in fibrinolytic activation was due to secondary fibrinolysis. McVeen *et al* showed that PAI-1 levels are increased prior to starting ECMO in 13 children in comparison to controls[317]. These levels subsequently fell during ECMO. tPA levels were also elevated prior to ECMO in this cohort. These studies were in neonates and infants who are recognised as having impaired fibrinolytic activity in comparison to adults[318,319].

In the setting of CPB, plasmin generation and D-dimer production increase significantly during its use. Chandler *et al* showed *in vivo* increases of 100-200-fold within the first 5 minutes of CPB with a subsequent elevation of 20-30-fold during the remainder of its use[320]. However, plasmin generation was not affected earlier in surgery prior to CPB, including following sternotomy, suggesting a key role of the extracorporeal circuit in this process. tPA levels increased early during CPB with later increases in PAI-1 causing a reduction in tPA although

this remained elevated in comparison to baseline levels with heterogeneity between patients[321–323]. However, plasma uPA levels did not change in patients during CPB[323].

Antifibrinolytic drugs can be given to reduce bleeding. Their use is established in trauma-associated, post-partum and major haemorrhage although there is lack of randomised-controlled trials in ECMO. A case-control study by Wilson *et al* showed 56 infants given the antifibrinolytic agent, aminocaproic acid as a regular infusion during ECMO, compared to 52 without, had reduced rates of bleeding and blood product use[324]. Similarly, the use of antifibrinolytic agents has been demonstrated in multiple randomised control studies to safely reduce bleeding, blood product use and surgical reintervention during CPB and major surgery[325,326].

The most frequent haemostatic complications seen during VV ECMO are PE, DVT and ICH, which have been described in Chapter 2. Changes in fibrinolysis have been demonstrated in ICH as part of a bleeding phenotype as a possible consequence of blood-brain barrier injury. Hyperfibrinolysis is not seen as an early clinical feature in patients with spontaneous ICH and SAH[327]. However, early fibrinolytic activity is seen in those with progressive ICH following traumatic brain injury in both patients and murine models with correlation to elevated levels of tPA, uPA and D-dimers[328,329].

Table 21 Studies describing fibrinolytic changes during ECMO

Study	*Cohort Size*	*Events*	*Outcome(s)*	*Comments*
Hudalani, Ped Crit Care. 2014[288]	29 neonates	5% bleeding 14% thrombosis 10% bleeding & thrombosis	Day 5 PAP levels increased in neonates >30 days with bleeding Day 5 PAP levels increased in <30 days with/out bleeding D-dimers correlated with PAP & TAT on D1 & D5 in >30 days old	Case-control study Single centre
McVeen, Am J Hemat. 1991[317]	13 infants	tPA and PAI-1 levels pre-, during and post-ECMO	Elevated levels of PAI-1 and tPA prior to ECMO Decline in PAI-1 and tPA during ECMO	Single centre No correlation to clinical outcomes
Kreyer, J Thor Dis. 2017[330]	36 adults	Haemorrhage *74 events* *14 patients: severe* *22 patients: mild*	Lower survival with severe haemorrhage Maximum daily D-dimer levels does not correlate with RBC transfusion	No assessment of other fibrinolytic proteins
Lubnow, J Crit Care. 2014[304]	24 adults	34 membrane oxygenator (MO) changes	Increased D-dimer levels prior to MO change	No assessment of other fibrinolytic proteins
Dornia, Artif Organs. 2015[303]		Presence of thrombus within MO	Elevated D-dimers with an absence of other causes was associated with presence of MO thrombus Decrease in D-dimers with circuit change after 48 hours	D-dimer was only marker assessed for MO thrombus
Passmore, Crit Care. 2017[134]	27 sheep	Changes in haemostatic factors over 24-hours in smoke-lung injury vs. sham injury and ventilation, ECMO vs. no ventilation	No changes in D-dimers after 24 hours of ECMO and no differences between ECMO and controls Decrease in fibrinogen following 24 hours use of ECMO	Haemostatic events were not described/occurred

7.2 Aim

4) To assess the changes in fibrinolysis over 24-hours in an *ex vivo* ECMO circuit
5) To assess the changes in fibrinolysis in adult patients prior to, during and following decannulation from ECMO
6) To assess whether there is a relationship between fibrinolysis and the presence of thrombotic and haemorrhagic events during ECMO

7.3 Methods

The methods described pertain to both samples collected from an *ex vivo* ECMO circuit and serial samples collected from patients. The details of the design and set-up of the *ex vivo* circuit have previously been described in Chapter 4. The methods for performing ELISA to assess fibrinolysis during ECMO are described in Chapter 5.

7.3.1 Outcome Variables

The primary outcome for both the *ex vivo* circuits and patients were the changes in each fibrinolytic parameter from the baseline level prior to starting ECMO. Secondary outcomes for the patient samples were the changes in each fibrinolytic parameter in those developing thrombosis, haemorrhage and membrane occlusion to those not developing these complications.

7.4 Results

7.4.1 Fibrinolytic factors in an *ex vivo* model of ECMO

Haemodilution

Haemodilution following circuit priming was seen in all the assessed fibrinolytic assays although some varied in comparison to haematocrit. Table 22 shows the percentage changes following haemodilution of the circuit representing circuit priming *in vivo*. The largest percentage change was seen in fibrinogen, likely due to adhesion of the fibrinogen too the circuit in addition to haemodilution, however this was not statistically significant due to small valid sample size (n = 3). In comparison to haematocrit, there were smaller percentage changes following haemodilution in D-dimer levels, PAP complexes and TAFI. Haemodilution of tPA and PAI-1 was similar to the haematocrit.

Table 22 Median percentage change in fibrinolytic parameters after dilution for circuit priming compared the time of blood collection

Fibrinolytic Assay	*Median % change*
Haematocrit	-52.1%
Fibrinogen	**-73.4%**
D-dimer	**-11.8%**
tPA	-56.5%
PAP	**-5.7%**
PAI-1	-55.1%
TAFI	-**35.5%**

D-Dimers

There was an increase over the 24-hour period in D-dimer level in comparison to initiation although this was not statistically significant (461 ng/mL FEU [Fibrinogen Equivalent Units] at initiation 505 ng/mL FEU after 24-hours, p = 0.35) as shown in Figure 34(a). There was no observed difference at matched time points between the circuit and controls (505 ng/mL circuit FEU and 465 ng/mL control at 24-hours respectively, p = 0.67).

Plasmin-Antiplasmin Complexes

There were no significant changes in PAP complex levels over the 24-hour period in the *ex vivo* circuits (55.0 µg/L at initiation and 54.5 µg/L after 24-hours) show in Figure 34(b). There was no significant difference over the 24-hour period between the samples from *ex vivo* circuits and controls (54.5 µg/L vs 57.8 µg/L respectively at 24 hours).

Clauss Fibrinogen

There were no changes over the 24-hour period in fibrinogen levels in comparison with initiation of the circuit and the subsequent samples (1.0g/L at initiation and 1.1g/L at 24-hours) shown in Figure 34(c). There was no observed difference at matched time points between the circuit and controls (1.1 g/L circuit and 0.9 g/L control at 24-hours).

tPA Antigen

There was a decrease over time in tPA levels that was seen after 4 hours in the *ex vivo* circuit samples (1185 pg/mL at initiation to 815 pg/mL at 24-hours, p = 0.028) and 24-hours in the control samples (1185 pg/mL at initiation to 1103 pg/mL at 24-hours, p = 0.028) shown in Figure 34(d). There was a smaller decrease with controls over the 24-hour period in comparison to the *ex vivo* circuit (1103 vs 571 pg/mL respectively at 24 hours, p = 0.028).

PAI-1

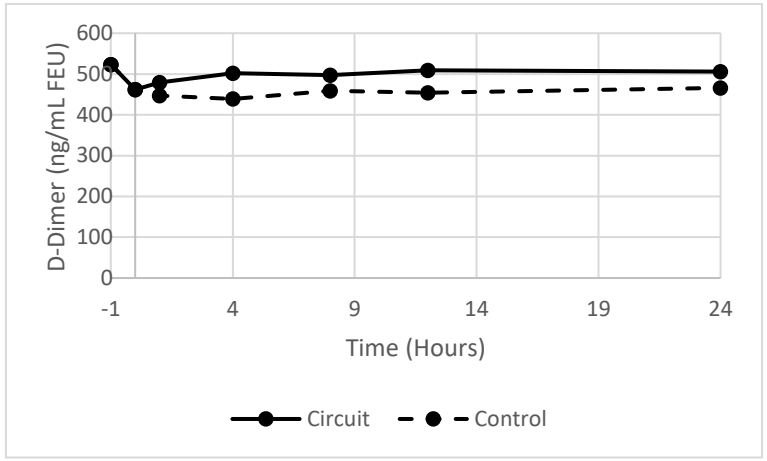
There was an increase in PAI-1 levels over time in both the *ex vivo* circuits (27.6 ng/mL at initiation and 52.1 ng/mL at 24-hours, p=0.028) and controls (27.6 ng/mL initiation and 37.8 ng/mL at 24-hours, p=0.027) at 24 hours shown in Figure 34(e). The median levels of PAI-1 were higher in the *ex vivo* circuits (52.1 ng/mL) in comparison to the controls (37.8 ng/mL, p = 0.028). There was a progressive increase in PAI-1 over time in all circuits.

TAFI Antigen

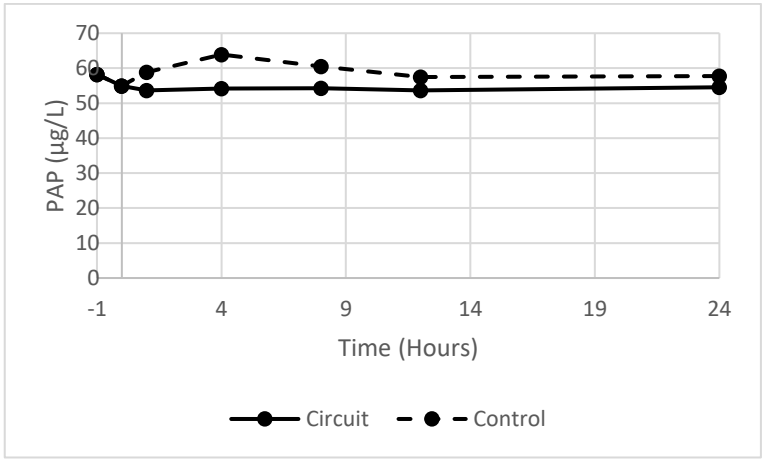
There was a non-statistically significant increase in TAFI levels from initiation to 24-hours in the *ex vivo* circuit (65.3 to 73.4%, p = 0.12) and control samples (65.3 to 76.8%, p = 0.16) shown in Figure 34(f). There was no statistically significant difference at any of the time point between the *ex vivo* circuits and control samples (73.4% circuits and 76.8% controls at 24-hours).

Figure 34 Changes in fibrinolysis over 24-hours in an ex vivo extracorporeal model (a) D-dimer (b) Plasmin-Antiplasmin (c) Fibrinogen (d) tPA (e) PAI-1 (f) TAFI

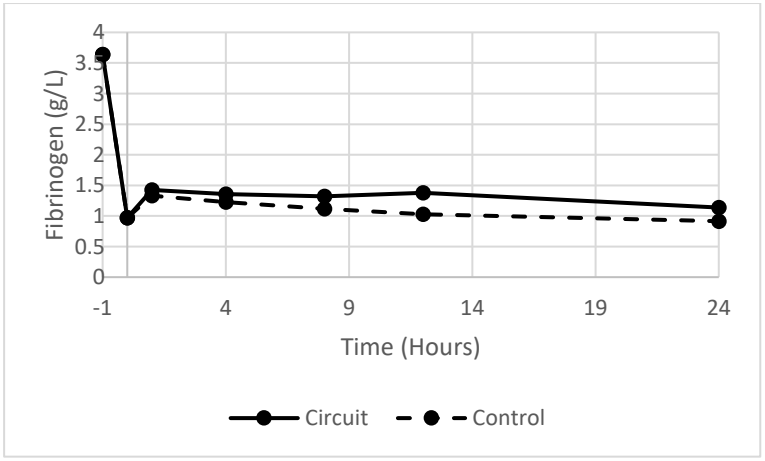
(a)



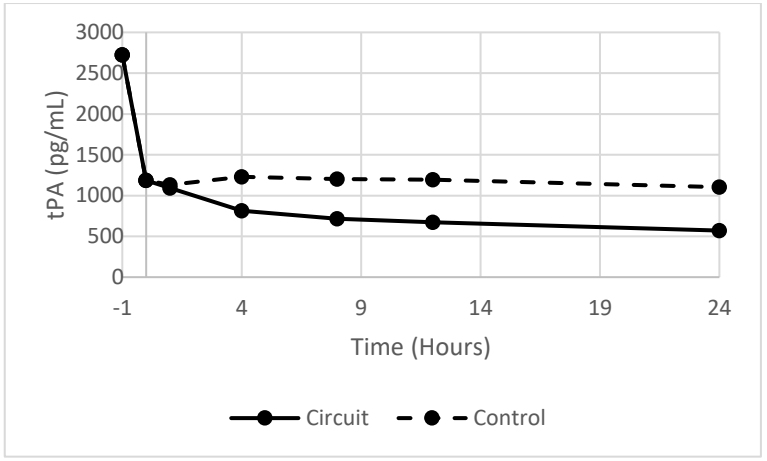
(b)



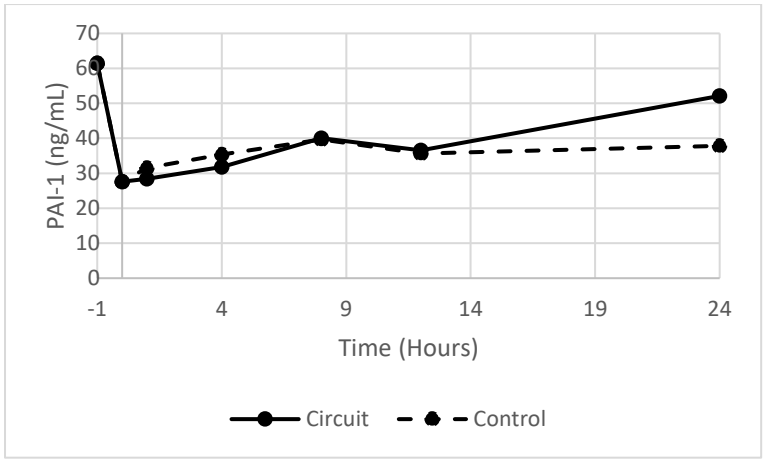
(c)



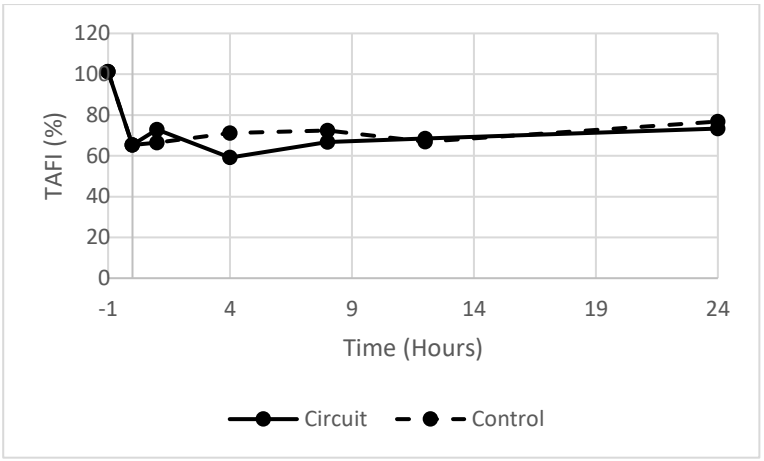
(d)



(e)


70
60
50
40
30
20
10
0
PAI-1 (ng/mL)
-1
4
9
14
19
24
Time (Hours)
Circuit
Control

(f)


120
100
80
60
40
20
0
TAFI (%)
-1
4
9
14
19
24
Time (Hours)
Circuit
Control

7.4.2 Fibrinolysis in adult patients during ECMO

Seventeen participants were reviewed in this analysis.

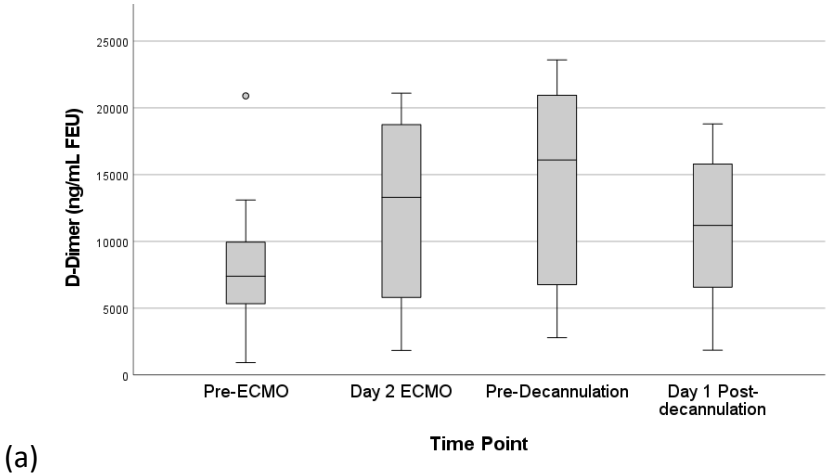
Temporal Changes in D-Dimer during ECMO

Normal reference range for D-dimer levels derived from 38 control samples were <400ng/mL. D-dimer levels were elevated at initiation of ECMO (median 7398 ng/mL FEU, IQR 5360 – 9721) shown in Figure 35. These levels continued to increase during the first two days of ECMO in comparison pre-ECMO (median D-dimer on Day 1: 9903 ng/mL FEU, IQR 4454 – 15,800, p = 0.036, median D-dimer on Day 2: 13,300 ng/mL FEU, IQR 7031 – 18,275, p = 0.023) but did not change after one week on ECMO. At the time of decannulation, D-dimer levels significantly decreased one day after but were elevated in comparison to normal levels (median prior to decannulation: 16,100 ng/mL FEU and day following decannulation: 11,200 ng/mL FEU, p = 0.05).

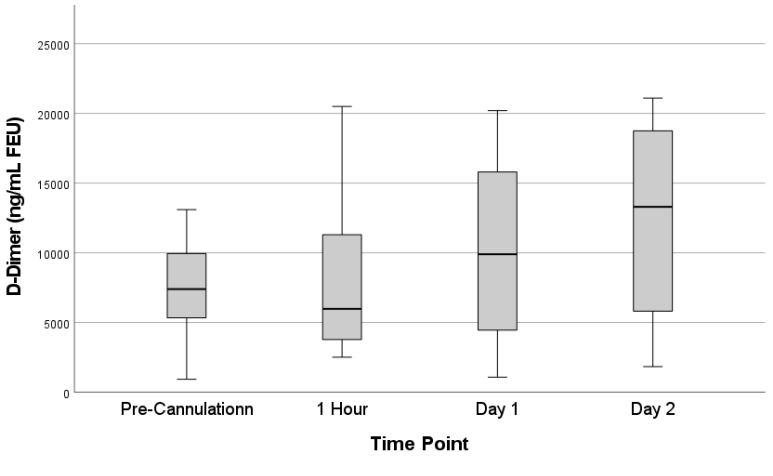
Temporal Changes in Plasmin-Antiplasmin Complexes

Normal PAP levels were 150-800 μg/L. PAP levels were mildly increased at the initiation of ECMO (median 1022 μg/L, IQR 757 - 2023) shown in Figure 36. These levels began to increase from day 2 of ECMO use (median 1797 μg/L, IQR 1309 - 2434, p = 0.023) and continued to increase further after 7 days (median 2776 μg/L, IQR 1400 – 3772, p = 0.05). There was a significant fall in PAP levels one day after decannulation (median prior to decannulation: 3378 μg/L and day following decannulation: 1756 μg/L, p = 0.006).

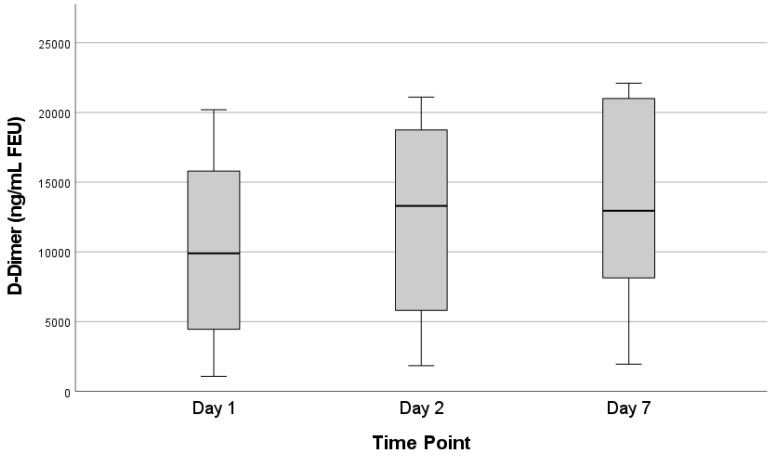
Figure 35 *Box plots for changes over time in D-Dimer levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation*



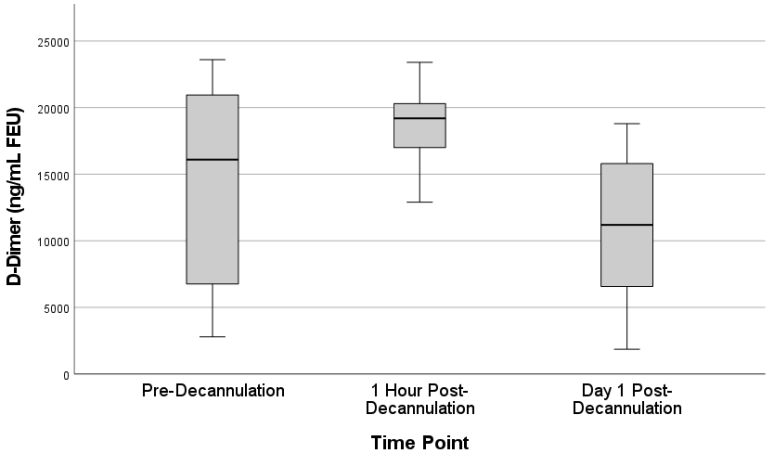
(a)



(b)

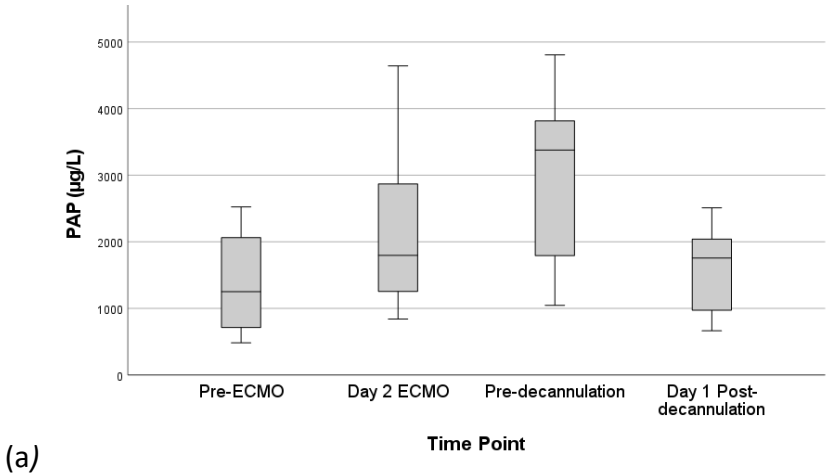


(c)

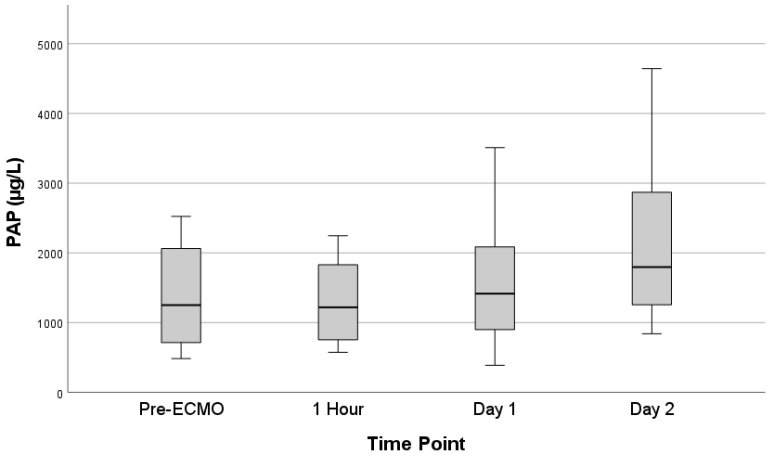


(d)

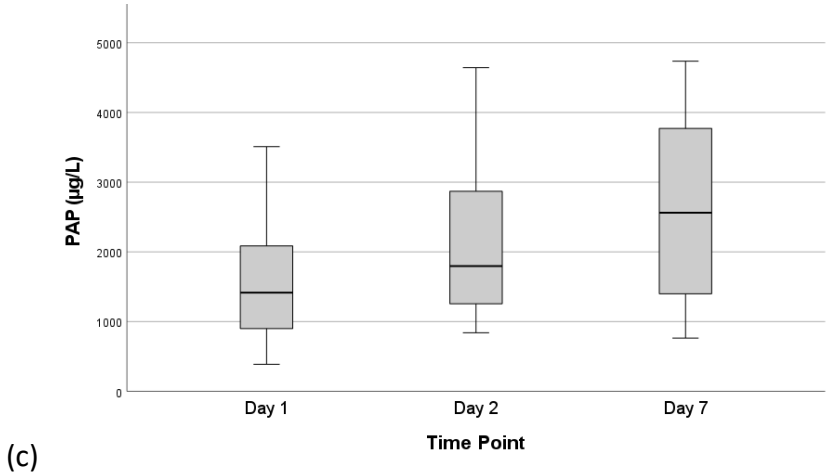
Figure 36 Box plots for changes over time in PAP levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



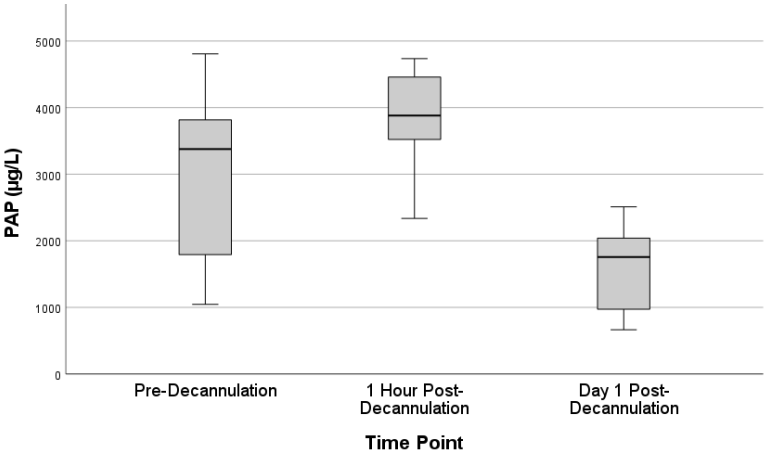
(a)



(b)



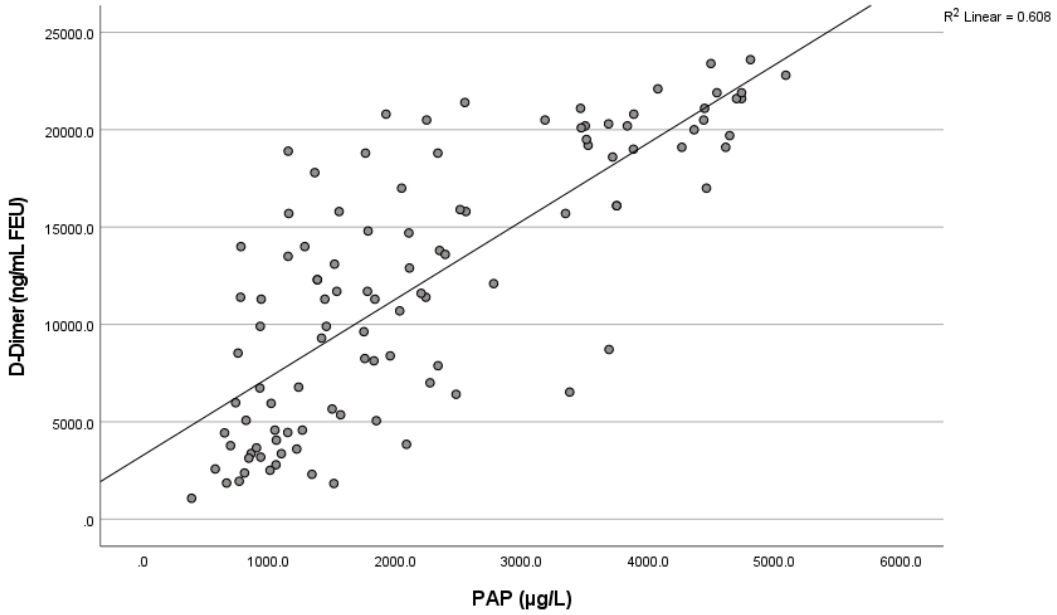
(c)



(d)

There was a strongly positive correlation show in Figure 37 between D-dimer levels and PAP in all samples (R = +0.6, p < 0.001). This did not change whether the samples were assessed from the time of ECMO initiation, during its use and around decannulation.

Figure 37 *Scatter plot comparing D-dimer to PAP levels during ECMO*



Temporal Changes in Fibrinogen

Normal levels of Clauss fibrinogen were 2.0 – 4.1 g/L. Fibrinogen levels were elevated prior to the initiation of ECMO (median 4.6 g/L, IQR 3.5 – 6.1) as expected in critical illness shown in Figure 38. These levels fell significantly following the first hour of ECMO use (median 3.3 g/L, IQR 2.5 – 4.3, p = 0.02), reflecting circuit adherence or haemodilution from circuit priming. Fibrinogen levels continued to remain low subsequently (median at Day 2: 3.34 g/L, p = 0.20 and median at Day 7: 2.51 g/L, p = 0.58). There was no significant change in fibrinogen levels following decannulation (median prior to decannulation: 2.3 g/L, one day after decannulation: 2.3 g/L, p = 0.19). Cryoprecipitate was given to 7/17 (41.2%) patients during ECMO.

Temporal Changes in tPA Antigen

Normal levels of tPA were <6531 pg/mL. tPA antigen levels were mildly elevated prior to ECMO use (median 9687 pg/mL, IQR 4265 - 14603) shown in Figure 39. These levels did not change following ECMO initiation nor during the subsequent 7 days (median 16,283 pg/mL, IQR 7619 - 19272). At decannulation, there was a small increase in tPA levels after 1 hour (median prior to decannulation: 6275 pg/mL, 1-hour after decannulation: 6385 pg/mL, p = 0.04) although this change did not persist to one day following this (median 5711 pg/mL).

Temporal Changes in uPA

Normal levels of uPA were 150 – 4799 pg/mL. uPA antigen levels were not increased prior to the initiation of ECMO (median 2517 pg/mL, IQR 1413 – 2780) as shown in Figure 40. These levels did not change following ECMO initiation, during its use and following decannulation. Following decannulation, uPA levels remained within normal ranges (median prior to decannulation: 2423 pg/mL, day following decannulation: 2090 pg/mL).

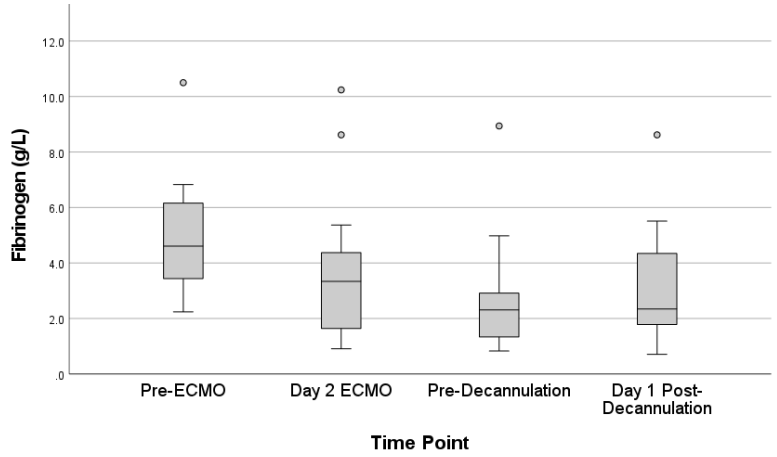
Temporal Changes in PAI-1

Normal levels of PAI-1 were <33 ng/mL. PAI-1 level were elevated prior to ECMO use (median 59.7 ng/mL, IQR 50.8 - 94) as expected in critically ill patients shown in Figure 41. Following initiation of ECMO, these levels did not change over the first two days (median at Day 2: 42.6 ng/mL, p = 0.10 and after one week: 66.6 ng/mL). There were no significant changes in PAI-1 levels following decannulation (median prior to decannulation: 35.5 ng/mL and day follow decannulation: 30.4 ng/mL, p = 0.89).

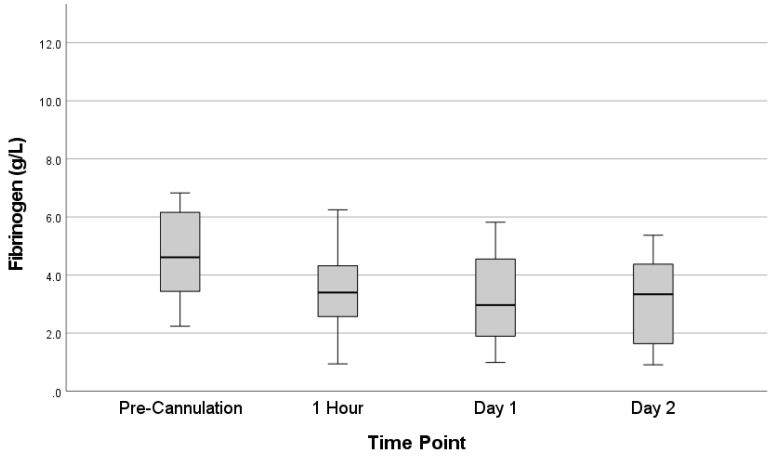
Temporal Changes in TAFI

Normal levels of TAFI were 42 – 154%. TAFI antigen levels were not increased prior to the initiation of ECMO (122%, IQR 113 – 148%) shown in Figure 42. These levels did not change following ECMO initiation (median at day 2 149%), during its use (median at Day 7 164%) and prior to decannulation (median day following decannulation 168%). Following decannulation, TAFI levels were mildly elevated (165%).

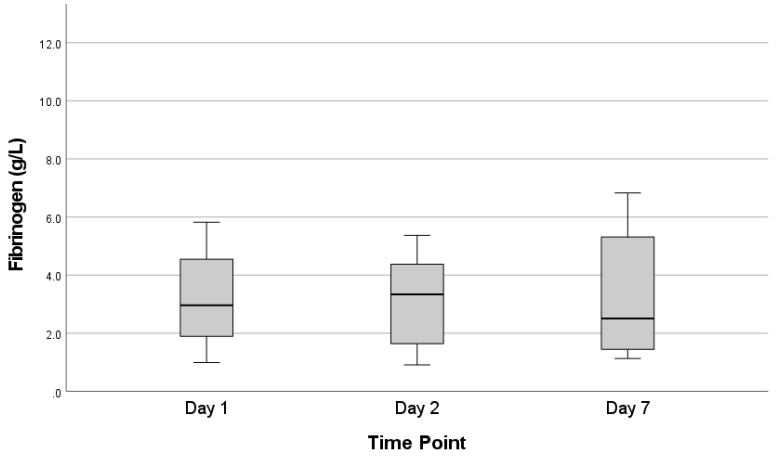
Figure 38 Box plots for changes over time in fibrinogen levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



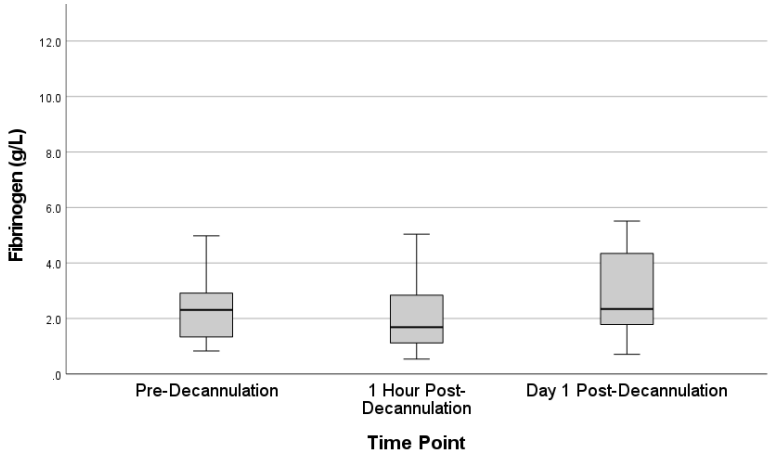
(a)



(b)

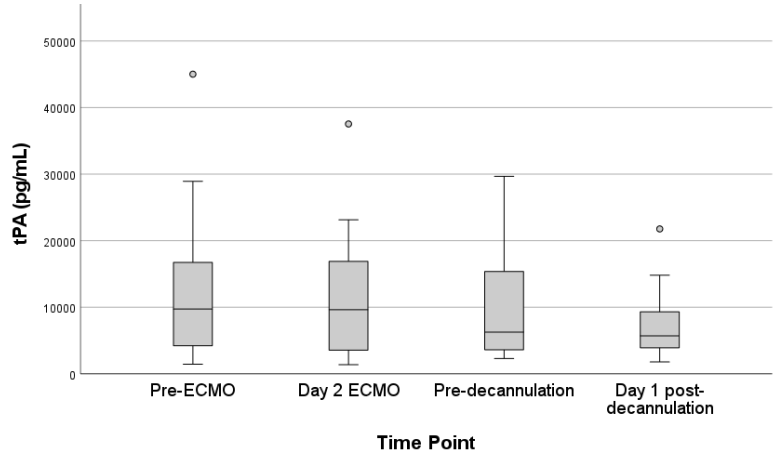


(c)

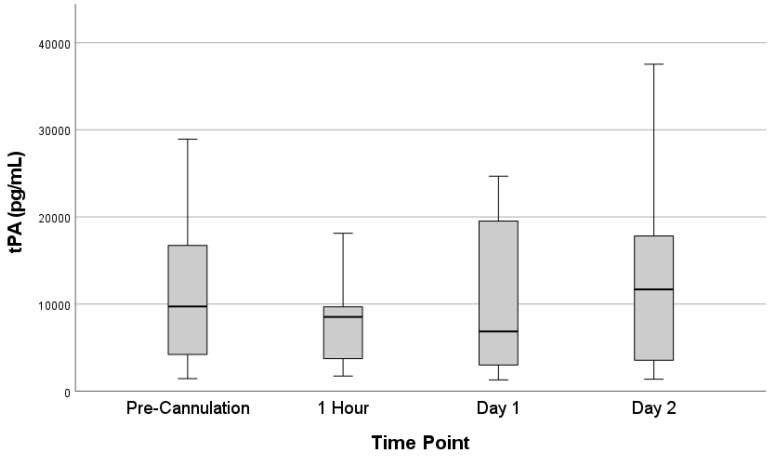


(d)

Figure 39 Box plots for changes over time in tPA levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



(a)



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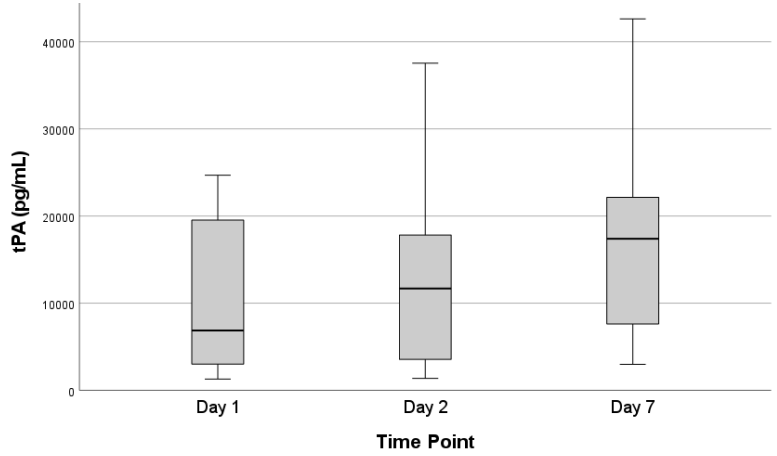
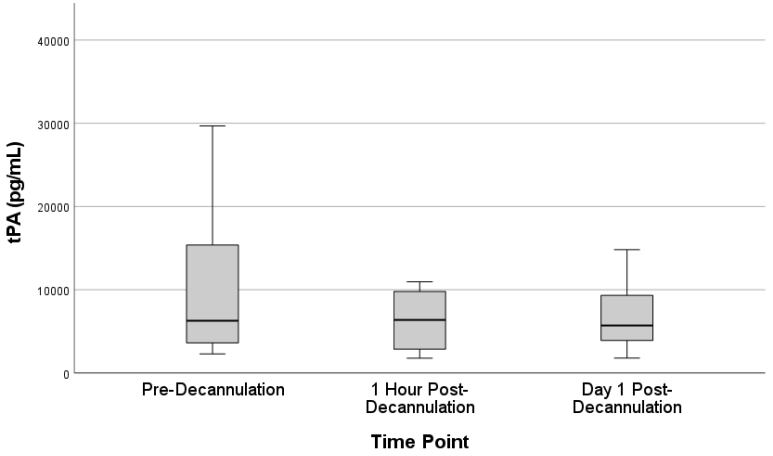
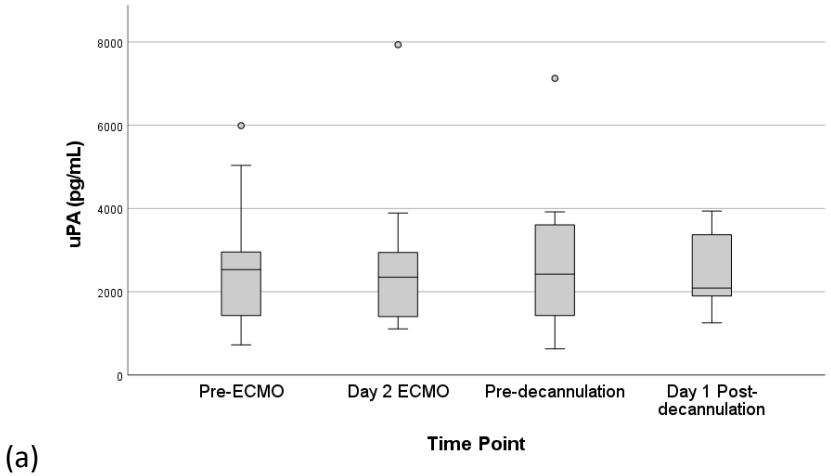


(c)

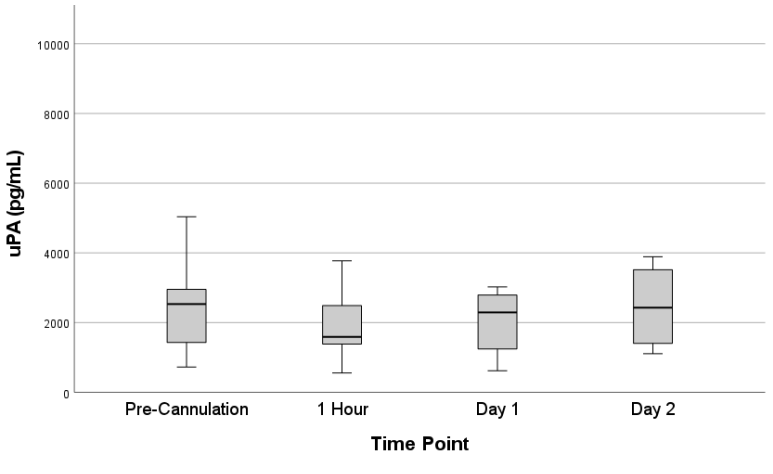


(d)

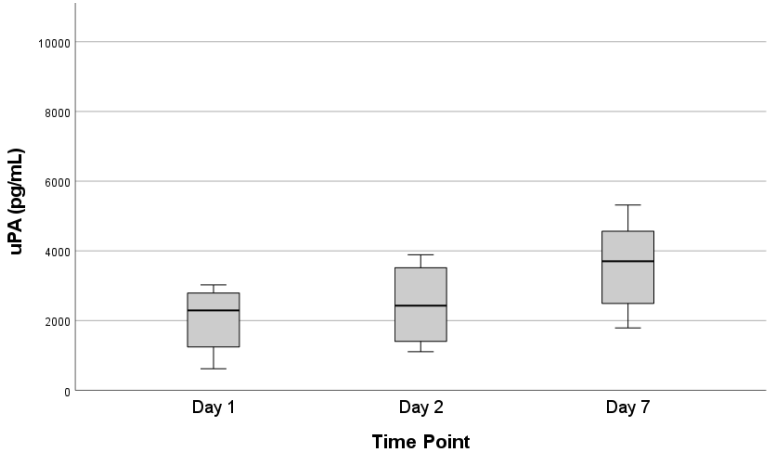
Figure 40 *Box plots for changes over time in uPA levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation*



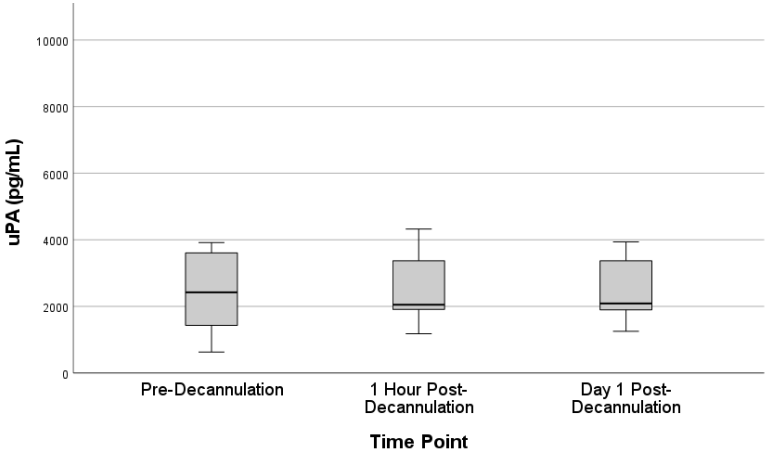
(a)



(b)



(c)



(d)

Figure 41 *Box plots for changes over time in PAI-1 levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation*

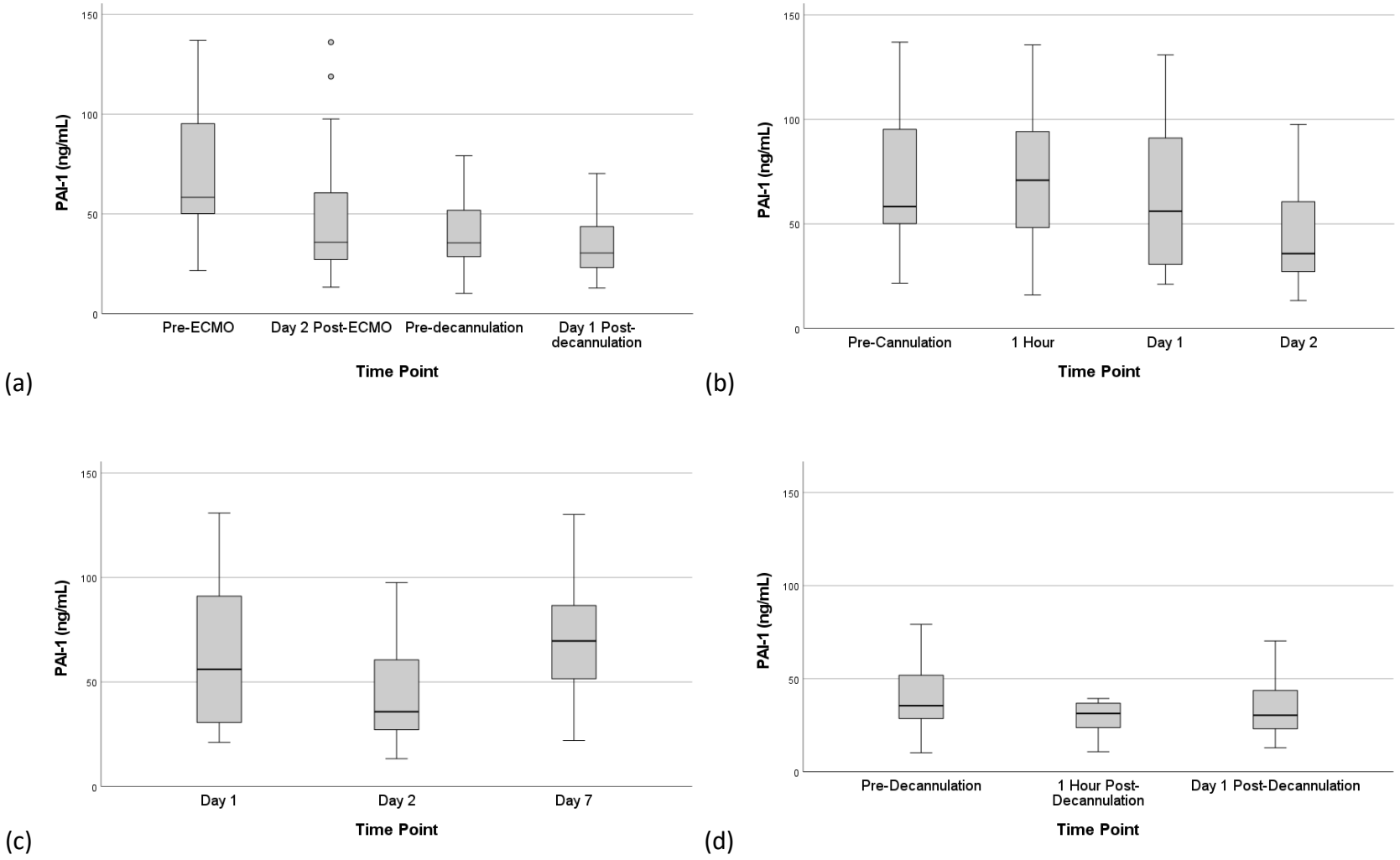
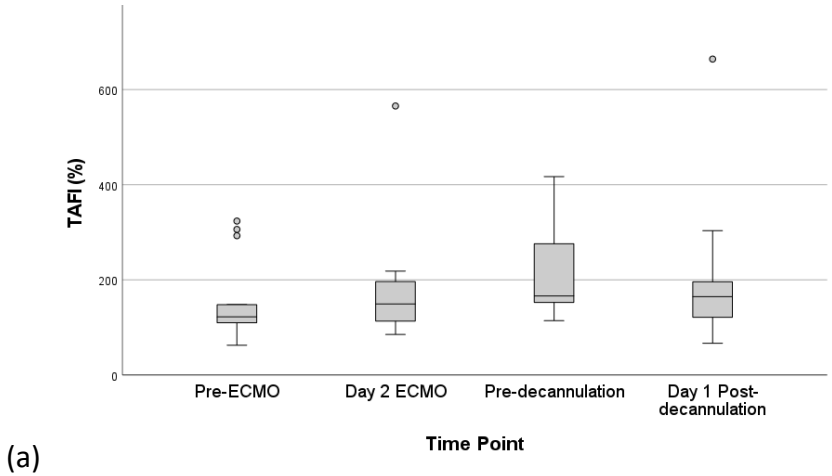
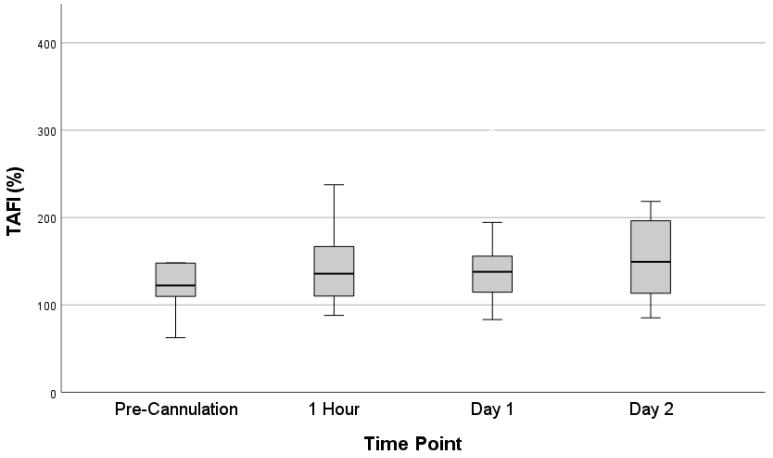


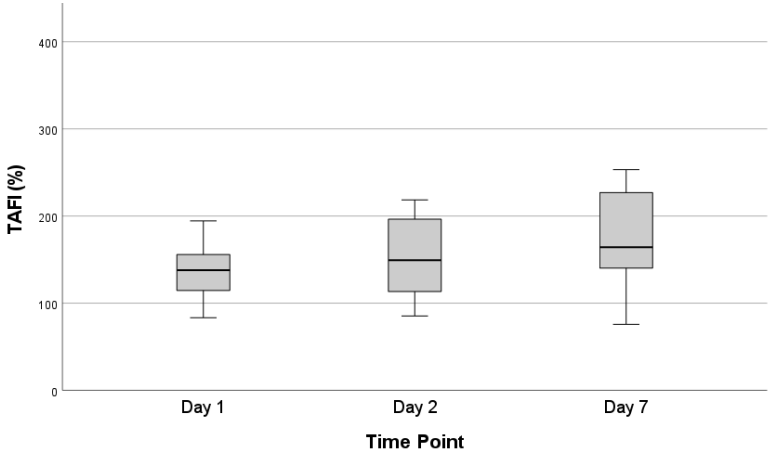
Figure 42 Box plots for changes over time in TAFI levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



(a)



(b)



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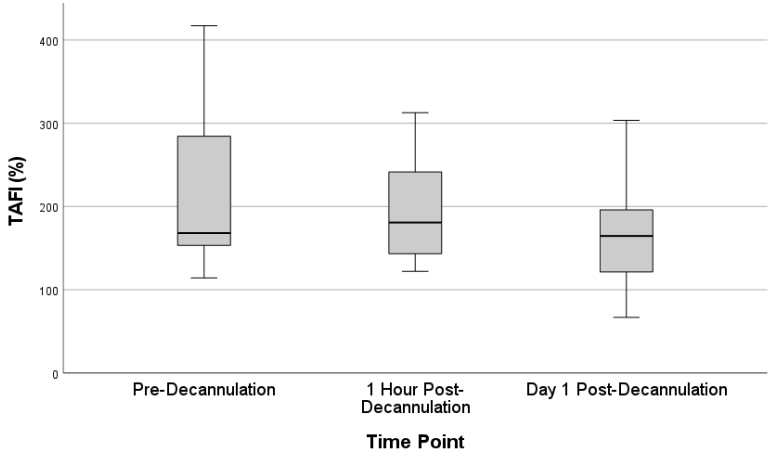


(d)

7.4.3 Fibrinolysis during haemorrhagic and thrombotic complications of Extracorporeal Membrane Oxygenation

Intracranial Haemorrhage

Blood samples used were those prior to starting ECMO and 1-hour after starting ECMO. All ICH were detected at screening imaging within 6-hours of ECMO initiation. There was no significant difference between the previously described fibrinolytic parameters prior to ECMO or 1-hour after the commencement of ECMO in those with ICH (n=4) to those without (n=13). When comparing the difference in fibrinolytic parameters between these time points there was no significant difference. There was a trend to significance for the change in D-dimer levels prior to and 1-hour following ECMO being increased in those with ICH (median change with ICH +2421 ng/mL FEU, median change without ICH = -705 ng/mL FEU, p = 0.069, Z-test statistic -1.82) (Figure 43). This was not reflected by a similar change in PAP (median change with ICH 202 µg/L and without ICH 70 µg/L, p = 0.68)

Pulmonary Embolism

Blood samples reviewed were those taken prior to initiation or 1-hour after initiating ECMO. All PEs were detected on initial screening CT scans. D-dimer levels were significantly increased in those with PE (n = 4) to those without PE (n = 13) after 1 hour of starting ECMO (median with PE 9948 ng/mL FE and median without PE 6192 ng/mL, p = 0.047) with a trend to significance prior to ECMO (p = 0.10) shown in Figure 44. There was a trend to significance for higher levels of PAP prior to and 1-hour after starting ECMO in those with PE (Median prior to ECMO with PE 2194 µg/L and without PE 1003 µg/L, p = 0.078. Median 1-hour after ECMO with PE 1836 µg/L and without PE 1008 µg/L, p = 0.10) and fibrinogen levels prior to ECMO (median with PE 4.42 g/L and without PE 4.25 g/L, p = 0.08).

Deep Vein Thrombosis

Fibrinogen levels prior to decannulation were higher in those with Ca-DVT (n = 7, median = 3.9 g/L) diagnosed after decannulation from ECMO at the cannula insertion sites than those without (n = 11, median = 1.5 g/L) (p = 0.05, Z-test statistic = -1.96) (Figure 45(a)). There were no other significant differences in fibrinolytic parameters including D-dimer (16100ng/L FEU without DVT vs 16350 ng/L FEU with DVT, p = 0.85, Figure 45(b)) prior to decannulation in those with or without a subsequent diagnosis of Ca-DVT.

Non-Intracranial Haemorrhage

Blood samples occurring on the same day or within 24-hours preceding a bleeding event were included. These were compared to all available blood samples from patients who did not have bleeding events. Samples were excluded if prior to and 1-hour after starting ECMO in the absence of bleeding, following decannulation and within 1-day of a circuit change.

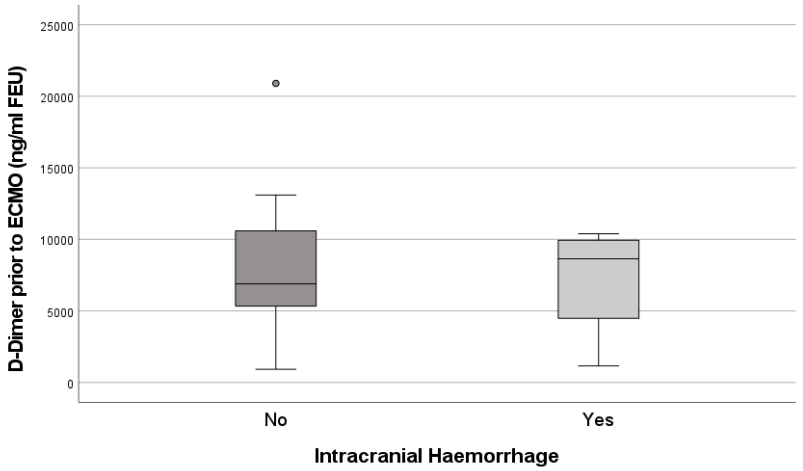
There were 3 patients with NICMH with 6 relevant blood samples to assess shown in Figure 46. In those with NICMH, TAFI levels were lower in those episodes (n = 3, median = 114.5%, p = 0.004) to those who did not have bleeding (n = 14, median = 154.5%). PAI-1 levels were higher in those with NICMH during these episodes (median = 111%, p = 0.003) to those who did not (median = 54.3%). There was no other statistically significant different between the other fibrinolytic parameters for patients with and without NICMH.

There were 10 patients who had CRNMB with 14 relevant blood samples taken to assess, which are shown in Figure 47. At the time of CRNMB, levels of uPA were significantly higher in than those that had bleeding (median 3874 pg/mL, p = 0.013) than those without bleeding (1439 pg/mL). There was a trend to significance for lower fibrinogen levels in those CRNMB than those without (1.5 g/L vs 2.6 g/L, p = 0.10).

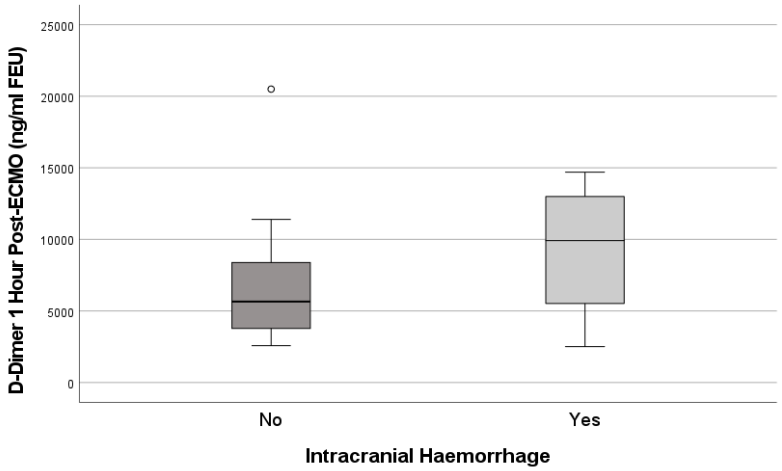
Circuit Change

There were five patients who had six circuit changes during their ECMO runs, although the samples were limited to four patients. There was no identified difference in fibrinolytic factors in those who had circuit changes and those that did not on matched days. There was a trend to significance for PAP levels to be increased preceding a circuit change to those who did not ($p = 0.09$). There was a decrease in the level of PAP and D-dimer pre- and post-circuit change, although this was not statistically significant due to small patient numbers shown in Figure 48.

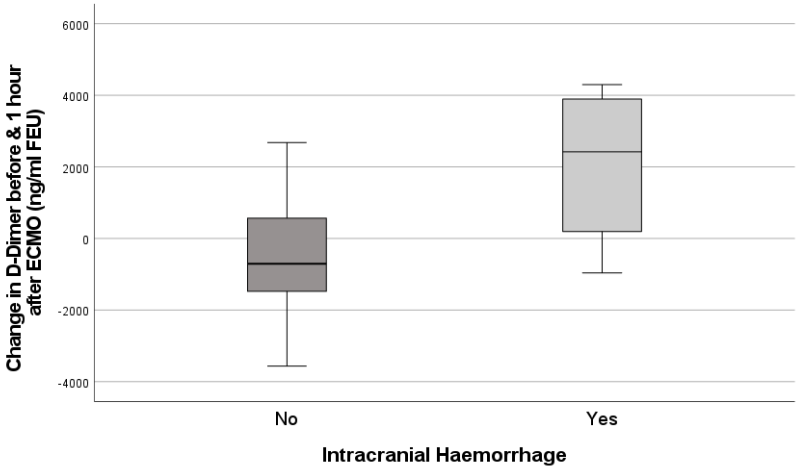
Figure 43 Box plots of D-dimer levels in patients with and without ICH (a) Prior to ECMO (b) 1-Hour after ECMO (c) Change between prior to and 1-hour after ECMO



(a)

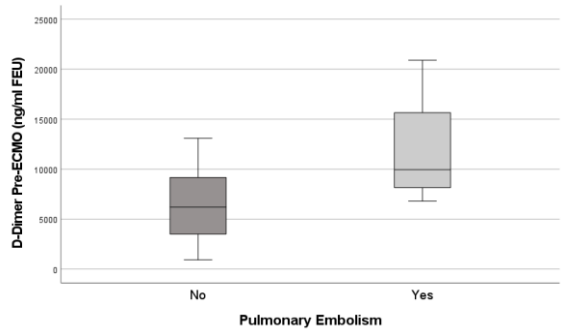


(b)

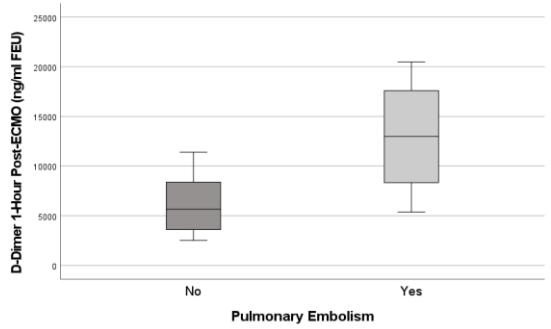


(c)

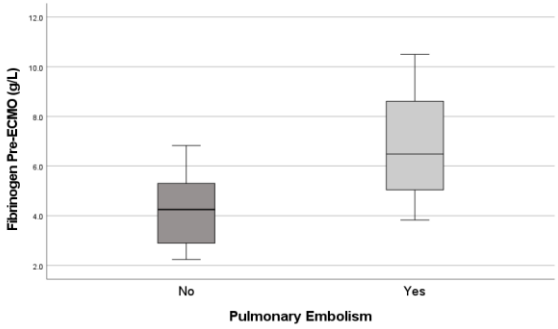
Figure 44 Box plots of fibrinolytic factor levels in patients with and without PE (a) D-dimers prior to ECMO (b) D-dimers 1-Hour after ECMO (c) Fibrinogen prior to ECMO (d) Fibrinogen 1-Hour after ECMO (e) PAP prior to ECMO (f) PAP 1-Hour after ECMO



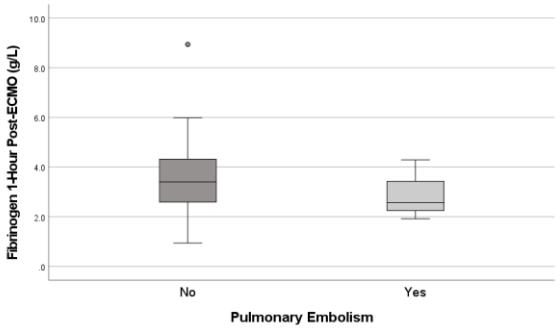
(a)



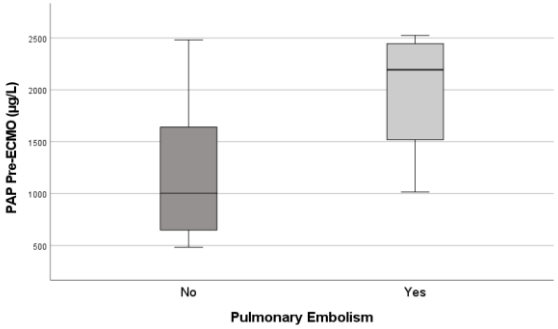
(b)



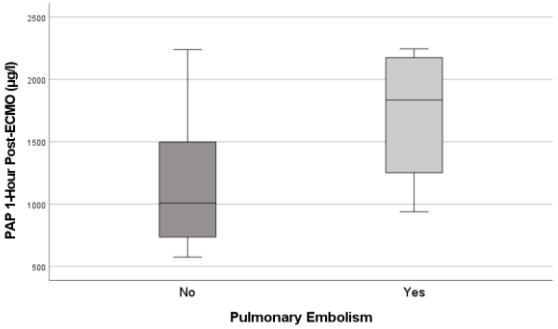
(c)



(d)

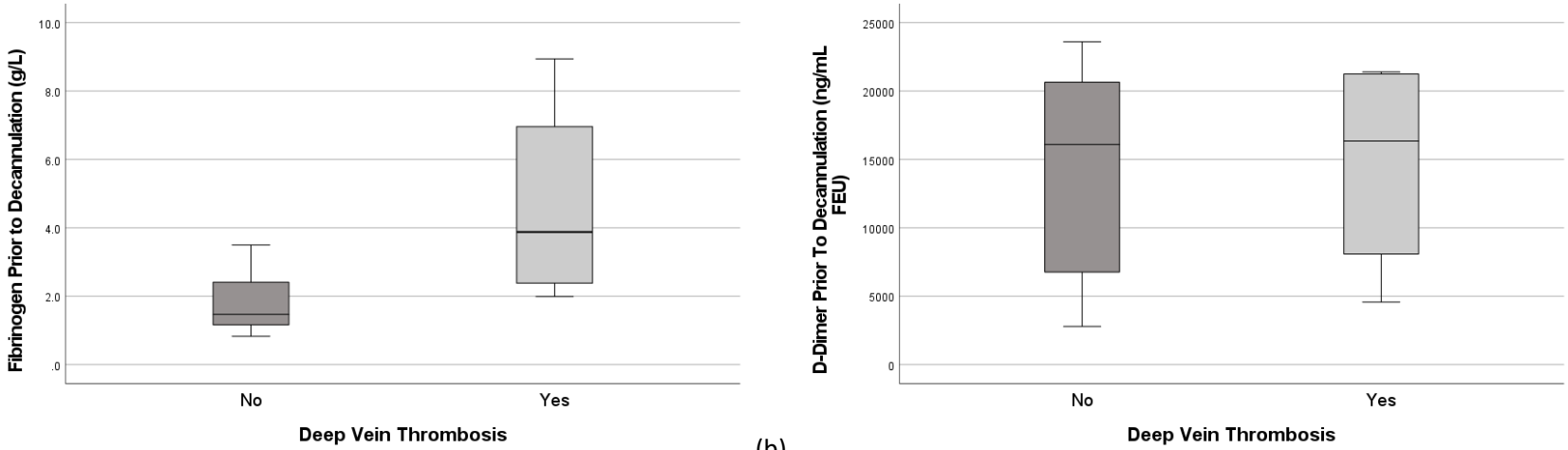


(e)



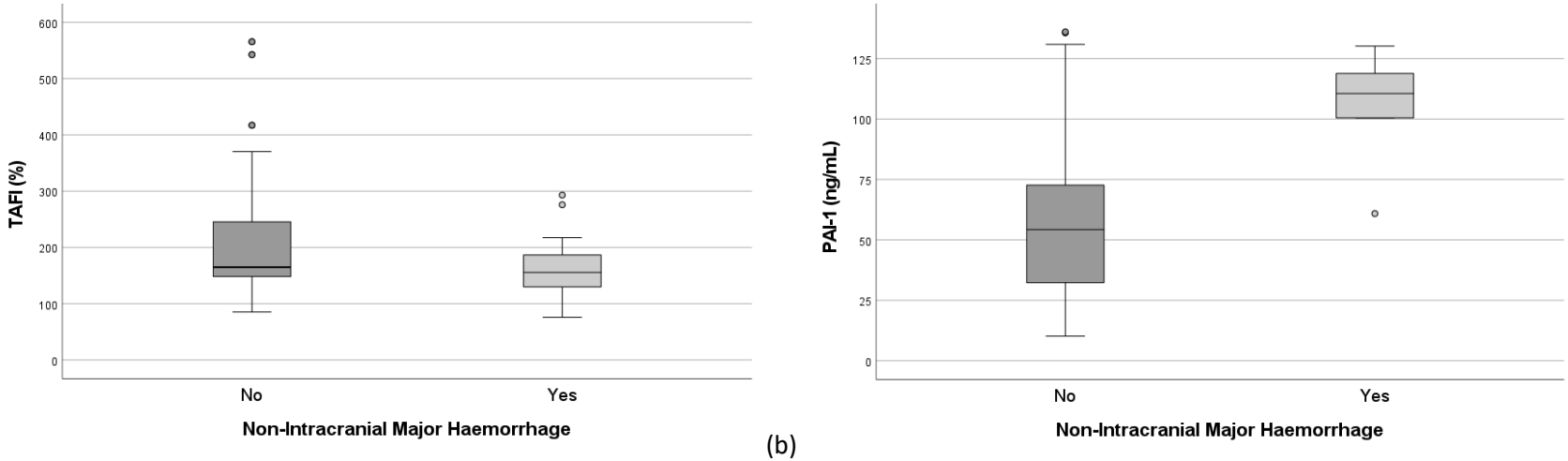
(f)

Figure 45 Box plots of fibrinolytic factor levels in patients with and without DVT prior to decannulation (a) Fibrinogen (b) D-Dimer



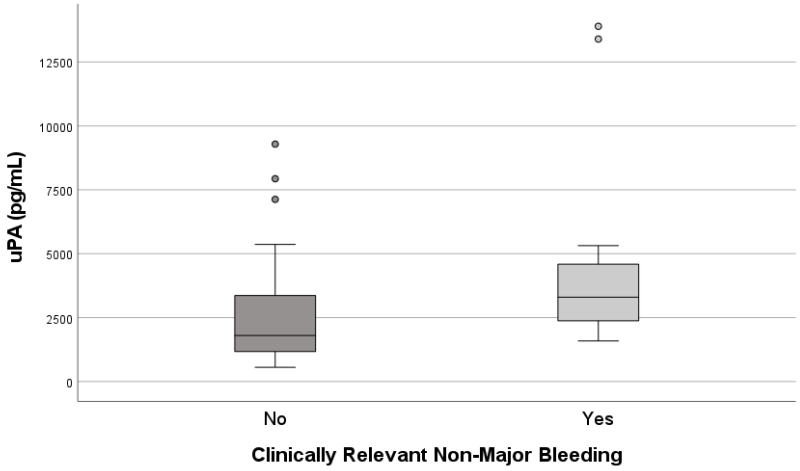
(a) (b)

Figure 46 Box plots of fibrinolytic factor levels in patients preceding and without NICMH (a) TAFI (b) PAI-1

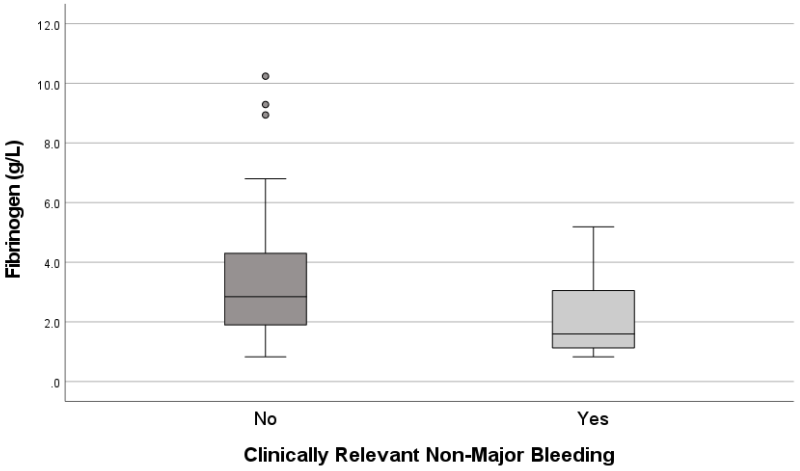


(a) (b)

Figure 47 Box plots of fibrinolytic factor levels in patients preceding and without CRNMB (a) uPA (b) Fibrinogen

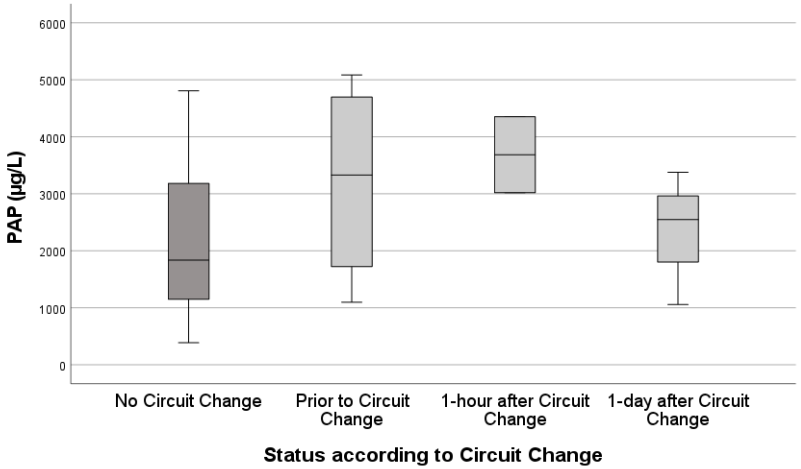


(a)

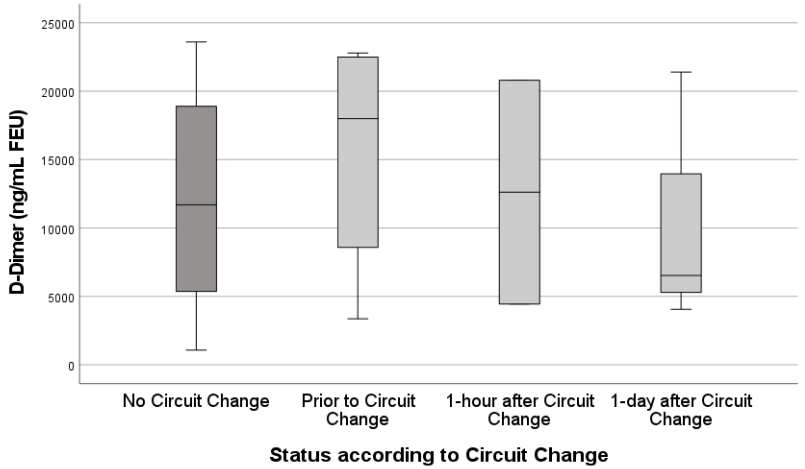


(b)

Figure 48 Box plots of fibrinolytic factor levels in patients around ECMO circuit changes (a) PAP (b) D-dimer



(a)



(b)

7.5 Discussion

The role of fibrinolysis during ECMO remains poorly understood along with its subsequent impact upon the development of bleeding and thrombosis. The purpose of this study was to assess these changes and to identify potential contributing factors.

Prior to the initiation of ECMO, there were several altered fibrinolytic factors attributable to the pro-inflammatory state of the patients. In this cohort, there was a global activation of all components of the fibrinolytic pathway with elevated levels of D-dimer, PAP, fibrinogen, tPA and PAI-1. This pattern is recognised in sepsis, hypoxia and inflammatory state, in this case released from the endothelium as a result of severe lung injury release [331–334]. This concurs with similar results of elevated tPA and PAI-1 described by McVeen *et al* in neonates requiring ECMO[317]. In contrast, in elective patients undergoing cardiac surgery using cardiopulmonary bypass with normal preceding inflammatory markers, levels of PAP and D-dimer are not elevated until the procedure and CPB commences[320].

Subsequently, various changes were seen in fibrinolysis with the use of ECMO. At initiation, the key findings were a decrease in fibrinogen levels during first 2 days of ECMO use in patients and in the *ex vivo* circuits, particularly in the first few hours, and a continued increase in D-dimer levels. The decrease in fibrinogen was due to haemodilution of fibrinogen in the *ex vivo* circuits but the fall was greater than seen with the haematocrit, similar to what is seen in CPB[335]. Additionally, there is adhesion of fibrinogen to the extracorporeal circuit explaining a larger percentage fall.

Early elevation of D-dimer levels was seen with relatively normal PAP levels initially. However, increases in PAP were seen after the first 2 days of ECMO use with on-going increases in D-

dimer levels. Hundalini *et al* also showed a correlation to PAP and D-dimer levels during ECMO in neonates and by Chander and Velan during CPB[288]. However, this change in PAP was not seen after 24 hours in the *ex vivo* circuits suggesting that fibrinolytic activation is secondary to thrombin generation. This can be considered in the context of trauma and sepsis were PAP levels are elevated in the majority of patients[336,337]. However, the median PAP levels described by Raza *et al* in severe trauma were also grossly elevated, which reflected the pre-ECMO state. PAP levels were twice as high at the time of decannulation in this cohort than at ECMO initiation. It appears that a combination of the extracorporeal circuit and the inflammatory state of the patient aboth interplay during ECMO use to cause hyperfibrinolysis.

D-dimer levels and PAP subsequently decreased following decannulation in patients demonstrating a key role in the extracorporeal circuit in activating fibrinolysis with circuit removal or change. Possible causes of this may the secondary activation of the fibrinolytic system due to excess thrombin generation because of microclot formation occurring within the oxygenator membrane or from the direct activation of blood cellular components such as platelet and NETs release or impairing fibrinolytic factors [304,338,339]. TAFI for example although principally secreted by the liver is released by activated platelets[340]. There was significant change in the individual fibrinolytic markers during ECMO use in patients although there was an increase in PAI-1 levels and reduction in t-PA in the circuit model over 24-hours. The reduction of the latter may be due to the formation of complexes between tPA and PAI- 1 although the tPA ELISA assay detected tPA in these complexes. An increase in PAI-1 may be attributed to release from α-granules in platelets from shear stress[148,149,341,342]. Following decannulation, tPA and PAI-1 levels had generally normalised whereas PAP and D-dimer levels remained elevated although lower than the peak levels during ECMO use.

The results are largely similar to those recently described by Hékimian *et al* when comparing fibrinolytic changes in patients with COVID-19 to other causes of respiratory failure during ECMO[298]. One key difference was a statistically significant reduction in PAI-1 levels, which only showed a trend to significance in these results. However, in the study by Hékimian *et al* ECMO duration was a median of five days with blood sampling occurring after 1, 7 and 14 days from ECMO cannulation with some patients not receiving ECMO at those time points.

This study looked at the role of uPA as a potential contributing factor to fibrinolytic activation during lung injury. Previous studies have shown that uPA is upregulated in the lungs in ALI as a result of release by activated macrophages in the injured pulmonary parenchyma[312]. Increased plasma levels of uPA were not demonstrated in this patient cohort requiring ECMO for SRF, although it was seen in one patient with haemophagocytic syndrome, a condition with gross macrophage activation. However, uPA was measured as a circulating protein rather than localised to the pulmonary vasculature, where fibrinolysis is likely to be required due to fibrin deposition in the damaged lung[312,343]. Furthermore, this study has not looked at the role of the receptor for uPA, uPAR and the co-factor of S100A which can be upregulated in lung injury and sepsis contributing to fibrinolysis[344,345]. Although not elevated in comparison to normal controls, uPA levels were higher in those with a milder bleeding phenotype than those without bleeding.

Despite a small event rate, several fibrinolytic changes were shown to be significant in relation to clinical bleeding. The change in D-dimer levels prior to and 1-hour after starting ECMO was higher in those with ICH than those without. This may suggest that increased fibrinolysis may be a contributing mechanism during the development of ICH as well other risk factors such as thrombocytopenia, hypoxic brain injury and anticoagulation. Elevated D-dimer levels have

previous been described in those with progression of bleeding following traumatic brain injury[305]. Lower TAFI levels were also seen in NICMH bleeding events conferring an increased fibrinolytic state. Of interest, PAI-1 levels were increased following major haemorrhage during ECMO although this may be a secondary response to maintain haemostasis[346].

With regards to thrombosis, D-dimer levels were elevated in patients with PE at initiation of ECMO. This is in keeping the findings described in Chapter 2. A hyperfibrinogenaemic state was seen in those with DVT prior to decannulation. Higher rates of DVT were seen in those with cryoprecipitate transfusions as described in Chapter 3, and high rates of DVT in patients with COVID-19 who had increased levels of fibrinogen in comparison to other viral and bacterial pneumonias described in Chapter 2. Hypofibrinolysis as shown by low PAP levels was not seen in this cohort, in keeping with hypofibrinolysis not being a recognised a risk factor for thrombosis.

Several limitations of this study were identified to assess fibrinolytic changes during ECMO. There was a small sample size in the patient cohort, which may be under powered to detect significant changes, such as PAP, that showed only a trend significance despite different median levels. However, as a pilot study this has provided insight into further potential markers to assess in larger cohorts. This study is also limited by the lack of global fibrinolytic activity such as the euglobulin lysis time and TEG. These assays were not possible due to the long transportation time of samples from hospitals where ECMO was initiated; therefore, it was decided to use an alternate assay of fibrinolytic activity - PAP complexes. The use of cryoprecipitate and FFP may have also distorted results, and a larger change due to haemodilution was seen with the *ex vivo* circuits when compared to patient samples, making direct comparison inappropriate as suggested in Chapter 5.

7.6 Conclusions

Fibrinolytic activity changes were observed in both an *ex vivo* model of ECMO and in patients with SRF receiving ECMO. These data show that the continued activation of fibrinolysis during ECMO with pilot data suggesting that fibrinolytic activation may be associated with bleeding.

Chapter 8 – Circulating Histones during ECMO

8.1 Introduction

The role of intracellular histones has been well established as the protein scaffold that allows DNA packaging within the nucleus in the form of nucleosomes. However, their role as extracellular circulating proteins has become of increasing interest in understanding the response to organ injury and infection and their pathogenic role in sepsis, multiorgan failure and trauma[43,347]. They have become implicated in the prothrombotic state that can be seen in these conditions.

Histones can be considered in the subgroups of core histones, H2A, H2B, H3 and H4 and linker histones, H1 and H5. The core histones form dimers with either H2A and H2B or H3 and H4. These dimer structures subsequently bind together as 4 units to form an octameric nucleosome. DNA coils around the nucleosome and is tethered by the linker histone H1. Histones, particularly H3 and H4 which have extra-nucleosomal tails, can undergo post-transcriptional modification by various enzymes. Citrullination by the intranuclear enzyme peptidylarginine deiminase 4 (PAD4) adds a keto group to arginine residues on these histone tails to form citrulline[348]. Due to the change in charge following citrullination, DNA can unbind from the nucleosome[349].

Upon cellular injury, histones, DNA and other intracellular proteins are released acting a DAMPSs allowing for localisation and activation of the innate immune system at the site of cellular damage. Neutrophils are typically activated by bacterial lipopolysaccharides, complement and toll-like receptors (TLR)-2 and -4 undergo the process of NETosis, a form of cell death distinct from apoptosis. During this process, uncondensed neutrophilic DNA is

extruded from the cell forming an extracellular matrix. These are known as neutrophil extracellular traps (NETs). This process is dependent on the citrullination of histones to allow for the extracellular release of neutrophilic DNA. NETs can bind either intracellular or circulating histones and other bactericidal proteins, such as neutrophil elastases and myeloperoxidase, acting as a surface for the breakdown of pathogens and to act as a physical intravascular barrier to stop their spread. Megakaryocytes and platelets have also been found to be sources of extranuclear histones although not involved in NETosis[350].

At present, there are no specific assays for NETosis. Total circulating histones and cell-free DNA as DAMPs can be reflective of cellular damage or NETosis, which occur as concurrent processes in critically unwell patients. However, the measure of citrullinated histones reflects the presence of NETosis although citrullination may not occur in all disease conditions involving NETosis[347,351].

Circulating histones have been demonstrated as cytotoxic to pulmonary tissues and others following trauma and sepsis[43,334,352]. Xu *et al* demonstrated that H3 and H4 are the histones subtypes associated with cytotoxicity. This effect was reduced with the use of infusions of APC in murine models and human studies. APC was suggested to be an *in vivo* inhibitor by directly cleaving H3 and H4 histones[43]. Circulating histone levels have also been correlated with organ injury and illness severity scores in sepsis and trauma[352–354]. Recently, levels of total H3 and citrullinated H3 have been correlated with disease severity in patients with COVID-19 infections and shown to be higher than in other causes of critical illness[355,356]. Injury to the blood-brain barrier due to the presence of NETs has been shown to contribute to tPA-induced cerebral haemorrhage following stroke in murine models[357].

Histones have been directly implicated in the activation of coagulation, which may contribute to mechanisms of organ injury during critical illness. Pozzi *et al* described the effect of direct prothrombin activation and inhibition of the prothrombinase complex by H4 histones[358]. They showed that H4 caused autoactivation of prothrombin in the presence of FXa and absence of phospholipids. However, this was inhibited in the presence of FVa. Similarly, Abrams *et al* showed that histones can form an alternative prothrombinase complex with the presence of FXa and phospholipid resulting in increased thrombin generation. They also showed that histone levels were higher in patients with DIC than those without in ICU, which they suggest was mediated by the aforementioned mechanism. A dose-dependent effect of histones is seen in thrombin generation with platelet dependence with a reduction of Protein C activation by thrombomodulin[359,360]. Histones also attenuate the effects of different types of heparins by predominantly affecting their anti-Xa activity[44,361].

Histones have been shown to cause platelet dysfunction and activation. Thrombocytopenia is a common abnormality in patients during critical illness. Alhamdi *et al* described higher levels of circulating histones inversely correlated to platelet counts on admission to critical care.[45] At a histone levels of >30 μg/mL, there was a sensitivity and specificity of 79% and 89% respectively for the subsequent development of thrombocytopenia. Extracellular histones, in particular H3 and H4, have also been demonstrated to cause platelet activation and aggregation with the secretion of P-selectin, von Willebrand factor and phosphatidylserine[44,360,362]. TLR2 and TLR4 are implicated with histone binding to platelets, causing platelet aggregation by fibrinogen binding to GPIIb/IIIa leading to the subsequent formation of microaggregates and thrombocytopenia[44,362]. Protection from this process was achieved by a dose-dependent use of unfractionated heparin, which may adsorb circulating histones[44]. Murine models with histone infusions have shown protection from lung injury if

heparin was co-administered[44]. In the setting of trauma, H4 has been demonstrated to cause platelet ballooning and platelet microparticle release[54].

Apart from platelets, other cells are affected by circulating histones adding to a prothrombotic tendency. Gould *et al* have shown that histones can cause monocyte activation leading to increased tissue factor secretion contributing to thrombin generation, which again can be attenuated by heparin[363]. Histones can cause endothelial activation with upregulation of tissue factor and downregulation of thrombomodulin[364]. These processes have been shown again to be dependent on TLR2 and TLR4.

There are limited studies describing the role of histones in extracorporeal circuits such as ECMO and CPB, which have been summarised in Table 23. These studies highlight that histone levels correspond to underlying disease severity. Additionally, the use of the extracorporeal circuits during surgery can lead to an increase in levels of histones. At present, it is unclear in the setting of ECMO whether circulating histones are due to cellular damage from SRF and the circuit, or due to neutrophil activation causing NETosis by infectious and inflammatory processes. Additionally, histone levels have not been reviewed in those having haemostatic complications during ECMO.

Table 23 Studies describing extracellular histones during use of extracorporeal circuits

Study	*Cohort Size*	*Events*	*Outcome(s)*	*Comments*
Winnersbach. Perfusion. 2021[270]	4 *ex vivo* circuits: 2 with PRP and 2 with PPP	NET formation (MPO-DNA level), clot stability (TEG), platelet activation (surface P-selectin) at 1, 2, 4 & 6 hours. Clot structure in oxygenator fibres at 6 hours	Reduced NET formation, maximal clot firmness and fibre density in PPP. Increased platelet activation at 1 hour in PRP.	Significant haemodilution demonstrated
Wen. Perfusion. 2019[365]	56 patients requiring ECMO	Disease severity, inflammatory markers and mortality in patients with low (<48 μg/mL by Western Blot) and high (≥48 μg/mL) histone levels	Higher mortality rate in those with high levels than low levels (59 vs 50%), higher inflammatory markers and higher SOFA scores	Single centre Inflammatory markers assessed: IL-2, IL-6, IL-8, IL-10, IL-18, IFN-γ and TNF-α
Beaubien-Souligny. CJC Open. 2019[366]	24 patients requiring CPB : 12 high and 12 low-risk EuroSCORE	Change in NET markers pre- and 3-hours post-CPB	Increase in NET markers following CPB with correlation between markers and duration of CPB	Single centre Markers assessed: MPO-DNA complexes, citrullinated H3 and MPO
Gao. Interact Cardiovasc Thorac Surg. 2016[367]	48 patients undergoing CPB	Changes in circulating histones, CRP, procalcitonin and NT-proBNP every 24 hours for 72 hours	Histone levels correlated positively with other laboratory parameters, ICU stay and ventilation time	Single centre

8.2 Aims

1) To assess the changes in circulating histones over 24-hours in an *ex vivo* ECMO circuit
2) To assess the changes in circulating histones in adult patients prior to, during and following decannulation from ECMO.
3) To assess the changes in circulating histones in correlation with thrombotic and haemorrhagic events.

8.3 Methods

The methods described pertain to both samples collected from an *ex vivo* ECMO circuit and serial samples collected from patients. The methods for performing ELISA to assess circulating histones during ECMO are described in Chapter 4. The details of the design and set-up of the *ex vivo* circuit have previously been described in Chapter 5.

8.3.1 Outcome Variables

The primary outcome for both the *ex vivo* circuits and patients were the changes in circulating histone levels (H3.1 and H3R8, a citrullinated form of H3) from the baseline level prior to starting ECMO in both the *ex vivo* circuits and patient samples. Secondary outcomes for the patient samples were the changes in circulating histone levels in those developing thrombosis, membrane occlusion and thrombocytopenia to those not developing these complications.

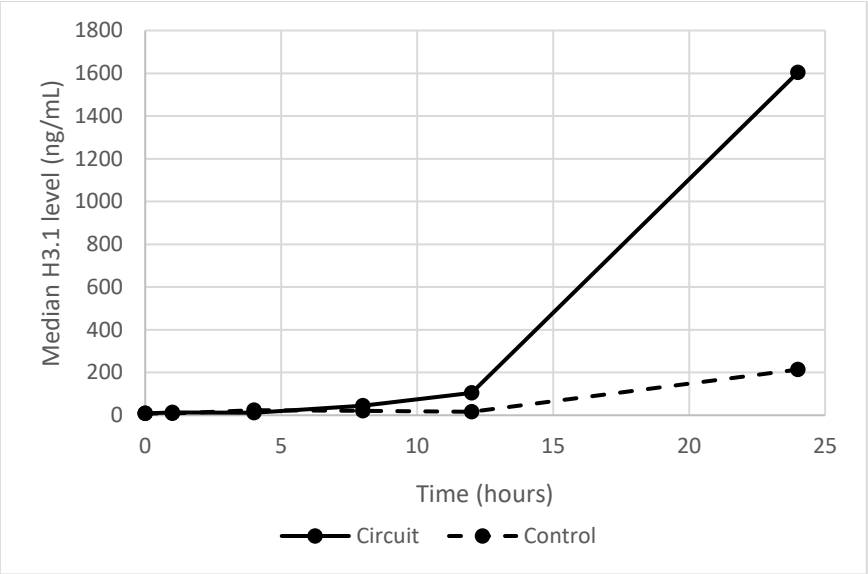
8.4 Results

8.4.1 Circulating histones in an *ex vivo* model of ECMO

H3.1

Following the initiation of the *ex vivo* circuit, there was a statistically significant increase in H3.1 levels after 8 hours (median H3.1 levels 8.7 ng/mL at initiation to 44.9 ng/mL at 8 hours, p = 0.028) shown in Figure 49. The levels of H3.1 continued to increase significantly to a median level of 1604 ng/mL at 24 hours. In the control samples, there was an increase in H3.1 levels after 24-hours (8.7 ng/mL at initiation and 214 ng/mL at 24-hours, p = 0.028). The median levels were significantly higher at 24-hours in the circuit than control samples (p = 0.028).

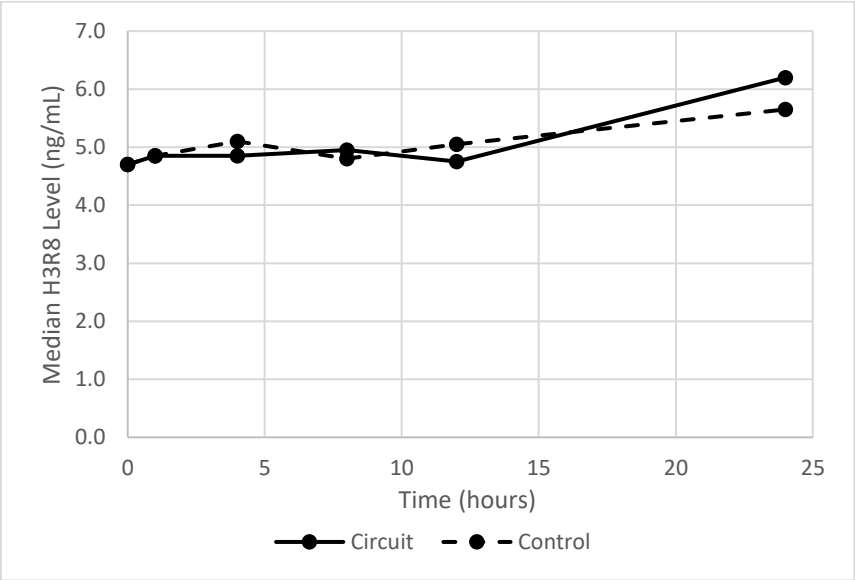
Figure 49 Changes in H3.1 levels over 24-hours in an ex vivo extracorporeal model



H3R8

There was a small increase in H3R8 levels over time from the initiation of the *ex vivo* circuits, which was statistically different after 24-hours (4.7 ng/mL to 6.2 ng/mL respectively, p = 0.028) shown in Figure 50. A similar change was also seen in the control samples over the same period (4.7 ng/mL to 5.7 ng/mL respectively, p = 0.028). There was no statistically significant difference between controls and circuit samples at 24-hours (p = 0.67).

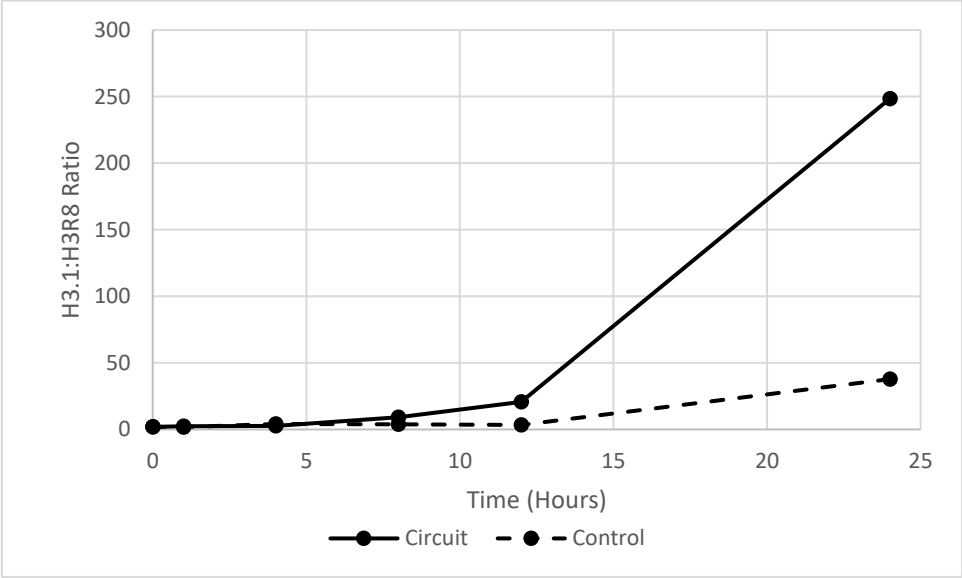
Figure 50 Changes in H3R8 levels over 24-hours in an ex vivo extracorporeal model



H3.1/H3R8 Ratio

The ratio of H3.1 to H3R8 was calculated to indicate if circulating H3.1 was in proportion to citrullinated H3R8. There was an increased in the ratio over 24-hours in both the *ex vivo* circuits (1.9 at initiation and 249 at 24-hours, p = 0.028) and controls (1.9 at initiation and 37.9 at 24-hours, p = 0.028) shown in Figure 51. However, the proportion of H3.1 to H3R8 was significantly higher in the *ex vivo* circuits than control samples (median ratio in circuits – 249, controls – 37.8 at 24-hours, p = 0.028).

Figure 51 Changes in H3.1/H3R8 ratio over 24-hours in an ex vivo extracorporeal model



8.4.2 Circulating histones changes in adult patients receiving ECMO

Seventeen participants were reviewed in this analysis.

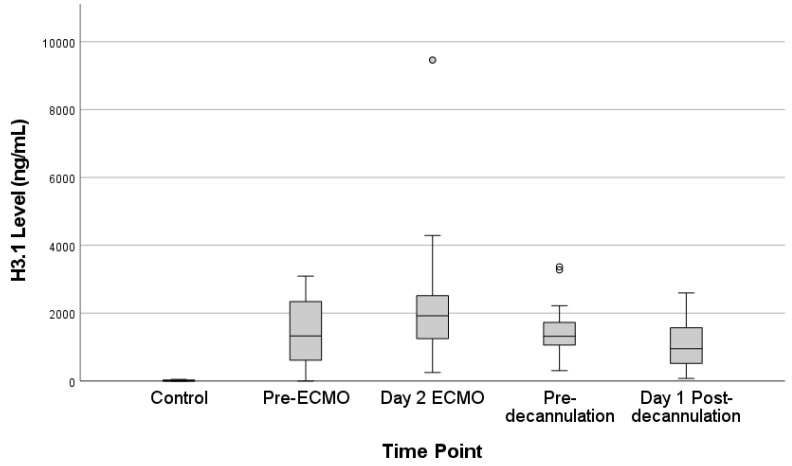
Temporal Changes in H3.1

The normal reference range for H3.1 derived from 38 control samples was 0 – 48 ng/mL. H3.1 levels were elevated at initiation of ECMO (median = 1271 ng/mL, IQR 330 - 2380) (Figure 52(a)). These levels continued to increase during the first two days of ECMO in comparison pre-ECMO (median H3.1 on Day 1 1686 ng/mL, IQR 586 – 2330, p=0.034, median H3.1 on Day 2 1924 ng/mL, IQR 1150 - 2541, p = 0.031) (Figure 52(b)) and after one week on ECMO, they did not change in comparison to day 1 after starting ECMO (median H3.1 on Day 7 2067 ng/mL, p=0.78) (Figure 52(c)). At the time of decannulation, H3.1 levels remained elevated with a decrease 1 day afterwards (median prior to decannulation 1321 ng/mL and day following decannulation 953 ng/mL, p=0.030) (Figure 52(d)).

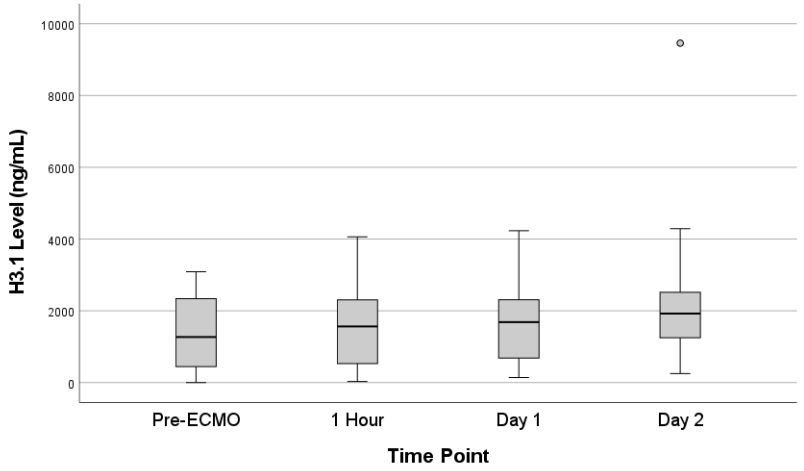
Temporal Changes in H3R8

The normal reference range derived from 30 control samples was 0 – 4.7 ng/mL. H3R8 levels were elevated at initiation of ECMO (median = 49.6 ng/mL, IQR 16.9 – 135.7) (Figure 53(a)). These levels continued to increase during the first two days of ECMO in comparison to pre-ECMO (median H3R8 on Day 1 130.2 ng/mL, IQR 56.5 - 191, p= 0.005, median H3.1 on Day 2 123.9 ng/mL, IQR 66.8 – 216.8, p = 0.012) (Figure 53(b)) and after one week on ECMO, they remainder similar to Day 1 after starting ECMO (median 189.5 ng/mL, IQR 135.1 – 238.6, p = 0.89) (Figure 53(c)). At the time of decannulation, H3R8 levels remained elevated with a decrease 1 day afterwards (median prior to decannulation 58.2 ng/mL and day following decannulation 36.0 ng/mL, p=0.01) (Figure 53(d)).

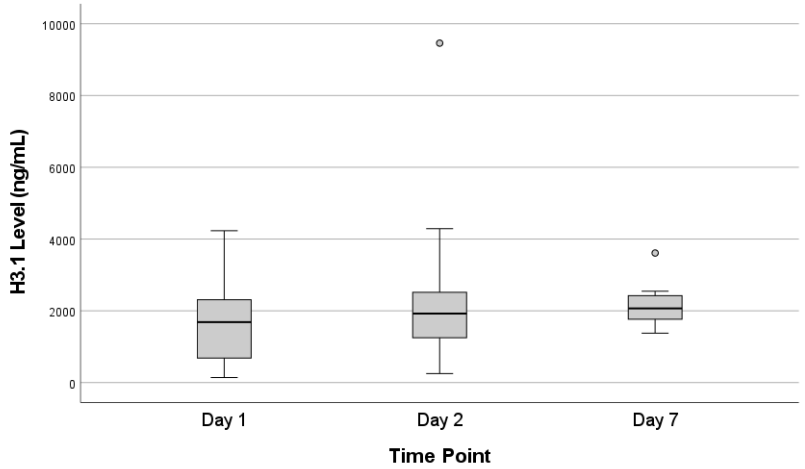
Figure 52 Box plots for changes over time in H3.1 levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



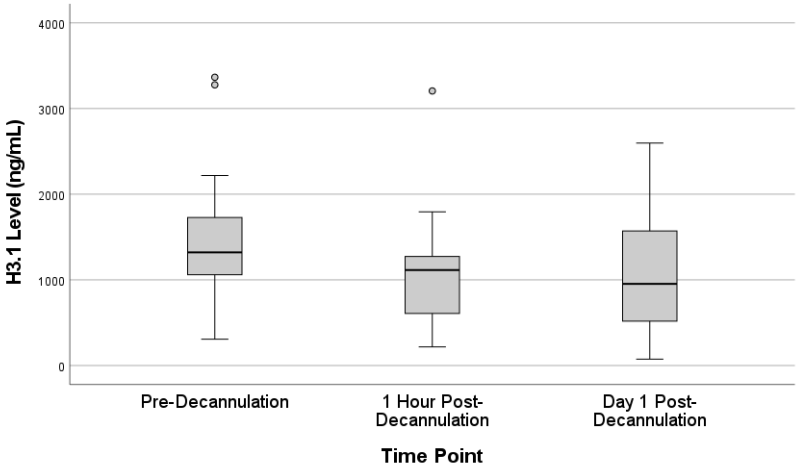
(a)



(b)

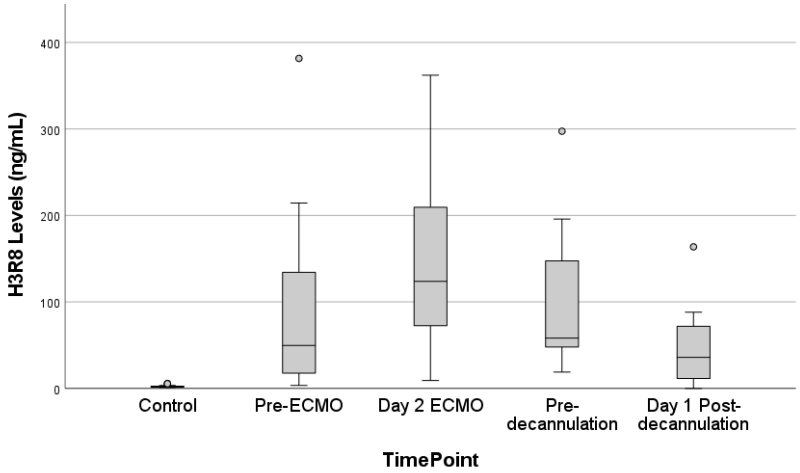


(c)

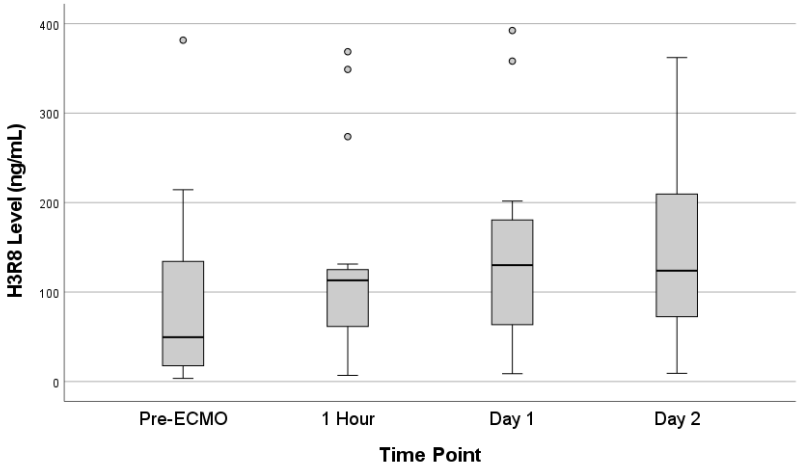


(d)

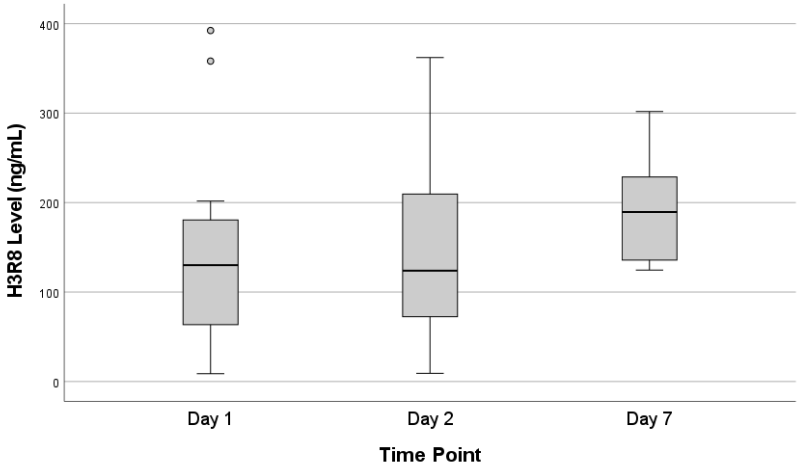
Figure 53 Box plots for changes over time in H3R8 levels (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



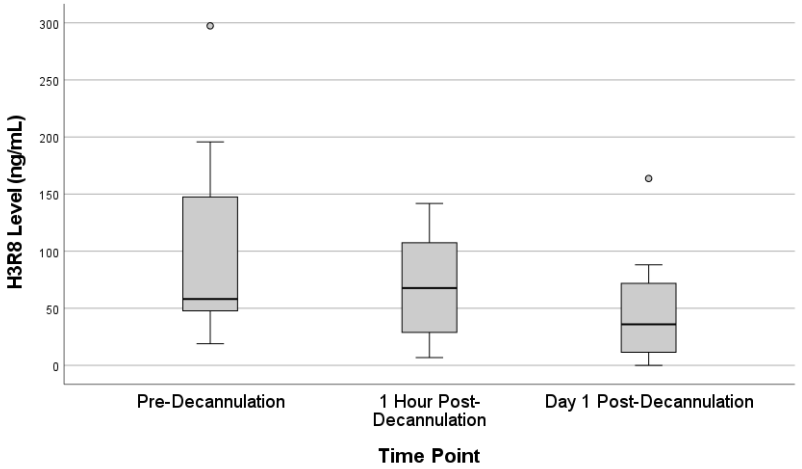
(a)



(b)



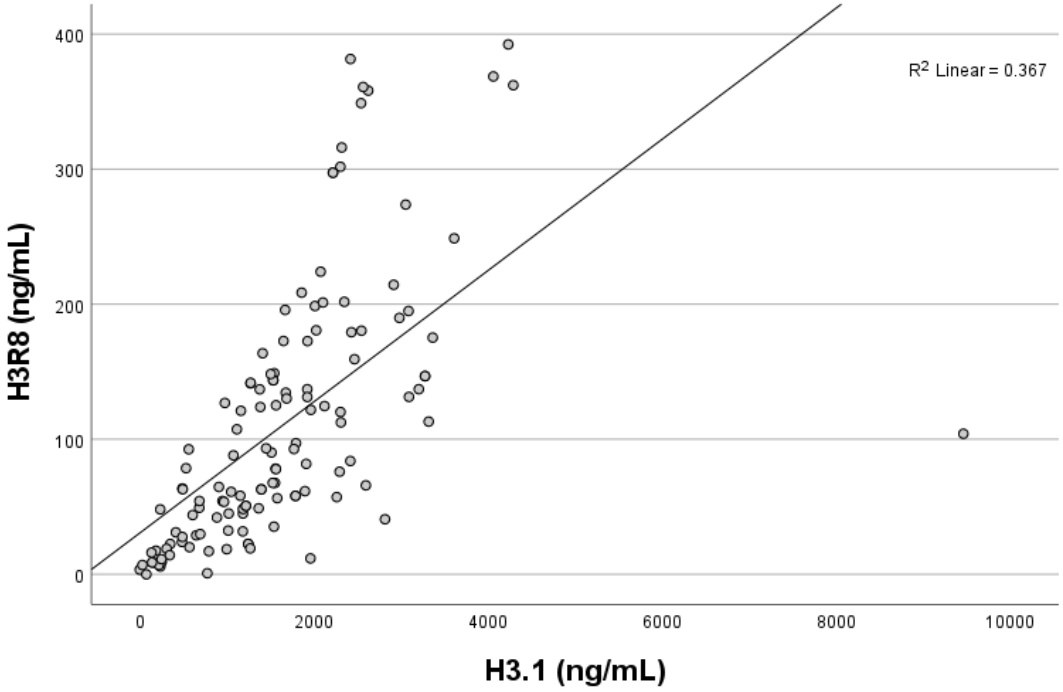
(c)



(d)

There was a strong positive correlation between H3.1 and H3R8 levels (Figure 54) in all patients were samples throughout ECMO.

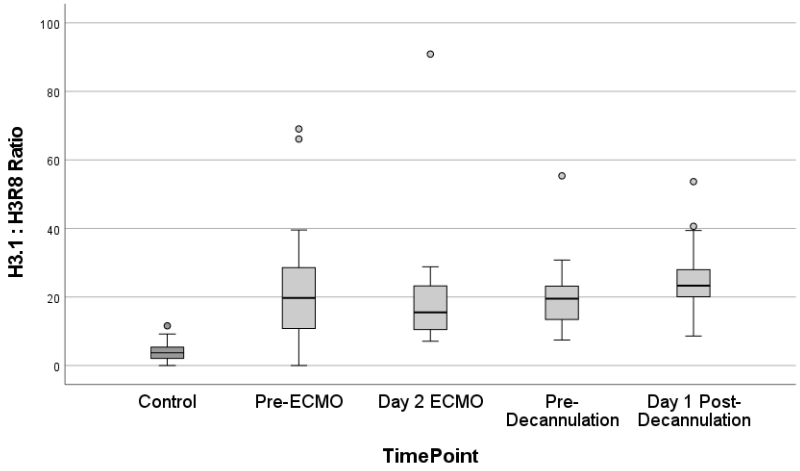
Figure 54 Scatter plot comparing H3.1 to H3R8 levels during ECMO



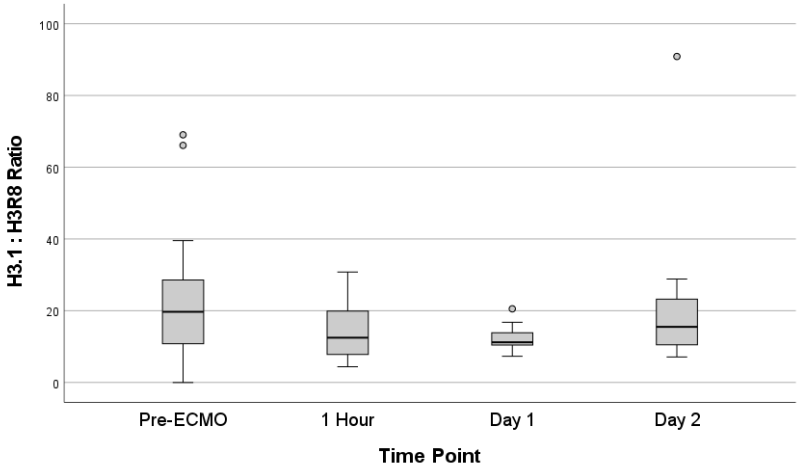
H3.1/H3R8 Histone Ratio

The normal reference range derived from 30 control samples was 0 – 7.1. The H3.1/H3R8 histone ratio was elevated at initiation of ECMO (median = 19.7 ng/mL, IQR 9.9 – 30.0) (Figure 55(a)). These ratios decreased during the first two days of ECMO in comparison to pre-ECMO (median ratio on Day 1 11.2, IQR 10.0 – 14.7, p= 0.010, median ratio on Day 2 12.5 ng/mL, IQR 7.5 – 22.2, p = 0.26) (Figure 55(b)) and after one week on ECMO, they remained similar to Day 1 after starting ECMO (median 11.3 ng/mL, IQR 9.2 – 14.4, p = 0.89) (Figure 55(c)). At the time of decannulation, the histone ratio did not change following decannulation (median prior to decannulation 19.5 and day following decannulation 23.3, p=0.25) (Figure 55(d)).

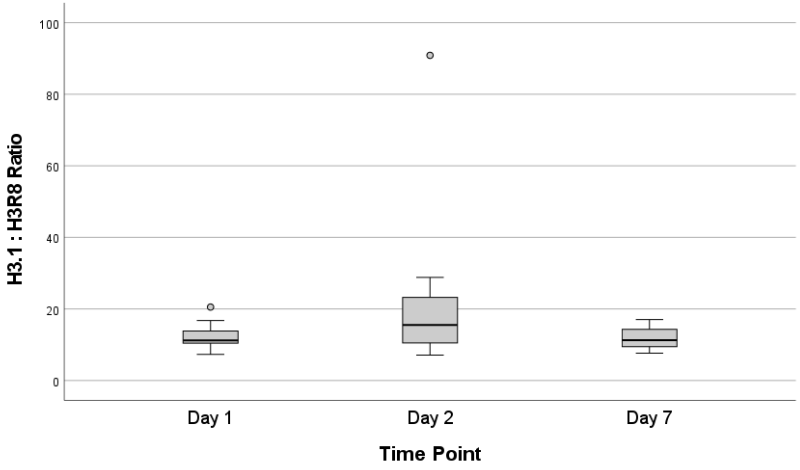
Figure 55 Box plots for changes over time in histone ratio (a) Throughout ECMO (b) Initiation of ECMO (c) During ECMO use (d) Around decannulation



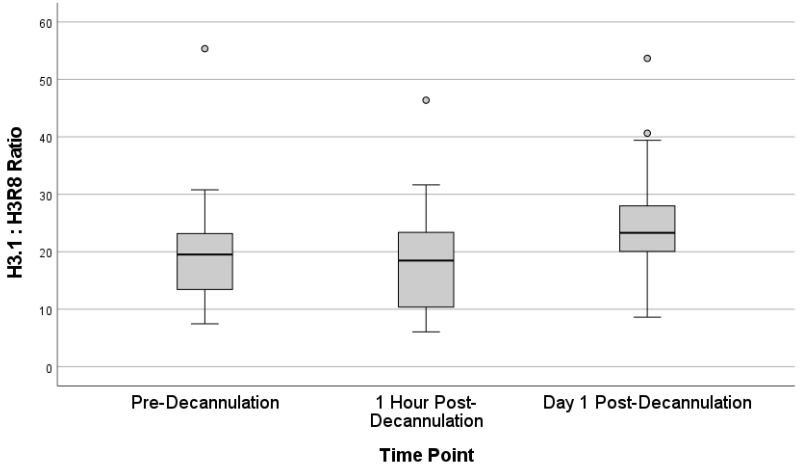
(a)



(b)



(c)



(d)

8.4.3 Circulating Histones during haemorrhagic and thrombotic complications of Extracorporeal Membrane Oxygenation

Disease Severity

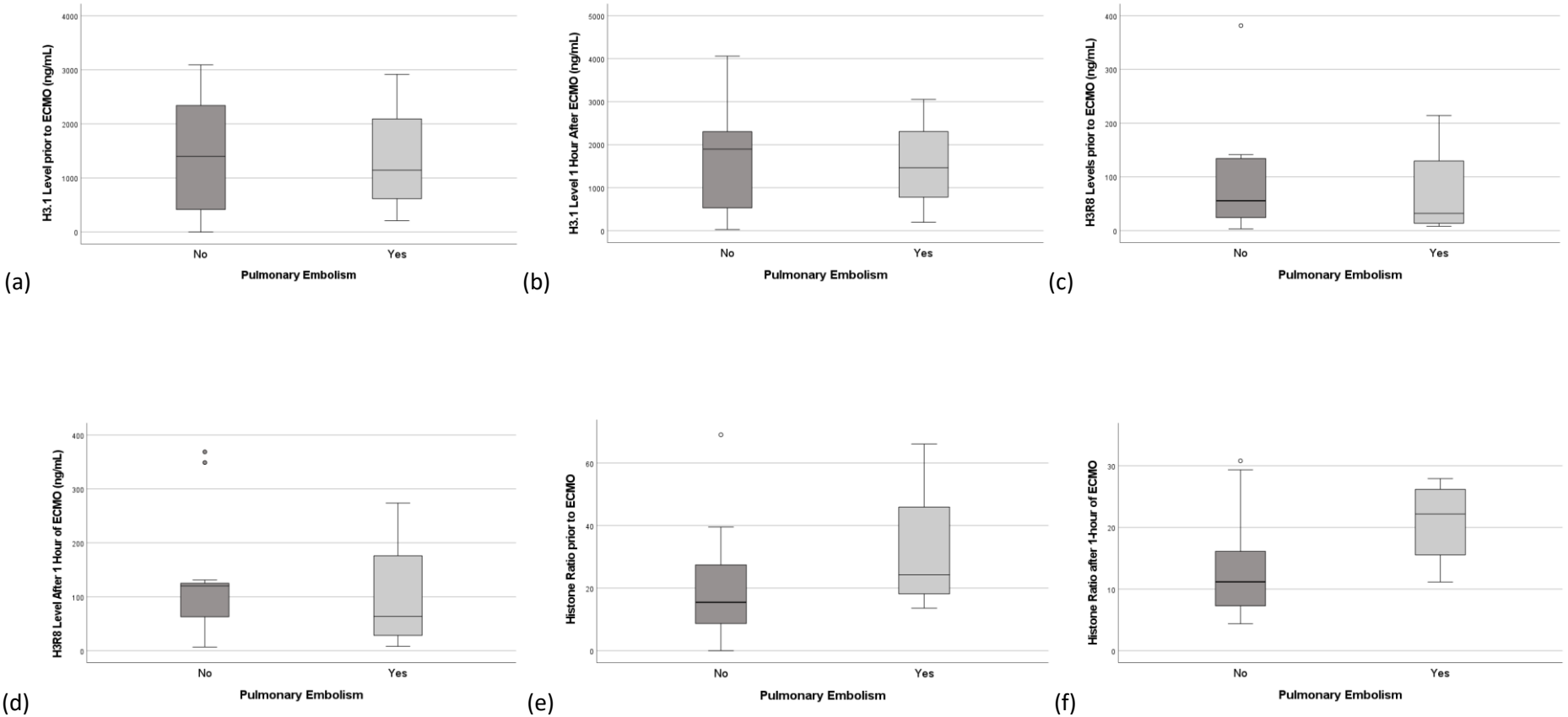
The SOFA and APACHE II scores of patients at the time of ECMO cannulation were assessed according H3.1 and H3R8 levels prior to cannulation. The median SOFA score was 7 (IQR 5.5 – 8) and median APACHE II score was 16 (IQR 14 – 20) in this cohort.

Although levels were higher, there was no statistical difference in the H3.1 in those with a SOFA score of ≤7 (n = 10) to those with >7 (n = 7) (1145 vs 1726 ng/mL, p = 0.71) or APACHE II score ≤ 17 (n = 9) to those with >17 (n = 8) (1398 vs 1147 ng/mL, p = 0.96). Similarly, there was no statistical difference in the H3R8 in according to SOFA score (36.6 vs 62.5 ng/mL, p = 0.31) and APACHE II score (36.6 vs 92.9 ng/mL, p = 0.33).

Pulmonary Embolism

Blood samples were taken prior to initiation or 1-hour after initiating ECMO as shown in Figure 56. H3.1 (median 1400 vs 1145 ng/mL, p = 1.0) and H3R8 levels (median 55.8 vs 32.1 ng/mL, p = 0.68) and histone ratios (median 15.5 vs 24.2, p = 0.32) were not significantly different in those without PE (n = 13) to those with PE (n = 4) before starting ECMO. There was no difference after 1-hour after starting ECMO (H3.1 1897 vs 1463 ng/mL, p = 0.62) (H3R8 120.2 vs 63.6 ng/mL, p = 0.41) (histone ratio 11.2 vs 22.2, p = 0.16).

Figure 56 Box plots of histone levels in patients with and without PE (a) H3.1 prior to ECMO (b) H3.1 1-hour after ECMO (c) H3R8 prior to ECMO (d) H3R8 1-hour after ECMO (e) Histone ratio prior to ECMO (f) Histone ratio 1-hour after ECMO



Deep Vein Thrombosis

Blood samples reviewed were those taken prior to decannulation from ECMO. H3.1 (1290 vs 1374 ng/mL, p = 0.68), and H3R8 levels (54.0 vs 58.2 ng/mL, p = 0.77) and histone ratios (19.2 vs 19.5, p = 0.77) were not statistically different in those with Ca-DVT (n = 7) to those without (n = 13) prior to ceasing ECMO.

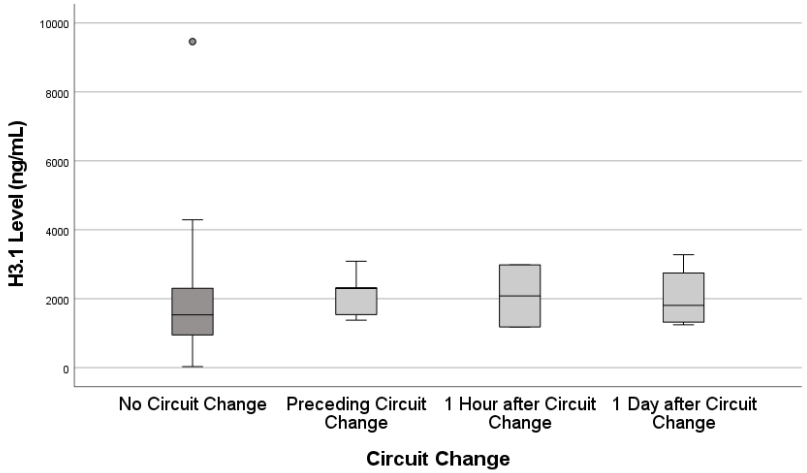
Intracranial Haemorrhage

Blood samples reviewed were those taken prior to initiation and 1-hour after initiating ECMO. H3.1 (median 1398 vs 1147 ng/mL, p = 0.95), and H3R8 levels (median 30.0 vs 55.8 ng/mL, p = 0.38) and histone ratios (median 68.3 vs 13.8 ng/mL, p = 0.10) were not statistically different in those with ICH (n = 4) to those without ICH (n = 13) before starting ECMO. There was no difference in these levels 1-hour after starting ECMO (H3.1 1665 vs 1565 ng/mL, p = 0.79) (H3R8 95.9 vs 120.2 ng/mL, p = 0.70) (histone ratio 30.0 vs 11.2, p = 0.35).

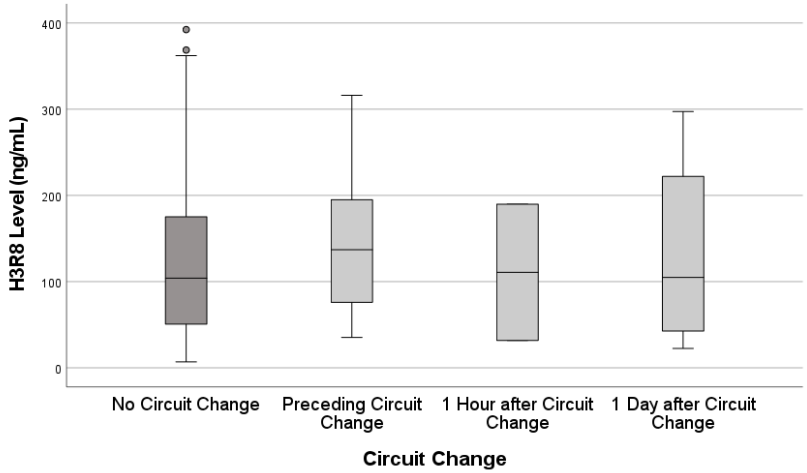
Circuit Change

Blood samples reviewed were those taken prior to circuit changes to those not requiring circuits with samples pre- and post-cannulation not included. Blood samples were also compared pre- and 1-hour 1-day following circuit changes in those requiring circuit changes (n = 5) to those not (n = 17). H3.1 (medians levels 2296, 2083, 1807,1532 ng/mL respectively, p = 0.58, Figure 57(a)), and H3R8 (median levels 137, 110.9, 104.9, 104.1 ng/mL respectively, p = 0.95, Figure 57(b)) levels and histone ratios (median 15.8, 26.5, 22.3, 14.5 respectively, p = 0.36) were not statistically different show in Figure 57(c).

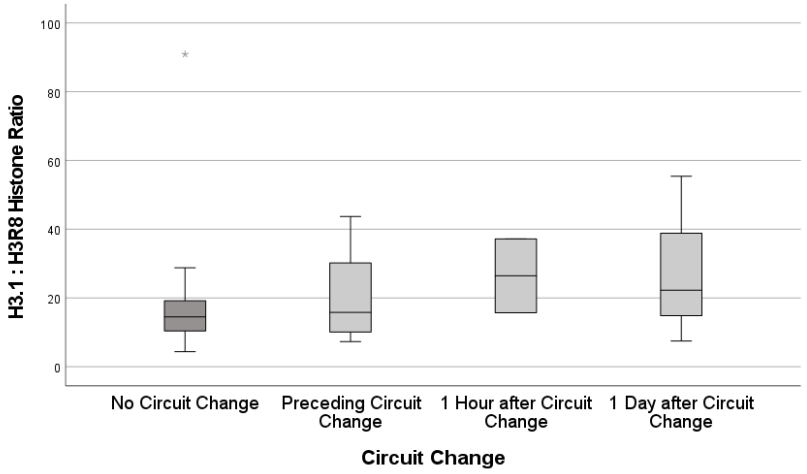
Figure 57 Box plots of histone levels in patients around circuit change (a) H3.1 (b) H3R8 (c) Histone ratio



(a)



(b)



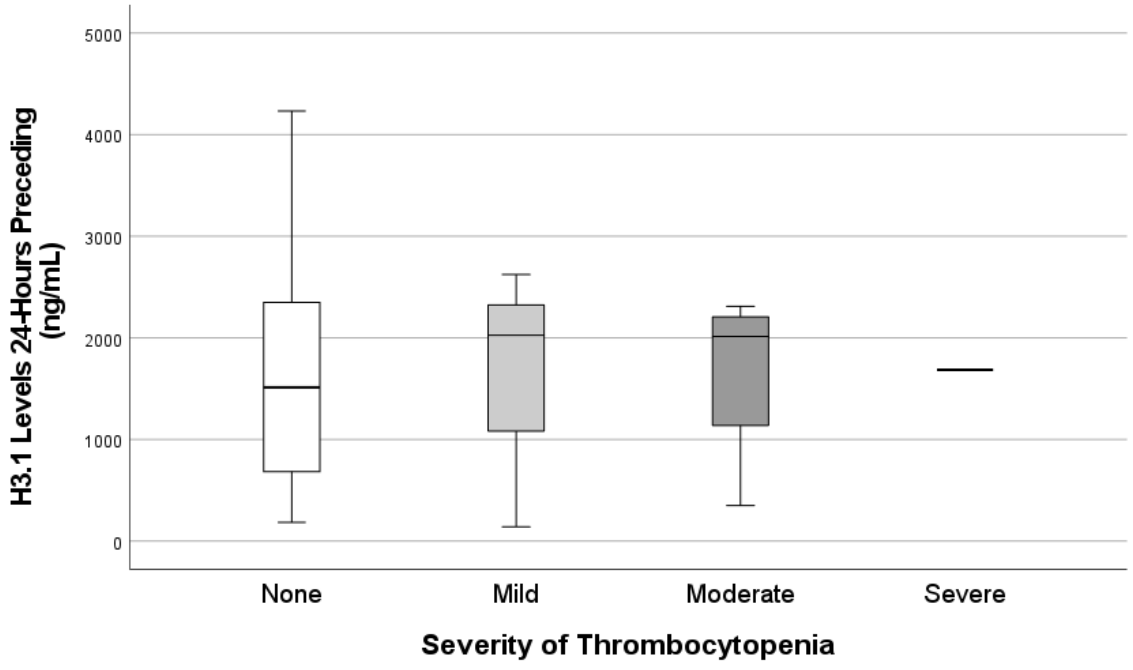
(c)

Thrombocytopenia

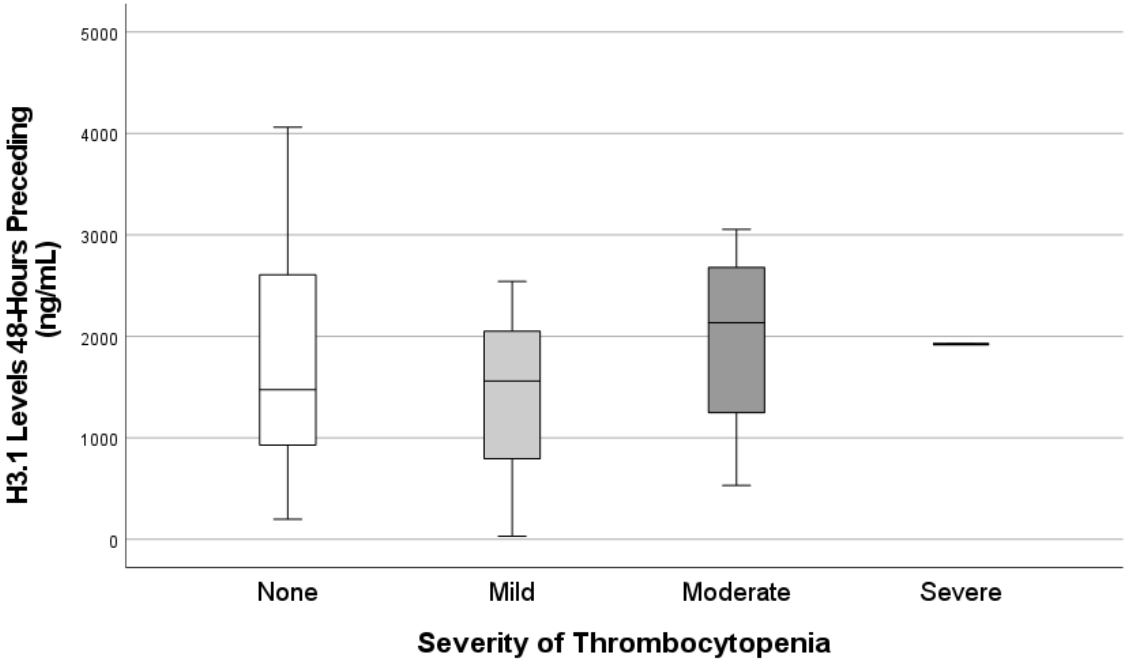
Thirteen of 17 patients developed thrombocytopenia during ECMO use. Two had mild thrombocytopenia (platelet 100-149x10^9/L, seven had moderate thrombocytopenia (50-99 x10^9/L) and four had severe thrombocytopenia (< 50 x10^9/L) at their lowest platelet counts. Seven had thrombocytopenia at the time of ECMO cannulation and the median time to most severe thrombocytopenia was day 5 (IQR 4-8 days).

Histone levels at the time of ECMO cannulation and after 1-day of ECMO were compared to the development of subsequent thrombocytopenia after 2-days of ECMO as shown in Figure 58. There was no difference in H3.1 levels at 24-hour (1564 vs 2043 p = 0.70) and 48-hours (1327 vs 2043, p = 0.64) preceding the development of severe thrombocytopenia (platelets < 50x10^9/L) after 2-days of ECMO. There was no difference in the severity of thrombocytopenia after 2-days of ECMO in the preceding 24-hours (p = 0.98) and 48-hours (p = 0.80).

Figure 58 Box plots of H3.1 levels in patients with and without thrombocytopenia (a) 24-hour and (b) 48-hours preceding (none – platelet count ≥150 x10^9/L, mild –100-149 x10^9/L, moderate – 50-99 x10^9/L, severe - <50 x10^9/L)



(a)



(b)

8.5 Discussion

The changes in circulating extracellular histone levels during ECMO use at present have yet to be described. The purpose of this study was to assess their changes during ECMO and whether there was a correlation to haemorrhagic and thrombotic events during its use. This study used H3.1 and H3R8 as markers of total and citrullinated histones respectively.

The *ex vivo* circuit model demonstrated a significant total increase in H3 histones with relatively stable levels of citrullinated histones. These results are in keeping with increases seen during CPB[366,367]. This suggests that cellular damage by the extracorporeal circuits cause the increase in histones levels as opposed to NETosis. As the only nucleated cells within this model, it would appear likely that leucocytes would be a significant source of circulating histones although platelets may also contribute[350]. H3 and H4 histones are present diffusely throughout the cytoplasm as opposed to within platelet granules and are higher in patients during the early stages of sepsis. Human red blood cells are not a source of histones although they are affected by their presence extracellularly[368].

In patients during ECMO use, the levels of histones were significantly elevated prior to initiating ECMO in comparison to control samples. The levels subsequently increased in the first two days of ECMO use and decreased following decannulation although were stable at later time points of ECMO use. It is likely that circulating extracellular histones are from damaged lung tissue in ARDS, other organs in multi-organ failure and cellular damage from blood cells by the circuit. In patients with COVID-19 pneumonia, the levels of H3 histones were related to disease severity and mortality[356]. The H3.1/H3R8 histone ratio remained high throughout ECMO suggest that NETosis may not be the predominant process for extracellular histone release. As discussed in Chapter 6, thrombin generation markers were not different according to anti-Xa levels prior to decannulation from ECMO. As H3 histone levels were higher during ECMO from day 2 onwards, histone-attenuation of heparin activity may be a proposed mechanism for this.

With high levels of circulating histones acting as prothrombotic DAMPs, it is hypothesised that increased levels of histones would be associated with increased thrombotic complications during ECMO. However, there was no statistically significant differences in levels in patients with PE at ECMO initiation, or Ca-DVT at decannulation or preceding and following circuit change. This may be due to several considerations: firstly, histone levels were elevated in 16/17 patients prior to ECMO initiation so therefore this may not be discriminatory between those developing haemostatic complication but may still be implicated in the pathogenesis. Alhamdi *et al* showed that thrombocytopenia was more prevalent in those with elevated histone levels as opposed to those with normal levels, which was not a feature of this cohort with significantly elevated levels seen in all but one case[45]. Secondly, this study looked at only DNA-bound H3 histones and not has reviewed H4 histone and unbound circulating histones, which have been implicated in these complications[54,352]. Thirdly, the sample size is small with

a low frequency of haemostatic events. Finally, it appears that ALI with cellular damage is already well established by the timing that ECMO is initiated so during ECMO, H3 histone levels may not be predictive of established changes.

Further analysis to assess these would include H4 histones and to assess the role of both unbound and DNA-bound histones. Histological assessment of clots within the circuit for histones may also give further support for this hypothesis.

8.6 Conclusions

These data have shown for the first time that circulating histones are released by cellular damage due to the extracorporeal circuit itself and are also present in patients preceding ECMO as part of DAMP of ALI and multiorgan failure. In this small study, there is no clear link between the presence of circulating H3 histones and the development of haemostatic complication during ECMO, but further larger studies are required.

Chapter 9 – Conclusions and Future Directions

9.1 Rationale of Study

Bleeding and thrombosis are recognised as key complications during the use of ECMO, which remain a concern with the expansion of the technology globally. Therefore, understanding the patterns of these events and potentially contributing changes that may allow better screening, monitoring and therapeutic targets will be key in improving survival outcomes. The aims of this thesis were to understand the natural history of thrombotic and bleeding complications during ECMO, the impact of blood product transfusions on patient outcomes and to assess the contributions of haemostatic changes from both patients and the extracorporeal circuit in these events.

9.2 Summary of Findings

The study has focussed on three components: 1) the prevalence of haemostatic complications and the use of blood product transfusions during ECMO and their effects on survival from a high-volume, single centre retrospective registry, 2) the use of an *ex vivo* circuit to assess haemostatic changes during ECMO, and 3) the temporal assessment of haemostatic changes during ECMO and their association with haemostatic changes in a small observational study of patients with SRF.

In the first component, a large patient cohort was identified that adopted an imaging pathway that screened patients for thromboembolism and haemorrhage at initiation and DVT at decannulation as part of the care pathway and included additional imaging based upon clinical assessment. As part of this, the data demonstrated a 11% incidence of intracranial haemorrhage with a predominance of small volume, subarachnoid haemorrhage. Survival

rates in these patients were not different unless progression of the haemorrhage was seen in the subsequent days (median time to progression – 3 days), which demonstrated poor survival rates similar to ICH detected based upon clinical deterioration at similar timepoints. In contrast, NICMH was seen throughout ECMO use and was shown to have an impact upon survival to ICU discharge. There was a high incidence of small filling defects in pulmonary vessels (12% of patients) seen at screening imaging, which given the lack of clinical correlation with DVT and temporal relationship to severe lung injury, seemed likely to be due to immunothrombosis as opposed to embolisation. The underlying disease aetiology appears to be a driver of this with the highest incidence of these filling defects in patients with COVID-19 infection (33% of patients), which has a significantly pro-inflammatory state. At present, it is unclear if individualised anticoagulation strategies are needed according due to disease aetiology but merits further exploration.

In terms of the use of RBC transfusion in this large retrospective cohort, the outcomes also reflected that of other studies with no improvement in survival outcomes with higher transfusion targets. There was a recognition of a limited evidence base for the use of platelet, cryoprecipitate and FFP use during ECMO, and these data suggest concern regarding the use of large numbers of platelet transfusions upon short-term ECMO survival.

The use of an *ex vivo* circuit demonstrated the significant release of H3 histones likely to be from cellular injury as opposed to NETosis demonstrated by a similar ratio increase in citrullinated H3 levels. Other molecules such as PAI-1 may also be released from platelets by this mechanism of blood cell damage although further evaluation is required. Thrombin generation was not shown to be increased with this model, which may be a result of non-critically ill donor blood or citrate anticoagulation use.

In contrast, there was a significant increase in both markers of thrombin generation and fibrinolysis in samples from patients requiring ECMO, showing a profile similar to an acute phase response prior cannulation as a result of severe ALI. These markers increased with circuit use and reduced with its removal. An increase in fibrinolytic activity occurred later during the circuit use (typically after day 2) whereas thrombin generation occurred earlier (within first two days). Specific factor abnormalities in the fibrinolytic pathway were not identified to drive hyperfibrinolysis. Additionally, there was no clear relationship between bleeding and thrombosis to haemostatic factors. Elevated D-dimer levels were seen in both the retrospective cohort and observational studies with ICH with an increase in PF1+2 in the latter, suggestive of a reactive increase in thrombin generation to ICH.

This study explored the hypothesis that circulating H3 histones may be a potential marker of innate immune system activation, due to neutrophil activation from infection and inflammation, or cellular injury and may be associated with a prothrombotic state and thrombocytopenia. However, there was no clear correlation when using two novel H3 ELISA assays. Further analysis to look at histone and DNA deposition within the circuit thrombosis is needed.

9.3 Critiques and Weaknesses of Study

The results obtained from the registry analysis for thrombotic and bleeding complications and transfusion-related outcomes were obtained from a single centre and the data were retrospective. Internationally, anticoagulation practice during ECMO is variable between centres with different methods of monitoring and administration. The anticoagulation practice for ECMO in this thesis used a ‘low intensity’ for those without thrombosis, ‘high intensity’ for those with concurrent thrombosis and no anticoagulation for those with

intracranial haemorrhage, major haemorrhage or deemed to be at high-bleeding risk such as in a post-operative period. In comparison, the EOLIA study had a less intense anticoagulation strategy with lower rates of bleeding[204]. Additionally, the retrospective nature of the study meant that the assessment of bleeding had reporting bias, particularly for clinically relevant non-major bleeding.

Clinical definitions of haemorrhage are difficult to apply in the setting of ECMO given additional causes of anaemia such as haemodilution and haemolysis. In this cohort between 0.4 and 0.7 RBC units were transfused per day depending on transfusion targets. Therefore, a definition of ≥4 units of RBC units within a 24-hour period was used in keeping with definitions by ELSO as opposed to the current ISTH definition of ≥2 units. A statistically significant difference in ICU survival rates was seen with this definition showing 63% in patients with NICMH vs 82% without. Further exploration is warranted to assess this definition for research purposes based upon survival outcomes to help standardisation between studies. In addition, due to the lack of an evidence base for transfusion targets in the setting of ECMO, clinician preferences and experience may have altered these rates.

Because of the COVID-19 pandemic, the CYCLE study was suspended for further recruitment for a period of 6 months with a smaller than intended recruitment target of >20 patients. Additionally, samples of patients with COVID-19 infection were unable to be analysed at the time that the study was open for recruitment. There was also concern that the significantly prothrombotic state of the condition may have distorted results.

In terms of the analysis of the haemostatic factors, it was decided to use ELISA-based methods due to the prolonged time of processing samples from prior to cannulation and 1-hour post-cannulation. It was felt that TGA and clot lysis assays would be inaccurate, particularly if there

were delays in transportation of the samples and participants in the early timepoints. This study also looked at a novel H3 ELISA based assay but did not assess histone H4 and circulating free DNA as additional components of NETosis and DAMP.

As recognised in the evaluation of the *ex vivo* circuit for assessing haemostasis during ECMO, there are several limitations in using the design. Development of a small volume circuit, potentially from a paediatric device, would ameliorate the concern of haemodilution whilst using a single blood donor. The use of non-human blood may be another alternative. The use of a non-heparin coated circuit may also be considered to propagate the process of thrombin generation due to the artificial surface although these are now not used in clinical practice.

9.4 Implications of Study

The registry cohort study demonstrated that further prospective studies of transfusion targets for ECMO are required given adverse outcomes seen in more liberal transfusion approaches. This would reflect previously discussed observations seen in RBC transfusion in critical care and cardiac surgery and for platelet transfusion in critical care and bleeding with antiplatelet agents. This study which is currently underway as a prospective, multi-centre study in ECMO may provide these answers to clinicians[369].

These results support the use of screening imaging for bleeding and thrombosis due to an absence of specific biomarkers or predictors for thrombosis and haemorrhage during ECMO. The observational study showed that although D-dimers were sensitive for identifying pulmonary embolism and potentially ICH, they were not specific. Additionally in a small patient cohort, PF1+2 and TAT ELISA assays did not demonstrate statistical significance. D-dimer, PF1+2 and TAT levels were also not different in those with DVT at the time of

decannulation despite being commonly seen. Therefore, detailed imaging remains important for patient management.

Fibrinolysis was shown to be activated during ECMO particularly during the later stages of its use and although thrombin generation peaked early and remained elevated, there was a lack of correlation to anti-Xa monitoring for anticoagulation. Therefore, anticoagulation strategies may need to be optimised to consider more intense anticoagulation during the first few days of ECMO to prevent circuit occlusion and then reduced or titrated to avoid the risk of bleeding at non-intracranial sites, which can occur throughout the use of ECMO.

9.5 Future Work

This study reiterates the identification of high rates of bleeding and thrombosis during ECMO with a temporal nature to these events with associated changes in fibrinolysis and thrombin generation over time.

One of the key findings was the identification of early, small volume ICH in the registry cohort with similar survival rates if extension of bleeding did not occur during ECMO. Other than early cranial CT imaging, the potential to develop a biomarker to assess for this prior to ECMO use may help to identify patients at high-risk of ICH, and guide when anticoagulation may later be reintroduced if ICH has occurred. Specific cerebral biomarkers, such as S100-ß, warrant further evaluation as a diagnostic test for this event.

The role of histones, neutrophil injury and NETosis needs further analysis, particularly in the development of thrombosis during ECMO and the DAMP of ALI during its use. Immunohistostaining for neutrophil deposition, histones, and DNA in clots from ECMO circuits may help to understand alternate mechanisms and subsequent targets to prevent

circuit occlusion. Therapeutic targets may be of use if this is demonstrated to avoid the need for anticoagulation to avoid the risk of haemorrhage.

The role of platelet transfusion remains of interest during ECMO. As both thrombocytopenia and platelet activation occur during ECMO, clinical consideration of transfusion targets and the age of donated units may be of consideration to improve survival outcomes. The underlying cause of poorer survival outcomes in those using platelet transfusions also requires further evaluation – is the use of platelet transfusion a marker of disease severity and a poor prognostic feature or do the transfused platelets play an active part in thromboinflammation during ALI in ECMO? Assessing platelet activation markers and platelet extracellular vesicles temporally around the time of platelet transfusion during ECMO may provide further details of this.

Finally, larger, multicentre prospective analysis of imaging strategies are needed to identify potential biomarkers for thrombosis and haemorrhage in ECMO. As highlighted, the approach in this registry cohort focused on an intensive imaging strategy, which has implications on resource provision and high service demands. Comparative retrospective analysis of data on thrombotic and bleeding complications between this imaging approach, and data from centres that provide imaging based upon when clinical events occur should be evaluated. Alternatively, a comparative prospective study to assess these approaches could be considered.

To summarise, this thesis suggests a temporal pattern to bleeding and thrombotic events during ECMO with disease aetiology affecting their frequency. The perception of a more 'liberal' transfusion approach for blood product transfusion may not be advantageous and needs to be explored further. Finally, thrombin generation and fibrinolysis are increased in

both critical illness and by the ECMO circuit as suggested by these exploratory data although in this study, their changes during the bleeding and thrombotic events remain unclear.

Publications and Abstracts

Publications related to Thesis

Doyle AJ, Hunt BJ. Current Understanding of How Extracorporeal Membrane Oxygenators Activate Haemostasis and Other Blood Components. Front Med (Lausanne). 2018 Dec 12;5:352. doi: 10.3389/fmed.2018.00352. PMID: 30619862; PMCID: PMC6299009.AML and ECMO

Doyle AJ, Richardson C, Sanderson B, Wong K, Wyncoll D, Camporota L, Barrett NA, Hunt BJ, Retter A. Restrictive Transfusion Practice in Adults Receiving Venovenous Extracorporeal Membrane Oxygenation: A Single-Center Experience. Crit Care Explor. 2020 Jan 29;2(1):e0077. doi: 10.1097/CCE.0000000000000077. PMID: 32166297; PMCID: PMC7063903.

Desborough MJR, **Doyle AJ**, Griffiths A, Retter A, Breen KA, Hunt BJ. Image-proven thromboembolism in patients with severe COVID-19 in a tertiary critical care unit in the United Kingdom. Thromb Res. 2020 Sep;193:1-4. doi: 10.1016/j.thromres.2020.05.049. Epub 2020 May 29. PMID: 32485437; PMCID: PMC7256551.

Zhang J, Merrick B, Correa GL, Camporota L, Retter A, **Doyle A**, Glover GW, Sherren PB, Tricklebank SJ, Agarwal S, Lams BE, Barrett NA, Ioannou N, Edgeworth J, Meadows CIS. Veno-venous extracorporeal membrane oxygenation in coronavirus disease 2019: a case series. ERJ Open Res. 2020 Oct 26;6(4):00463-2020. doi: 10.1183/23120541.00463-2020. PMID: 33257913; PMCID: PMC7520944.

Doyle AJ, Danaee A, Furtado CI, Miller S, Maggs T, Robinson SE, Retter A. Blood component use in critical care in patients with COVID-19 infection: a single-centre experience. Br J Haematol. 2020 Nov;191(3):382-385. doi: 10.1111/bjh.17007. Epub 2020 Oct 21. PMID: 32640484; PMCID: PMC7362152.

Doyle AJ, Hunt BJ, Sanderson B, Zhang J, Mak SM, Benedetti G, Breen KA, Camporota L, Barrett NA, Retter A. A Comparison of Thrombosis and Hemorrhage Rates in Patients With Severe Respiratory Failure Due to Coronavirus Disease 2019 and Influenza Requiring Extracorporeal Membrane Oxygenation. Crit Care Med. 2021 Jul 1;49(7):e663-e672. doi: 10.1097/CCM.0000000000004971. PMID: 33861545.

Abstracts related to Thesis

Doyle A, Gooby N, Parmar K, Breen KA, Barrett NA, Retter A, Hunt BJ. Changes in Fibrinolytic Factors in a Simulated Extracorporeal Membrane Oxygenator Circuit [abstract]. Res Pract Thromb Haemost. 2020; 4 (Suppl 1). https://abstracts.isth.org/abstract/changes-in-fibrinolytic-factors-in-a-simulated-extracorporeal-membrane-oxygenator-circuit/. Accessed June 7, 2022.

Doyle A, Sanderson B, Vasques F, Wyncoll D, Barrett NA, Hunt BJ, Camporota L, Retter A. The Use of Platelet Transfusions during Extracorporeal Membrane Oxygenation in a Large Tertiary Centre [abstract]. Res Pract Thromb Haemost. 2020; 4 (Suppl 1). https://abstracts.isth.org/abstract/the-use-of-platelet-transfusions-during-extracorporeal-membrane-oxygenation-in-a-large-tertiary-centre/. Accessed June 7, 2022.

Doyle AJ, Pandey M, Shah R, Breen KA, Barrett N, Hunt BJ, Retter A. Outcomes of Patients with Anti-Xa versus APTTr Monitoring for Unfractionated Heparin Infusions during

Extracorporeal Membrane Oxygenation [abstract]. Res Pract Thromb Haemost. 2020; 4 (Suppl 1). https://abstracts.isth.org/abstract/outcomes-of-patients-with-anti-xa-versus-apttr-monitoring-for-unfractionated-heparin-infusions-during-extracorporeal-membrane-oxygenation/. Accessed June 7, 2022.

Doyle A, Parmar K, Aswani A, Breen K, Barrett N, Retter A, Hunt B. Changes in circulating H3 Histone levels during Extracorporeal Membrane Oxygenation in patients with severe respiratory failure with correlation to haemostatic complications [abstract]. https://abstracts.isth.org/abstract/changes-in-circulating-h3-histone-levels-during-extracorporeal-membrane-oxygenation-in-patients-with-severe-respiratory-failure-with-correlation-to-haemostatic-complications/. Accessed June 30, 2022.

Doyle A, Parmar K, Gooby N, Breen K, Barrett N, Retter A, Hunt B. A comparison of thrombin generation ex vivo circuit and in critically ill patients requiring Extracorporeal Membrane Oxygenation [abstract]. https://abstracts.isth.org/abstract/a-comparison-of-thrombin-generation-ex-vivo-circuit-and-in-critically-ill-patients-requiring-extracorporeal-membrane-oxygenation/. Accessed June 30, 2022.

Doyle A, Parmar K, Breen K, Barrett N, Retter A, Hunt B. Changes in fibrinolysis during Extracorporeal Membrane Oxygenation in critically ill adult patients [abstract]. https://abstracts.isth.org/abstract/changes-in-fibrinolysis-during-extracorporeal-membrane-oxygenation-in-critically-ill-adult-patients/. Accessed June 30, 2022.

References

1. Cahill, P. A. & Redmond, E. M. Vascular endothelium – Gatekeeper of vessel health. *Atherosclerosis* **248**, 97–109 (2016).

2. Esmon, C. T. Possible involvement of cytokines in diffuse intravascular coagulation and thrombosis. *Best Pract. Res. Clin. Haematol.* **12**, 343–359 (1999).

3. Long, A. T., Kenne, E., Jung, R., Fuchs, T. A. & Renné, T. Contact system revisited: an interface between inflammation, coagulation, and innate immunity. *J. Thromb. Haemost.* **14**, 427–437 (2016).

4. Hoffman, M. & Monroe, D. M. A cell-based model of hemostasis. *Thromb. Haemost.* **85**, 958–65 (2001).

5. Kappelmayer, J., Bernabei, A., Edmunds, L. H., Edgington, T. S. & Colman, R. W. Tissue factor is expressed on monocytes during simulated extracorporeal circulation. *Circ. Res.* **72**, 1075–1081 (1993).

6. Østerud, B. Tissue factor expression in blood cells. *Thromb. Res.* **125**, S31–S34 (2010).

7. Luchtman-Jones, L. & Broze, G. J. The Current Status of Coagulation. *Ann. Med.* **27**, 47–52 (1995).

8. Subramaniam, S. *et al.* Distinct contributions of complement factors to platelet activation and fibrin formation in venous thrombus development. *Blood* **129**, 2291–2302 (2017).

9. Conway, E. M. Complement-coagulation connections. *Blood Coagul. Fibrinolysis* **29**, 243–251 (2018).

10. Ellery, P. & Adams, M. Tissue Factor Pathway Inhibitor: Then and Now. *Semin. Thromb. Hemost.* **40**, 881–886 (2014).

11. Jordan, R. E., Oosta, G. M., Gardner, W. T. & Rosenberg, R. D. The kinetics of hemostatic enzyme-antithrombin interactions in the presence of low molecular weight heparin. *J. Biol. Chem.* **255**, 10081–90 (1980).

12. Esmon, C. T. The Protein C Pathway. *Chest* **124**, 26S-32S (2003).

13. Andersen, H. *et al.* Protease-activated receptor 1 is the primary mediator of thrombin-stimulated platelet procoagulant activity. *Proc. Natl. Acad. Sci.* **96**, 11189–11193 (1999).

14. Levin, E. G., Marzec, U., Anderson, J. & Harker, L. A. Thrombin stimulates tissue plasminogen activator release from cultured human endothelial cells. *J. Clin. Invest.* **74**, 1988–1995 (1984).

15. Smith, D., Gilbert, M. & Owen, W. G. Tissue plasminogen activator release in vivo in response to vasoactive agents. *Blood* **66**, 835–9 (1985).

16. Amara, U. *et al.* Molecular Intercommunication between the Complement and Coagulation Systems. *J. Immunol.* **185**, 5628–5636 (2010).

17. Huber-Lang, M. *et al.* Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* **12**, 682–687 (2006).

18. Ikeda, K. *et al.* C5a induces tissue factor activity on endothelial cells. *Thromb. Haemost.* **77**, 394–8 (1997).

19. KASTL, S. P. *et al.* The complement component C5a induces the expression of

plasminogen activator inhibitor-1 in human macrophages via NF-?B activation. *J. Thromb. Haemost.* **4**, 1790–1797 (2006).

20. Wojta, J. *et al.* C5a stimulates production of plasminogen activator inhibitor-1 in human mast cells and basophils. *Blood* **100**, 517–523 (2002).

21. Cook, D. J. *et al.* Risk Factors for Gastrointestinal Bleeding in Critically Ill Patients. *N. Engl. J. Med.* **330**, 377–381 (1994).

22. Chu, Y.-F. *et al.* Incidence and risk factors of gastrointestinal bleeding in mechanically ventilated patients. *World J. Emerg. Med.* **1**, 32–6 (2010).

23. Cook, D. J. *et al.* The attributable mortality and length of intensive care unit stay of clinically important gastrointestinal bleeding in critically ill patients. *Crit. Care* **5**, 368–75 (2001).

24. Cook, D. J., Pearl, R. G., Cook, R. J. & Guyatt, G. H. Incidence of Clinically Important Bleeding in Mechanically Ventilated Patients. *J. Intensive Care Med.* **6**, 167–174 (1991).

25. Oppenheim-Eden, A., Glantz, L., Eidelman, L. A. & Sprung, C. L. Spontaneous intracerebral hemorrhage in critically ill patients: incidence over six years and associated factors. *Intensive Care Med.* **25**, 63–67 (1999).

26. Ryu, J.-A. *et al.* Spontaneous intracranial haemorrhage in critically ill patients with malignancies. *Support. Care Cancer* **24**, 2971–8 (2016).

27. Cook D, Meade M, Guyatt G, Walter S, Heels-Ansdell D, Warkentin TE, Zytaruk N, Crowther M, Geerts W, Cooper DJ, Vallance S, Qushmaq I, Rocha M, Berwanger O, V. N. Dalteparin versus Unfractionated Heparin in Critically Ill Patients. *N. Engl. J. Med.*

364, 1305–1314 (2011).

28. Samama, M. M. *et al.* A Comparison of Enoxaparin with Placebo for the Prevention of Venous Thromboembolism in Acutely Ill Medical Patients. *N. Engl. J. Med.* **341**, 793–800 (1999).

29. Leizorovicz, A. *et al.* Randomized, Placebo-Controlled Trial of Dalteparin for the Prevention of Venous Thromboembolism in Acutely Ill Medical Patients. *Circulation* **110**, 874–879 (2004).

30. Pariser, D. N. *et al.* Lung megakaryocytes are immune modulatory cells. *J. Clin. Invest.* **131**, (2021).

31. Zarychanski, R. & Houston, D. S. Assessing thrombocytopenia in the intensive care unit: the past, present, and future. *Hematology* **2017**, 660–666 (2017).

32. Wu, Q. *et al.* Effect of Persistent Thrombocytopenia on Mortality in Surgical Critical Care Patients. *Clin. Appl. Thromb.* **23**, 84–90 (2017).

33. Edwards, R. L., Rickles, F. R. & Bobrove, A. M. Mononuclear cell tissue factor: cell of origin and requirements for activation. *Blood* **54**, 359–70 (1979).

34. McDonald, B. *et al.* Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood* **129**, 1357–1367 (2017).

35. Delabranche, X. *et al.* Early Detection of Disseminated Intravascular Coagulation During Septic Shock. *Crit. Care Med.* **44**, e930–e939 (2016).

36. TOH, C. H. & HOOTS, W. K. The scoring system of the Scientific and Standardisation

Committee on Disseminated Intravascular Coagulation of the International Society on Thrombosis and Haemostasis: a 5-year overview. *J. Thromb. Haemost.* **5**, 604–606 (2007).

37. Wada, H. *et al.* Modified non-overt DIC diagnostic criteria predict the early phase of overt-DIC. *Am. J. Hematol.* **85**, 691–694 (2010).

38. Zakynthinos, S. G. *et al.* Sepsis severity is the major determinant of circulating thrombopoietin levels in septic patients. *Crit. Care Med.* **32**, 1004–1010 (2004).

39. Valade, S., Mariotte, E. & Azoulay, E. Coagulation Disorders in Hemophagocytic Lymphohistiocytosis/Macrophage Activation Syndrome. *Crit. Care Clin.* **36**, 415–426 (2020).

40. Bockmeyer, C. L. *et al.* Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor. *Haematologica* **93**, 137–140 (2008).

41. Aibar, J. *et al.* ADAMTS-13 in Critically Ill Patients With Septic Syndromes and Noninfectious Systemic Inflammatory Response Syndrome. *Shock* **43**, 556–562 (2015).

42. Peigne, V. *et al.* The prognostic value of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13) deficiency in septic shock patients involves interleukin-6 and is not dependent on disseminated intravascular coagulation. *Crit. Care* **17**, R273 (2013).

43. Xu, J. *et al.* Extracellular histones are major mediators of death in sepsis. *Nat. Med.* **15**, 1318–1321 (2009).

44. Fuchs, T. A., Bhandari, A. A. & Wagner, D. D. Histones induce rapid and profound thrombocytopenia in mice. *Blood* **118**, 3708–3714 (2011).

45. Alhamdi, Y., Abrams, S. T., Lane, S., Wang, G. & Toh, C.-H. Histone-Associated Thrombocytopenia in Patients Who Are Critically Ill. *JAMA* **315**, 817 (2016).

46. Ito, T. *et al.* Serum histone H3 levels and platelet counts are potential markers for coagulopathy with high risk of death in septic patients: a single-center observational study. *J. Intensive Care* **7**, 63 (2019).

47. Wildhagen, K. C. A. A. *et al.* Extracellular histone H3 levels are inversely correlated with antithrombin levels and platelet counts and are associated with mortality in sepsis patients. *Thromb. Res.* **136**, 542–547 (2015).

48. Crowther, M. A. *et al.* The 4Ts scoring system for heparin-induced thrombocytopenia in medical-surgical intensive care unit patients. *J. Crit. Care* **25**, 287–293 (2010).

49. Boldt, J., Menges, T., Wollbrück, M., Sonneborn, S. & Hempelmann, G. Platelet Function in Critically Ill Patients. *Chest* **106**, 899–903 (1994).

50. van Hezel, M. E. *et al.* The effect of red blood cell transfusion on platelet function in critically ill patients. *Thromb. Res.* **184**, 115–121 (2019).

51. Hase, T. *et al.* Platelet dysfunction in critically ill patients. *Blood Coagul. Fibrinolysis* **28**, 475–478 (2017).

52. Lundahl, T. H. *et al.* Activated platelets and impaired platelet function in intensive care patients analyzed by flow cytometry. *Blood Coagul. Fibrinolysis* **7**, 218–220 (1996).

53. Protti, A. *et al.* Platelet mitochondrial dysfunction in critically ill patients: comparison between sepsis and cardiogenic shock. *Crit. Care* **19**, 39 (2015).

54. Vulliamy, P. *et al.* Histone H4 induces platelet ballooning and microparticle release during trauma hemorrhage. *Proc. Natl. Acad. Sci.* **116**, 17444–17449 (2019).

55. Ekaney, M. L. *et al.* Impact of plasma histones in human sepsis and their contribution to cellular injury and inflammation. *Crit. Care* **18**, 543 (2014).

56. Boldt, J., Menges, T., Wollbruck, M., Sonnebokn, S. & Hempelmann, G. Continuous hemofiltration and platelet function in critically ill patients. *Crit. Care Med.* **22**, 1155–1160 (1994).

57. Hedges, S. J., Dehoney, S. B., Hooper, J. S., Amanzadeh, J. & Busti, A. J. Evidence-based treatment recommendations for uremic bleeding. *Nat. Clin. Pract. Nephrol.* **3**, 138–53 (2007).

58. Woźnica, E., Inglot, M., Woźnica, R. & Łysenko, L. Liver dysfunction in sepsis. *Adv. Clin. Exp. Med.* **27**, 547–552 (2018).

59. Kobashi, H., Toshimori, J. & Yamamoto, K. Sepsis-associated liver injury: Incidence, classification and the clinical significance. *Hepatol. Res.* **43**, 255–266 (2013).

60. Ohmann, C., Stöltzing, H., Wins, L., Busch, E. & Thon, K. Prognostic Scores in Oesophageal or Gastric Variceal Bleeding. *Scand. J. Gastroenterol.* **25**, 501–512 (1990).

61. Tripodi, A. *et al.* The coagulopathy of cirrhosis assessed by thromboelastometry and its correlation with conventional coagulation parameters. *Thromb. Res.* **124**, 132–136 (2009).

62. Tripodi, A. *et al.* Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. *Hepatology* **41**, 553–558 (2005).

63. Bos, S. *et al.* Haemostatic Profiles are Similar across All Aetiologies of Cirrhosis. *Thromb. Haemost.* **119**, 246–253 (2019).

64. Collins, P. W. *et al.* Global tests of haemostasis in critically ill patients with severe sepsis syndrome compared to controls. *Br. J. Haematol.* **135**, 220–227 (2006).

65. Winstedt, D., Solomon, C., Hillarp, A., Lundahl, T. & Schött, U. Intraoperative Hydroxyethyl Starch and its Effects on Different Fibrinogen Measurements. *Clin. Appl. Thromb.* **22**, 641–647 (2016).

66. Schlimp, C. J., Cadamuro, J., Solomon, C., Redl, H. & Schöchl, H. The effect of fibrinogen concentrate and factor XIII on thromboelastometry in 33% diluted blood with albumin, gelatine, hydroxyethyl starch or saline in vitro. *Blood Transfus.* **11**, 510–7 (2013).

67. Ranucci, M., Baryshnikova, E., Ciotti, E., Ranucci, M. & Silvetti, S. Hemodilution on Cardiopulmonary Bypass: Thromboelastography Patterns and Coagulation-Related Outcomes. *J. Cardiothorac. Vasc. Anesth.* **31**, 1588–1594 (2017).

68. Murray, D. J., Pennell, B. J., Weinstein, S. L. & Olson, J. D. Packed Red Cells in Acute Blood Loss. *Anesth. Analg.* **80**, 336–342 (1995).

69. Lorente, J. A. *et al.* Time Course of Hemostatic Abnormalities in Sepsis and its Relation to Outcome. *Chest* **103**, 1536–1542 (1993).

70. Mavrommatis, A. C. *et al.* Activation of the fibrinolytic system and utilization of the coagulation inhibitors in sepsis: comparison with severe sepsis and septic shock. *Intensive Care Med.* **27**, 1853–1859 (2001).

71. Watanabe, R. *et al.* Activity and Antigen Levels of Thrombin-Activatable Fibrinolysis

Inhibitor in Plasma of Patients With Disseminated Intravascular Coagulation. *Thromb. Res.* **104**, 1–6 (2001).

72. Panigada, M. *et al.* Assessment of Fibrinolysis in Sepsis Patients with Urokinase Modified Thromboelastography. *PLoS One* **10**, e0136463 (2015).

73. Müller, M. C., Meijers, J. C., Vroom, M. B. & Juffermans, N. P. Utility of thromboelastography and/or thromboelastometry in adults with sepsis: a systematic review. *Crit. Care* **18**, R30 (2014).

74. Leiper, K. *et al.* Tissue plasminogen activator, plasminogen activator inhibitors, and activator-inhibitor complex in liver disease. *J. Clin. Pathol.* **47**, 214–217 (1994).

75. GUNAWAN, B. & RUNYON, B. The efficacy and safety of e-aminocaproic acid treatment in patients with cirrhosis and hyperfibrinolysis. *Aliment. Pharmacol. Ther.* **23**, 115–120 (2006).

76. Cook, D. J. & Crowther, M. A. Thromboprophylaxis in the intensive care unit: Focus on medical–surgical patients. *Crit. Care Med.* **38**, S76–S82 (2010).

77. Cohen, A. T. *et al.* Efficacy and safety of fondaparinux for the prevention of venous thromboembolism in older acute medical patients: randomised placebo controlled trial. *BMJ* **332**, 325–329 (2006).

78. Malato, A. *et al.* The impact of deep vein thrombosis in critically ill patients: a meta-analysis of major clinical outcomes. *Blood Transfus.* **13**, 559–68 (2015).

79. Cook, D. J., Crowther, M. A., Meade, M. O. & Douketis, J. Prevalence, incidence, and risk factors for venous thromboembolism in medical-surgical intensive care unit patients. *J. Crit. Care* **20**, 309–313 (2005).

80. McLeod, A. G. & Geerts, W. Venous Thromboembolism Prophylaxis in Critically Ill Patients. *Crit. Care Clin.* **27**, 765–780 (2011).

81. Moser, K. M., LeMoine, J. R., Nachtwey, F. J. & Spragg, R. G. Deep venous thrombosis and pulmonary embolism. Frequency in a respiratory intensive care unit. *JAMA* **246**, 1422–4 (1981).

82. Tomashefski, J. F. *et al.* The pulmonary vascular lesions of the adult respiratory distress syndrome. *Am. J. Pathol.* **112**, 112–26 (1983).

83. Greene, R. *et al.* Early bedside detection of pulmonary vascular occlusion during acute respiratory failure. *Am. Rev. Respir. Dis.* **124**, 593–601 (1981).

84. Miyazawa, T., Tanemori, N., Utsumi, T., Katayama, S. & Furukawa, N. [Balloon occlusion pulmonary angiography and anticoagulant-antithrombotic therapy in ARDS-associated pulmonary vascular thrombosis]. *Nihon Kyobu Shikkan Gakkai Zasshi* **29**, 439–43 (1991).

85. Vesconi, S., Rossi, G. P., Pesenti, A., Fumagalli, R. & Gattinoni, L. Pulmonary microthrombosis in severe adult respiratory distress syndrome. *Crit. Care Med.* **16**, 111–113 (1988).

86. Frantzeskaki, F., Armaganidis, A. & Orfanos, S. E. Immunothrombosis in Acute Respiratory Distress Syndrome: Cross Talks between Inflammation and Coagulation. *Respiration* **93**, 212–225 (2017).

87. Pillai, P., Joseph, J. P., Fadzillah, N. H. M. & Mahmod, M. COVID-19 and Major Organ Thromboembolism: Manifestations in Neurovascular and Cardiovascular Systems. *J. Stroke Cerebrovasc. Dis.* **30**, 105427 (2021).

88. Tóth, J. *et al.* Characteristics of thrombin generation in a fulminant porcine sepsis model. *Thromb. Res.* **158**, 25–34 (2017).

89. Gando, S., Nanzaki, S., Sasaki, S., Aoi, K. & Kemmotsu, O. Activation of the extrinsic coagulation pathway in patients with severe sepsis and septic shock. *Crit. Care Med.* **26**, 2005–2009 (1998).

90. Brinkmann, V. Neutrophil Extracellular Traps Kill Bacteria. *Science (80-.).* **303**, 1532–1535 (2004).

91. Gould, T. J. *et al.* Neutrophil Extracellular Traps Promote Thrombin Generation Through Platelet-Dependent and Platelet-Independent Mechanisms. *Arterioscler. Thromb. Vasc. Biol.* **34**, 1977–1984 (2014).

92. Yang, S. *et al.* Neutrophil Extracellular Traps Promote Hypercoagulability in Patients With Sepsis. *Shock* **47**, 132–139 (2017).

93. Czaikoski, P. G. *et al.* Neutrophil Extracellular Traps Induce Organ Damage during Experimental and Clinical Sepsis. *PLoS One* **11**, e0148142 (2016).

94. Patel, J. M. *et al.* Sepsis Induces a Dysregulated Neutrophil Phenotype That Is Associated with Increased Mortality. *Mediators Inflamm.* **2018**, 1–10 (2018).

95. Warkentin, T. E. *et al.* Heparin-Induced Thrombocytopenia in Medical Surgical Critical Illness. *Chest* **144**, 848–858 (2013).

96. Hitos, K., Cannnon, M., Cannon, S., Garth, S. & Fletcher, J. P. Effect of leg exercises on popliteal venous blood flow during prolonged immobility of seated subjects: implications for prevention of travel-related deep vein thrombosis. *J. Thromb. Haemost.* **5**, 1890–1895 (2007).

97. Minet, C. *et al.* Pulmonary embolism in mechanically ventilated patients requiring computed tomography. *Crit. Care Med.* **40**, 3202–3208 (2012).

98. Gandotra, S. *et al.* Physical Function Trajectories in Survivors of Acute Respiratory Failure. *Ann. Am. Thorac. Soc.* **16**, 471–477 (2019).

99. Arabi, Y. M. *et al.* Adjunctive Intermittent Pneumatic Compression for Venous Thromboprophylaxis. *N. Engl. J. Med.* **380**, 1305–1315 (2019).

100. Yau, J. W. *et al.* Mechanism of catheter thrombosis: Comparison of the antithrombotic activities of fondaparinux, enoxaparin, and heparin in vitro and in vivo. *Blood* **118**, 6667–6674 (2011).

101. Vogler, E. A. & Siedlecki, C. A. Contact activation of blood-plasma coagulation. *Biomaterials* **30**, 1857–1869 (2009).

102. Forauer, A. R., Theoharis, C. G. A. & Dasika, N. L. Jugular Vein Catheter Placement: Histologic Features and Development of Catheter-related (Fibrin) Sheaths in a Swine Model. *Radiology* **240**, 427–434 (2006).

103. Nifong, T. P. & McDevitt, T. J. The Effect of Catheter to Vein Ratio on Blood Flow Rates in a Simulated Model of Peripherally Inserted Central Venous Catheters. *Chest* **140**, 48–53 (2011).

104. Geerts, W. Central venous catheter–related thrombosis. *Hematology* **2014**, 306–311 (2014).

105. Kaplan, D. *et al.* VTE Incidence and Risk Factors in Patients With Severe Sepsis and Septic Shock. *Chest* **148**, 1224–1230 (2015).

106. Cook, D. *et al.* Venous thromboembolic disease: An observational study in medical-surgical intensive care unit patients. *J. Crit. Care* **15**, 127–132 (2000).

107. Gibbon, J. H. Artificial Maintenance of Circulation During Experimental Occlusion of Pulmonary Artery. *Arch. Surg.* **34**, 1105 (1937).

108. Spencer, F. C. The Development of Valvular Heart Surgery Over the Past 50 Years (1947–1997): Personal Recollections. *Ann. Thorac. Surg.* **64**, 1549–1554 (1997).

109. DeWall, R. A. *et al.* A Simple, Expendable, Artificial Oxygenator for Open Heart Surgery. *Surg. Clin. North Am.* **36**, 1025–1034 (1956).

110. Bartlett, R. H. & Gazzaniga, A. B. Extracorporeal circulation for cardiopulmonary failure. *Curr. Probl. Surg.* **15**, 1–96 (1978).

111. Zapol, W. M. Extracorporeal membrane oxygenation in severe acute respiratory failure. A randomized prospective study. *JAMA J. Am. Med. Assoc.* **242**, 2193–2196 (1979).

112. Peek, G. J. *et al.* Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. *Lancet* **374**, 1351–1363 (2009).

113. Thiagarajan, R. R. *et al.* Extracorporeal Life Support Organization Registry International Report 2016. *ASAIO J.* **63**, 60–67 (2017).

114. Burns, J. *et al.* Retrospective Observational Review of Percutaneous Cannulation for Extracorporeal Membrane Oxygenation. *ASAIO J.* **62**, 325–328 (2016).

115. Wahba, A., Philipp, A., Behr, R. & Birnbaum, D. E. Heparin-coated equipment reduces the risk of oxygenator failure. *Ann. Thorac. Surg.* **65**, 1310–1312 (1998).

116. Camporota, L. & Barrett, N. Current Applications for the Use of Extracorporeal Carbon Dioxide Removal in Critically Ill Patients. *Biomed Res. Int.* **2016**, 1–8 (2016).

117. Flecher, E. *et al.* Current aspects of extracorporeal membrane oxygenation in a tertiary referral centre: determinants of survival at follow-up. *Eur. J. Cardio-Thoracic Surg.* **46**, 665–671 (2014).

118. Cooper, E. *et al.* Prevalence of venous thrombosis following venovenous extracorporeal membrane oxygenation in patients with severe respiratory failure. *Crit. Care Med.* **43**, e581–e584 (2015).

119. Menaker, J. *et al.* Incidence of Cannula-Associated Deep Vein Thrombosis after Veno-Venous Extracorporeal Membrane Oxygenation. *ASAIO J.* **63**, 588–591 (2017).

120. Al-Bawardy, R. *et al.* Extracorporeal membrane oxygenation in acute massive pulmonary embolism: a case series and review of the literature. *Perfusion* **34**, 22–28 (2019).

121. Hartley, E. L., Singh, N., Barrett, N., Wyncoll, D. & Retter, A. Screening pulmonary angiogram and the effect on anticoagulation strategies in severe respiratory failure patients on venovenous extracorporeal membrane oxygenation. *J. Thromb. Haemost.* **18**, 217–221 (2020).

122. Sutter, R., Tisljar, K. & Marsch, S. Acute Neurologic Complications During Extracorporeal Membrane Oxygenation. *Crit. Care Med.* **46**, 1506–1513 (2018).

123. Omar, H. R. *et al.* Incidence and predictors of ischemic cerebrovascular stroke among

patients on extracorporeal membrane oxygenation support. *J. Crit. Care* **32**, (2016).

124. Rastan, A. J. *et al.* Autopsy Findings in Patients on Postcardiotomy Extracorporeal Membrane Oxygenation (ECMO). *Int. J. Artif. Organs* **29**, 1121–1131 (2006).

125. SCHULMAN, S. & KEARON, C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J. Thromb. Haemost.* **3**, 692–694 (2005).

126. Lockie, C. J. A. *et al.* Severe Respiratory Failure, Extracorporeal Membrane Oxygenation, and Intracranial Hemorrhage. *Crit. Care Med.* **45**, 1 (2017).

127. Arachchillage, D. *et al.* Intracranial Hemorrhage and Early Mortality in Patients Receiving Extracorporeal Membrane Oxygenation for Severe Respiratory Failure. *Semin. Thromb. Hemost.* **44**, 276–286 (2018).

128. Mateen, F. J. Neurological Injury in Adults Treated With Extracorporeal Membrane Oxygenation. *Arch. Neurol.* **68**, 1543 (2011).

129. Fletcher-Sandersjöö, A. *et al.* Incidence, Outcome, and Predictors of Intracranial Hemorrhage in Adult Patients on Extracorporeal Membrane Oxygenation: A Systematic and Narrative Review. *Front. Neurol.* **9**, (2018).

130. Fletcher Sandersjöö, A. *et al.* Predictors of intracranial hemorrhage in adult patients on extracorporeal membrane oxygenation: An observational cohort study. *J. Intensive Care* **5**, 1–10 (2017).

131. Kreyer, S. *et al.* Hemorrhage under veno-venous extracorporeal membrane oxygenation in acute respiratory distress syndrome patients: A retrospective data analysis. *J. Thorac. Dis.* **9**, 5017–5029 (2017).

132. Adams, A. L. in Plasma at. **55**, (2018).

133. Turbill, P., Beugeling, T. & Poot, A. A. Proteins involved in the Vroman effect during exposure of human blood plasma to glass and polyethylene. **17**, (1996).

134. Passmore, M. R. *et al.* Evidence of altered haemostasis in an ovine model of venovenous extracorporeal membrane oxygenation support. *Crit. Care* **21**, 1–9 (2017).

135. Moganasundram, S. *et al.* The Relationship Among Thromboelastography, Hemostatic Variables, and Bleeding After Cardiopulmonary Bypass Surgery in Children. **110**, 995–1002 (2010).

136. Millar, J. E., Fanning, J. P., McDonald, C. I., McAuley, D. F. & Fraser, J. F. The inflammatory response to extracorporeal membrane oxygenation (ECMO): A review of the pathophysiology. *Crit. Care* **20**, 1–10 (2016).

137. Poot, A., Beugeling, T., Cazenave, J. P., Bantjes, A. & van Aken, W. G. Platelet deposition in a capillary perfusion model: quantitative and morphological aspects. *Biomaterials* **9**, 126–132 (1988).

138. Watanabe, H., Hayashi, J. & Ohzeki, H. Biocompatibility of a Silicone-Coated Polypropylene Hollow Fiber Oxygenator in an In Vitro Model. **4975**, 3–7 (1999).

139. Bannan, S. *et al.* Cell activation and thrombin generation in heparin bonded cardiopulmonary bypass circuits using a novel in vitro model. *Eur. J. Cardiothorac. Surg.* **12**, 268–75 (1997).

140. Cohen, H. C., Joyce, E. J. & Kao, W. J. Biomaterials selectively modulate interactions between human blood-derived polymorphonuclear leukocytes and monocytes. *Am. J.*

Pathol. **182**, 2180–2190 (2013).

141. Keuren, J. F. W. *et al.* Thrombogenicity of polysaccharide-coated surfaces. *Biomaterials* **24**, 1917–1924 (2003).

142. Malfertheiner, M. V. *et al.* Hemostatic Changes during Extracorporeal Membrane Oxygenation: A Prospective Randomized Clinical Trial Comparing Three Different Extracorporeal Membrane Oxygenation Systems. *Crit. Care Med.* **44**, (2016).

143. Hang, K. *et al.* Molecular Design of Antifouling Polymer Brushes Using Sequence-Specifi c Peptoids. 1–10 (2014) doi:10.1002/admi.201400225.

144. de los Santos Pereira A, Sheikh S, Blaszykowski C, Pop-Georgievski O, Fedorov K, Thompson M, R.-E. C. Antifouling Polymer Brushes Displaying Antithrombogenic Surface Properties. *Biomacromolecules* **17**, 1179–85 (2016).

145. Obstals, F. *et al.* Improving Hemocompatibility of Membranes for Extracorporeal Membrane Oxygenators by Grafting Nonthrombogenic Polymer Brushes. **1700359**, 1–11 (2018).

146. Sun, W. *et al.* Impact of high mechanical shear stress and oxygenator membrane surface on blood damage relevant to thrombosis and bleeding in a pediatric ECMO circuit. *Artif. Organs* **44**, 717–726 (2020).

147. Lukito, P. *et al.* Mechanical circulatory support is associated with loss of platelet receptors glycoprotein Ibα and glycoprotein VI. *J. Thromb. Haemost.* **14**, 2253–2260 (2016).

148. Cheung, P. Y. *et al.* The mechanisms of platelet dysfunction during extracorporeal membrane oxygenation in critically ill neonates. *Crit. Care Med.* **28**, 2584–2590

(2000).

149. Kalbhenn Johannes, Schlagenhauf Axel, Rosenfelder Simone, Schmutz Axel, Z. B. Acquired von Willebrand syndrome and impaired platelet function during venovenous extracorporeal membrane oxygenation: Rapid onset and fast recovery. *J. Hear. Lung Transplant.* **37**, 985–91 (2018).

150. Kalbhenn, J. *et al.* Early Diagnosis of Acquired von Willebrand Syndrome (AVWS) is Elementary for Clinical Practice in Patients Treated with ECMO Therapy. *J. Atheroscler. Thromb.* **22**, 265–271 (2015).

151. Dewanjee, M. K. *et al.* Emboli from an extraluminal blood flow hollow fiber oxygenator with and without an arterial filter during cardiopulmonary bypass in a pig model. *ASAIO Journal* vol. 42 1010–1018 (1996).

152. Pugsley W, Klinger L, P. C. et al. Microemboli and cerebral impairment during cardiac surgery. *Vasc. Surg.* **24**, 34–43 (1990).

153. Membrane, E. & Systems, O. HHS Public Access. **61**, 37–42 (2016).

154. Miyazaki, Y. *et al.* High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. *Blood* **88**, 3456–3464 (1996).

155. Heilmann, C. *et al.* Acquired von Willebrand syndrome in patients with extracorporeal life support (ECLS). *Intensive Care Med.* **38**, 62–68 (2012).

156. Pasala, S., Fiser, R. T., Stine, K. C., Swearingen, C. J. & Prodhan, P. Von willebrand factor multimers in pediatric extracorporeal membrane oxygenation support. *ASAIO J.* **60**, 419–423 (2014).

157. Tauber, H. *et al.* Extracorporeal Membrane Oxygenation Induces Short-Term Loss of High-Molecular-Weight von Willebrand Factor Multimers. *Anesth. Analg.* **120**, 730–736 (2015).

158. Adachi, I., Kostousov, V., Hensch, L., Chacon-Portillo, M. A. & Teruya, J. Management of Hemostasis for Pediatric Patients on Ventricular-Assist Devices. *Semin. Thromb. Hemost.* **44**, 30–37 (2018).

159. Vincent, F. *et al.* Arterial Pulsatility and Circulating von Willebrand Factor in Patients on Mechanical Circulatory Support. *J. Am. Coll. Cardiol.* **71**, 2106–2118 (2018).

160. Halaweish, I., Cole, A., Cooley, E., Lynch, W. R. & Haft, J. W. Roller and Centrifugal Pumps. *ASAIO J.* **61**, 496–501 (2015).

161. Lou, S., MacLaren, G., Best, D., Delzoppo, C. & Butt, W. Hemolysis in pediatric patients receiving centrifugal-pump extracorporeal membrane oxygenation: Prevalence, risk factors, and outcomes. *Crit. Care Med.* **42**, 1213–1220 (2014).

162. Omar, H. R., Mirsaeidi, M., Socias, S. & Sprenker, C. Plasma Free Hemoglobin Is an Independent Predictor of Mortality among Patients on Extracorporeal Membrane Oxygenation Support. 1–10 (2015) doi:10.1371/journal.pone.0124034.

163. Marcus, Aaron J, Broekman, M. J. Cell-Free Hemoglobin as an Oxygen Carrier Removes Nitric Oxide, Resulting in Defective Thromboregulation. *Circulation* **92**, 208–9 (1996).

164. Da, Q. *et al.* Free hemoglobin increases von Willebrand factor-mediated platelet adhesion in vitro: Implications for circulatory devices. *Blood* **126**, 2338–2341 (2015).

165. Cate, H. T. E. N. Platelet- and erythrocyte-derived microparticles trigger thrombin

generation via factor XIIa. 1355–1362 (2012) doi:10.1111/j.1538-7836.2012.04758.x.

166. Dornia, C. *et al.* Analysis of thrombotic deposits in extracorporeal membrane oxygenators by multidetector computed tomography. *ASAIO J.* **60**, 652–656 (2014).

167. Hastings, S. M., Ku, D. N., Wagoner, S., Maher, K. O. & Deshpande, S. Sources of Circuit Thrombosis in Pediatric Extracorporeal Membrane Oxygenation. *ASAIO J.* **63**, 86–92 (2017).

168. Kimmoun, A. *et al.* Prevalence and outcome of heparin-induced thrombocytopenia diagnosed under veno-arterial extracorporeal membrane oxygenation: a retrospective nationwide study. *Intensive Care Med.* **44**, 1460–1469 (2018).

169. Laurance Lequier, Gail Annich, Omar Al-Ibrahim, Melania Bembea, D. B., Tom Brogan, Shannon Buckvold, Louis Chicoine, Steve Conrad, D. C., Heidi Dalton, Jason Frischer, Bill Harris, Robert Mazor, Matthew Paden, N. & Rintoul, Lindsay Ryerson, Phil Spinella, Jun Teruya, Annie Winkler, Trisha Wong, M. P. M. ELSO Anticoagulation Guidelines. 10 https://www.elso.org/portals/0/files/elsoanticoagulationguideline8-2014-table-contents.pdf (2014).

170. Bembea, M. M. *et al.* Variability in Anticoagulation Management of Patients on Extracorporeal Membrane Oxygenation. *Pediatr. Crit. Care Med.* **14**, e77–e84 (2013).

171. Arnouk, S. *et al.* Evaluation of Anti-Xa and Activated Partial Thromboplastin Time Monitoring of Heparin in Adult Patients Receiving Extracorporeal Membrane Oxygenation Support. *ASAIO J.* **66**, 300–306 (2020).

172. Liveris, A. *et al.* Anti-Factor Xa Assay Is a Superior Correlate of Heparin Dose Than Activated Partial Thromboplastin Time or Activated Clotting Time in Pediatric

Extracorporeal Membrane Oxygenation*. *Pediatr. Crit. Care Med.* **15**, e72–e79 (2014).

173. Saifee, N. H. *et al.* Monitoring Hemostasis During Extracorporeal Life Support. *ASAIO J.* **66**, 230–237 (2020).

174. Parzy, G. *et al.* Prevalence and Risk Factors for Thrombotic Complications Following Venovenous Extracorporeal Membrane Oxygenation. *Crit. Care Med.* **48**, 192–199 (2020).

175. Chi, G. *et al.* Venous Thromboembolism among Hospitalized Patients with COVID-19 Undergoing Thromboprophylaxis: A Systematic Review and Meta-Analysis. *J. Clin. Med.* **9**, 2489 (2020).

176. Avnon, L. S., Munteanu, D., Smoliakov, A., Jotkowitz, A. & Barski, L. Thromboembolic events in patients with severe pandemic influenza A/H1N1. *Eur. J. Intern. Med.* **26**, 596–598 (2015).

177. Shaw, R. J., Bradbury, C., Abrams, S. T., Wang, G. & Toh, C. COVID-19 and immunothrombosis: emerging understanding and clinical management. *Br. J. Haematol.* **194**, 518–529 (2021).

178. Parzy, G. *et al.* Venous Thromboembolism Events Following Venovenous Extracorporeal Membrane Oxygenation for Severe Acute Respiratory Syndrome Coronavirus 2 Based on CT Scans. *Crit. Care Med.* **48**, e971–e975 (2020).

179. Fisser, C. *et al.* Incidence and Risk Factors for Cannula-Related Venous Thrombosis After Venovenous Extracorporeal Membrane Oxygenation in Adult Patients With Acute Respiratory Failure. *Crit. Care Med.* **47**, e332–e339 (2019).

180. Cavayas, Y. A., Munshi, L., del Sorbo, L. & Fan, E. The Early Change in Pa CO 2 after

Extracorporeal Membrane Oxygenation Initiation Is Associated with Neurological Complications. *Am. J. Respir. Crit. Care Med.* **201**, 1525–1535 (2020).

181. Vaquer, S., de Haro, C., Peruga, P., Oliva, J. C. & Artigas, A. Systematic review and meta-analysis of complications and mortality of veno-venous extracorporeal membrane oxygenation for refractory acute respiratory distress syndrome. *Ann. Intensive Care* **7**, 51 (2017).

182. Zangrillo, A. *et al.* A meta-analysis of complications and mortality of extracorporeal membrane oxygenation. *Crit. Care Resusc.* **15**, 172–8 (2013).

183. Anton-Martin, P. *et al.* Pre-ECMO Coagulopathy does not Increase the Occurrence of Hemorrhage during Extracorporeal Support. *Int. J. Artif. Organs* **40**, 250–255 (2017).

184. Lotz, C. *et al.* Therapeutic Interventions and Risk Factors of Bleeding During Extracorporeal Membrane Oxygenation. *ASAIO J.* **63**, 624–630 (2017).

185. Engelmann, B. & Massberg, S. Thrombosis as an intravascular effector of innate immunity. *Nat. Rev. Immunol.* **13**, 34–45 (2013).

186. Komeyama, S. *et al.* The Early Initiation of Extracorporeal Life Support May Improve the Neurological Outcome in Adults with Cardiac Arrest due to Cardiac Events. *Intern. Med.* **58**, 1391–1397 (2019).

187. Hunsicker, O. *et al.* Timing, Outcome, and Risk Factors of Intracranial Hemorrhage in Acute Respiratory Distress Syndrome Patients During Venovenous Extracorporeal Membrane Oxygenation. *Crit. Care Med.* **49**, e120–e129 (2021).

188. Luyt, C.-E. *et al.* Brain injury during venovenous extracorporeal membrane oxygenation. *Intensive Care Med.* **42**, 897–907 (2016).

189. Foerch, C. *et al.* Elevated Serum S100B Levels Indicate a Higher Risk of Hemorrhagic Transformation After Thrombolytic Therapy in Acute Stroke. *Stroke* **38**, 2491–2495 (2007).

190. Castellanos, M. *et al.* Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. *Stroke* **34**, 40–6 (2003).

191. Fletcher-Sandersjöö, A. *et al.* Serial S100B Sampling Detects Intracranial Lesion Development in Patients on Extracorporeal Membrane Oxygenation. *Front. Neurol.* **10**, (2019).

192. Gazzolo, D., Masetti, P., Meli, M., Grutzfeld, D. & Michetti, F. Elevated S100B protein as an early indicator of intracranial haemorrhage in infants subjected to extracorporeal membrane oxygenation. *Acta Paediatr.* **91**, 218–221 (2002).

193. Panholzer, B. *et al.* Acquired von Willebrand syndrome in ECMO patients: A 3-year cohort study. *Blood Cells, Mol. Dis.* **87**, 102526 (2021).

194. Mazzeffi, M. *et al.* Bleeding, Transfusion, and Mortality on Extracorporeal Life Support: ECLS Working Group on Thrombosis and Hemostasis. *Ann. Thorac. Surg.* **101**, 682–689 (2016).

195. Schmidt, M. *et al.* Predicting Survival after Extracorporeal Membrane Oxygenation for Severe Acute Respiratory Failure. The Respiratory Extracorporeal Membrane Oxygenation Survival Prediction (RESP) Score. *Am. J. Respir. Crit. Care Med.* **189**, 1374–1382 (2014).

196. Le Berre, A. *et al.* Dual-energy CT angiography reveals high prevalence of perfusion defects unrelated to pulmonary embolism in COVID-19 lesions. *Insights Imaging* **12**,

24 (2021).

197. Calfee, C. S. *et al.* Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir. Med.* **2**, 611–620 (2014).

198. Escher, R., Breakey, N. & Lämmle, B. ADAMTS13 activity, von Willebrand factor, factor VIII and D-dimers in COVID-19 inpatients. *Thromb. Res.* **192**, 174–175 (2020).

199. Goshua, G. *et al.* Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol.* **7**, e575–e582 (2020).

200. Magro, C. *et al.* Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl. Res.* **220**, 1–13 (2020).

201. Cauchie, P. *et al.* Diagnosis and prognosis of overt disseminated intravascular coagulation in a general hospital—Meaning of the ISTH score system, fibrin monomers, and lipoprotein–C-reactive protein complex formation. *Am. J. Hematol.* **81**, 414–419 (2006).

202. Kreger, B. E., Craven, D. E. & McCabe, W. R. Gram-negative bacteremia. *Am. J. Med.* **68**, 344–355 (1980).

203. Spero, J. A., Lewis, J. H. & Hasiba, U. Disseminated intravascular coagulation. Findings in 346 patients. *Thromb. Haemost.* **43**, 28–33 (1980).

204. Combes, A. *et al.* Extracorporeal membrane oxygenation for acute respiratory distress syndrome in adults. *N. Engl. J. Med.* **378**, 1965–1975 (2018).

205. Doyle, A. J. *et al.* Blood component use in critical care in patients with COVID-19 infection: a single-centre experience. *Br. J. Haematol.* **191**, 382–385 (2020).

206. Stiller, B. *et al.* Consumption of blood products during mechanical circulatory support in children: comparison between ECMO and a pulsatile ventricular assist device. *Intensive Care Med.* **30**, (2004).

207. Abbasciano, R. G., Yusuff, H., Vlaar, A. P. J., Lai, F. & Murphy, G. J. Blood Transfusion Threshold in Patients Receiving Extracorporeal Membrane Oxygenation Support for Cardiac and Respiratory Failure—A Systematic Review and Meta-Analysis. *J. Cardiothorac. Vasc. Anesth.* (2020) doi:10.1053/j.jvca.2020.08.068.

208. ELSO Guidelines for Cardiopulmonary Extracorporeal Life Support. Extracorporeal Life Support Organization (ELSO) Guidelines for Adult Respiratory Failure. *ELSO Guidel. Adult Respir. Fail. Guidel.* **Version 1.**, 1–32 (2017).

209. Singh, G. *et al.* Transfusion Thresholds for Adult Respiratory Extracorporeal Life Support: An Expert Consensus Document. *Can. J. Cardiol.* **36**, 1550–1553 (2020).

210. Holcomb, J. B. *et al.* The Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) Study. *JAMA Surg.* **148**, 127 (2013).

211. Holcomb, J. B. *et al.* Transfusion of Plasma, Platelets, and Red Blood Cells in a 1:1:1 vs a 1:1:2 Ratio and Mortality in Patients With Severe Trauma. *JAMA* **313**, 471 (2015).

212. Guimbretière, G. *et al.* Prognostic impact of blood product transfusion in VA and VV ECMO. *Perfusion* **34**, 246–253 (2019).

213. Winkler, A. M. Transfusion Management during Extracorporeal Support. in *Extracorporeal Life Support: The ELSO Red Book* 105–122 (2017).

214. Cashen, K. *et al.* Platelet Transfusion Practice and Related Outcomes in Pediatric Extracorporeal Membrane Oxygenation*. *Pediatr. Crit. Care Med.* **21**, 178–185 (2020).

215. Henríquez-Henríquez, M. *et al.* Blood Component Usage during Extracorporeal Membrane Oxygenation: Experience in 98 Patients at a Latin-American Tertiary Hospital. *Int. J. Artif. Organs* **37**, 233–240 (2014).

216. Smith, A. H., Hardison, D. C., Bridges, B. C. & Pietsch, J. B. Red blood cell transfusion volume and mortality among patients receiving extracorporeal membrane oxygenation. *Perfus. (United Kingdom)* **28**, 54–60 (2013).

217. Swol, J., Marschall, C., Strauch, J. T. & Schildhauer, T. A. Hematocrit and impact of transfusion in patients receiving extracorporeal life support. *Perfus. (United Kingdom)* **33**, 546–552 (2018).

218. Esper, S. A. *et al.* Platelet transfusion is associated with 90-day and 1-year mortality for adult patients requiring veno-arterial extracorporeal membrane oxygenation. *Vox Sang.* vox.13016 (2020) doi:10.1111/vox.13016.

219. Hughes, T., Zhang, D., Nair, P. & Buscher, H. A Systematic Literature Review of Packed Red Cell Transfusion Usage in Adult Extracorporeal Membrane Oxygenation. *Membranes (Basel).* **11**, 251 (2021).

220. Martucci, G. *et al.* Hemoglobin trigger and approach to red blood cell transfusions during veno-venous extracorporeal membrane oxygenation: the international TRAIN-ECMO survey. *Perfusion* **34**, 39–48 (2019).

221. ELSO. Extracorporeal Life Support Organization (ELSO). Guidelines for Adult

Respiratory Failure. 1–32 https://www.elso.org/Portals/0/ELSO Guidelines For Adult Respiratory Failure 1_4.pdf (2017).

222. Spinelli, E. & Bartlett, R. H. Anemia and Transfusion in Critical Care. *J. Intensive Care Med.* **31**, 295–306 (2016).

223. Corwin, H. L. & Napolitano, L. M. Anemia in the Critically Ill. *Crit. Care Med.* **42**, 2140–2141 (2014).

224. Spinelli, E. & Bartlett, R. H. Relationship Between Hemoglobin Concentration and Extracorporeal Blood Flow as Determinants of Oxygen Delivery During Venovenous Extracorporeal Membrane Oxygenation. *ASAIO J.* **60**, 688–693 (2014).

225. Cahill, C. M. *et al.* Implementation of a Standardized Transfusion Protocol for Cardiac Patients Treated With Venoarterial Extracorporeal Membrane Oxygenation Is Associated With Decreased Blood Component Utilization and May Improve Clinical Outcome. *Anesth. Analg.* **126**, 1262–1267 (2018).

226. Agerstrand, C. L., Burkart, K. M., Abrams, D. C., Bacchetta, M. D. & Brodie, D. Blood conservation in extracorporeal membrane oxygenation for acute respiratory distress syndrome. *Ann. Thorac. Surg.* **99**, 590–595 (2015).

227. Mazer, C. D. *et al.* Restrictive or Liberal Red-Cell Transfusion for Cardiac Surgery. *N. Engl. J. Med.* **377**, 2133–2144 (2017).

228. Murphy, G. J. *et al.* Liberal or Restrictive Transfusion after Cardiac Surgery. *N. Engl. J. Med.* **372**, 997–1008 (2015).

229. Hebert, P. C. *et al.* A Multicenter, Randomized, Controlled Clinical Trial of Transfusion Requirements in Critical Care. *J. Urol.* **162**, 280–280 (2003).

230. Voelker, M. T. *et al.* Restrictive transfusion practice during extracorporeal membrane oxygenation therapy for severe acute respiratory distress syndrome. *Artif. Organs* **39**, 374–378 (2015).

231. Lo Pinto H; Allyn J; Persichini R; Bouchet B; Brochier G; Martinet O; Brulliard C; Valance D; Delmas B; Braunberger E; Dangers L; Allou N. Predictors of red blood cell transfusion and its association with prognosis in patients undergoing extracorporeal membrane oxygenation. *Int. J. Artif. Organs* **41**, 644–652 (2018).

232. Ang, A. L. *et al.* Blood transfusion requirements and independent predictors of increased transfusion requirements among adult patients on extracorporeal membrane oxygenation - a single centre experience. *Vox Sang.* **96**, 34–43 (2009).

233. Karam, O., Goel, R., Dalton, H. & Nellis, M. E. Epidemiology of Hemostatic Transfusions in Children Supported by Extracorporeal Membrane Oxygenation. *Crit. Care Med.* **48**, e698–e705 (2020).

234. Chevuru, S. C. *et al.* Multicenter Analysis of Platelet Transfusion Usage Among Neonates on Extracorporeal Membrane Oxygenation. *Pediatrics* **109**, e89–e89 (2002).

235. Abrams, D., Baldwin, M. R, Champion, M., Agerstrand, C., Eisenberger, A., Bacchetta, M., & Brodie, D. Thrombocytopenia and Extracorporeal Membrane Oxygenation in Adults with Acute Respiratory Failure: A Cohort Study. *Intensive Care Med.* **42**, 844–52 (2016).

236. Wu, M.Y., Chou, P.L., Wu, T.I. & Lin, P.J. Predictors of hospital mortality in adult trauma patients receiving extracorporeal membrane oxygenation for advanced life support: a retrospective cohort study. *Ina. J. Trauma, Resusc. Emerg. Med.* **26**, 1–12

(2018).

237. Murphy, G. J. *et al.* Increased Mortality, Postoperative Morbidity, and Cost After Red Blood Cell Transfusion in Patients Having Cardiac Surgery. *Circulation* **116**, 2544–2552 (2007).

238. Paden, M. L., Conrad, S. A., Rycus, P. T. & Thiagarajan, R. R. Extracorporeal life support organization registry report 2012. *ASAIO J.* **59**, 202–210 (2013).

239. Bartlett RH, C. S. The Physiology of Extracorporeal Life Support. in *Extracorporeal Life Support: The ELSO Red Book* 31–47 (2017).

240. Vasques, F., Romitti, F., Gattinoni, L. & Camporota, L. How I wean patients from veno-venous extra-corporeal membrane oxygenation. *Crit. Care* **23**, 316 (2019).

241. Vincent, J.L. *et al.* Sepsis in European intensive care units: Results of the SOAP study. *Crit. Care Med.* **34**, 344–353 (2006).

242. Spinella, P. C. *et al.* Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in hospital mortality in patients with traumatic injuries. *Crit. Care* **13**, R151 (2009).

243. Aubron, C. *et al.* Factors associated with outcomes of patients on extracorporeal membrane oxygenation support: a 5-year cohort study. *Crit. Care* **17**, R73 (2013).

244. Keene, S. D. *et al.* Blood product transfusion and mortality in neonatal extracorporeal membrane oxygenation. *Transfusion* **60**, 262–268 (2020).

245. Curley, A. *et al.* Randomized Trial of Platelet-Transfusion Thresholds in Neonates. *N. Engl. J. Med.* **380**, 242–251 (2019).

246. Baharoglu, M. I. *et al.* Platelet transfusion versus standard care after acute stroke due to spontaneous cerebral haemorrhage associated with antiplatelet therapy (PATCH): a randomised, open-label, phase 3 trial. *Lancet* **387**, 2605–2613 (2016).

247. Thorn, S., Güting, H., Mathes, T., Schäfer, N. & Maegele, M. The effect of platelet transfusion in patients with traumatic brain injury and concomitant antiplatelet use: a systematic review and meta-analysis. *Transfusion* **59**, 3536–3544 (2019).

248. Ming, Y. *et al.* Transfusion of Red Blood Cells, Fresh Frozen Plasma, or Platelets Is Associated With Mortality and Infection After Cardiac Surgery in a Dose-Dependent Manner. *Anesth. Analg.* **130**, 488–497 (2020).

249. Khorana, A. A. *et al.* Blood Transfusions, Thrombosis, and Mortality in Hospitalized Patients With Cancer. *Arch. Intern. Med.* **168**, 2377 (2008).

250. Christou, G. *et al.* Impact of platelet transfusion on toxicity and mortality after hematopoietic progenitor cell transplantation. *Transfusion* **55**, 253–258 (2015).

251. Schmidt, A. E., Henrichs, K. F., Kirkley, S. A., Refaai, M. A. & Blumberg, N. Prophylactic Preprocedure Platelet Transfusion Is Associated With Increased Risk of Thrombosis and Mortality. *Am. J. Clin. Pathol.* **149**, 87–94 (2018).

252. Shams Hakimi, C., Singh, S., Hesse, C. & Jeppsson, A. Effects of fibrinogen and platelet transfusion on coagulation and platelet function in bleeding cardiac surgery patients. *Acta Anaesthesiol. Scand.* **63**, 475–482 (2019).

253. Rosenfeld, B. A., Herfel, B., Faraday, N., Fuller, A. & Braine, H. Effects of Storage Time on Quantitative and Qualitative Platelet Function after Transfusion. *Anesthesiology* **83**, 1167–1172 (1995).

254. Vulliamy, P. *et al.* Platelet transfusions reduce fibrinolysis but do not restore platelet function during trauma hemorrhage. *J. Trauma Acute Care Surg.* **83**, 388–397 (2017).

255. Layios, N. *et al.* Sepsis prediction in critically ill patients by platelet activation markers on ICU admission: a prospective pilot study. *Intensive Care Med. Exp.* **5**, 32 (2017).

256. Russwurm, S. *et al.* Platelet and leukocyte activation correlate with the severity of septic organ dysfunction. *Shock* **17**, 263–268 (2002).

257. Lonergan, T. *et al.* The HAT Score—A Simple Risk Stratification Score for Coagulopathic Bleeding During Adult Extracorporeal Membrane Oxygenation. *J. Cardiothorac. Vasc. Anesth.* **31**, 863–868 (2017).

258. Triulzi, D. *et al.* A multicenter study of plasma use in the United States. *Transfusion* **55**, 1313–1319 (2015).

259. Warner, M. A., Chandran, A., Jenkins, G. & Kor, D. J. Prophylactic Plasma Transfusion Is Not Associated With Decreased Red Blood Cell Requirements in Critically Ill Patients. *Anesth. Analg.* **124**, 1636–1643 (2017).

260. McMichael, A. B. V., Zimmerman, K. O., Kumar, K. R. & Ozment, C. P. Evaluation of effect of scheduled fresh frozen plasma on <scp>ECMO</scp> circuit life: A randomized pilot trial. *Transfusion* **61**, 42–51 (2021).

261. Chen, F.T. *et al.* Impact of massive blood transfusion during adult extracorporeal membrane oxygenation support on long-term outcomes: a nationwide cohort study in Taiwan. *BMJ Open* **10**, e035486 (2020).

262. Malfertheiner, MD. *et al*. Hemostatic Changes During Extracorporeal Membrane Oxygenation: A Prospective Randomized Clinical Trial Comparing Three Different

Extracorporeal Membrane Oxygenation Systems. *Crit. Care Med.* **44**, 747–54 (2016).

263. Ghazi, L., Schwann, T. A., Engoren, M. C. & Habib, R. H. Role of blood transfusion product type and amount in deep vein thrombosis after cardiac surgery. *Thromb. Res.* **136**, 1204–1210 (2015).

264. Abu-Rustum, N. R. *et al.* Transfusion utilization during adnexal or peritoneal cancer surgery: Effects on symptomatic venous thromboembolism and survival. *Gynecol. Oncol.* **99**, 320–326 (2005).

265. Emuakhagbon, V., Philips, P., Agopian, V., Kaldas, F. M. & Jones, C. M. Incidence and risk factors for deep venous thrombosis and pulmonary embolus after liver transplantation. *Am. J. Surg.* **211**, 768–771 (2016).

266. Chung, Y. S. *et al.* Is Stopping Heparin Safe in Patients on Extracorporeal Membrane Oxygenation Treatment? *ASAIO J.* **63**, 32–36 (2017).

267. Kurihara, C. *et al.* Feasibility of Venovenous Extracorporeal Membrane Oxygenation Without Systemic Anticoagulation. *Ann. Thorac. Surg.* **110**, 1209–1215 (2020).

268. Kaatz, S., Ahmad, D., Spyropoulos, A. C. & Schulman, S. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. *J. Thromb. Haemost.* **13**, 2119–2126 (2015).

269. Malfertheiner, M. V. *et al.* Ex vivo models for research in extracorporeal membrane oxygenation: a systematic review of the literature. *Perfusion* **35**, 38–49 (2020).

270. Winnersbach, P. *et al.* Platelet count reduction during in vitro membrane oxygenation affects platelet activation, neutrophil extracellular trap formation and clot stability,

but does not prevent clotting. *Perfus. (United Kingdom)* 026765912198923 (2021) doi:10.1177/0267659121989231.

271. Bleilevens, C. *et al.* Twelve Hours In Vitro Biocompatibility Testing of Membrane Oxygenators. *ASAIO J.* **61**, 548–555 (2015).

272. Meyer, A. D. *et al.* Effect of blood flow on platelets, leukocytes, and extracellular vesicles in thrombosis of simulated neonatal extracorporeal circulation. *J. Thromb. Haemost.* **18**, 399–410 (2020).

273. Ki, K. K. *et al.* Low flow rate alters haemostatic parameters in an ex-vivo extracorporeal membrane oxygenation circuit. *Intensive Care Med. Exp.* **7**, 51 (2019).

274. Teligui, L. *et al.* Ex vivo simulation of cardiopulmonary bypass with human blood for hemocompatibility testing. *Perfus. (United Kingdom)* **31**, 376–383 (2016).

275. Meyer, A. D. *et al.* Hemolytic and thrombocytopathic characteristics of extracorporeal membrane oxygenation systems at simulated flow rate for neonates*. *Pediatr. Crit. Care Med.* **13**, e255–e261 (2012).

276. Van Poucke, S. *et al.* Platelet Function During Hypothermia in Experimental Mock Circulation. *Artif. Organs* **40**, 288–293 (2016).

277. Huyzen, R. J. *et al.* In vitro effect of hemodilution on activated clotting time and high-dose thrombin time during cardiopulmonary bypass. *Ann. Thorac. Surg.* **62**, 533–7 (1996).

278. Darlington, D. N. *et al.* Effect of Hemodilution on Coagulation and Recombinant Factor VIIa Efficacy in Human Blood In Vitro. *J. Trauma Inj. Infect. Crit. Care* **71**, 1152–1163 (2011).

279. Zakhary, B., Sheldrake, J. & Pellegrino, V. Extracorporeal membrane oxygenation and V/Q ratios: an ex vivo analysis of CO 2 clearance within the Maquet Quadrox-iD oxygenator. *Perfusion* **35**, 29–33 (2020).

280. Dirkmann, D., Hanke, A. A., Görlinger, K. & Peters, J. Hypothermia and Acidosis Synergistically Impair Coagulation in Human Whole Blood. *Anesth. Analg.* **106**, 1627–1632 (2008).

281. Paluszkiewicz, A., Kellner, J., Elshehabi, M. & Schneditz, D. Effect of Hemolysis and Free Hemoglobin on Optical Hematocrit Measurements in the Extracorporeal Circulation. *ASAIO J.* **54**, 181–184 (2008).

282. Weerwind PW, van der Veen FH, Lindhout T, de Jong DS, C. P. Ex vivo testing of heparin-coated extracorporeal circuits: bovine experiments. *Int. J. Artif. Organs* **21**, 291–8 (1998).

283. Wolberg, A. S. & Campbell, R. A. Thrombin generation, fibrin clot formation and hemostasis. *Transfus. Apher. Sci.* **38**, 15–23 (2008).

284. LUTSEY, P. L., FOLSOM, A. R., HECKBERT, S. R. & CUSHMAN, M. Peak thrombin generation and subsequent venous thromboembolism: the Longitudinal Investigation of Thromboembolism Etiology (LITE) study. *J. Thromb. Haemost.* **7**, 1639–1648 (2009).

285. Capecchi, M. *et al.* Relationship between thrombin generation parameters and prothrombin fragment 1 + 2 plasma levels. *Int. J. Lab. Hematol.* **43**, (2021).

286. Chandler, W. L. & Velan, T. Estimating the rate of thrombin and fibrin generation in vivo during cardiopulmonary bypass. *Blood* **101**, 4355–4362 (2003).

287. Sniecinski, R. M. & Chandler, W. L. Activation of the Hemostatic System During

Cardiopulmonary Bypass. *Anesth. Analg.* **113**, 1319–1333 (2011).

288. Hundalani, S. G. *et al.* Age-based difference in activation markers of coagulation and fibrinolysis in extracorporeal membrane oxygenation. *Pediatr. Crit. Care Med.* **15**, (2014).

289. Bartoszko, J. *et al.* The association of thrombin generation with bleeding outcomes in cardiac surgery: a prospective observational study. *Can. J. Anaesthesia/Journal Can. d'anesthésie* (2021) doi:10.1007/s12630-021-02165-1.

290. Rifón, J. *et al.* Thrombin-antithrombin complexes and prothrombin fragment 1+2 in aorto-coronary bypass surgery: relation to graft occlusion. *Hematol. Pathol.* **8**, 35–42 (1994).

291. Bosch, Y. *et al.* Preoperative thrombin generation is predictive for the risk of blood loss after cardiac surgery: a research article. *J. Cardiothorac. Surg.* **8**, 154 (2013).

292. Boisclair, M. D. *et al.* Mechanisms of thrombin generation during surgery and cardiopulmonary bypass. *Blood* **82**, 3350–3357 (1993).

293. Lison, S. *et al.* Enhanced Thrombin Generation After Cardiopulmonary Bypass Surgery. *Anesth. Analg.* **112**, 37–45 (2011).

294. Hunt, B.J. *et al*. Activation of coagulation and fibrinolysis during cardiothoracic operations. *Ann. Thorac. Surg.* **65**, 712–8 (1998).

295. Knudsen, L. *et al.* Monitoring Thrombin Generation with Prothrombin Fragment 1.2 Assay During Cardiopulmonary Bypass Surgery. *Thromb. Res.* **84**, 45–54 (1996).

296. Wendel, H. P. FXII levels , FXIIa-like activities and kallikrein activities in normal

subjects and patients undergoing cardiac surgery. 141–144 (1999).

297. Arnold, P. *et al.* Coagulation factor activity during neonatal extra-corporeal membrane oxygenation. *Intensive Care Med.* **27**, 1395–1400 (2001).

298. Hékimian, G. *et al.* Extracorporeal Membrane Oxygenation Induces Early Alterations in Coagulation and Fibrinolysis Profiles in COVID-19 Patients with Acute Respiratory Distress Syndrome. *Thromb. Haemost.* **121**, 1031–1042 (2021).

299. Mazzeffi, M. *et al.* Coagulation Factor Levels and Underlying Thrombin Generation Patterns in Adult Extracorporeal Membrane Oxygenation Patients. *Anesth. Analg.* **129**, 659–666 (2019).

300. Larsson, M. *et al.* Cardiovascular disease: A factor XIIa inhibitory antibody provides thromboprotection in extracorporeal circulation without increasing bleeding risk. *Sci. Transl. Med.* **6**, (2014).

301. Wiegele, M. *et al.* Thrombin Generation and Platelet Function in ICU Patients Undergoing CVVHD Using Regional Citrate Anticoagulation. *Front. Med.* **8**, (2021).

302. Petros, S., Kliem, P., Siegemund, T. & Siegemund, R. Thrombin generation in severe sepsis. *Thromb. Res.* **129**, 797–800 (2012).

303. Dornia, C. *et al.* D-dimers Are a Predictor of Clot Volume Inside Membrane Oxygenators During Extracorporeal Membrane Oxygenation. *Artif. Organs* **39**, (2015).

304. Lubnow, M. *et al.* D-dimers as an early marker for oxygenator exchange in extracorporeal membrane oxygenation. *J. Crit. Care* **29**, 473.e1-473.e5 (2014).

305. Fair, K. A. *et al.* Fibrinolytic Activation in Patients with Progressive Intracranial

Hemorrhage after Traumatic Brain Injury. *J. Neurotrauma* **38**, 960–966 (2021).

306. Esnault, P. *et al.* Ability of Fibrin Monomers to Predict Progressive Hemorrhagic Injury in Patients with Severe Traumatic Brain Injury. *Neurocrit. Care* **33**, 182–195 (2020).

307. Hulka, F. Blunt Brain Injury Activates the Coagulation Process. *Arch. Surg.* **131**, 923 (1996).

308. Kawahara, Y. *et al.* Use of laboratory testing for prediction of postoperative bleeding volume in cardiovascular surgery. *Thromb. J.* **19**, (2021).

309. Ernofsson, M., Thelin, S. & Siegbahn, A. Thrombin Generation During Cardiopulmonary Bypass Using Heparin-Coated or Standard Circuits. *Scand. J. Thorac. Cardiovasc. Surg.* **29**, 157–165 (1995).

310. Moore, H. B. *et al.* Fibrinolysis Shutdown in Trauma. *Anesth. Analg.* **129**, 762–773 (2019).

311. Eitzman, D. T. *et al.* Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J. Clin. Invest.* **97**, 232–237 (1996).

312. Gross, T. J., Simon, R. H., Kelly, C. J. & Sitrin, R. G. Rat alveolar epithelial cells concomitantly express plasminogen activator inhibitor-1 and urokinase. *Am. J. Physiol. Cell. Mol. Physiol.* **260**, L286–L295 (1991).

313. Chapman, H. A., Yang, X. L., Sailor, L. Z. & Sugarbaker, D. J. Developmental expression of plasminogen activator inhibitor type 1 by human alveolar macrophages. Possible role in lung injury. *J. Immunol.* **145**, 3398–405 (1990).

314. Jansen, P. M. *et al.* Contribution of interleukin-1 to activation of coagulation and fibrinolysis, neutrophil degranulation, and the release of secretory-type phospholipase A2 in sepsis: studies in nonhuman primates after interleukin-1 alpha administration and during lethal ba. *Blood* **86**, 1027–34 (1995).

315. Dong, J. *et al.* IL-1 and IL-6 induce hepatocyte plasminogen activator inhibitor-1 expression through independent signaling pathways converging on C/EBPδ. *Am. J. Physiol. Physiol.* **292**, C209–C215 (2007).

316. van Hinsbergh, V. W. *et al.* Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo. *Blood* **72**, 1467–73 (1988).

317. McVeen, R. V. *et al.* Letter to the editor: Changes in fibrinolytic factors in newborns during extracorporeal membrane oxygenation (ECMO). *Am. J. Hematol.* **38**, 254–255 (1991).

318. Estelles, A., Aznar, J., Gilabert, J. & Parrilla, J. J. Dysfunctional Plasminogen in Full-Term Newborn. *Pediatr. Res.* **14**, 1180–1185 (1980).

319. Hilali, M. M. & Gilliver, B. E. Physiological activation of plasminogen in full term newborn infants. *J. Clin. Pathol.* **37**, 1264–1267 (1984).

320. Chandler, W. L. & Velan, T. Plasmin generation and D-dimer formation during cardiopulmonary bypass. *Blood Coagul. Fibrinolysis* **15**, 583–91 (2004).

321. Chandler, W. L. *et al.* Individual variations in the fibrinolytic response during and after cardiopulmonary bypass. *Thromb. Haemost.* **74**, 1293–7 (1995).

322. Chandler, W. L. & Velan, T. Secretion of tissue plasminogen activator and

plasminogen activator inhibitor 1 during cardiopulmonary bypass. *Thromb. Res.* **112**, 185–192 (2003).

323. Valen, G., Eriksson, E., Risberg, B. & Vaage, J. Fibrinolysis during cardiac surgeryRelease of tissue plasminogen activator in arterial and coronary sinus blood. *Eur. J. Cardio-Thoracic Surg.* **8**, 324–330 (1994).

324. Wilson, J. M. *et al.* Aminocaproic acid decreases the incidence of intracranial hemorrhage and other hemorrhagic complications of ECMO. *J. Pediatr. Surg.* **28**, 536–541 (1993).

325. Sigaut, S. *et al.* Comparison of Two Doses of Tranexamic Acid in Adults Undergoing Cardiac Surgery with Cardiopulmonary Bypass. *Anesthesiology* **120**, 590–600 (2014).

326. Henry, D. A. *et al.* Anti-fibrinolytic use for minimising perioperative allogeneic blood transfusion. in *Cochrane Database of Systematic Reviews* (ed. Henry, D. A.) (John Wiley & Sons, Ltd, 2011). doi:10.1002/14651858.CD001886.pub4.

327. Lauridsen, S. V. *et al.* No hyperfibrinolysis following subarachnoid or intracerebral haemorrhage. *Blood Coagul. Fibrinolysis* **30**, 341–349 (2019).

328. Karri, J. *et al.* Early Fibrinolysis Associated with Hemorrhagic Progression Following Traumatic Brain Injury. *Shock* **48**, 644–650 (2017).

329. Hijazi, N. *et al.* Endogenous plasminogen activators mediate progressive intracerebral hemorrhage after traumatic brain injury in mice. *Blood* **125**, 2558–2567 (2015).

330. Kreyer, S. *et al.* Hemorrhage under veno-venous extracorporeal membrane oxygenation in acute respiratory distress syndrome patients: a retrospective data analysis. *J. Thorac. Dis.* **9**, 5017–5029 (2017).

331. Biemond, B. J. *et al.* Plasminogen Activator and Plasminogen Activator Inhibitor I Release during Experimental Endotoxaemia in Chimpanzees: Effect of Interventions in the Cytokine and Coagulation Cascades. *Clin. Sci.* **88**, 587–594 (1995).

332. van Deventer, S. J. *et al.* Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. *Blood* **76**, 2520–6 (1990).

333. Pinsky, D. J. *et al.* Coordinated induction of plasminogen activator inhibitor-1 (PAI-1) and inhibition of plasminogen activator gene expression by hypoxia promotes pulmonary vascular fibrin deposition. *J. Clin. Invest.* **102**, 919–928 (1998).

334. Zhang, Y. *et al.* Pulmonary endothelial activation caused by extracellular histones contributes to neutrophil activation in acute respiratory distress syndrome. *Respir. Res.* **17**, 155 (2016).

335. Chandler, W. L. Effects of hemodilution, blood loss, and consumption on hemostatic factor levels during cardiopulmonary bypass. *J. Cardiothorac. Vasc. Anesth.* **19**, 459–467 (2005).

336. Raza, I. *et al.* The incidence and magnitude of fibrinolytic activation in trauma patients. *J. Thromb. Haemost.* **11**, 307–314 (2013).

337. Semeraro, F. *et al.* D-dimer corrected for thrombin and plasmin generation is a strong predictor of mortality in patients with sepsis. *Blood Transfus.* **18**, 304–311 (2020).

338. BAETEN, K. M. *et al.* Activation of single-chain urokinase-type plasminogen activator by platelet-associated plasminogen: a mechanism for stimulation of fibrinolysis by platelets. *J. Thromb. Haemost.* **8**, 1313–1322 (2010).

339. Gould, T. J. *et al.* Cell-Free DNA Modulates Clot Structure and Impairs Fibrinolysis in Sepsis. *Arterioscler. Thromb. Vasc. Biol.* **35**, 2544–2553 (2015).

340. Suzuki, Y., Sano, H., Mochizuki, L., Honkura, N. & Urano, T. Activated platelet-based inhibition of fibrinolysis via thrombin-activatable fibrinolysis inhibitor activation system. *Blood Adv.* **4**, 5501–5511 (2020).

341. Fuchs, G., Berg, N., Broman, L. M. & Prahl Wittberg, L. Flow-induced platelet activation in components of the extracorporeal membrane oxygenation circuit. *Sci. Rep.* **8**, (2018).

342. Kawabata, K. *et al.* Soluble P-Selectin Is Released from Activated Platelets in vivo during Hemodialysis. *Nephron* **78**, 148–155 (1998).

343. Shetty, S. & Idell, S. Post-transcriptional Regulation of Urokinase mRNA. *J. Biol. Chem.* **275**, 13771–13779 (2000).

344. Okulu, E. *et al.* Serum Levels of Soluble Urokinase Plasminogen Activator Receptor in Infants with Late-onset Sepsis. *J. Clin. Lab. Anal.* **29**, 347–352 (2015).

345. Hoenigl, M. *et al.* Diagnostic accuracy of soluble urokinase plasminogen activator receptor (suPAR) for prediction of bacteremia in patients with systemic inflammatory response syndrome. *Clin. Biochem.* **46**, 225–229 (2013).

346. Yamashita, M., Darlington, D. N., Weeks, E. J., Jones, R. O. & Gann, D. S. Plasminogen activator inhibitor-1 rises after hemorrhage in rats. *Am. J. Physiol. Metab.* **268**, E1065–E1069 (1995).

347. Alhamdi, Y. & Toh, C.H. Recent advances in pathophysiology of disseminated intravascular coagulation: the role of circulating histones and neutrophil extracellular

traps. *F1000Research* **6**, 2143 (2017).

348. Wang, Y. *et al.* Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J. Cell Biol.* **184**, 205–213 (2009).

349. Thiam, H. R. *et al.* NETosis proceeds by cytoskeleton and endomembrane disassembly and PAD4-mediated chromatin decondensation and nuclear envelope rupture. *Proc. Natl. Acad. Sci.* **117**, 7326–7337 (2020).

350. Frydman, G. H. *et al.* Megakaryocytes contain extranuclear histones and may be a source of platelet-associated histones during sepsis. *Sci. Rep.* **10**, 4621 (2020).

351. Masuda, S. *et al.* NETosis markers: Quest for specific, objective, and quantitative markers. *Clin. Chim. Acta* **459**, 89–93 (2016).

352. Abrams, S. T. *et al.* Circulating Histones Are Mediators of Trauma-associated Lung Injury. *Am. J. Respir. Crit. Care Med.* **187**, 160–169 (2013).

353. Li, Y. *et al.* Circulating Histones in Sepsis: Potential Outcome Predictors and Therapeutic Targets. *Front. Immunol.* **12**, (2021).

354. Ulusoy, E. *et al.* Acute Traumatic Coagulopathy: The Value of Histone in Pediatric Trauma Patients. *Turkish J. Hematol.* **35**, 122–128 (2018).

355. Cavalier, E. *et al.* Circulating Nucleosomes as Potential Markers to Monitor COVID-19 Disease Progression. *Front. Mol. Biosci.* **8**, (2021).

356. Shaw, R. J. *et al.* Circulating histones play a central role in COVID-19-associated coagulopathy and mortality. *Haematologica* **106**, 2493–2498 (2021).

357. Wang, R. *et al.* Neutrophil extracellular traps promote tPA-induced brain hemorrhage

via cGAS in mice with stroke. *Blood* **138**, 91–103 (2021).

358. Pozzi, N. & Di Cera, E. Dual effect of histone H4 on prothrombin activation. *J. Thromb. Haemost.* **14**, 1814–1818 (2016).

359. Ammollo, C. T., Semeraro, F., Xu, J., Esmon, N. L. & Esmon, C. T. Extracellular histones increase plasma thrombin generation by impairing thrombomodulin-dependent protein C activation. *J. Thromb. Haemost.* **9**, 1795–1803 (2011).

360. Semeraro, F. *et al.* Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* **118**, 1952–1961 (2011).

361. Ammollo, C. T., Semeraro, N., Carratu, M. R., Colucci, M. & Semeraro, F. Histones Differentially Modulate the Anticoagulant and Profibrinolytic Activities of Heparin, Heparin Derivatives, and Dabigatran. *J. Pharmacol. Exp. Ther.* **356**, 305–313 (2016).

362. Carestia, A. *et al.* Functional responses and molecular mechanisms involved in histone-mediated platelet activation. *Thromb. Haemost.* **110**, 1035–1045 (2013).

363. Gould, T. J. *et al.* Extracellular Histones Increase Tissue Factor Activity and Enhance Thrombin Generation by Human Blood Monocytes. *Shock* **46**, 655–662 (2016).

364. Kim, J. E., Yoo, H. J., Gu, J. Y. & Kim, H. K. K. Histones induce the procoagulant phenotype of endothelial cells through tissue factor up-regulation and thrombomodulin down-regulation. *PLoS One* **11**, (2016).

365. Wen, Z. *et al.* Extracellular histones indicate the prognosis in patients undergoing extracorporeal membrane oxygenation therapy. *Perfusion* **34**, 211–216 (2019).

366. Beaubien-Souligny, W., Neagoe, P. E., Gagnon, D., Denault, A. Y. & Sirois, M. G. Increased Circulating Levels of Neutrophil Extracellular Traps During Cardiopulmonary Bypass. *CJC Open* **2**, 39–48 (2020).

367. Gao, H. *et al.* Circulating histones for predicting prognosis after cardiac surgery: a prospective study. *Interact. Cardiovasc. Thorac. Surg.* **23**, 681–687 (2016).

368. Kordbacheh, F., O'Meara, C. H., Coupland, L. A., Lelliott, P. M. & Parish, C. R. Extracellular histones induce erythrocyte fragility and anemia. *Blood* **130**, 2884–2888 (2017).

369. Gennaro Martucci. ClinicalTrials.gov: PRospective Multicenter Observational Study on Transfusion Practice in Vv-ECMO Patients: The PROTECMO Study (PROTECMO). *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03815773?te https://clinicaltrials.gov/ct2/show/NCT03815773?term=transfusion&cond=ECMO&draw=2&rank=1 (2019).

Appendix

Clinical features of participants recruited in CYCLE Study during ECMO

Clinical Feature	**Frequency/Median (Interquartile Range)**
Age at cannulation (years)	45 (22 – 64)
Gender (Male:Female)	11:6
Weight (kg)	80 (65 – 90)
Cause of Severe Respiratory Failure	Infection – 7 Non-infective ARDS – 3 Autoimmune Lung Disease – 2 Trauma – 2 Other – 3
APACHE II Score	16 (9 – 23)
SOFA Score	7 (2 – 13)
Duration of ECMO (days)	9 (4 – 25)
Mode of ECMO	VV – 16 VVA – 1
Haemoglobin (g/L)	84 (81 – 88)
Platelets (x 10^9/L)	142 (104 – 165)
White Cell Count (x 10^6/L)	13.6 x 10^6/L (10.2 – 16.4)
APTTr	1.2 (1.1 – 1.6)
PTr	1.1 (1.1 – 1.1)
Mortality during ECMO	1/17 (6%)

Haemorrhagic and Thrombotic Complications during ECMO of participants recruited in CYCLE Study

Complications	**Frequency**
Pulmonary artery filling defect	4 (24%)
Deep vein thrombosis at cannula sites	7 (41%)
Thrombosis at other sites	4 (24%)
Intracranial Haemorrhage	4 (24%)
Major non-intracranial haemorrhage	3 (18%)
Clinically relevant non-major bleeding	9 (53%)
ECMO Circuit Change	5 (29%)

Macroscopic Analysis for Thrombus in ECMO Circuits of participants in CYCLE Study

Sites of visible thrombus on Membrane Surface*	**Estimated Pump Chamber Coverage (%)**	**Thrombus Length in Red Cannulae/Connector**	**Thrombus Length in Blue Cannulae/Connector**
RC & LC	50	2cm	6cm
FC	75	3cm	1cm
None	50	7cm	1cm
IC & LC	25	5cm	10cm
IC	100	0	3cm
RC, IC & LC	0	30cm	100cm
None	0	0	0
RC & LC	50	36cm	0
IC & LC	25	61cm	31cm

* SC – Superior corner, IC – Inferior corner, RC – Right corner, LC – Left corner, FC – Full cover of membrane

Blood samples collected of participants in CYCLE Study

Participant	Pre-ECMO	1-Hour	Day 1	Day 2	Day 7	Day 14	Day 21	Pre-Decannulation	1-Hour	Day 1	Bleeding	Pre-Change	1-Hour	Day 1
01	X	X	X	X				X	X	X				
02	X	X	X					X		X	MH & CRNMB	X	X	X
03	X	X	X	X	X	X		X	X	X		X		X
04	X	X	X	X	X			X	X	X				
05	X	X	X	X				X	X	X	CRNMB			
06	X	X	X	X				X	X	X				
07	X	X	X	X	X	X	X	X	X	X	CRNMB			
08	X	X	X	X	X						MH & CRNMB			
09	X	X	X					X	X	X				
10	X	X	X	X				X	X	X	CRNMB			
11	X	X	X	X	X			X	X	X	CRNMB			
12	X	X	X	X	X			X		X	CRNMB	X	X	X
13		X	X	X	X			X		X	MH & CRNMB	X	X	X
14	X	X	X	X				X	X	X	CRNMB			
15	X	X	X	X				X	X	X	MH			
16	X	X	X	X	X	X		X	X	X	CRNMB			
17	X	X	X	X				X	X	X				
Total	16	17	17	15	8	3	1	16	13	16	11 participants 20 samples	4	4	4