Optimal tests to minimise bleeding and ischaemic complications in patients on extracorporeal membrane oxygenation

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Running title: Thrombosis and bleeding assessment in ECMO

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Take home message:

Patients receiving extracorporeal membrane oxygenation (ECMO) have a very high frequency of bleeding and ischaemic complications that often jeopardize the patient's outcome and monitoring of antithrombotic therapy can be very challenging.
We describe the selection of complementary tests which can collectively assess heparin-effect, coagulation, platelet function and platelet aggregation, with supporting evidence in ECMO, to enable personalised management and optimise outcomes.

Abbreviations

ACT – activated clotting time
anti-Xa – anti-Factor Xa level
aPTT – activated partial thromboplastin time
AT – antithrombin
AVWS – acquired von Willebrand syndrome
ECMO – extracorporeal membrane oxygenation
ETP – endogenous thrombin potential
HMWM – high molecular weight multimers
HIT – heparin-induced thrombocytopenia
HR – heparin resistance
LTA – light transmission aggregometry
MCF – maximum clot firmness
MCS – mechanical circulatory support
MEA – multiple electrode aggregometry
TG – thrombin generation
UFH – unfractionated heparin
VA – veno-arterial
VV – veno-venous
vWF – von Willebrand Factor

Abstract

Patients supported with extracorporeal membrane oxygenation (ECMO) experience a very high frequency of bleeding and ischaemic complications, including stroke and systemic embolism. These patients require systemic anticoagulation, mainly with unfractionated heparin (UFH) to prevent clotting of the circuit and reduce the risk of arterial or venous thrombosis. Monitoring of UFH can be very challenging. Whilst most centres routinely monitor the activated clotting time and activated partial thromboplastin time (aPTT) to assess UFH, measurement of anti-Xa level best correlates with heparin dose, and appears predictive of circuit thrombosis, although aPTT may be a better predictor of bleeding. Although monitoring of prothrombin time, platelet count and fibrinogen is routinely undertaken to assess haemostasis, there is no clear guidance available regarding the optimal test.

Additional tests, including antithrombin level and thromboelastography can be used for risk stratification of patients to try and predict the risks of thrombosis and bleeding. Each has their specific role, strengths and limitations. Increased thrombin generation may have a role in predicting thrombosis. Acquired von Willebrand syndrome is frequent with ECMO, contributing to bleeding risk and can be detected by assessing the von Willebrand factor activity to antigen ratio, whilst the Platelet Function Analyzer can be used in urgent situations to detect this, with high negative predictive value. Tests of platelet aggregation can aid the prediction bleeding.

In order to personalise management, a selection of complementary tests to collectively assess heparin-effect, coagulation, platelet function and platelet aggregation is proposed, in order to optimise clinical outcomes in these high-risk patients.

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Introduction

Both bleeding and ischaemic complications are frequent in patients on mechanical circulatory support (MCS). A meta-analysis of 1,866 adult patients with cardiogenic shock treated with extracorporeal membrane oxygenation (ECMO) reported major or significant bleeding in 40.8% of patients, lower limb ischemia in 16.9% and stroke in 5.9% of patients.[1] In patients receiving MCS, the artificial surface of the circuit activates the contact pathway of the coagulation cascade and, in combination with the inflammatory response, is an ongoing driver of thrombosis (Figure 1). At the same time, over-anticoagulation, low platelet count and/or acquired platelet dysfunction due to loss of platelet receptors through the MCS and acquired von Willebrand syndrome (AVWS), all contribute to an increased risk of bleeding. AVWS is frequent with ECMO and can greatly exacerbate bleeding.

Maintaining optimal haemostasis and preventing significant bleeding and thrombosis are key aspects of managing patients receiving ECMO. Anticoagulation is essential for left-sided support to prevent thrombotic complications and reduce the risk of systemic embolism, including stroke or limb ischemia.[2] This is usually achieved with intravenous unfractionated heparin (UFH),[2] or the direct thrombin inhibitors bivalirudin or argatroban when UFH is contraindicated, such as in the case of allergy to UFH/heparin-induced thrombocytopenia (HIT), or in patients with severe acute antithrombin (AT) deficiency when treatment with AT concentrate is also an option. There are no randomised controlled trials to guide anticoagulation targets in patients on ECMO.

Maintaining optimal anticoagulation is challenging, as monitoring of UFH can be difficult in patients on MCS[2] and the ideal strategy for monitoring remains unknown. In addition to assessing the effect of anticoagulation, it is also important to assess the overall haemostatic profile, in order to assess bleeding and thrombosis risk (Figure 1). Platelets play a major role in both primary and secondary haemostasis, and thrombocytopoenia and/or impaired platelet function can contribute to the excess bleeding risk.

Depending on the centre, the method used for monitoring UFH varies, and may relate to the availability of the tests, as well as to clinician preference. The most commonly used tests include the activated clotting time (ACT), activated partial thromboplastin time (aPTT) and

heparin levels as measured by anti-factor Xa level (anti-Xa). Furthermore, to assess haemostasis, most centres offering ECMO routinely check prothrombin time (PT) and fibrinogen.[2] Additionally, measurement of AT level and thromboelastography (TEG), as an assessment of global haemostasis, are reported to be used variably, or on an "as needed" basis. Newer tests are also available, but their role is often not well understood.

There are currently no guidelines regarding the optimal assessment of patients on ECMO. The aim of this review is to present available tests to assess the anticoagulant intensity of UFH and other haemostatic tests to predict bleeding and thrombosis, in order to provide guidance to practicing clinicians.

We performed a literature search using the MEDLINE/PubMed database, including articles published online from inception until January 2020. The search strategy is described in Appendix 1. As this is a narrative review, included articles were selected based on the authors' joint subjective determination of relevance to the overall theme of this paper and the most pertinent articles informing clinical practice were referenced.

Assessing anticoagulant effect

The optimal anticoagulant monitoring strategy for UFH in patients receiving ECMO is undefined (Table 1). Overall, the dose of UFH best correlates with the anti-Xa level, however, whether levels relate to bleeding and thrombosis is not established in large studies. A study in paediatric ECMO patients reported poor correlation between UFH dose and either anti-Xa level ($\rho=0.1$, $p<0.0001$) or aPTT ($\rho=0.26$, $p<0.0001$). Anti-Xa level and aPTT were weakly correlated ($\rho=0.38$, $p<0.0001$) and neither was related to survival or haemorrhagic and thrombotic complications.[3]

Activated Clotting Time (ACT)

In the critical care setting, UFH dose has previously been titrated to the measurement of the ACT, a widely available point-of-care assay.[2] Performed on whole blood after addition of an activator (e.g. kaolin), it provides a rapid assessment of the adequacy of anticoagulation when high dose UFH is used. However, it may not provide an accurate measure of UFH

anticoagulant effect as a result of various confounding factors which are more marked in critically-ill patients, including liver failure, haemodilution, thrombocytopenia, hypothermia, extreme fibrinogen levels, inflammation, lupus anticoagulant, and consumptive coagulopathy.[4] Although routinely used, its effectiveness to guide anticoagulation in this patient group has never been prospectively studied. Numerous reports indicate that monitoring UFH with ACT alone leads to suboptimal anticoagulation during MCS. In 46 adult patients on veno-arterial (VA) or veno-venous (VV) ECMO, heparin dose correlated better with aPTT than ACT.[5] In paediatric patients receiving ECMO with comparable ACT levels, a 0.01 IU/ml decrease in anti-Xa level was shown to increase the risk of circuit thrombosis by 5%.[6] In a retrospective analysis of 604 children on ECMO monitored with ACT in the range 180-220 seconds, there was an improved survival with increased heparin dose and ACT did not correlate with heparin dose, implying that ACT may be too insensitive to guide systemic anticoagulation.[7]

Activated Partial Thromboplastin Time (aPTT)

Although the aPTT is a commonly-used, inexpensive and readily available test, it may not provide an accurate measure of the amount of UFH present because of various confounding factors including both pre-analytical and analytical variables. The aPTT is measured by adding a surface activator and calcium to citrated plasma. Both laboratory and point-of-care aPTT tests are available. In the intensive care setting, aPTT correlates better with heparin concentration than ACT, although the optimal aPTT target range and the correlation between aPTT and bleeding risk in patients on ECMO remains unclear.[5,8] Both laboratory and point-of-care aPTT tests correlate poorly with heparin anti-Xa levels. Although a number of studies have shown that elevated aPTT level is a better predictor of bleeding than anti-Xa level. In a study of 539 adult in-patients treated with UFH, discordant aPTT and anti-Xa values were found in 42% of paired samples.[9] In these patients, a disproportionately higher aPTT was associated with increased bleeding (9% vs 3%, p=0.03) and 30-day mortality (14% vs 5%, p=0.02). In a prospective study of 202 paediatric patients undergoing cardiac surgery, an elevated aPTT, but not anti-Xa level was associated with bleeding.[10] In a retrospective analysis of 149 adult patients on ECMO (111 VA and 38 VV), higher aPTT levels were independently associated with bleeding.[11] These findings may be a reflection of intrinsic coagulation abnormalities rather than the direct effect of UFH.

There is significant variability in aPTT readings between laboratories, different instruments and even between reagents. The American College of Chest Physicians recommends the determination of aPTT goal ranges for individual institutions based on heparin (anti-Xa) levels, indicating that heparin level monitoring may be a superior approach.[12]

Anti-Factor Xa level (anti-Xa)

The anti-Xa assay evaluates the dose of heparin available in the circulation and may be considered a surrogate measurement of the overall anticoagulant activity of UFH. The assay measures the ability of a patient's plasma to inhibit exogenous added FXa, hydrolyzing its synthetic substrate (chromogenic assay) or the anti-Xa activity is measured by performing an aPTT-based FX assay on each dilution without exogenous FXa (clotting based assay). Heparin anti-Xa assay that does not contain exogenous AT may give a more accurate estimation of anticoagulation in plasma, thus reflecting the *in vivo* UFH effect for the specific patient.[13] However, it may not detect all the heparin present in that sample. Some chromogenic anti-Xa assays involve the addition of exogenous AT, which may increase the measured anti-Xa activity, whilst ones that do not add exogenous AT, and the latter maybe considered a more physiological assessment.[14] However, although anti-Xa assays with added AT trend higher than those without, a recent paper found good correlation between the two types of anti-Xa platforms in paediatric patients treated with low molecular weight heparin even when AT activity was <70%.[15]
However, while the anti-Xa level is a direct measure of heparin effect, it does not reflect overall haemostatic status, which may depend on the amount of thrombin/AT and pro-coagulant drivers. It is an expensive test and not widely available. However, unlike aPTT, anti-Xa levels are not affected by the presence of lupus anticoagulant, liver disease or consumptive coagulopathy.

Small case series in paediatric patients suggest that anti-Xa levels correlate better with heparin dosing, and probably the overall anticoagulation state during ECMO than does ACT or a combination of ACT and aPTT.[16–18] Small retrospective studies demonstrated that anti-Xa level was predictive of circuit thrombosis in paediatric[6] and adult patients[19] on ECMO, where for every unit decrease in anti-Xa level, there was a seven-fold increase in deep venous thrombosis.[19] However, some studies indicate that for the assessment of bleeding risk, aPTT is superior to anti-Xa for monitoring UFH in paediatric patients undergoing cardiac surgery,[10]

and in adult patients receiving ECMO.[20] This could be due the fact that aPTT is reflective of both heparin and the other intrinsic coagulation factors, whereas anti-Xa levels indicate only the heparin levels present. In a retrospective single-centre study of 34 adults on ECMO, in whom heparin was monitored by anti-Xa and/or aPTT, some 15% of patients experienced a major thrombotic event and 27% experienced a haemorrhagic event.[20] The anti-Xa assay better correlated with weight-based heparin dose than aPTT. Low anti-Xa values were predictive of thrombosis whereas high aPTT values were predictive of bleeding. In a prospective study of 202 pediatric patients treated with UFH after cardiac surgery, in those who experienced bleeding events, the highest aPTT and corresponding anti-Xa for the 24 hours before bleeding events was used to assess the predictive value of these tests for bleeding. Whilst there was moderate correlation between aPTT and anti-Xa, aPTT >150 seconds was significantly related to bleeding (odds ratio 1.71 per 10-second increase in aPTT, 95% confidence interval 1.21-2.42; p=0.003), whereas anti-Xa was not associated with bleeding.[10] Therefore, a combination of anti-Xa and aPTT monitoring approach to detect thrombotic and bleeding risk may be preferable to either alone.

Antithrombin III (AT) level and anticoagulant effect of UFH

AT is essential as a cofactor for UFH to exert its anticoagulant effect. AT deficiency may result in inadequate anticoagulation with UFH at usual doses. Acquired AT deficiency is common in acutely unwell patients due to either decreased production or increased consumption, especially with MCS. Whilst a consensus definition is lacking, "heparin resistance" (HR) refers to the requirement of very high doses of heparin usually in excess of 35,000 units within a 24 hour period to maintain the anti-Xa level or aPTT within the therapeutic range.[21] The prevalence of HR with extracorporeal circuits was originally derived from large case series of patients undergoing cardiopulmonary bypass, including those supported with intra-aortic balloon pumps. In these studies, the prevalence of HR was around 20%,[22–24] depending on the definition of HR used, and was associated with adverse outcomes.[25] A large retrospective study in paediatric patients on ECMO showed that subtherapeutic aPTT was associated with increased mortality and increased heparin dosing conferred a survival advantage.[26] However, a more recent small study of 69 neonatal and paediatric ECMO patients showed that neither dose of UFH nor time at therapeutic anti-Xa or aPTT levels affected the risk of bleeding or survival.[27] The prevalence of HR is reported in only 2 studies of patients on ECMO. In one study of 67 adult patients on VA or VV ECMO,

half the patients had HR,[21] and there was no apparent relationship between HR and thrombotic or bleeding complications.[21] A recent study of 81 adult patients with COVID-19 receiving VV ECMO and renal replacement therapy indicated that all patients met the criteria for HR.[28] The Extracorporeal Life Support Organization recommends maintaining AT levels within the normal range (80-120% of control) during ECMO,[13] although AT assays are not readily available in all hospitals. Low AT can be treated by giving fresh frozen plasma, cryoprecipitate, human plasma derived AT or recombinant AT concentrate, although the latter is expensive. Although not formally evaluated in patients supported with ECMO, AT concentrate is preferable to cryoprecipitate or fresh frozen plasma, since the latter requires carries the potential risk of transfusion-transmitted infections and contains only small concentration of AT unit per volume of plasma (fresh frozen plasma contains only 1 U/mL of AT), necessitating large volumes to normalise AT levels, which could lead to volume overload or transfusion-related acute lung injury.[29]

Low AT may be a particular problem in neonates, who have a physiologic deficiency of AT, with levels as low as 40% of the adult range and this can be exacerbated by sepsis and MCS. In adults, there are no studies showing the clear benefit of closely monitoring and maintaining the AT level within the recommended range, with recent data suggesting that AT supplementation may not decrease heparin requirments, not diminish the incidence of bleeding and/or thrombosis in adult patients on ECMO.[30]

Tests to assess the haemostatic state of patients on MCS

Thromboelastography and thromboelastometry

Both thromboelastography (TEG) and rotational thromboelastometry (ROTEM) provide information on the characteristics of clot produced *ex vivo* under low shear conditions. As the clot starts to form, changes in impedance reflect the dynamics of clot formation, viscoelastic clot strength and clot lysis. Both are point-of-care tests and utilise either native blood, which must be tested immediately, or citrated whole blood, which is stable for up to two hours at room temperature and requires recalcification prior to analysis.

Drawbacks include the necessity for staff training and inter-operator variability with TEG, which unlike ROTEM, is not automated; and it can take up to two hours to obtain results. Use of activators of coagulation such as tissue factor can accelerate the test producing results in 15 minutes, although this compromises the physiological relevance of the test.

Both TEG and ROTEM are used in cardiopulmonary bypass to assess haemostasis and guide blood transfusion requirements. Hyperfibrinolysis based on the manufacturers' definitions of maximum lysis >15% in the ROTEM or Lysis30 >8% in the TEG, a measurement derived from the reduction of clot firmness over time, has been shown to be associated with increased bleeding. However, data supporting their usefulness in the setting of ECMO are less robust. A retrospective evaluation of 27 paediatric patients on MCS, predominantly VA ECMO, showed a weak correlation between TEG variables and aPTT, and between TEG and ACT; with a stronger correlation between TEG (maximum amplitude, MA) and platelet count.[31] A retrospective analysis of prospective data to assess UFH effect in adults on VV or VA ECMO showed weak correlation between ROTEM clotting time and aPTT, with no agreement between the directional changes of aPTT and ROTEM clotting time results on successive days.[32]

Small studies showed that reduced maximum clot firmness (MCF) was predictive of bleeding in adult patients on ECMO, whilst a low MCF was observed in only 17% of non-bleeders.[33] In 23 patients initiated on ECMO and 24 on ventricular assist (Berlin Heart Excor®), MCF was predictive of bleeding and 30-day mortality.[34] In 57 adult patients on VV ECMO, clotting time, but not MCF correlated with severity of bleeding.[35] In terms of transfusion requirements, a prospective trial of 42 adult patients on VV ECMO randomised to TEG-guided or aPTT-guided anticoagulation protocols showed no significant difference in bleeding or thrombotic events.[36] Furthermore, a study in 57 adult patients showed that VV ECMO leads to a continuous increase in clotting time and a decrease in MCF the longer ECMO lasts, but addition of ROTEM to aPTT or fibrinogen measurement did not aid the prediction of bleeding.[35] The role of these viscoelastic tests in assessing thrombosis is even weaker. In a retrospective chart review of 30 paediatric patients on VA or VV ECMO, a TEG R time greater than 17.85 min and anti-Xa activity greater than 0.25 IU/mL were independent predictors of thrombosis.[37] However, a meta-analysis including 9 studies of paediatric patients on ECMO concluded that none of the measures of heparin anticoagulation, including TEG-R, correlated with either bleeding or thrombotic episodes.[38]

Thus, there are limited data indicating a role for TEG/ROTEM in predicting bleeding and possibly to guide anticoagulation and transfusion in patients on MCS, but not in predicting thrombotic complications.

Thrombin generation (TG)

Thrombin (human activated coagulation factor II [FIIa]) is one of the key players in the haemostatic system. Activation of both the extrinsic (tissue factor) and intrinsic (contact) coagulation pathways result in conversion of prothrombin to thrombin *via* factor Xa, resulting in fibrin formation. Whereas most tests focus on a particular part of the thrombotic process (e.g. d-dimer levels on fibrin degradation, aPTT on the intrinsic pathway), TG provides a more global assessment of coagulation which assesses the capacity of blood to generate thrombin after stimulating the coagulation pathway with exogenous tissue factor. Specifically lag time (time to minimum thrombin formation), peak height (maximum thrombin concentration) and endogenous thrombin potential, which is the total amount of thrombin generated (ETP; area under the curve [AUC]) are reported. The greater the TG, the greater the potential for thrombosis and the less for bleeding. TG can be assessed in platelet-poor, platelet-free or platelet-rich plasma, where the latter enables the investigation of interaction between platelets and coagulation factors, that closely mimics *in vivo* conditions. An advantage of the TG assay is that it measures the full potential of the plasma sample to generate thrombin, whereas conventional coagulation tests (PT, aPTT) terminate with fibrin clot formation when 95% of thrombin is yet to be formed. However, TG is an expensive specialized laboratory test, not rapidly available in the acute setting and more widely used in research. Automated tests such as the Thrombinoscope version of the calibrated automated thrombogram (CAT) are becoming available, but data are scarce.

TG is mainly used to asses congenital or acquired bleeding disorders related to haemostasis, or to assess antithrombotic treatment. Outwith the setting of ECMO, a number of retrospective and prospective observational studies have shown a strong correlation between high ETP and the occurrence of venous thrombosis,[39–41] although data in ECMO patients is sparse. In a small study, peak TG and ETP were significantly higher in samples from 20 patients on ECMO compared to control plasma.[42] However, although half the cohort experienced a pulmonary embolism, there were no significant differences in lag time, peak TG, or ETP between patients with and without pulmonary embolism.
Although there is a strong correlation between increased TG and the occurrence of venous thrombosis,[43] a relationship with arterial thrombosis has not been established. Reduced TG in patients on cardiopulmonary bypass is related to peri-operative bleeding.[44] A small study assessing TG in adult patients on VA ECMO showed increased peak TG, and increased ETP

compared to controls, indicative of a pro-coagulant profile.[42] Tests of TG may therefore have a role both in the prediction of bleeding and thrombosis, but convincing clinical data in the setting of ECMO are lacking.

D-dimer

D-dimer is a fibrin degradation product, produced as the result of fibrin formation and degradation. Although marked elevations may indicate the presence of venous thromboembolism or disseminated intravascular coagulation, D-dimer is a non-specific marker that is elevated in other conditions including sepsis. Individual D-dimer levels are not helpful in patients on ECMO, but daily measurement is recommended since an increase in D-dimer in the absence of other explaining pathology during ECMO therapy may reflects coagulation activity within the membrane oxygenator,[45] and decrease after circuit exchange.[46] Rising levels of D-dimer are usually considered in the context of other markers, such as a rising plasma-free haemoglobin and falling fibrinogen, which together should prompt the need for urgent consideration of circuit change.

Assessment of platelet count and platelet function

Regular assessment of platelet count is essential, with vigilance for the occurrence of HIT, which occurs in some 4-7% of ECMO patients.[47] A low platelet count or a fall in platelet count should prompt consideration of HIT and various scores are available to gauge the likelihood of this.[48] In individuals with suspected HIT, heparin should be discontinued and replaced with a non-heparin anticoagulant, whilst awaiting laboratory confirmation of HIT, which is initially assessed with enzyme-linked immunosorbent assay for anti-platelet factor 4 antibodies.

<u>Light Transmission Aggregometry and Multiple Electrode Aggregometry</u>

Light transmission aggregometry (LTA) is considered the gold standard to assess platelet aggregation. However, it involves multiple steps and specialist laboratory expertise. It utilises anticoagulated platelet-rich plasma to which various agonists are added to stimulate platelet aggregation, including collagen, thrombin and thromboxane A2. Multiple Electrode Aggregometry (MEA) is a later derivative of impedance aggregometry, which employs

anticoagulated whole blood, can be used as a point-of-care technique and has been shown to predict bleeding in patients undergoing cardiopulmonary bypass.[49]

In an experimental artificial circuit perfused with human blood, initiation of mechanical circulation was associated with reduction in platelet count and platelet aggregation as measured using LTA and MEA.[50]

There are small studies showing reduced platelet aggregation in adult patients on ECMO.[51] However, assessment of platelet function in patients on ECMO can be challenging, since it can be compounded by the presence of thrombocytopenia, present in some 25% of VV and 23% of VA ECMO patients, which begins within 2-3 days and is not related to duration of ECMO.[52] A recent meta-analysis of 21 studies evaluating patients on ECMO revealed impaired platelet function, predominantly impaired platelet aggregation, in a number of studies, and a few studies also demonstrated reduced platelet adhesion and activation, including granule secretion.[52] A meta-analysis of 3 paediatric ECMO studies demonstrated reduced platelet function as assessed using platelet aggregometry, flow cytometry, and TEG-platelet mapping, which in 2 studies was irreversible by platelet transfusion.[53] However, this should be interpreted with caution since another recent small study of 33 adults on VA or VV ECMO showed that both platelet count and platelet aggregation were reduced on ECMO, but when aggregation was assessed relative to platelet count, this did not differ from that of healthy controls.[54] Furthermore, mild haemolysis is common in patients on ECMO, with severe haemolysis in 2-20% of patients, which can further compound platelet function analysis performed using whole blood.[55]

If the LTA or MEA shows significant platelet dysfunction in the presence of major bleeding, antiplatelet should be withheld and consideration given to platelet transfusion, even in the presence of normal platelet count. In the case of minor bleeding, the risk of withholding antiplatelet treatment should be balanced against the risk of bleeding. If the bleeding risk exceeds ischaemic risk, antiplatelet therapy should be withheld until the bleeding settles. If the thrombosis risk is high, antiplatelet therapy should be continued and tranexamic acid administered for 24-48 hours.

Role of Von Willebrand factor (vWF) in haemostasis

VWF is a high-molecular-weight multimer (HMWM), synthesized and released by endothelial cells. It plays a crucial role in platelet-subendothelium adhesion and platelet-

platelet interaction, and functions as a carrier protein for coagulation factor VIII. VWF alters from an irregularly coiled to extended thread-like state in the transition from shear to elongational flow at sites of haemostasis and thrombosis. Transition from pulsatile to continuous blood flow, as seen in patients with continuous-flow MCS, leads to cleavage of large vWF multimers into monomers by the matrix metalloproteinase ADAMTS13. Once cleaved, lower-molecular-weight multimers (LMWM) lose their affinity for binding platelets, increasing the susceptibility to bleeding. This imbalance between degradation induced by shear stress and the endothelial release of new vWF triggered by pulsatile flow during MCS, results in overall reduction in HMWM, leading to device-induced AVWS, characterized by bleeding mainly from the mucosal surfaces, including gastrointestinal bleeding.[56] The high shear in MCS appears to cause shedding of the platelet glycoproteins Ib/IX and VI, reducing vWF binding on the platelet surface and increasing the propensity for bleeding.[57]

The diagnosis of AVWS is made by detecting abnormally low vWF activity in relation to vWF antigen (vWF:Ag) levels, reflected in a deficiency of HMWM on gel electrophoresis. Although the latter is the gold standard for diagnosing AVWS, this is generally not readily available, and most centres use the ristocetin cofactor assay (vWF:RCo) to assess vWF activity in relation to vWF:Ag. Normal vWF:RCo/vWF:Ag ratio is >0.7, and a ratio of <0.6–0.7 indicates a loss of HMWM and AVWS. Although assessment of VWF:RCo with platelet aggregometry has been regarded as the "gold standard", this demonstrates high variability and relatively low sensitivity for AVWS. Automated tests for vWF:RCo using turbidimetry or chemiluminescence have greatly improved precision, with markedly reduced coefficients of variation.[58] Other automated tests include the vWF:GPIbR in which platelets are replaced with latex beads coated with recombinant wild type GPIb and the vWF:Ab test which uses polystyrene beads coated with monoclonal antibody directed against the GPIb binding A1 domain of vWF.[58] These tests may eventually replace the vWF:RCo performed by aggregometry in many laboratories. To date, there are no point-of-care tests specifically for AVWS especially in the setting of MCS, but adaptations of the platelet function analyser and viscoelastic tests may hold promise (*vide infra*).

There is evidence that use of continuous-flow mechanical support devices and ECMO lead to substantial reduction in HMWM of vWF and AVWS-related gastrointestinal bleeding.[56,59] Indeed, the permanent high shear stress and continuous blood flow not only induce proteolysis of HMWMs, but also result in platelet inhibition and reorganization of endothelial

cells, particularly in the blood vessels of the gastrointestinal tract, leading to gastrointestinal dysplasia and subsequently bleeding.[59,60] ECMO is generally associated with loss of HMWMs within 24 hours of treatment, with rapid reversal after withdrawal of MCS.[59] In more than 200 patients on long term pulsatile and continuous flow MCS, development of AVWS, vWF:Ag and vWF:RCo levels were predictive of bleeding.[61] The incidence of bleeding is significantly higher with non-pulsatile, continuous flow assist devices than that with pulsatile flow devices. Even using the same non-pulsatile device (*ex-vivo* ECMO circuit), lowering the flow rate may enhance the loss of HMWMs.[62] Importantly, patients supported with the latest generation left ventricular assist devices (lower pump speed, different textured interior surfaces, physiological pulsatile flow) had more intact HMWMs and higher vWF activity compared to patients treated with older devices.[63]

Possible future monitoring approaches

Point-of-care tests to assess AVWS

Although not evaluated in the setting of MCS, the PFA-100 appears to be a good screening test for von Willebrand disease (vWD), with high sensitivity. A study in 41 paediatric outpatients showed that prolonged closure time with the collagen/ADP cartridge had an overall sensitivity of 90% to detect vWD.[64] Specifically, all patients with types 2 or 3 vWD had prolonged PFA-100 closure times with both cartridge types, whereas amongst those with type 1 vWD, 83% had prolonged closure times with the collagen/ADP cartridge and 79% with collagen/epinephrine. A recent review confirms that the PFA-100/200 can be used in urgent situations to exclude the presence of AVWS with high sensitivity if closure time is normal, with a negative predictive value >99%,[65] although validation specifically in the setting of ECMO is lacking. However, a prolonged closure time in either the collagen/epinephrine or the collagen/ADP channel has very low positive predictive value of for AVWS, since such closure time prolongation is also seen with thrombocytopenia, low haematocrit and acquired platelet function defects.[65]

The latest version of MEA incorporates a RISTOtest, containing ristocetin for the quantitative determination of vWF- and glycoprotein Ib-dependent platelet aggregation, but is available for research use only.

The performance of a modified TEG assay to screen for vWD was evaluated in 328 patients, assessing the ratio of clot strength (MA) with and without preincubation of blood with ristocetin. Decrease in MA with a cut-off of 25% for the area under the curve (AUC) ratio, resulted in a sensitivity of 53-100% for different types of vWD.[66] The same group subsequently compared the performance of TEG and ROTEM in 100 patients with vWD and 89 healthy controls.[67] Prolonged TEG R-time had a positive predictive value (PPV) of 0.84 and a negative predictive value (NPV) of 0.68 respectively, while the clotting index (CI) had a PPV of 1.00 and an NPV of 0.60. Both R-time and CI had a high specificity and accurately discriminated VWD patients from healthy controls, with an AUC of 0.85 and 0.99, respectively. On the other hand, ROTEM parameters could not differentiate patients with vWD from healthy controls.[67] However, a recent modification of the ROTEM assay with preincubation of the blood sample with ristocetin and commercially-available vWF has shown promise in identifying vWD in a small study of 27 out-patients,[68] but there has been no further validation.

Quantification of vWF cleavage

A relatively recent development is a mass spectrometry method to quantify vWF cleavage as the ratio of the ADAMTS-13-cleaved peptide MVTGNPASDEIK to the ILAGPAGDSNVVK peptide. Whilst this has not been evaluated in ECMO patients, increased vWF cleavage was detected in samples from patients on left ventricular assist devices who had developed bleeding.[69] However, this requires special laboratory expertise and is time-consuming, such that it may not be practical for routine clinical use.

Global Thrombosis Test (GTT)

This automated point-of-care test assesses the time taken to form an occlusive thrombus under high shear stress (occlusion time), and in the subsequent phase of the test, measures the time taken for spontaneous restart of flow, as a measure of endogenous fibrinolysis (lysis time). It assesses native, non-anticoagulated whole blood and is a global test of thrombosis. It has been shown to identify patients who are prothrombotic due to impaired endogenous fibrinolysis, in the setting of acute coronary syndromes.[70,71] As it provides a global assessment of thrombotic status, including platelet reactivity and coagulation in the setting of high shear, it would seem an ideal point-of-care test in patients on ECMO. It could be used to assess the risk of

thrombosis (short occlusion time and/or long lysis time) and bleeding (long occlusion time and/or short lysis), but studies to date have not been performed in this patient group.

Total Thrombus-Formation Analysis System (T-TAS)

This is a relatively novel point-of-care flow chamber system which employs two microchips; the platelet (PL) chip AUC assesses platelet function and primary haemostasis, while the atheroma (AR) chip assesses overall haemostatic ability. It has been shown to be of use in monitoring the effects of antiplatelet therapy and the severity of vWD type 1, with small AUC predictive of periprocedural bleeding in patients undergoing stenting.[72] In four patients on continuous flow left ventricular assist devices, HMWM vWF and T-TAS PL_{24}-AUC_{10} and AR_{10}-AUC_{30} were significantly reduced compared to anticoagulated control patients.[72] Future studies are required to see whether it could be used to detect AVWS in patients on ECMO.

Growth Differentiation Factor 15 (GDF-15)

GDF-15, a member of the transforming growth factor-beta superfamily, is upregulated in many disease states including cardiovascular disease, has been shown to reduce ADP-induced platelet aggregation in a dose-dependent manner and is a marker of bleeding. In large studies in patients on dual antiplatelet therapy or oral anticoagulation, elevated GDF-15 levels were independently associated with major bleeding during follow-up.[73,74] Circulating GDF-15 level has been shown to be elevated in patients with heart failure prior to left ventricular assist device implantation, and to fall significantly after 1 month of mechanical unloading,[75] but there are no studies assessing its utility to predict bleeding in patients on ECMO.

Possible novel anticoagulant approaches

Exposure of blood to artificial surfaces potentiates thrombosis through *contact activation*, namely the activation of factor XII (FXII), XI (FXI) and plasma prekallikrein. Targeted inhibition of FXIa or FXIIa may reduce thrombotic risk associated with contact activation, whilst leaving haemostasis largely intact;[76] an avenue that seems particularly attractive in patients on ECMO who require anticoagulation but are prone to excessive bleeding.

In rabbits connected to a paediatric ECMO circuit, a recombinant fully human FXIIa activity neutralizing antibody (3F7) dose-dependently reduced thrombus formation at arterial shear rates, without an increase in bleeding.[77] In primates, monoclonal antibodies against FXII reduced thrombosis in collagen-coated arteriovenous grafts.[78] In rabbits, antisense oligonucleotides (ASO) inhibiting the production of FXII or FXI, prolonged the time to catheter-induced venous thrombosis,[79] whilst a monoclonal IgG against FXIa was as effective as rivaroxaban in preventing venous thrombosis, without increasing cuticle bleeding.[80] It remains to be established if FXII or FXI is the better target for thromboprophylaxis in patients supported by devices with artificial surfaces. Whilst FXII inhibition may not increase the risk of bleeding, inhibition of FXI may produce a more potent antithrombotic effect. No clinical trials employing FXII inhibition in humans have been reported, however there are several different approaches being developed to inhibit FXI, including ASOs, monoclonal antibodies, aptamers and small molecules. Small molecule inhibitors can be delivered orally or parenterally, whereas ASOs, antibodies and aptamers require parenteral administration. Antibodies, aptamers and small molecules can achieve rapid FXI inhibition whilst ASOs can take 3 to 4 weeks to achieve therapeutic anticoagulation. In phase II trials in patients undergoing knee surgery, an ASO FXI inhibitor[81] administered subcutaneously and an anti-FXIa antibody osocimab,[82] given intravenously have both been shown to be superior to enoxaparin for thromboprophylaxis without an increase in bleeding.[81] A small molecule oral FXIa-inhibitor has commenced a Phase IIb program (PACIFIC), enrolling more than 4,000 patients with atrial fibrillation, myocardial infarction, or stroke. Several ongoing phase II studies will assess the safety of FXI inhibition in patients with ESRD on haemodialysis. There are no studies in patients on MCS, although onset of effect, half-life and offset/reversibility will be key issues when considering such strategies in patients on ECMO.

Guide to haemostasis assessment and management

We provide a summary and schematic for assessing thrombosis and bleeding risk in patients on ECMO (Figure 2), based on our experience combined with literature review. Anti-Xa level best correlates with heparin dose, and appears predictive of thrombosis, although aPTT may be a better predictor of bleeding. In the absence of data to guide optimal anticoagulation, the 2014 ELSO anticoagulation guideline states that the majority of ELSO centres that use the anti-Xa levels aim for a target of 0.3-0.7 IU/mL, which is a very broad range.[13] We use the lower end of the range, aiming for 0.2-0.5 IU/mL to reduce the risk of bleeding, with a lower

target of 0.2-0.3 IU/mL for patients on VV-ECMO, based on our experience[47] and supported by protocol in the EOLIA trial.[83] Heparin resistance should be considered in patients who are not achieving the desired anti-Xa level despite escalating doses of UFH. In such cases, the AT level should be checked and preferably maintained in the normal range, with consideration given to switching from UFH to a direct thrombin inhibitor such as argatroban. Patients with thrombocytopenia or those who are bleeding should be promptly investigated for HIT, with consideration of alternative anticoagulation. The patient should be assessed for AVWS and ECMO flow rates should be adjusted to minimise AVWS whenever possible. Viscoelastic tests may be used to assess bleeding risk. Platelet aggregation should be assessed relative to platelet count, and in patients experiencing major bleeding with markedly impaired platelet aggregation on antiplatelet medication, consideration should be given to the risks and benefits of withholding antiplatelet medication as indicated by the clinical scenario (such as in the case of acute coronary syndrome, recent stent implantation in a major artery or last remaining conduit) in consultation with the wider clinical team(s). In addition, platelet transfusion should be considered, even in the presence of normal platelet count. If the ischaemic risk is high, antiplatelet therapy should be continued and tranexamic acid administered concomitantly for 1-2 days. In patients experiencing thrombosis, anti-Xa levels and possibly TG may be useful, where rising ETP may indicate a risk of venous thrombosis.

Discussion

There are no prospective randomised controlled studies assessing clinical outcomes in patients on ECMO receiving UFH, which compare monitoring with aPTT versus heparin anti-Xa level, to guide recommendations for anticoagulant monitoring. Measurement of anti-Xa level best correlates with heparin dose, and appears predictive of thrombosis, although aPTT level is a better predictor of bleeding. Platelets play a major role both primary and secondary haemostasis. In addition to thrombocytopenia due to reduced bone marrow production, sepsis, pooling of platelets in the liver or spleen, and consumption of platelets through the MCS, there is also a high risk of platelet dysfunction which is directly related to the degree of shear and the duration of exposure to the artificial circuit. The high shear in MCS appears to cause vWF binding on the platelet surface and increasing the propensity for bleeding. AVWS, best detected by assessing the vWF:RCo ratio, is frequent, almost universal in patients on MCS,

contributing to bleeding. Tests of platelet aggregation may be additionally used to predict bleeding. High shear stress also causes platelet activation and simultaneously increases the risk of thrombosis. Despite conventional monitoring of UFH, there is a high rate of thrombotic and bleeding complications with MCS. A selection of complementary tests to collectively assess heparin-effect, coagulation, platelet function and platelet aggregation would be most useful for optimal early detection of bleeding and thrombosis risk, in order to personalise management and optimise outcomes in patients on MCS.

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Figure 1. Aetiology of thrombosis and bleeding in patients on mechanical circulatory support and tests available to identify these risks.

Contact of blood with the artificial circuit results in activation of coagulation, whilst platelet activation occurs due to the foreign surface and high shear flow conditions. Activation of coagulation and platelet aggregation result in platelet thrombus formation. Meanwhile, MCS particularly with continuous (as opposed to pulsatile) flow, leads to cleavage of HMWM of vWF and development of AVWS, increasing the susceptibility to bleeding. Endogenous fibrinolysis, mediated predominantly by tissue-activated plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI), is responsible for prevention of lasting thrombotic occlusion and when enhanced can increase bleeding risk.

Conventional tests to assess UFH shown in blue rectangles, more novel tests of haemostasis and fibrinolysis shown in green rectangles.

Figure 2. Proposal for monitoring patients on mechanical circulatory support.

Baseline assessment and monitoring of anticoagulation is required in all patients as shown. Patients on MCS are at risk of both thrombosis (green panels) and bleeding (red panels), requiring requiring regular assessment (turquoise panels). To prevent bleeding whilst preventing thrombosis, we aim to keep heparin anti-Xa level in the target 0.2-0.5 IU/mL, at the lower end of the therapeutic range. Patients on MCS are at risk of developing platelet dysfunction due to loss of platelet receptors and AVWS despite normal platelet count. Platelet function tests allow the identification of these patients and if the patient is bleeding despite normal coagulation assays and platelet count, assessment of platelet function can help reveal platelet dysfunction. In patients with recurrent thrombotic events, particularly arterial events, despite adequate anticoagulation, and normal or activated platelet response, the addition of antiplatelet treatment to anticoagulation can be considered.

* dose range is given to reflect the variation in practice.

Abbreviations: anti-PF4=anti-platelet factor 4 antibodies, aPTT=activated partial thromboplastin time, AT=antithrombin, AVWS=acquired von Willebrand syndrome, CRP=C-reactive protein, CT=computerised tomography, DIC=disseminated intravascular coagulation, ELISA=enzyme-linked immunosorbent assay , HIT=heparin-induced thrombocytopenia, ICH=intracranial haemorrhage, LTA=light transmittance aggregometry, MEA=multiple electrode aggregometry, pf-Hb = plasma free haemoglobin, PT=prothrombin time, ROTEM=rotational thromboelastometry, TEG=thromboelastography, t.d.s.= ter die sumendum (3 times a day), UFH=unfractionated heparin.

Table 1. Tests available to monitor anticoagulation with unfractioned heparin.

Test	**Advantages**	**Disadvantages**
ACT	• Point-of-care test • Rapid to perform • Whole blood test	• No clear validation in ECMO • Affected by haemodilution, hypofibrinogenemia, thrombocytopenia, liver failure, inflammation, lupus anticoagulant and consumptive coagulopathy • Used alone may lead to suboptimal anticoagulation with ECMO
aPTT	• Point-of-care test available (not routinely) • Best predictor of bleeding • Relatively inexpensive	• Often central lab: time delay • High variation between reagents and labs • Affected by many biologic factors, often disturbed in critically ill patients • Range based on anti-Xa
Anti-Xa	• Considered gold standard for assessing heparin effect • Good correlation with heparin dose • Unaffected by presence of lupus anticoagulant, liver disease or consumptive coagulopathy	• Expensive • Difficult to automate • Not universally available • Not point-of-care; central lab required: time delay

Appendix

1. Literature search strategy

The literature search was performed by interrogating the MEDLINE/PubMed database combining the search terms (“extracorporeal membrane oxygenation” OR “ECMO” OR “mechanical circulatory support”) AND the terms (“heparin” OR “anticoagulation” OR “anticoagulant” OR “ACT” OR “APTT” OR “anti-Factor X level” OR “Antithrombin III” OR “von Willebrand syndrome” or “con Willebrand factor” OR “TEG” OR “ROTEM” OR “platelet function ” OR “multiplate” or “MEA” OR “thrombin generation” OR “endogenous thrombin potential” OR “platelet aggregation” OR “light transmittance aggregometry” OR “PFA” OR “VerifyNow” OR “Global Thrombosis Test” OR “T-TAS” OR “GDF-15” OR “Biomarkers of bleeding” OR “Biomarkers of thrombosis”) in any combination. Articles were filtered based on title and abstract, and reference lists were traced to identify additional articles of relevance. We included case series, observational or interventional studies and review articles, but excluded isolated case reports. There were no language restrictions.