Peer Review File
Article information: https://dx.doi.org/10.21037/aob-23-28

REVIEWER A

COMMENT 1:
Title: Not only anticoagulation but also thrombosis and bleeding management are covered. The title should reflect this. Maybe better "Coagulation management in patients requiring ECMO support…"
REPLY: Thank you for the comment
CHANGES IN THE TEXT: "Coagulation management in patients requiring ECMO support: a comprehensive narrative review"

COMMENT 2:
Background: "Several risk factors for bleeding and thrombosis events have been identified, including vasopressor use, longer treatment duration, single-site cannulation, older age, overweight, initial low pH, PaCO2 levels, and AKI." I suggest to add a reference to this sentence.
REPLY: Thank you to raise this point. We added a citation.
CHANGES IN THE TEXT: Several risk factors for bleeding and thrombosis events have been identified, including vasopressor use, longer treatment duration, single-site cannulation, older age, overweight, initial low pH, PaCO2 levels, and AKI (2).
Nunez JI, Gosling AF, O'Gara B, Kennedy KF, Rycus P, Abrams D, Brodie D, Shaefi S, Garan AR, Grandin EW. Bleeding and thrombotic events in adults supported with venovenous extracorporeal membrane oxygenation: an ELSO registry analysis. Intensive Care Med. 2022 Feb;48(2):213-224. doi: 10.1007/s00134-021-06593-x. Epub 2021 Dec 18. Erratum in: Intensive Care Med. 2022 Jan 18;: PMID: 34921625; PMCID: PMC9178906.

COMMENT 3:
Line 138-161. The paragraph covers coagulation physiology and contact activation. I´d wish for an instructive (yet not too complex) figure to illustrate contact activation (which is a challenge, I admit).
REPLY: Thank you for the suggestion
CHANGES IN THE TEXT: We included a new figure (Figure 1)

COMMENT 4:
The first part of the main text body (as far as "Bleeding complications and management") contains a number of statements without reference. If you refer to previously cited reviews, please clarify, otherwise please add references to the following phrases:
REPLY: We clarified the references to each statement to be clearer

CHANGES IN THE TEXT:

Line 166 ff: "One of the most apparent signs of factor consumption is a reduction of platelet count by 25–40%, not attributable to heparin-induced thrombocytopenia (HIT) (7).
Balle CM, Jeppesen AN, Christensen S, Hvas AM. Platelet Function During Extracorporeal Membrane Oxygenation in Adult Patients. Front Cardiovasc Med. 2019 Aug 8;6:114. doi: 10.3389/fcvm.2019.00114. PMID: 31440518; PMCID: PMC6694790.

Line 169 ff: "Platelet function can also be altered, leading to acquired von Willebrand syndrome and factor XIII deficiency during ECMO support (8).
Noitz M, Brooks R, Szasz J, Jenner D, Böck C, Krenner N, Dünser MW, Meier J. Acquired Factor XIII Deficiency Is Common during ECMO Therapy and Associated with Major Bleeding Events and Transfusion Requirements. J Clin Med. 2023 Jun 18;12(12):4115. doi: 10.3390/jcm12124115. PMID: 37373805; PMCID: PMC10299514.

Line 171ff: "Independent of the consumption and the subsequent decrease of pro-hemostatic factors, antithrombin levels decrease in about 50% of the cases during the first days of therapy the first days of therapy. Still, they return to normal after this period (9, 10).

Panigada M, Artoni A, Passamonti SM, Maino A, Mietto C, L'Acqua C, Cressoni M, Boscolo M, Tripodi A, Bucciarelli P, Gattinoni L, Martinelli I. Hemostasis changes during veno-venous extracorporeal

membrane oxygenation for respiratory support in adults. Minerva Anestesiol. 2016 Feb;82(2):170-9. Epub 2015 May 20. PMID: 25990432.
Malfertheiner MV, Philipp A, Lubnow M, Zeman F, Enger TB, Bein T, Lunz D, Schmid C, Müller T, Lehle K. Hemostatic Changes During Extracorporeal Membrane Oxygenation: A Prospective Randomized Clinical Trial Comparing Three Different Extracorporeal Membrane Oxygenation Systems. Crit Care Med. 2016 Apr;44(4):747-54. doi: 10.1097/CCM.0000000000001482. PMID: 26646464.

Line 291ff: "The advantages of their use include a more predictable anticoagulant effect independently of the fluctuating antithrombin activity and more predictable dosing regimens" (27).
Lee CJ, Ansell JE. Direct thrombin inhibitors. Br J Clin Pharmacol. 2011 Oct;72(4):581-92. doi: 10.1111/j.1365-2125.2011.03916.x. Erratum in: Br J Clin Pharmacol. 2011 Oct;72(4):718. Dosage error in article text. PMID: 21241354; PMCID: PMC3195735.

Line 301f: "DTIs such as argatroban and bivalirudin have been previously used in patients with HIT" (27)
Lee CJ, Ansell JE. Direct thrombin inhibitors. Br J Clin Pharmacol. 2011 Oct;72(4):581-92. doi: 10.1111/j.1365-2125.2011.03916.x. Erratum in: Br J Clin Pharmacol. 2011 Oct;72(4):718. Dosage error in article text. PMID: 21241354; PMCID: PMC3195735.

COMMENT 5:
Line 170: Altered platelet function leading to aVWS? I´m not sure. Some say, it´s the centrifugal pump (Lopez et al. Blood Coagulation and Fibrinolysis 2005, 16(Suppl 1):S11–S16)

REPLY: We added two recent refences to support the statement of platelet dysfunction and acquired VWS.

CHANGES IN THE TEXT:

Line 168: acquired von Willebrand syndrome and factor XIII deficiency during ECMO support (8-10)
Kalbhenn J, Schlagenhauf A, Rosenfelder S, Schmutz A, Zieger B. Acquired von Willebrand syndrome and impaired platelet function during venovenous extracorporeal membrane oxygenation: Rapid onset and fast recovery. J Heart Lung Transplant. 2018 Aug;37(8):985-991. doi: 10.1016/j.healun.2018.03.013. Epub 2018 Mar 17. PMID: 29650295.

Tamura T, Horiuchi H, Obayashi Y, Fuki M, Imanaka M, Kuroda M, Nishimura S, Amano M, Sakamoto J, Tamaki Y, Enomoto S, Miyake M, Kondo H, Izumi C, Nakagawa Y. Acquired von Willebrand syndrome in patients treated with veno-arterial extracorporeal membrane oxygenation. Cardiovasc Interv Ther. 2019 Oct;34(4):358-363. doi: 10.1007/s12928-019-00568-y. Epub 2019 Jan 17. PMID: 30656612.

COMMENT 6:

Line 189f: "Eighty percent of the patients had bleeding events, of which 19% were major bleeding episodes" These are two different studies, better "…bleeding events, 19% are major bleeding episodes"

REPLY: We clarified as suggested.

CHANGES IN THE TEXT: Eighty percent of the patients had bleeding events (14), 19% are major bleeding episodes (15).

COMMENT 7:

Line 217ff, paragraph on hemolysis: Major hemolysis is mostly due to pump clotting or very negative pressures at the drainage cannula (Mulholland, Perfusion 2000; 15: 485–494). These facts should be mentioned.

REPLY: We clarified as suggested.

CHANGES IN THE TEXT: Major hemolysis is mostly due to pump clotting or very negative pressures at the drainage cannula (20).

Mulholland JW, Massey W, Shelton JC. Investigation and quantification of the blood trauma caused by the

combined dynamic forces experienced during cardiopulmonary bypass. Perfusion. 2000 Nov;15(6):485-94. doi: 10.1177/026765910001500603. PMID: 11131211.

COMMENT 8: Line 285: Delete "However"
REPLY: Deleted
CHANGES IN THE TEXT: …dose regimens. An increased rate of thromboembolic events…

COMMENT 9: Line 288: "Alternative anticoagulants…" The paragraph should change prior to this sentence.
REPLY: We changed the statement to finalize accordingly and to introduce the next paragraph.
CHANGES IN THE TEXT: Other anticoagulant regimens that have been used include Direct Thrombin Inhibitors (DTIs) and Nafamostat Mesylate.

COMMENT 10: Line 290ff, discussion on DTIs: Pharmacological differences between Bivalirudin and Argatroban should be mentioned (half life, elimination).
REPLY: We added this information and the respective citations.
CHANGES IN THE TEXT:
Line 303: Bivalirudin is an specific and reversible DTI that binds to the catalytic and anionic exosite of both circulating and clot-bound thrombin, preventing thrombin-mediated cleavage of fibrinogen to fibrin monomers, and further activation of factors V, VIII and XIII. Its onset of action is immediate with a half-life of 25 minutes if renal function is preserved, though it can be double in severe dysfunction and as long as 3.5 h in dialysis patients. Its elimmintaion is mainly through urine (20%) (32).

Line 313: Argatroban is direct DTI that reversibly binds to the active thrombin site of free and clot-associated thrombin, therefore inhibiting fibrin formation and activation of factors V, VIII and XIII, protein C and platelet aggregation (35). This drug is available…

COMMENT 11: Line 322f: Anti-platelet strategies: There are options of adding reversible anti-platelet agents: Please refer to Buchtele et al. Am J Respir Crit Care Med (2022)206, 170–177
REPLY: We added this information and the respective citation.
CHANGES IN THE TEXT: Some platelet antiaggregants have been studied for their use in ECMO. Prostaglandin E1 (PGE1), in addition to low-dose unfractionated heparin, augments the biocompatibility of ECMO circuits without increasing bleeding risk. In a previous pilot study, add-on treatment with PGE1 was safe but did not reduced the rate of red blood cell transfusions in ECMO patients. The potential role of antiplatelets (e.g., aspirin) in ECMO must be elucidated (40).

Buchtele N, Schörgenhofer C, Schwameis M, Jilma B, Schellongowski P, Herkner H, Riss K, Schmid M, Hermann A, Robak O, Nagler B, Traby L, Bojic A, Staudinger T. Add-On Prostaglandin E_1 in Venovenous Extracorporeal Membrane Oxygenation: A Randomized, Double-Blind, Placebo-controlled Pilot Trial. Am J Respir Crit Care Med. 2022 Jul 15;206(2):170-177. doi: 10.1164/rccm.202110-2359OC. PMID: 35426776.

COMMENT 12: Line 329ff: ACT: Bad correlation of ACT with aPTT and heparin dose. Please mention. (e.g. Saife et al. ASAIO J 2020; 66:230-237).
REPLY: We added this information and the respective citation.
CHANGES IN THE TEXT: In a previous study, ACT and aPTT had low correlation with coagulation factor levels and heparin dose. The authors found Anti-Factor Xa Activity being the most specific for heparin levels, and PT being most specific for monitoring coagulation and hemostasis for patients on ECMO (43).

Saifee NH, Brogan TV, McMullan DM, Yalon L, Matthews DC, Burke CR, Chandler WL. Monitoring Hemostasis During Extracorporeal Life Support. ASAIO J. 2020 Feb;66(2):230-237. doi: 10.1097/MAT.0000000000000993. PMID: 30913107.

COMMENT 13: Line 342ff: aPTT: Please mention possible confounding factors (F XII deficiency, Lupus anticoagulans, etc.) Refer to: Levy et al. Intensive Care Med 2022;48:1076-1079, Buchtele et al. Crit Care Med 2021;49:e1206-e1211, Kornfehl et al. Clin Appl Thromb Hemost 2023, epub

REPLY: We added this information and the respective citation.
CHANGES IN THE TEXT: Confounding factors for the interpretation of aPPT during anticoagulation treatment in ECMO include inherited factor deficiencies (e.g., hemophilia A or B, vWD, factor II, V, X, XI,

or XII deficiencies), liver disease, combined vitamin K-dependent factor deficiency, disseminated intravascular coagulation, and lupus anticoagulant-type ihnibitors (43-45).

43. Buchtele N, Schwameis M, Schellongowski P, Quehenberger P, Knöbl P, Traby L, et al. Prevalence and Clinical Impact of Reduced Coagulation Factor XII Activity in Patients Receiving Extracorporeal Membrane Oxygenation. Crit Care Med. 2021;49(12):e1206-e11.
44. Favaloro EJ. Welcome to Seminars in Thrombosis & Hemostasis 2023. Semin Thromb Hemost. 2023;49(1):1-2.
45. Levy JH, Staudinger T, Steiner ME. How to manage anticoagulation during extracorporeal membrane oxygenation. Intensive Care Med. 2022;48(8):1076-9

COMMENT 14: Line 350: Anti-Xa range: Depends on center, assay and strategy. 0.7 can be very high –I advice to be cautious. (The EOLIA trial for instance used 0.2-0.3 and did well!)
REPLY: Thank you for pointing this out. We added this information to be considered and the respective citation. We added as well the information from EOLIA trial for aPTT.
CHANGES IN THE TEXT:
Line 358 : The EOLIA trial used a target activated partial-thromboplastin time of 40 to 55 seconds (42)

Line 37: … Of note, in the EOLIA trial, the study protocol considered a lower target Anti-Xa, between 0.2–0.3 IU/mL. Clinically important bleeding events were observed in 53% of ECMO patients vs. 26% in the non-ECMO group, with no differences observed in massive bleeding events between both groups (42).

COMMENT 15: Line 354ff: Discussion on TEG: Please refer to anticoagulation strategies based on TEG/ROTEM (e.g. Panigada et al. Ann. Intensive Care (2018) 8:7)
REPLY: We added this information and the respective citation.
CHANGES IN THE TEXT:
In a feasibility pilot study, Panigada et al randomized patients to one of two strategies, either TEG-based (target 16-24 min of R parameter) or aPTT-based (1.5-2 of aPTT ratio), to manage heparin protocol. TEG-based protocol was safe for guiding anticoagulation management in ECMO patients, and it was associated with the administration of lower heparin doses than the aPTT-based protocol (49).

Panigada M, E Iapichino G, Brioni M, Panarello G, Protti A, Grasselli G, Occhipinti G, Novembrino C, Consonni D, Arcadipane A, Gattinoni L, Pesenti A. Thromboelastography-based anticoagulation management during extracorporeal membrane oxygenation: a safety and feasibility pilot study. Ann Intensive Care. 2018 Jan 16;8(1):7. doi: 10.1186/s13613-017-0352-8. PMID: 29340875; PMCID: PMC5770349.

COMMENT 16: Table 1: Please indicate where this example of an algorithm comes from.
REPLY: We think the reviewer referred to figure 1, rather than Table 1 (table 1 displays the search strategy for the manuscript).
So, we specified that the algorithms in Figure 1 and and Figure 2 (we divided the algorithms), both came from our institutional protocols.
CHANGES IN THE TEXT: Figure 1 and Figure 2 detail an example of anticoagulation monitoring and a bleeding management protocols in ECMO used in our unit. However, prevention is the best strategy for bleeding management.

COMMENT 17: I miss an overview on HIT management. Maybe should be placed following the UFH chapter.
REPLY: We added this information and the respective citations.
CHANGES IN THE TEXT: Patients with heparin-induced thrombocytopenia are at risk of presenting arterial or venous thromboembolism. Therefore, they have an ongoing need for anticoagulation. Beyond holding heparin, a non-heparin anticoagulant should be administered. Fondaparinux and oral Xa inhibitors are commonly chosen initially. Depending on the urgency of anticoagulation, either a parenteral or oral anticoagulant can be used first. When there is a need for urgent reversal (high risk of bleeding, need for invasive procedures) or kidney impairment, bivalirudin and argatroban are the best choices for non-heparin anticoagulation due to their short half-lives. In the case of liver disease, argatroban should be avoided, and bivalirudin or fondaparinux are the preferred options (41). Concomitant use of heparin with plasma exchange or potent antiplatelet agents (e.g., prostacyclin analog, tirofiban, or high-dose IV) have only been studied in patients with cardiopulmonary bypass, and there is no evidence supporting the implementation of these strategies in ECMO patients (42).

Nicolas D, Nicolas S, Hodgens A, et al. Heparin-Induced Thrombocytopenia. [Updated 2023 May 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482330/ (Accessed on Jan, 2, 2024).

May J, Westbrook B, Cuker A. Heparin-induced thrombocytopenia: An illustrated review. Res Pract Thromb Haemost. 2023;7(5):100283.

REVIEWER B

COMMENT 1:
P7 Para2 L138: The authors make assumptions that the contact activation is the main pathway. This is highly likely but have not been proven and therefore the tone should be changed. There is limited original research highlighted clearly to support. The suggestion of progressive protein and cellular deposition is based largely upon glass surfaces although recent interesting data from immunohistostaining has shown various protein/cellular deposition within circuits that has not been discussed.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Beyond contact activation phenomena, the coagulopathy in ECMO is a complex syndrome due to high shear forces, hemostatic activation, clotting factor consumption and platelet activation and dysfunction".**

COMMENT 2:
P8 L164: It is inappropriate to say that D-dimer reflects fibrinolytic response. D-dimer is a degradation production and there reflects coagulation activation and/or fibrinolytic activity, of which its likely both are increased in ECMO.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"The fibrinolytic system is activated to keep clot formation localized, and the progressive increases in D-dimer levels reflect the ongoing process of thrombosis and thrombolysis, so it can be used as a marker of the intensification of this event in ECMO"**.

COMMENT 3:
P8 L165: DIC is difficult to manage and it is difficult to fully perscribe this process to the circuit although it is likely that there is a localised consumptive coagulopathy in those with high clot burden in the membrane. There is also no mention that DIC is more likely to be attributable to the patient causes e.g. sepsis and surgery.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Furthermore, it is important to note that there are patient-related causes, such as sepsis, acute liver failure, acute kidney failure, etc., that can cause different degrees of disseminated intravascular coagulation".**

COMMENT 4:
P8 L168: Platelet dysfunction does not cause an acquired von Willebrand Syndrome. Platelet dysfunction is due to platelet glycoprotein shedding in the circuit and aVWS is due to loss of HMW multimers due to sheer stress within the circuit. Again there are recent interest articles that have not been acknowledged on this.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Some studies suggest that AvWS is one of the etiologies of bleeding. It is believed to be caused by cleavage of large vWF multimers by the metalloprotease a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 under ECMO-associated shear stress conditions Thereby the platelet adhesion-promoting potential of vWF decreases, which contributes to bleeding".**

COMMENT 5:
P9 Para 1: Consider discussing inhibition of intrinsic pathway factors. There are described animal models of this. What are the outcomes of using the different circuit types in comparison to each other?
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"The intrinsic coagulation pathway predominantly mediates the coagulation during ECMO, except in patients with recent surgery, where the extrinsic coagulation pathway has an important role. Important advances and progress in terms of biocompatibility and hemocompatibility in the ECMO system have improved patient outcomes. However, no specific bioactive surface is currently better than another".**

COMMENT 6:
P9 L209: Need to be clear that there is 1) risk of thrombosis in the patient and 2) circuit thrombosis/occlusion.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"ECMO patients experience an increased risk of thrombosis, but mostly ECMO circuit thrombosis".**

COMMENT 7:
P9 L217: The author do not elaborate how thrombotic events cause haemolysis. Please elaborate if you are talking about pump thrombosis or membrane occlusion causing more stress and hence red cell damage.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Hemolysis is usually produced within the high-shear stress zones within the ECMO circuit (but also inside cannulas), especially by thrombosis of the pump head leading to occlusion of membrane oxygenation, and represents a factor that further contributes to homeostasis dysregulation".**

COMMENT 8:
P10 L237: Stasis in the patient's vasculature and reduce flow around the large bore cannula are also contributable to catheter-associated thrombosis, for which there is citable literature.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **We added 2 references related to the issue.**

COMMENT 9:
P10 L239: I am unclear why patients with severe respiratory failure alone are increased risk of CA-DVT. There is an increased risk generally in patients requiring ECMO.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"The prevalence of DVT following decannulation in patients whose indication for V-V ECMO was severe respiratory failure is clinically significant, but its relationship is still unclear".**

COMMENT 10:
P10 L241: This is not correct. Anticoagulation does not inhibit platelet function. It inhibits the activation of thrombin generation.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Ideally, anticoagulation should inhibit clotting formation, which simultaneously generates platelet activation, within the ECMO circuit while maintaining platelet function and clotting activity preserved not to cause patient bleeding".**

COMMENT 11:
P11 L251-254: This sentence is not clear and needs rephrasing.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"UFH binds to the enzyme inhibitor antithrombin III (ATIII), causing the activation of ATIII with a conformational change which potentiates the anticoagulant activity. The activated antithrombin then inactivates thrombin, factor Xa, and other proteases."**

COMMENT 12:
P11 L253-257: I would suggest that this is moved to the beginning of the section on Anticoagulation Monitoring.
REPLY: Thank you for the comment. We prefer to keep the phrase in this position.
CHANGES IN THE TEXT: **None.**

COMMENT 13:
P14 L296: Sentence is repeated with L292.
REPLY: Thank you for the comment. We are not able find the similarity in sentences.
CHANGES IN THE TEXT: **None.**

COMMENT 14:
P14 L305: Review cautions for each drug. Argatroban should be avoided in liver impairment and bivalirudin should be avoided in renal impairment.

REPLY: Thank you for the comment. There is no contraindication for the use of these agents in case of liver (Argatroban) or renal (Bivalirudin) dysfunction.
CHANGES IN THE TEXT: **Bivalirudin: "This agent could be used as an initial anticoagulant in ECMO therapy and for patients with HIT or UFH resistance and liver or renal dysfunction (34), but it may be necessary the dosage reduction in case of renal impairment". Argatroban: "Argatroban is approved in the United States and Europe for anticoagulation in patients with or at risk of HIT, while bivalirudin is used "off-label" for this indication. Besides, a dosage reduction may be necessary in the case of liver impairment".**

COMMENT 15:
P14 L317-319: DOACs are oral medication and relatively long-lasting. They should not be used in ECMO and this statement is not appropriate.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Still, there is currently limited *in vivo* evidence for its use and it is not routinely recommended".**

COMMENT 16:
P14 L320: Antiplatelets are already used during VA-ECMO in patient following coronary artery treatment.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"An indication for dual antiplatelet therapy due to coronary stent implantation is present in a considerable number of V-A ECMO patients in addition to anticoagulation treatment, but increasing the risk of bleeding".**

COMMENT 17:
P15 L321: What immunotherapies are to be considered? Surely this would be and interesting area to elaborate on.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Immunotherapy is expected to have a place in ECMO anticoagulation as new evidence evolves in the near future, for example, the use of Factor XIIa Inhibitory Antibody as a thromboprotection treatment in ECMO patients without increasing bleeding risk".**

COMMENT 18:
'Anticoagulation Monitoring': There should be more studies described in this area particularly with the advantage and disadvantage of each assay. The ACT is becoming generally more obsolete in high-volume ECMO centres and anti-Xa is becoming the standard of care for heparin monitoring. The authors should elaborate the differences in the techniques used particularly in critical illness and ECMO as this will be of interest to haematologists.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **Modified text.**

COMMENT 19:
P17 L377: This line does not make sense. Please can it be clarified whether mortality is due to bleeding and thrombosis or whether those that have bleeding and thrombosis have this risk of mortality.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"In the recent ELSO registry analysis, bleeding events were associated with higher mortality (46.1%) than thrombotic events (36.1%)".**

COMMENT 20:
P17 L387: I am unclear of '<1 year of support'. If this is ECMO/ITU support, one would assume that this is all patients.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Longer ECMO runs, younger age, alkalosis, and < 1 year of ECMO support were associated with bleeding and thrombosis".**

COMMENT 20:
Table 2 & 3: It should be made clear whether this is a suggested strategy from the authors or if this is one from a study that has provided outcomes. In Table 2, I have concerns about the routine infusion of AT replacement without evidence of heparin resistance and this is also suggested by some of the data you have presented. In Table 3, you have not discussed desmopressin and tranexamic acid in the rest of your article but have included it as treatment option. I also raise question why you would put 'third level - give rFVIIa'

and ‘fourth level - IV protamine’. It seems illogical to give a reversal agent after consider a potent thrombin activator.
REPLY: Thank you for the comment. Tables 2 and 3 were modified by Figures 1 and 2 and represent the strategy we use in our center.
CHANGES IN THE TEXT: **Modified figures.**

COMMENT 21:
P17 L387: I am unclear of ‘<1 year of support’. If this is ECMO/ITU support, one would assume that this is all patients.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **“Longer ECMO runs, younger age, alkalosis, and < 1 year of ECMO support were associated with bleeding and thrombosis”.**

COMMENT 22:
P19 L399: Change title to include ‘reduced anticoagulation strategies’ and need to reference ISTH guidance (2023).
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **“Patients with severe bleeding and coagulopathy are candidates to receive anticoagulation-free treatment or reduced anticoagulation strategies”. “Many of these cases involve VA-ECMO in high-risk patients with ongoing hemorrhage, cardiogenic shock, postCPR, or postcardiopulmonary bypass, postcardiotomy shock, and thus routine anticoagulation is omitted. Instead, heparin-coated ECMO circuits are generally used”.**

COMMENT 23:
P20 Para 1: The authors need to highlight and make clear the proportion of patients that had anticoagulation held temporarily and what were the typical durations, which is the majority. The paragraph inadvertently suggests that these patients had full anticoagulation-free ECMO runs and leads to some bias in the data they are presenting.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **“Olson et al. in their systematic review, reported a total incidence of thrombosis of 22.9% in a group of 201 patients with ECMO without systemic anticoagulation for a median of 4.75 days and a total duration of anticoagulant-free ECMO of 304.7 days”.**

COMMENT 24:
P20 L438: Do you have any evidence to substantiate increasing flow rates to maintain circuit patency - please include.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **“However, considering the limitations of the retrospective data, we recommend increasing the ECMO flow as much as possible when prescribing an anticoagulant-free strategy because thrombosis in the ECMO circuit is more likely to occur during periods of low flow”.**

COMMENT 25:
P20 L441: Please change ‘hematological disease’ to ‘severe thrombocytopenia [due to hematological disease]’. However, platelet transfusion can be given successfully in these patients such as conditions like acute myeloblastic leukemia and MDS. Additionally, patients with haemophilia can be given ECMO however they need additional coagulation factor support and non-coagulation based assays for heparin monitoring. There are published cases reports of this.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **“Two groups of patients on ECMO that usually receive anticoagulation-free ECMO runs are those with severe thrombocytopenia due hematological diseases and recent trauma”.**

COMMENT 26:
P20-21: This may be more useful if separated in to a new section looking at ECMO in specific patient cohorts and to include COVID-19 within this.
REPLY: Thank you for the comment. We prefer to dedicate a specific section for COVID-19 due to important available data like ECMOVIBER study.
CHANGES IN THE TEXT: **None.**

COMMENT 27:

P27 L600: Activation of fibrinolysis does not lead to a thrombotic phenotype. In COVID-19 there was a suggestion of hypofibrinolysis causing excess thrombus burden.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: **"Sepsis-induced coagulopathy, immunothrombosis, and decresead activation of the fibrinolytic cascade (suspected hypofibrinolysis) are mechanisms promoting thrombus formation during SARS-CoV-2 infection".**

REVIEWER C

COMMENT 1: I believe it is generally viewed that the preponderance of activation of clotting in patients with foreign surfaces devices is initiated by the intrinsic system and not the extrinsic system. In the article it is kind of presented the opposite way with von Willebrand factor and platelets activating tissue factor and then the extrinsic pathway.
REPLY: Thank you for the comment.
CHANGES IN THE TEXT: NONE.

COMMENT 2: The article is a very good review of the activation of clotting by ECMO and also of secondary bleeding and there are helpful suggestions offered about maintaining the platelet count at a certain level particularly after cardiac surgery. However, the authors basically acknowledge that they do not really have that much in the way of new recommendations for anticoagulation and in the monitoring of anticoagulation and the use of Antithrombin and other modalities like tranexamic acid to offer.
REPLY: Thank you for your kindness. Yes, in this regard, evidence is scares, but still it is interesting to merge the best available evidence on this topic.
CHANGES IN THE TEXT: NONE.

COMMENT 3: In the article They should emphasize some of the advantages of ROTEM and TEG in that while they measure coagulation factor levels through the R phase they can also simultaneously measure platelet function and fibrinolytic activity and it is also a point-of-care device for people in the hospital where all ECMO patients will be located.
REPLY: We have added a review about anticoagulation and ROTEM.
CHANGES IN THE TEXT: line 386: TEG and ROTEM may also provide a view of clot formation dynamics, clot strength, and clot lysis. In a feasibility pilot study, Panigada et al randomized patients to one of two strategies, either TEG-based (target 16-24 min of R parameter) or aPTT-based (1.5-2 of aPTT ratio), to manage heparin protocol. The TEG-based protocol was safe for guiding anticoagulation management in ECMO patients, and it was associated with the administration of lower heparin doses than the aPTT-based protocol (51). Thus, these tests are useful for the primary treatment for early disseminated intravascular coagulation (DIC). For primary hyperfibrinolysis, tranexamic acid is the preferred antifibrinolytic agent. Maintaining fibrinogen levels (>100 gr/L) and platelet counts (>80,000 cells/mm3) during ECMO support is recommended and should be closely monitored during therapy.

COMMENT 4: A limitation of the PTT is that any other process going on that impacts the PTT like DIC would make the PTT prolongation not necessarily a standalone accurate reflection of the ECMO device's participation in its prolongation. I do not think the article adds a lot to add to help a clinician in wrestling with questions like anticoagulation dosage and handling of bleeding. What it does add is it is a very good review of clotting in particular and in relationship to an ECMO device.
REPLY: We believe anticoagulation monitoring section was improved already by following previous reviewer´s recommendations. We gathered the best available evidence on bleeding management, though it is scare. We believe adding our institutional algorithm for bleeding management in ECMO patients adds interesting practical information for physicians at bedside. Thank you for your kind words.
CHANGES IN THE TEXT: Please refer to anticoagulation and bleeding changes already done following the recommendations of Reviewer A.

COMMENT 5: I think the article could be rendered more reader friendly to the practicing clinician by organizing it in the form of offering a series of recommendations in various situations such as the person with a clotted circuit, a person with a VTE, a person with an arterial clot, a person with bleeding, a person with thrombocytopenia and offer recommendations in each specific area even though they may not be fully evidence-based upon pivotal trials. I think that mode of presenting the valuable basic information would be more useful and easier to follow for to the reader.
REPLY: Thank you for your point of view. We chose this way of presenting the information following the

methodology for that purpose, presenting the most important evidence of a general aspect for ECMO management, that is the general management of anticoagulation to prevent thrombosis in ECMO, and the potential complications of anticoagulation therapies in ECMO. We think that presenting hypothetical clinical patients with specific thrombotic complications during ECMO is out of the scope of this review. Moreover, ECMO patients are complex and, most of the times, show combined features of different complications. Many aspects of anticoagulation and bleeding management are variable among institutions and are undergoing continuous development, we gathered the information giving the added value of presenting our institutional protocols which has led to good outcomes for patients.
CHANGES IN THE TEXT: NONE.

REVIEWER D

The authors are to be congratulated on a thorough review of anticoagulation in ECMO as this is no simple task. Two points to consider:

COMMENT 1: While target Xa and ACTs levels were mentioned on page 15, aPTT levels were not. It would be prudent to include there, typically goal of 60-80 seconds
REPLY: We included this information, according to previous reviewers comments on the protocol used in EOLIA trial.
CHANGES IN THE TEXT:
line 372: The EOLIA trial used a target aPTT time of 40 to 55 seconds (44). Anticoagulation monitoring based on aPTT was associated with more significant bleeding complications but fewer thrombotic events (45). Confounding factors for the interpretation of aPPT during anticoagulation treatment in ECMO include inherited factor deficiencies (e.g., hemophilia A or B, vWD, factor II, V, X, XI, or XII deficiencies), liver disease, combined vitamin K-dependent factor deficiency, disseminated intravascular coagulation, and lupus anticoagulant-type inhibitors (46-48).

46. Buchtele N, Schwameis M, Schellongowski P, Quehenberger P, Knöbl P, Traby L, et al. Prevalence and Clinical Impact of Reduced Coagulation Factor XII Activity in Patients Receiving Extracorporeal Membrane Oxygenation. Crit Care Med. 2021;49(12):e1206-e11.
47. Favaloro EJ. Welcome to Seminars in Thrombosis & Hemostasis 2023. Semin Thromb Hemost. 2023;49(1):1-2.
48. Levy JH, Staudinger T, Steiner ME. How to manage anticoagulation during extracorporeal membrane oxygenation. Intensive Care Med. 2022;48(8):1076-9.

COMMENT 2: One of the cruxes about monitoring AC is the discordance between ACT and Xa/PTT levels, which would be important to address
REPLY:
CHANGES IN THE TEXT: We addressed this issue and included the most important reference.
Line 379: In a previous study, ACT and aPTT had a low correlation with coagulation factor levels and heparin dose. The authors found Anti-Factor Xa Activity to be the most specific for heparin levels, and PT was most specific for monitoring coagulation and hemostasis for patients on ECMO (49).

49. Saifee NH, Brogan TV, McMullan DM, Yalon L, Matthews DC, Burke CR, et al. Monitoring Hemostasis During Extracorporeal Life Support. Asaio j. 2020;66(2):230-7.